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Journal of Medicinal Plants Research

Full Length Research Paper

Characterization of thirteen microsatellite loci from the Ghanian antimalarial plant *Cryptolepis sanguinolenta*

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Cryptolepis sanguinolenta **(Lindl.) Schlechter (Periplocaceae) is an herbaceous plant used in traditional medicine to treat malaria and populations of the species are diminishing due to overharvesting and lack of conservation. Codominant microsatellite markers that can be used to characterize genetic diversity and population structure are currently not available. Therefore, the study isolated 75 microsatellite loci from genomic sequence data, which were then screened for the ability to reveal polymorphisms. From the 75 candidate loci, 13 polymorphic microsatellite loci were optimized for future population genetics studies. Twenty-two *C. sanguinolenta* samples were collected from eight different geographical locations in Ghana. Alleles per locus ranged from 3 to 7 with a mean of 4.4. Expected heterozygosity ranged from 0.24 to 0.77, and all but one locus deviated significantly from Hardy-Weinberg equilibrium. Genetic differentiation mean was 0.06 among all loci, indicating relatively low genetic diversity in these samples. These microsatellite loci should be useful to study genetic diversity, gene flow and population structure as well as in a project involving breeding and conservation of *C. sanguinolenta.***

Key words: *Cryptolepis sanguinolenta*, genetic diversity, malaria, medicinal plant, microsatellites.

INTRODUCTION

Cryptolepis sanguinolenta (Lindl.) Schlechter (Periplocaceae) is an important perennial medicinal plant species indigenous to West and Central Africa with a long history of use in the treatment of malaria (Ankrah, 2010; Tempesta, 2010; Osei-Djarbeng et al., 2015). An aqueous extraction of its roots, the portion of the plant with the highest concentration of active antimalarial ingredients, yields indoloquinoline alkaloids, primarily cryptolepine, an N-methyl derivative of the indoloquinoline compound quindoline (Dwuma-Badu, 1978; Tachie et al., 1991). The extract may also have some anticancer properties as well (Ansah and Mensah, 2013). The harvested roots are sold in local markets.Non-sustainable destructive harvesting of the plants for the roots has resulted in a substantial decrease in wild populations (Jansen and Schmelzer, 2010).

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Its widespread use as a medicinal plant coupled with over-harvesting, calls for the formulation of effective management plans and conservation through cultivation with the ultimate goal of selecting high active ingredient producing plants for breeding (Amissah et al., 2016).

There is very limited information about the genetic diversity in populations of *C. sanguinolenta*. Amplified Fragment Length Polymorphism (AFLP) revealed low genetic diversity among 116 plants sampled from three regions in Ghana, but did not characterize the population structure or gene flow within the collected sites (Amissah et al., 2016). Microsatellites or single sequence repeats (SSRs) are codominant molecular markers, as compared to dominant AFLPs markers, that typically are polymorphic (Gupta et al., 1996) and well-suited for studying population genetic diversity and dynamics. They are most often employed to evaluate population structure, gene flow and inbreeding (Arnold et al., 2002; Zhang and Hewitt, 2003).

Currently, microsatellite molecular markers are not available to genetically characterize *C. sanguinolenta* individuals and populations. The study generated genome sequence data for *C. sanguinolenta,* and identified 13 polymorphic microsatellites that were used to characterize individuals from eight populations in Ghana, West Africa. The microsatellites from this study will be used to characterize a larger sample of the population and provide information on genetic diversity, population structure and gene flow of the species. The microsatellites should be very useful for a breeding program to create elite genotypes that produce high quantities of antimalarial compounds.

MATERIALS AND METHODS

A DNA library (target 400 bp lengthreads) was constructed from a *C. sanguinolenta* individual that was collected in Hweehwee Oboyan, Ghana. One microgram of DNA was used for library preparation using the Ion Xpress™ Plus gDNA Fragment Library Preparation kit (Life Technologies, Carlsbad, CA). Prior to sequencing, the library was quantified with the Ion Library Quantitation Kit (Life Technologies) and diluted to 20 pM. The library was then prepared for sequencing using the Ion PGM™ Template OT2 400 Kit (Life Technologies). The library was loaded on an Ion 318™ Chip v2 (Life Technologies) and sequenced on the Ion PGM™ System (Life Technologies) using the Ion PGM™ Sequencing 400 Kit (Life Technologies). Raw sequencing reads were trimmed and then assembled *de novo* into contigs using the default parameters in CLC Genomics Workbench (Qiagen). Only contigs ≥ 20X coverage were searched for microsatellite motifs (di- to hexa-nucleotide) and primers were designed using BatchPrimer3 (You et al., 2008) using default settings.

