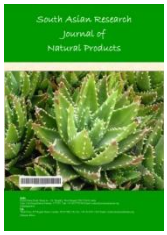

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Antimicrobial, Antioxidant Activities and Phytochemical Screening of Extracts and Essential Oil of *Cymbopogon schoenanthus* Flowers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Antibiotic resistance represents one of the major worldwide health problems. Though many medicinal plants are traditionally used to treat microbial infections. The antimicrobial potential of *Cymbopogon schoenanthus* flowers has not been investigated yet. Furthermore, exploring novel antioxidants has been a major goal for the management of various human health problems.
Objectives: To evaluate *in vitro* antimicrobial and antioxidant activities as well as a phytochemical screening of *Cymbopogon schoenanthus* flowers extracts and essential oil.
Materials and Methods: The methanol, aqueous extracts and essential oil of *Cymbopogon schoenanthus* were tested for antimicrobial activity against standard gram positives bacteria

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(*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungus of *Candida albicans*. Antioxidant activity was determined by using DPPH radical scavenging assay, and then extracts were subjected to qualitative phytochemical screening for the major secondary metabolites.

Results: Extracts and volatile oils showed promising activities against standard microorganisms with inhibition zone values 20-14 mm/20µL and minimum inhibitory concertation values 3.12 to 12.5 mg/ml. The highest activity was associated with methanol extract and volatile oil. Antioxidant activity found in order; methanol extract > aqueous extract > essential oil with DPPH radical scavenging activity (%) 77.0 ± 0.06, 64.0 ± 0.03, 40.0 ± 0.04 respectively. Phytochemical screening indicates the presence of, alkaloids, tannins, flavonoids, saponins, steroids, and triterpenes.

Conclusion: *Cymbopogon schoenanthus* flowers extract and volatile oils showed promising antimicrobial and antioxidant activities. However, a detailed phytochemical study of these extracts and the essential oil is essential to standardize the extract and isolate the active principle(s) responsible for their activities.

Keywords: Cymbopogon schoenanthus; antimicrobial; antioxidant; phytochemical.

1. INTRODUCTION

Antibiotic resistance represents one of the major health problems worldwide, and it is responsible for 700,000 to several million deaths per year [1]. The resistance of bacteria to antibiotics can occur by different mechanisms such as chromosomal mutation or by an exchange of genetic material throughout the transformation, transduction, or conjugation via plasmids [2,3]. Also, bacteria may possess transposons, which are able to enter transmissible plasmids or chromosomes [4]. Moreover, resistance can diffuse between Gram-positive species through inter genus transformation [5]. Multiple antibacterial drugs resistance is attributed to the irrational use of antibiotics for the management of the infectious disease [6,7]. In addition, the use of many antimicrobial agents is associated with side effects involving hypersensitivity, allergic reactions and immune-suppression [8]. This situation initiates researchers to search for new antimicrobial molecules from medicinal plants to be used as an alternative for the management of infectious diseases [9,10].

Medicinal plants are considered as a rich source of secondary metabolites with characteristics of high chemical diversity and biochemical specificity. Therefore, phytoproducts continue to offer great promise as potentially effective antimicrobial and antioxidant agents. Up to date, medicinal plants are utilized as a source of many effective therapeutic and preventive agents including antimicrobial and antioxidants [11]. Antioxidants, in addition to their applications for the prevention of many diseases and maintaining good health, they have an indirect role in protecting humans against infections by stimulating the immune system [12].

Cymbopogon schoenanthus (L. Spreng) of the Poaceae family is used in folk medicine as anti-spasmodic, antimalarial, and protection against fever, [13]. In addition, it is widely used in the north of Africa as an aromatic tea [14]. However, *C. schoenanthus* flowers are not subjected to sustained scientific evaluations of for their antimicrobial and antioxidant potentials to date. Therefore, the aim of this study is to evaluate in vitro antimicrobial and antioxidant activities as well as the phytochemical screening of the plant extracts.

2. MATERIALS AND METHODS

2.1 Plant Material

The flowers of *C. schoenanthus* were collected from Kordofan state in March 2018. The plant was authenticated by a specialist at Medicinal and Aromatic Plants and Traditional Medicine Research Institute, National Center for Research, Khartoum, Sudan, and a voucher specimen was deposited at the institute herbarium.

