



Research Article

# Antimicrobial Activity of *Cymbopogon schoenanthus* Extracts against Selected Urinary Tract Infection (UTI)-causing Bacteria

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## Abstract

**Background:** Several plant species, such as *Cymbopogon schoenanthus*, have been traditionally employed for treating a range of illnesses. The medicinal potential of *C. schoenanthus*, presently traded in the Sudan market, is believed to be significant. The objective of this study is to examine the antibacterial activity of *C. schoenanthus* against both Gram-positive and Gram-negative bacteria that commonly cause urinary tract infections.

**Methods:** The antimicrobial activity of *C. schoenanthus* extracts was evaluated using well diffusion and broth dilution methods by measuring inhibition zones and minimum bactericidal concentrations (MBCs).

**Results:** The largest inhibition zone was observed for *S. aureus* ( $17.0 \pm 0.58$  mm with diethyl ether extract), while the smallest was for *E. coli* ( $7.7 \pm 0.58$  mm with ethanol). MBCs ranged from 6.25 to 50 mg/mL, with both extracts showing the strongest bactericidal effect against *S. aureus* (MBC: 6.25 mg/mL), and the diethyl ether extract exhibiting enhanced activity against *E. coli* (MBC: 25 mg/mL).

**Conclusion:** Both ethanolic and diethyl ether extracts of *C. schoenanthus* exhibited antibacterial activity against common UTI-causing pathogens. These findings support the traditional use of the plant and suggest potential for further investigation into its bioactive compounds.

**Keywords:** *Cymbopogon schoenanthus*, urinary pathogens, agar well diffusion


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## 1. Introduction

Medicinal plants and their bioactive compounds have gained significant attention as potential therapeutic agents due to their effectiveness and minimal side effects [1, 2]. With the increasing resistance to conventional antibiotics, there is a growing need to explore alternative sources, such as plant extracts, for antimicrobial compounds [3]. Among these, aromatic plants have shown a wide range of pharmacological activities.

The genus *Cymbopogon* (family Poaceae) comprises over 50 species, many of which are known for their aromatic properties and traditional uses [4]. *Cymbopogon schoenanthus*, locally known as *El Mahareib* in Sudan, is one such species widely consumed in North Africa as a herbal tea for its health-promoting effects and refreshing taste [4]. It is a tall perennial herb with long, thin leaves that contain silica thorns along the edges (Figure 1) [5].

Traditionally, *C. schoenanthus* has been used to treat various ailments, including gastrointestinal disorders, fevers, and parasitic infections. Its essential oil has demonstrated antibacterial, antifungal, anti-inflammatory, and sedative properties [7]. Several *in vitro* studies have reported its antimicrobial activity against common pathogens. The plant also has anti-intestinal ailment, antispasmodic, fever-reducing, anti-malarial, and anthelmintic (especially against Guinea worms) properties [8]. It is also found to have sedative, digestive, and antiparasitic effects, and is effectively used as a renal antispasmodic and diuretic agent [9–12]. Moreover, it is an antifungal and anti-inflammatory agent that can be used to prevent and treat acute inflammatory skin disorders [13]. The plant is more effective as an antifungal when in vapor form rather than as a liquid [14]. While the vapor-phase activity

suggests possible use in air decontamination, further validation is essential to confirm its practical applicability.

In the agar diffusion method, the growth of many bacteria (such as *Enterococcus faecium*, *Salmonella typhimurium*, *Escherichia coli*, *Streptococcus agalactiae*, and *Staphylococcus aureus*) and fungi (such as *Candida albicans*) was inhibited via the essential oil of *C. schoenanthus*, which originated in Algeria [15]. *C. citratus*' oil was studied, showing remarkable results against dermatophytes (such as *Microsporum gypseum*, *Trichophyton mentagrophytes*, *T. rubrum*, and *Epidermophyton floccosum*) [16]. In another study, the antibacterial activity against *E. coli*, *Shigella dysenteriae*, *S. aureus*, *Klebsiella pneumoniae*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* was studied using the essential oil of *C. nervatus*, which originated in Sudan [17]. Other species of *Cymbopogon* (such as *C. winterianus*) were also tested using the microdilution method for antimicrobial properties [18]. In addition to its use in food and perfume items, *C. schoenanthus* is also used for its ability to cure some diseases like abdominal cramps, colds, aches, and epilepsy [19].

While essential oils from *Cymbopogon* species such as *C. citratus*, *C. nervatus*, and *C. schoenanthus* have shown promise in antimicrobial assays, there remains limited data on their specific activity against bacteria causing urinary tract infections (UTIs). Considering the global rise in antibiotic-resistant UTI pathogens, there is a pressing need to evaluate alternative plant-based antimicrobials. Therefore, this study aims to evaluate the antibacterial activity of ethanolic and diethyl ether extracts of *C. schoenanthus* against selected Gram-positive and Gram-negative bacteria known to cause UTIs.