Genomic DNA was isolated from leaves of 22 *C. sanguinolenta* plants from 8 locations using a CTAB protocol (Porebski et al., 1997) and one DNA sample of *Pityopsis graminifolia* (Michx.) Small from Tennessee (USA) was used as a negative control for amplification. Ten µl reaction amplifications were completed as described in Hatmaker et al. (2015) using 75 primer pairs. The thermal cycler conditions consisted of 94°C for 3 min and 35 cycles of 94°C for 40 s, 55°C for 40 s and 72°C for 30 s, followed by 72°C for 4 min. Allelic products were separated via electrophoresis using the QIAxcel Capillary Electrophoresis System (Qiagen, Valencia, California USA) and the data binned into allelic classes according to the protocols described by Hatmaker et al. (2015). Data was analysed for number of alleles/locus, observed heterozygosity, expected heterozygosity, probability of deviation from Hardy-Weinberg equilibrium at $P<0.05$, Shannon's information index, and genetic differentiation (F_{ST}) using the program GenALEx 6.5 (Peakall and Smouse, 2012).

RESULTS AND DISCUSSION

A total of 4,910,467 reads (mode = 405 bp) and 1.3 Gb were generated from sequencing with a single Ion 318™ Chip (v2) on the Ion PGM™ System. After quality trimming, the reads were assembled *de novo* into 38,029 contigs with an average length of 1,721 bp and a N50 of 1,670 bp. Only contigs ≥ 20X coverage (n=1,928) were screened for microsatellites, and 821 were detected. Tri- and tetra-nucleotide motifs were the most common, with 36.1% of the motifs in each of these classes. The next most common class was the dinucleotide motifs at 13%. A total of 574 primer pairs were designed and of these 75 were randomly selected for amplification and detection of polymorphic alleles.

Seventy of the seventy-five primer pairs amplified microsatellite loci. Nineteen of the primers consistently yielded more than two products, twenty-five amplified only one allele/locus (monomorphic) for all plants included in the study and another thirteen primers amplified only two alleles (either as homozygotes or heterozygotes) per locus. Thirteen primers were polymorphic, revealed more than two alleles per locus, and were used for analysis in this study (Table 1). DNA from *P. graminifolia* did not amplify with any of the primer pairs, indicating accuracy of our results. The number of alleles/ locus was relatively low and ranged from 3 to 7 (mean 4.4), which has been observed in other endemic perennial species (Arroyo et al., 2016). The mean observed frequency of heterozygotes (H_o) in the total sample was 0.44, which deviated from the expected heterozygosity (H_e), indicating potential presence of population structure among the eight population collection areas (Hadziabdic et al., 2012). Expected heterozygosity ranged from 0.24 to 0.77, and all but one locus significantly deviated from Hardy-Weinberg equilibrium. However, more samples from each of these areas will be needed to determine population structure and gene flow. Shannon's diversity index averaged 0.85 among all loci. This index measures the number of unique genotypes in a sample population and how evenly the genotypes are distributed (Brown and Weir, 1983). Genetic differentiation (F_{ST}) value of 0.06 was calculated across the 13 loci Three of loci had a calculated F_{ST} ≥ 0.05, which showed moderate diversity, however 10 of the loci had a F_{ST} of ≤ 0.05 that indicated relatively low genetic diversity in these samples may portend the same for the general population (Hartl and Clark, 2007).

Annual and herbaceous perennial plants in

Table 1. Primer sequences, repeat motifs, annealing temperatures (T_a), number of alleles (A), allele size range, observed (H_o) and expected heterozygosity (H_e), Shannon's information index (I), and genetic differentiation (F_{ST}) of 13 microsatellite loci isolated from *Cryptolepis sanguinolenta*.