2.2 Standard Microorganisms

Gram-positive microorganisms used were *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923), Gram-positive micro-organisms used were *Escherichia coli,* (ATCC 25922) and *Pseudomonas aeruginosa*, (ATCC 27853), the fungus used was *Candida albicans* (ATCC7596).

2.3 Preparation of the Extracts

Shade dried plant material was grounded into coarse powder using mortar and pestle and extracted with methanol (sd fine-chemi limited)

according to the procedure described by Sukhdev et al. [15], briefly, 100 gm of the plant material was soaked in 1000 ml of absolute methanol at 25-30ºC for 2h hours and then filtered. The extraction was repeated three times with the same solvent. The extract was collected and the solvent was evaporated under reduced pressure to dryness using rotary evaporator (Buchi, Switzerland). The Aqueous extract was prepared by soaking 100 g of the plant material in 1000 ml hot distilled water for four hours with continuous steering. Then, filtered and evaporated using freeze drier. Then the yield percentage was calculated for each extract using the following formula:

Yield percentage (w/w) = (Weight of the dried extract × 100) / (Weight of the plant material)

2.4 Preparation of the Essential Oil

100 g of shade dried and powdered plant material was submitted to hydro-distillation for four hours in Clevenger apparatus (Duran West Germany) utilized for the production of essential oils lighter than water. The essential oil was pipetted and dried over sodium sulfate anhydrous and stored in a sealed, dark container in the refrigerator till required.

2.5 Preparation of Bacterial Inoculum

One ml aliquots of 24 hours, broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to give a suspension containing about 108- 109 C.F.U/ ml. The suspension was stored in the refrigerator at 4°C till used.

2.6 Preparation of the Fungal Suspension

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspension in 100 ml of sterile normal saline, and the suspension was stored in the refrigerator until used.

2.7 Evaluation of *in vitro* Antimicrobial Activity

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS) [16]. Microorganisms suspension was diluted with sterile physiological solution to 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of microorganism's suspension were swabbed uniformly on the surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (What man No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured and compared with a standard antibacterial and antifungal agent.

In addition, the minimum inhibitory concentration (MIC) was determined following the protocol of the National Committee for Clinical Laboratory Standards (NCCLS, 2000); each selected plant extract was subjected to a serial dilution using sterile nutrient broth medium as diluents. The volume of the plant extract to the broth medium (v/v) were 1:2, 1:2.5, 1:3, 1:3.5, and 1:4. Each plant extract dilution was inoculated with 20µl of an individual microorganism present in its log phase. All inoculated dilutions were set at 37ºC for 24 h. The highest dilution of the plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism is recorded as the MIC value of the extract.

2.8 Evaluation of Antioxidant Activity

The DPPH radical scavenging was determined according to the method of Shimada et al. [17] with minor modification; in 96- wells plate, the test samples were allowed to react with 2.2Di (4-test-octyl phenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. The concentration of DPPH was kept as (300µM). The test samples were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, the decrease in absorbance was measured in 517 nm using multiple reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. All tests and analysis were run in triplicate.

2.9 Phytochemical Screening

Qualitative phytochemical screening of methanol and aqueous extract for different secondary

metabolites, namely alkaloids, flavonoids, tannins, triterpenoids, saponins, steroids, and anthraquinone was carried out using standard protocols [18-21].

3. RESULTS AND DISCUSSION

3.1 Extraction and Hydrodistillation

C. schoenanthus flowers were extracted separately by maceration with methanol and infusion with water. The essential oil was produced by hydrodistillation, and the percentage yield was recorded (Table 1). The yield percentage of the aqueous extract is higher than the yield of methanol extracts; this may be attributed to the ability of hot water to dissolve a wide range of polar constituents.

3.2 Antimicrobial Activity

The two extracts and essential oil of *C. schoenanthus* were screened for their antimicrobial activity against standard organisms including gram-positive and gram-negative bacteria and fungus of *candida albicans*,, the average of the diameters of the growth inhibition zones obtained are shown in Table 2. (The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active).