**Figure 1:** *Cymbopogon schoenanthus*' (Herbarium No. 16950 / 1) culm nodes and their long, thin leaves [6].

## 2. Methods

The study was performed at the Microbiology Laboratory of the National Ribat University, Khartoum, Sudan. American Type Culture Collection (ATCC) strains of *K. pneumoniae*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *Proteus mirabilis* were obtained from the National Ribat University Faculty of Pharmacy Microbiology Laboratory, Khartoum, Sudan.

### 2.1. Preparation of the microbial cultures

The ATCC bacterial strains were confirmed by performing an indirect Gram stain test and standard biochemical tests (catalase test, indole test, citrate utilization test, Kligler iron agar test, mannitol fermentation test, gas-producing bacteria test, urease test, oxidase test, motility test, DNase (deoxyribonuclease) test, coagulase test, and H<sub>2</sub>S-producing bacteria test), following procedures outlined in standard microbiological references (e.g., CLSI [Clinical and Laboratory Standards Institute] M100 and Bailey & Scott's Diagnostic Microbiology, 14th ed.) [20, 21]. The ATCC bacterial strains used in this study were: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 53657, *P. aeruginosa* ATCC 27853, and *P. mirabilis* ATCC 12453. These were obtained as glycerol stocks

preserved at  $-80^{\circ}\text{C}$  in the Microbiology Laboratory, Faculty of Pharmacy, The National Ribat University, Khartoum, Sudan. Before experimentation, the strains were revived by inoculating 100  $\mu\text{L}$  of each glycerol stock into 5 mL of Nutrient Broth and incubating at  $37^{\circ}\text{C}$  for 18–24 hrs. A loopful of each revived culture was then streaked on Nutrient Agar (NA) plates and incubated under aerobic conditions at  $37^{\circ}\text{C}$  overnight for purification.

These bacterial strains were subcultured in NA for purification purposes. All NA culture plates were incubated aerobically overnight at  $37^{\circ}\text{C}$ . Mueller Hinton Agar (MHA) medium was prepared by adding 38 g to 1000 mL of distilled water, sterilized by autoclaving, dispensed into sterile 90-mm monoplate Petri dishes, and left to cool at  $4-8^{\circ}\text{C}$ .

The bacterial inoculum used for antimicrobial assays was standardized to match the 0.5 McFarland turbidity standard, equivalent to approximately  $1.5 \times 10^8$  colony-forming units (CFU)/mL, to ensure uniform and reproducible results.

### 2.2. Collection of plant material and its extracts

*C. schoenanthus* plant material was collected in November 2021 from Khartoum North, Sudan, during the dry season under clear weather conditions,

and identified by the National Center for Research, Khartoum, Sudan [22]. Voucher specimens were deposited at the National Herbarium of Sudan under accession number (Herbarium No. 16950 / 1), ensuring traceability and reproducibility. The plant (leaf) was washed and sliced into little pieces after being air-dried in the shade. A total of 100 g of dried, crushed plant material was used for each solvent (ethanol and diethyl ether) extraction. In a sterile conical flask, 800 mL of 100% ethanol and 100% diethyl ether were added to the clean, dry crushed plants and incubated for 48 hrs in an incubator with constant shaking. Ethanol (a polar solvent) and diethyl ether (a non-polar solvent) were selected to extract a broad range of phytochemicals with varying polarities, including both hydrophilic and lipophilic bioactive compounds. The obtained extracts were pooled and filtered using Whatman No.1 filter paper to remove plant residues. The ethanol and diethyl ether were evaporated using a hot air oven at 50°C. The solvents were evaporated entirely, and the extracts were weighed only after full drying to ensure accurate yield determination. Visual inspection and consistent final extract weights across replicates confirmed complete solvent evaporation. No residual solvent odor or mass loss was detected during post-drying handling. The resulting dried extracts were stored in airtight amber bottles at 4°C, protected from light until further use.

The weight of the extracts was 5.43 g and 2.5 g, respectively, using ethanol and diethyl ether. The percentage yield was calculated using the formula:  $(\text{weight of dried extract} / \text{weight of plant material used}) \times 100$ . This corresponds to a yield of 5.43% for ethanol and 2.5% for diethyl ether extract, based on 100 g of starting plant material. The collected amount of diethyl ether extract was dissolved in 10 mL of absolute dimethyl sulfoxide (DMSO). In comparison, 13.5 mL of DMSO was used to dissolve the ethanolic extract to

get stock concentrations (250 mg/mL and 400 mg/mL, respectively) [23]. Finally, serial dilutions by distilled water for both stock concentrations were done to give 200/100/50 mg/mL concentrations.