GenBank Accession No.	Locus	Primer sequences (5'-3')	Repeat Motif	Ta (°C)	A	Size range (bp)	Ho	He	I	F_{ST}
KU361063	Cry007	F: GACATAGCTTTGGAAGGGTAG R: TCAGGATCAATTTCCACTTG	$(AAAT)_3$	58	4	265-285	0.88	0.70*	1.27	0.03
KU361064	Cry021	R: GCTCCTGACCCCATATCAGTT F: GGTGACCCTCAATCTGATGAA	$(GAT)_4$	60	3	179-199	0.97	0.51*	0.75	0.00
KU361065	Cry025	F: GCCATTTGGAATGGTATCTCC R: TCACACAAGTGACCATGATTGA	$(TATTT)_3$	60	5	266-280	0.00	0.61*	1.12	0.07
KU361066	Cry030	F: GGCATTGGCCATATATACTCCT R: CATGTTCCAGGGCAGTAGAAA	$(AT)_7$	60	5	143-155	0.33	0.61*	1.06	0.01
KU361067	Cry031	F: AGGATACGTGGCTCCAAGATT R: AGGATGCCAATGAAAGGATCT	$(TAA)_4$	60	6	135-147	0.97	0.75*	1.47	0.04
KU361068	Cry032	F: GGAAGCATCCATAAGGAGGAG R: TGGATATTCTAGTTGCCTTGTGG	$(TTTA)_4$	60	5	107-122	0.97	0.64*	1.15	0.05
KU361069	Cry033	F: GAAGCCCCATTCTTTCGATAC R: TTAAACCTCCCAAGTGCTCAG	$(TTA)_5$	59	3	121-125	0.00	0.45*	0.74	0.09
KU361070	Cry035	F: CAACTCATAATCAGGGGTCAAA R: TGGAACTCTAAGGATTTGTGTC	$(ATTT)_3$	57	3	167-171	0.00	0.54*	0.82	0.02
KU361071	Cry036	F: GCGCAATTAAGAAATGCGTA R: CAGCGCATTTCCAACAATAA	$(TAT)_5$	59	4	244-262	0.13	0.56*	0.93	0.03
KU361072	Cry037	F: GCGCAATTAAGAAATGCGTA R: CAGCGCATTTCCAACAATAA	$(ATT)_5$	59	4	244-263	0.17	0.24*	0.45	0.12
KU361073	Cry 041	F: GAACAGTAGCGTAGCCCCAGT R: AAAGCACCAAGAAAAGATGAGG	$(ATAA)_3$	60	4	135-141	0.29	0.65	1.11	0.02
KU361074	Cry044	F: CGGTTCCCCTTCTACAATTTC R: CCCATCAACTAACGAAAAAGG	$(AAC)_5$	59	7	237-251	1.00	0.77*	1.55	0.03

Table 1. Cont'd.

KU361075	Cry047	F: TAGGGCTTCTTTCATGCGTTT R:CAGATTCAAGTGGACGAAACC	$(TTCC)_3$	60	4	134-146	0.03	0.39*	0.68	0.02
Means					4.4		0.44	0.57	0.85	0.06

*Significant deviation from Hardy-Weinberg equilibrium at P ≤ 0.05.

comparison to woody plant species have lower mean genetic diversity due to lack of polymorphic loci as well as narrow geographical distribution (Hamrick et al., 1992). The study finding agrees with the report by Amissah et al. (2016) that used AFLP analysis to reveal a low (25%) genetic diversity in 116 sampled plants. Additional samples and analyses need to be completed to confirm our preliminary findings using micro-satellite analysis.

Conclusion

The study provides defined codominant molecular markers for *C. sanguinolenta.* A total of 13 primer pairs can be used to amplify polymorphic microsatellite loci that should provide more complete assessment of genetic diversity, gene flow and population structure of this species. Additionally, these markers should be a valuable tool for breeding elite genotypes and conservation of *C. sanguinolenta*.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Antimicrobial and antioxidant activities of *Cymbopogon schoenanthus* (L.) spreng. essential oil, growing in Illizi - Algeria

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Hydrodistilled volatile oil obtained from the aerial parts of *Cymbopogon schoenanthus* cultivated near Illizi, Algeria, was analyzed by Gas Chromatography Mass Spectrometry (GC-MS) and Gas Chromatography – Flame Ionization Detector (GC-FID). More than twenty compounds were identified, representing 94.636% of the total oil. The major constituents of essential oil were piperitone (63.35%), β-eudesmol (9.305%) and elemol (6.915%). Isolated essential oil was tested for radical-scavenging ability using the stable 2,2-diphenylpicrylhydrazyl (DPPH) radical, the 2,2'-azino-bis (ABTS) radical and for reducing power ability with a test based on the reduction of ferric cations (FRAP). In all tests, oil did not show a prominent antioxidant activity. The screening of antimicrobial activity of oil was individually evaluated against representatives of gram-positive, gram-negative bacteria and fungi, using the agar diffusion method. All tested microorganisms were inhibited by the essential oil of *C. schoenanthus.*

Key words: *C. schoenanthus*, essential oil, antioxidant activity, antimicrobial activity.