Many antibiotics are deriving from different natural sources such as medicinal plants and others [22-23], therefore the initial step to develop a new antimicrobial agent from the plants is an in vitro antimicrobial activity assay [24]. The extracts and the essential oil showed good activity against tested microorganisms; the activity of three extracts is near and comparable to each other. The essential oil has similar effectiveness against pseudomonas aeruginosa and *C. albicans*, less active against *E. coli* and *S. aureus* and highly active against B. subtilis with large inhibition zone. The methanol and aqueous extract approximately have the same potency toward *E. coli, Ps. aeruginosa* and *C. albican*s, for *B. subtilis* methanol extract more active rather than the aqueous extract and vice versa in case of *S. aureus*. Gentamycin used as a standard antibacterial agent. In a comparing of gentamycin with the three extracts it had no activity against two-gram negative bacteria. This may be due to the gram-negative bacteria is high resistance. Gentamycin is strongly active against gram-positive *S.aurous*, also it more active toward gram positive *B. subtilis* rather than other extracts except for the essential oil. Nystatin also used as a standard antifungal agent, from the result in Table [2] nystatin is too more active than the three extracts in case of *C. albicans*. Finally, it is clear that; type and strain of microorganisms have a role on the activity variation of the plant extracts, there for identification of sensitivity of these microorganisms toward antimicrobial agents is important to the assessment of biological activity [25].

For further confirmation of antimicrobial activity, the minimum inhibitory concentration was measured and presented in Table 3. The MIC values of the extracts and essential oil on the five microorganisms varied from 3.125 to 12.5 mg/ml. The methanol extracts showed the highest activity against *Ps. aereuginosa, S. aureus* and *candida albicans* with MIC 3.125 mg/ml. The essential oil is the second most active and has

Table 1. The yield percentage of *C. schoenanthus* extracts and essential oil

Extract	Weight of the plant material	Weight of extract	Yield %
Methanol extract	100 gm	7.0 gm.	7.0 (w/w)
Aqueous extract	100 gm	9.5 gm.	9.5 (w/w)
Essential oil	100 gm	1.7 ml	1.7 (w/v)

Table 2. Results of susceptibility test for the *C. schoenanthus* extracts and essential oil (Inhibition zones; mm⁄20µL)

Microorganism	Methanol extract	Aqueous extract	Essential oil	Gentamycin	Nystatin
E. coli	15	15	16	0	-
P. aeruginosa	18	17	18	0	-
S. aureus	16	18	17	28	-
B. subtilus	17	14	20	19	-
C. albicans	15	14	18	-	26

the same MIC against all tested bacteria (6.25 mg/ml), and 3.125 mg/ml for *C. albicans.* which the oil exhibit low concentration of inhibition. The aqueous extract has poor activity with the same MIC against all tested microorganisms (12.5 mg/ml). Moreover, results obtained indicated that the antimicrobial activities were distributed into different extracts suggesting the existence of different antimicrobial principles. Methanol extract has different concentration against different microorganisms this is may be attributed to, methanol extract contains many different phytochemical groups lead to variation in the result.

By comparing of three extracts, methanol extract was more active rather than other extracts except in case of *E. coli* and *B. subtilus* which have the same activity of the aqueous extract.

3.3 Antioxidant Activity

DPPH radical scavenging assay revealed varying degrees of antioxidant activity (Fig. 1). In comparison to propyl gallate used as a positive standard. The highest activity was associated with methanol extracts, followed by the aqueous extract. The essential oil showed the lowest activity. The high this may be due to methanol extract contain many phenol groups or compound. Reactive oxygen species are contributed in a variety of pathological processes, including cellular signal transduction, cell necrosis, oxidative stress, cancer and immune response to microbial infections [26,27].

In DPPH radical scavenging assay, in the presence of an antioxidant which can donate an electron to DPPH, the purple color of free DPPH

Table 3. Result of a minimum inhibitory concentration of the *C. schoenanthus* extracts and essential oil(mg/ml)

Microorganisms	Essential oil	Methanol extract	Aqueous extract
E. coli	6.25	12.5	12.5
P. aeruginosa	6.25	3.125	12.5
S. aureus	6.25	3.125	12.5
B. subtilus	6.25	12.5	12.5
C. albicans	3.125	3.125	12.5