INTRODUCTION

The use of plants for treating diseases is as old as the human species. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known. Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases. Currently, data on the antimicrobial activity of numerous plants, so far considered empirical, have been scientifically confirmed (Silva et al., 2010).

In Sahara of Algeria, the flora is very rich in medicinal plants which produce valuable natural substances such as essential oil. Actually, essential oil and their

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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). Natural antioxidants are also in high demand for application as nutriceuticals as well as food additive because of consumer preferences (Neffati et al., 2009).

As part of the study evaluation of the biological effectiveness of the essential oil from the medicinal plants, the study presented a study of the antioxidant and antibacterial activities associated with the chemical composition of essential oil isolated from *Cymbopogon schoenanthus* (Poaceae). This plant is a sub-spontaneous grass, tropical-afro-asiatic, which is used as traditional medicines to treat digestive diseases: aerophagia, flatulence and urinary decrease, analeptic drink for new mother after childbirth, bad breath, gumboils and urinary incontinence (Hammiche et al., 2006).

MATERIALS AND METHODS

Plant material

The aerial parts of *C. schoenanthus* were collected from weddi tasset in Illlizi (Algeria), on April, 2014. The vernacular name of this plant is lemmad (the Arabic name) or tiberrimt (the Berber name). Samples of the plants were identified by Dr. Amar Eddoud department of Agricultural Sciences, university of Kasdi Merbah-Ouargla, Algeria

Essential oil extraction

The fresh aerial parts of *C. schoenanthus* were subjected to hydrodistillation in a modified Clevenger-type apparatus for 3 h. The essential oil was obtained with 2.695 % (w/w) of yield and was dried over anhydrous sodium sulfate and stored in sealed glass vials at 4 to 6°C prior to analyses.

Chemicals

All chemicals were of analytical grade. DPPH (2,2-diphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), potassium persulfate ($K_2S_2O_8$), methanol, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), TPTZ (2,3,5- triphenyl-1,3,4-triaza-2 azoniacyclopenta-1,4-diene), DMSO (dimethylsulfoxide). Were supplied from Sigma-Aldrich.

Essential oil chromatographic analysis

0.2 µL of sample was injected on a gas chromatography (Hewlett-packar computerized system, Agilent model 6890 GC coupled to a Agilent 5973 N mass selective detector , and equipped with an Agilent technologies capillary HP-5MS column (30 m, 0,25 mm I.d, 0.25µm thickness), a split/splitless injector used in the split mode (200:1), using Helium (20 mL/min). The initial temperature of the column was 40°C which was heated gradually to 325°C with a 2°C /min. High purity helium was used as carrier gas at 36 cm/s. The quadrupole, source and transfer line temperatures were maintained at 150, 230 and 280°C, respectively. A solvent delay of 3 min. Identification of components was assigned by matching their mass spectra of peaks with those obtained from authentic samples and/or the Wiley and NIST library data, and published data (Hellai and Hadj-Mahammed, 2008). The component concentration was obtained by semi-quantification by peak area integration from GC peaks.

Antioxidant activity determinations

Three methods have been applied for the antioxidant assessment of the *C. schoenanthus* essential oil in this study: the 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) assay (Brand-Williams et al.,1995), the 2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonate) radical cation ($ABTS^{\cdot +}$) assay (Miller et al.,1993) and the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). All measurements were performed in duplicate.

DPPH assay

The scavenging activity of $DPPH^{\cdot}$ was determined based on the tests described by Brand-Williams et al. (1995), with some modifications. Various concentrations of essential oil in methanol (1mL) were mixed with methanolic solution containing DPPH radicals (0.1mM). After vigorous agitation, the mixture was incubated for 1 h in the dark at room temperature, and then the absorbance is measured at 515 nm with a UV spectrophotometer screws (JASCO-V530). A solution containing 1 mL methanol and 2 mL of DPPH radicals was used as blank. The estimation of the antiradical activity is expressed by the value of the percent inhibition (IC (%)) according to the following formula:

$$IC\ (\%) = [(A_0 - A_x)/A_0] \times 100.$$

Where A_0 is the absorbance of analytical blank and A_x is absorbance in the presence of the extract solution.

Different sample concentrations were used in order to obtain antiradical curves for calculating the IC_{50} (= EC_{50}, the effective concentration) values. Antiradical curves were plotted referring to concentration on the x axis and their relative scavenging capacity on the y axis. The IC_{50} value, defined as the concentration of antioxidant that causes a 50% decrease in the $DPPH^{\cdot}$ absorbance or the extract concentration providing 50% inhibition. A lower IC_{50} value indicates greater antioxidant activity. Evaluation of free radical-scavenging activity was performed with Trolox equivalent antioxidant capacity (TEAC) assay (Neffati et al., 2009; Ćavar et al., 2012; Chen et al., 2013).

ABTS assay

The free-radical scavenging capacity was measured using the ABTS decoloration method (Re et al., 1999) with some modifications. Briefly, ABTS was dissolved in water to get a 7 mM concentration. ABTS radical ($ABTS^{\cdot +}$) was produced by reacting this stock solution with a 2.45 mM $K_2S_2O_8$ solution and allowing the mixture to stand in the dark at room temperature for 12 to 16 h. The $ABTS^{\cdot +}$ solution obtained was blue-green coloration which can be stored at -20°C. Before use, the formed solution was diluted with methanol to an absorbance of 0.70±0.02 at 734 nm. Samples were separately dissolved in methanol. In order to measure the

antioxidant activity of essential oils, 10 µL of each sample at various concentrations was added to 990 µL of diluted $ABTS^{\cdot+}$. The absorbance was measured spectrophotometrically at 734 nm using a UV spectrophotometer screws (JASCO-V530). Methanol was used to zero the spectrophotometer; $ABTS^{\cdot+}$ solution was used as blank sample. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of $ABTS^{\cdot+}$ (IC (%)), were calculated according to the formula

$$IC\ (\%) = [(A_0 - A_x)/A_0] \times 100$$

A_x and A_o were the absorbance at 734 nm of samples with and without essential oils, respectively.

IC_{50} values, defined as the inhibiting concentrations of substrate that causes 50% loss of ABTS activity (color), were calculated by regression analysis. A lower IC_{50} value indicates greater antioxidant activity. Evaluation of free radical-scavenging activity was performed with Trolox equivalent antioxidant capacity (TEAC) assay (Neffati et al., 2009; Ćavar et al., 2012; Chen et al., 2013).

FRAP assay

This method measures the ability of antioxidants to reduce the ferric iron. It is based on the reduction of the complex of ferric iron and 2,3,5-triphenyl-1,3,4-triaza-2 azoniacyclopenta-1,4-diene (TPTZ) in the ferrous form under acidic conditions. The reducing power is determined by the method described by Binsan et al. (2008). The FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM TPTZ in 40 mM HCl) and $FeCl_3 \cdot 6H2O$ (20 mM) in a ratio of 10:1:1 (Benzie and Strain, 1996). The FRAP solution was incubated at 37°C for 30 min. In a volume of 150 µL were prepared different concentrations of the sample to which was added 2850 µL of FRAP solution, incubated 30 min in the dark. Complex formation ferrous tripyridyltriazine (colored product) is measured by reading absorbance at 593 nm. The activity is expressed as Trolox equivalent (micromoles TE/g of extract).

Calculation of Trolox equivalent antioxidant capacity (TEAC)

The free radical-scavenging activity of each sample was expressed as Trolox equivalent antioxidant capacity (TEAC), which was obtained by comparing the absorbance change at 515 nm for DPPH essay, at 734 nm for ABTS essay and at 593 nm for FRAP essay, in a reaction mixture containing a sample of plant extract or test material with that containing Trolox. This index is defined as the millimolar concentration of a Trolox solution whose antioxidant capacity is equivalent to 1.0 mg of the extract (Re et al., 1999).

Antimicrobial screening

The antimicrobial activities were determined by using the drop agar diffusion method (Lopes-Lutz et al., 2008). The microorganisms tested were the fungi *Candida albicans ATCC 10231*, and the bacteria *Escherichia coli ATCC 8739, Salmonella typhimurium ATCC 14028, Staphylocous aureus ATCC 6538, Enterococcus feacium ATCC 19434, and Streptocoque B (Streptococcus agalactiae).* The oils were diluted in 10% DMSO/sterile H_2O solution. A suspension of the tested microorganisms was spread on the appropriate solid media plates and incubated overnight at 37°C (for the bacteria) or 25°C (for *conidia* of filamentous fungi). After 1 day, 4-5 loops of pure colonies were transferred to saline solution in a test tube for each bacterial strain and adjusted to the 0.5 McFarland turbidity standard (~10^8 cells/mL) (National Committee for Clinical Laboratory Standards (NCCLS), 1999). Sterile cotton dipped into the bacterial suspension and the agar plates were streaked three times, each time turning the plate at a 60° angle and finally rubbing the swab through the edge of the plate. Sterile paper discs (Glass Microfibre filters, Whatman; 6 mm in diameter) were placed onto inoculated plates and impregnated with the diluted solutions (15µL/ disc). Ampicillin (10µg/disc) was used as positive control for all strains except *C. albicans* for which Nystatin (100µg/disc) was used. Inoculated plates with discs were placed in a 37°C (or 25°C for *conidia*) incubator. After 24 h of incubation, the results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. The test was run in duplicate.