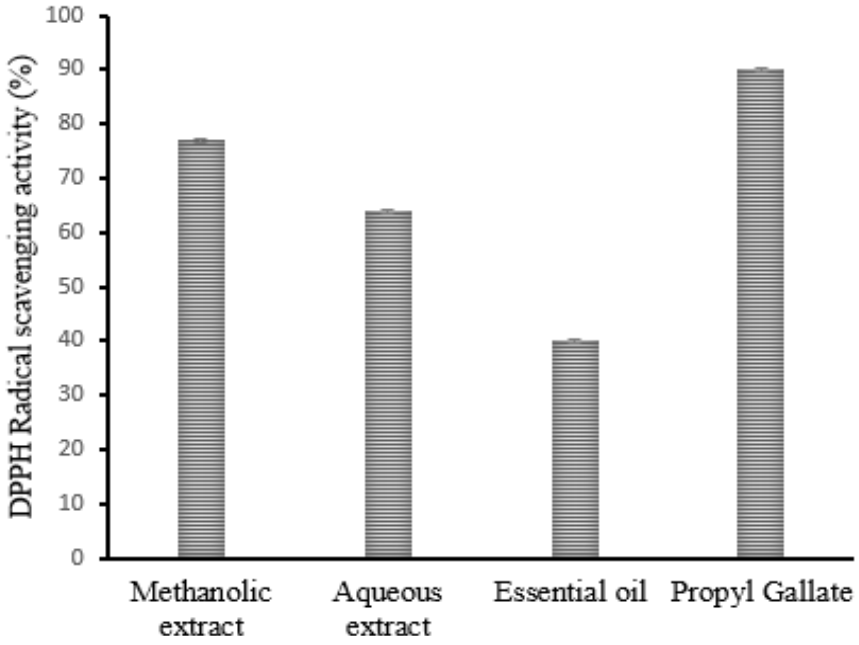

Fig. 1. DPPH Radical scavenging activity of *C. schoenanthus* flowers extracts, essential oil (0.5 mg/ml)and propyl Gallate used as standard antioxidant (0.2 mg/100µl)

Table 4. Results of phytochemical screening

Phytochemicals	Test/ Reagent	Results	
		Methanol extract	Aqueous extract
Saponins	Foam test	+ +*	+ + +
Alkaloids	Mayer's reagent	+	+ +
Flavonoids	Potassium hydroxide	+ + +	+ +
Tannins	Ferric chloride	++ +	+++
Sterols/ triterpenes	Liebermann's test	++	+
Anthraquinones	Borntrager's test	-	-

***** *+ Trace, ++ Moderate, +++ High and –Negative*

radical decays, and the extent of decrease in the absorbance correlates with the free radical scavenging potential of the plant extract. This represents one of the most commonly used methods due to its simplicity and compatibility with both hydrophilic and lipophilic antioxidant samples for their whoever it's sensitive to pH, temperature and light [28].

3.4 Phytochemical Screening

Qualitative phytochemical screening of *C. schoenanthus* extracts indicates the presence of many secondary metabolites (Table 4). The methanol extract revealed the presence of a relatively higher amount of flavonoids, steroids, and triterpenoids than the aqueous extract. The aqueous extract revealed the presence of a higher amount of tannins, saponin glycosides, and alkaloids. The two extracts are devoid of alkaloids anthraquinone glycosides. Phytochemical screening findings explain the correlation between the antimicrobial activity exhibited by extracts and the detected constituents. Phenolic compounds including flavonoids, tannins are known to have antimicrobial effects [29], and their mode of antibacterial activity may be related to their ability to complex with cellular proteins, often leading to inactivation of enzymes [30]. In addition, flavonoids have the ability to form a complex with bacterial cell walls, as well as disruption of membrane particularly with lipophilic flavonoids [31], and some of them are phytoalexins; produced by plants in response to microbial infection [32]. Polyphenolic compounds received a great deal of attention in recent years, since Due to their known antioxidant properties, was suggested that consumption of tannin-rich foods can cure or prevent a variety of illness [33].

The mechanism of antibacterial and antifungal activities of essential oils appears to be attributed to the mobile and lipophilic nature of essential oil components, especially the monoterpenes, which enables them to penetrate and disrupt cell membranes. Interestingly, essential oils have no specific cellular targets because of their great number of constituents he suggests a very low risk of the development of microbial resistance against essential oils [34].

4. CONCLUSION

Flowers of *C. scthoenanthus* plant have a potential antimicrobial and antioxidant activities against many microorganisms, this activity differs from one microorganism to another depending on many factors such as the type of extract and the strain of the microorganism. However, a detailed phytochemical, as well as biological studies of these extracts and essential oil, are required.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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