RESULTS AND DISCUSSION

Chemical composition of essential oil

A total of 35 compounds were detected in the study essential oil extracted from *C. schoenantus* but just 24 compounds were identified which accounted for 94.636 % of the essential oil (Table 1). The major component was piperitone (63.35%), the dominant compounds in lemmed oil were oxygenated monoterpenes (65.361 %) followed by oxygenated sesquiterpenes, monoterpens hydrocarbons and sesquiterpenes hydrocarbons. The same compounds have been obtained in a work done previously, when the study used the microwave-assisted hydrodistillation method to extract the essential oil of *C. schoenantus* in order to determine the impact of the extraction method on volatile constituents was used, 33 compounds were identified in this sample, the most abundant components were oxygenated monoterpenes (74.6 %) with high content of piperitone (72.6 %), monoterpens hydrocarbons (11.30 %) followed by oxygenated sesquiterpenes (9.79 %) and sesquiterpenes hydrocarbons (1.48 %) (Hellalli et al., unpublished data).

The literature review showed variation between chemical compositions of essential oil of *C. schoenantus*, depending on geographical origin. Due to the numerous published articles about chemical composition of *C. schoenantus* essential oil, in this section the recent findings will be discussed. The essential oil of the Togolese *C. schoenantus* (Ketoh et al., 2005, 2006; Koba et al., 2004) is characterized with high content of piperitone (61.01 to 69.01%) and the 2-carene (16.48 to 23.4%). While same species cultivated in Burkina Faso was not very rich in piperitone (42%) and the 2-carene (8.2%), but there is a significant presence of elemol (6.2%) (Onadja et al., 2007).

The same majority compounds were reported in the essential oil obtained from same plant species of Benin, with percentage of piperitone (58.9%), the 2-carene (15.5%) and elemol (5.3%) (Bossou et al., 2015). The Tunisians *C. schoenantus* essential oil showed variations in chemical composition of volatiles. According to Khadri et al. (2010), this species from Tunisia was characterized with high content of limonene (10.5 to 26%), and β-

Table 1. Chemical composition of essential oil of *C. schoenantus.*

N	RI	Component	Percentage (%)
1	1000	2-Carene	4.923
2	1025	Limonene	1.413
3	1031	cis-β-Ocimene	0.014
4	1042	trans-β-Ocimene	0.151
5	-	D-fenchone	0.037
6	1129	1-terpineol	0.429
7	1181	α-terpineol	1.447
8	-	Cis piperitol	0.106
9	-	Trans piperitol	0.029
10	1250	Piperitone	63.350
11	1377	β-elemene	0.616
12	1418	β-caryophyllene	0.060
13	1433	Calarene	0.094
14	1448	α-caryophyllene	0.032
15	1477	Germacrene D	0.210
16	1483	β-selinene	0.269
17	1490	α-selinene	0.242
18	1495	α-muurolene	0.562
19	1513	γ-cadinene	0.345
20	1517	δ-cadinene	0.946
21	1546	Elemol	6.915
22	1616	10-epi-γ-eudesmol	1.266
23	-	β-eudesmol	9.305
24	1656	α-eudesmol	1.875
-	-	monoterpens hydrocarbons	6.501
-	-	oxygenated monoterpenes	65.398
-	-	sesquiterpenes hydrocarbons	3.376
-	-	oxygenated sesquiterpenes	19.361

RI = retention index relative to n-alkanes (C6–C17) on SE 30 capillary column (25 m x 0.25 mm i.d., 0.22µm film thickness). **%** = percentage calculated by GC-FID on HP-5MS capillary column.

phellandrene (8.2 to 16.2%). In contrast, the content of sesquiterpenoid compounds was relatively low. Recently *C. schoenantus* from the eastern of Algeria was studied by Hadef et al. (2015), the most important compound detected in leaves was 4-isopropyl-1-methyl-2-cyclohexen-1-ol (cis) (15%).

As a result the study can say that the essential oil obtained from *C. schoenantus* showed significant variation in their chemical composition depending on geographical origin. Generally, *C. schoenantus* essential oils were characterized by high percentages of the monoterpenenoid compounds, such as piperitone, 2-carene and limonene.

Antioxidant activities

The antioxidant activity of *C. schoenantus* essential oil has been evaluated by three testing methods ABTS, DPPH and reducing power, the results is summarized in Table 2. It was found that the essential oil showed very different antioxidant capacities. The DPPH assay is most extensively used to evaluate antioxidant activity of plant extracts, foods and single compounds, thanks to its stability and its easiness. This assay is based on the measurement of the reducing ability of antioxidants toward DPPH radical, through electron spin resonance (EPR) detection or by measuring the decrease of its absorbance. DPPH is a stable and commercially available organic nitrogen radical, which reacts with hydrogen/electron donor compounds and has a maximum UV-V is absorption within the range of 515 to 520 nm. Upon reduction, the radical solution becomes discoloured according to the number of electrons paired (Chen et al., 2013).

The estimate of the anti-radical activity is based on a colorimetric test based on the measurement of the relative ability of an extract to trap preformed radical

Table 2. Antioxidant activity of *C. schoenanthus* expressed in TEAC and $IC_{50.}$

Variables	DPPH	ABTS	FRAP
TEAC (µmol/g)	1.032	11.685	3.33±0.3
IC_{50} (mg/mL)	44.213±3.78	18.91±0.17	-

Averages ± Standard Deviation were obtained from two different experiments.

Table 3. Antimicrobial activity of *C. schoenantus e*ssential oil.

Name of bacterial strain	Inhibition diameter (mm)*		Dilution ratio**
	Ampicillin/Nystatin 10 µg/100 µg	*C. schoenantus*	
Escherichia coli ATCC 8739	12±1	15±1.4	1
Salmonella typhimurium ATCC 14028	17.33±1.5	10.5 ±0.7	1
Staphylococcus aureus ATCC 6538	44.3±0.5	19.5 ±0.7	1/8
Enterococcus faecium ATCC 19434	45.3±1.5	21±1.4	1/32
Streptocoque B (Streptococcus agalactiae)	34.3±0.5	12.75 ±0.3	1/16
Candida albicans ATCC 10231	42±1	12±1.4	1/16

*Including disc diameter of 6 mm, Averages ± Standard Deviation were obtained from two different experiments; ** The essential oil was diluted in 10% DMSO/sterile H_2O solution

$ABTS^{\cdot+}$. The latter is generated by the oxidation of ABTS (2,2'-azinobis- acid (3-ethylbenzthiazoline-6-sulfonic acid) with potassium persulphate giving a blue-green solution. The radical $ABTS^{\cdot+}$ is reduced in the presence of an antioxidant electron donor compound (Re et al., 1999). This reduction results in a proportional bleach percent inhibition of the chromophore $ABTS^{\cdot+}$ and depending on the concentration of the antioxidant. These measurements are compared to the reactivity of a reference compound, generally Trolox. This defines the Trolox equivalent antioxidant capacity (TEAC). The ABTS method is used to study the activity of the compounds hydrophilic and lipophilic antioxidants, pure compounds and extracts for food (Re et al., 1999).

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma (FRAP, also Ferric ion reducing antioxidant power), which was first performed by Benzie and Strain (1996), as a measure of antioxidant power. The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored ferrous tripyridyltriazine (Fe^{II}-tripyridyltriazine) compound from the colorless oxidized Fe^{III} form by the action of electron donating antioxidants (Politeo et al., 2006).

Comparing IC_{50} and TEAC values obtained (Table 2) the *C. schoenantus* essential oil showed an antioxidant activity in the ABTS essay most important than in the DPPH essay, although DPPH and ABTS methods were based on the same principle, data obtained from ABTS assay are lower than those obtained from DPPH assay (Ćavar et al., 2012). In order to compare results given earlier, the study tested the ability of *C. schoenantus* essential oil of the reducing power of the ferric ion (F^{+3}) to corresponding ferrous ions (F^{+2}).

In general, the antioxidative effectiveness of essential oil depends on the content of phenolic compounds and the reaction activity of the phenol towards the chain-carrying peroxyl radicals and on the stability of the phenoxyl radical formed in the reaction (Ćavar et al., 2012). The essential oil obtained from *C. schoenantus* growing in Illizi is markedly rich in non-phenolic constituents, due to this fact the essential oil is known to be relatively weak antioxidant.

Antimicrobial activity

The antimicrobial activity of *C. schoenantus* essential oil was evaluated by a paper disc diffusion method. The data show that the essential oil of *C. schoenantus* exhibited strongly all the tested strains, but in variable degree. The results presented in Table 3, reveal that *C. schoenantus* essential oil inhibited strongly the growth of *E. faecium* and *S. aureus.* The study used antibiotic ampicillin as a positive probe.

Generally, the higher resistance among Gram-negative bacteria could be ascribed to the presence of their outer phospholipidic membrane, almost impermeable to lipophilic compounds (Rattanapitigorn et al., 2006; Boukhris et al., 2012). The absence of this barrier in Gram-positive bacteria allows the direct contact of the essential oil hydrophobic constituents with the phospholipids bilayer of the cell membrane, where they bring about their effect, causing either an increase of ion

permeability and leakage of vital intracellular constituents, or impairment of the bacteria enzyme (Dorman and Deans, 2000; Burt, 2004; Boukhris et al., 2012). That is way the results indicated that all the Gram-positive bacteria were the most sensitive strains tested to the oil of *C. schoenantus, especially E. faecium* (with dilution ration (1/32). But, it's known that Enterococci are normal commensals of the human intestinal tract and may cause various kinds of infections, including primary bacteremia, endocarditis, meningitis, and urinary tract infection.

E. faecalis and *E. faecium* account for the vast majority of clinical isolates in human enterococcal infections. These organisms have intrinsic and, often, acquired resistance to a number and high frequency of antibiotics, causing treatment of serious infection to be challenging (Murray, 1990; Arias and Murray, 2012). Tested oil showed a strong antimicrobial activity against all microorganism species, this can explained the effectiveness in the traditional treatment to heal the urinary tract diseases, digestives diseases and to post partum care for the new mother. This allows the study to confirmed the antimicrobial properties *in vitro* of *Cymbopogon schoenantus,* from Illizi (Extreme south-east of Algeria), through the effectiveness of its essential oil against the microorganisms tested. The high antimicrobial activity registered of *C. schoenantus* essential oil, can be explained by the high proportion of piperitone. This component was found in many plants like Mentha, Cymbopogon and *Eucalyptus* spp. Piperitone isolated from *C. schoenantus* in Togo was essentially a (+) enantiomer as generally observed in *Cymbopogon* spp. The good insecticidal properties of piperitone have been already reported. Indeed, piperitone, isolated from the essential oil of *Artemisia judaica*, has demonstrated a complete antifeedant activity at a concentration of 1000 µg/mL against the third instar larvae of *Spodoptera littoralis* using non-choice leaf discassay (Abdelgaleil et al., 2008; Bossou et al., 2015).

Furthermore when tested against *Callosobruchus maculatus,* piperitone, isolated from *C. schoenanthus*, was more toxic by fumigation to adults with a IC_{50} value of1.6 µL/L vs. 2.7 µL/L obtained with the crude extract (Ketoh et al., 2006; Bossou et al., 2015). Piperitone was also reported as a powerful repellent and antiappetent agent against ant of *Crematogaster* spp. Against *C. maculatus*, piperitone was very toxic to adults, newly laid eggs and to neonate larvae. But it was less efficient against individuals developing inside the seeds than the crude oil. The high larvicidal activity observed on 5-day old larvae with the crude oil was probably due to other compounds, which can act on various target sites at insect level like monooxygenases or acetylcholinesterases (Ketoh et al., 2006). The antimicrobial activities have been mainly explained through the presence of oxygenated sesquiterpenes and monoterpenes. The synergistic effect of essential oil components is a promising field that could lead to the optimization of a given bioactivity (Alitonou et al., 2012).

Conclusion

To the best of the study knowledge, this is the first study providing data on antibacterial, antifungal and antioxidant activities of the essential oil of *C. schoenanthus* from Algeria especially from the region of Illizi. Their antibacterial and antioxidant activities was studied *in vitro* on five bacterial strains and one fungal strain, all microbial strains were inhibited by the oil obtained from *C. schoenanthus*, but it strongly inhibits the growth of *Enterococcus faecium*. *C. schoenanthus* essential oil showed that it can be considered as a relatively weak antioxidant, this may be due to its lack of phenolic components.

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Conflict of interests

The authors have not declared any conflict of interests.

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