### 2.3. Agar well diffusion method

The agar well diffusion method is commonly used in plant extracts to examine their antimicrobial activity, an approach similar to that of disk diffusion. A loopful of the microbial inoculum was inoculated and streaked over the entire surface of the agar plate. Next, a hole with a diameter of 6 to 8 mm was pierced aseptically with a sterile cork borer. A fixed volume of 100  $\mu\text{L}$  of the extract solution was carefully pipetted into each agar well to ensure reproducibility and allowed to incubate based on the required growth temperature of the test bacterial strains. All incubation procedures were conducted under aerobic conditions for all tested bacterial strains. The growth of the tested bacterial strain was inhibited by the antimicrobial agent, which was diffused into the agar medium. A ruler was then used to measure the diameter of each inhibition zone in millimeters. Because CLSI breakpoints are not established for crude plant extracts, we report raw inhibition zone diameters (mm) without S/I/R categorization; comparisons were made statistically across groups [24, 25]. Negative control was performed using DMSO to ensure that it had no inhibitory effect. Ciprofloxacin was used as a positive control to provide a standard for comparison of antimicrobial activity.

### 2.4. Minimum bactericidal concentration and minimum inhibitory concentration

We followed CLSI guidance for broth dilution test conditions (media, inoculum standardization, incubation) as applicable [20]. Extract stocks were prepared as described earlier and twofold serially diluted in Mueller–Hinton broth. Due to the intense

extract coloration, visual turbidity endpoints for MIC were not reliable; therefore, we prespecified to report MBC only by subculturing each tube onto agar and recording the lowest concentration with no growth after 24 hr. The plant extracts were serially diluted into six concentrations in nutrient broth medium, with 200, 100, and 50 mg/mL showing antimicrobial activity [1]. Following the standardized 0.5 McFarland turbidity, the test bacterial strains were inoculated and incubated overnight at 37°C. The minimum inhibitory concentration (MIC) should be reported as the lowest concentration of extracts necessary to inhibit visible growth of the bacteria [26]. However, because the extracts were dark in color, observing turbidity to determine MIC was difficult. As a result, all tubes were sub-cultured on solid culture media to determine the minimum bactericidal concentration (MBC) of extracts at the lowest plant extraction concentration that completely prevents microorganism growth [27]. MHA was used as the medium for determining MIC values, following CLSI guidelines. MBC was confirmed by the complete absence of visible bacterial colonies on agar plates after incubation. Plates were incubated at 37°C under aerobic conditions for 24 hrs to allow sufficient recovery of viable bacteria if present. Ciprofloxacin was used as a positive control, while DMSO served as a negative control in MIC and MBC assays to ensure that observed inhibitory effects were due to the plant extracts alone. All antimicrobial assays, including MIC and MBC determinations, were performed in triplicate to ensure reproducibility and reliability of the results.

For each bacterial strain, all tests were conducted in three independent replicates, and the mean values were recorded. All laboratory procedures were performed following Biosafety Level 2 (BSL-2) guidelines and institutional biosafety protocols approved by the Microbiology Laboratory, National Ribat University.

## 2.5. Statistical analysis

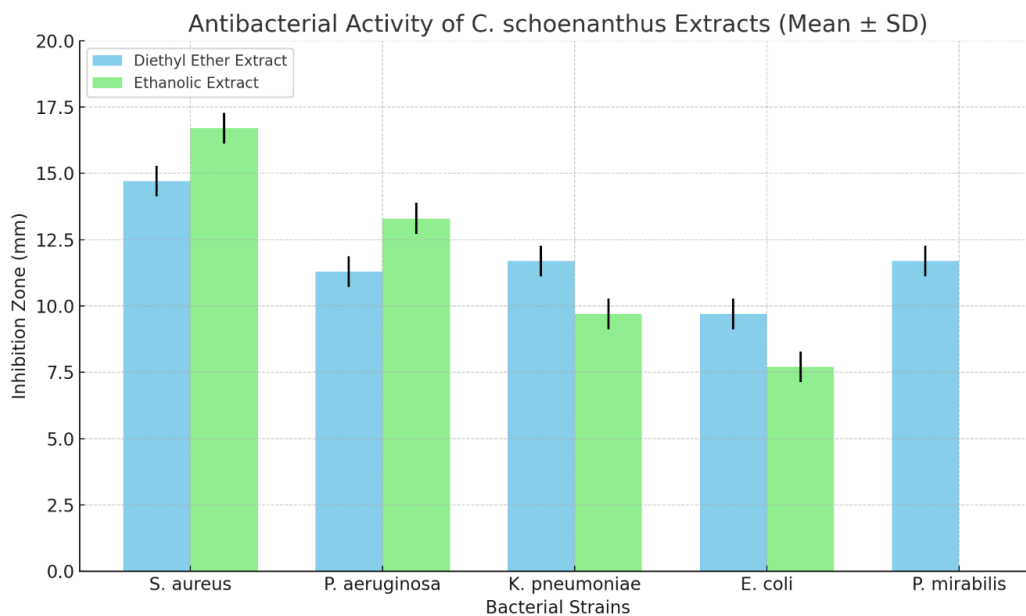
All antimicrobial assays, including inhibition zone measurements and MBC determinations, were performed in triplicate ( $n = 3$ ). Each replicate was conducted on an independently prepared agar plate to account for plate-to-plate variation. Results for inhibition zones and MBC values were expressed as mean  $\pm$  standard deviation (SD). The standard deviation was calculated for each inhibition zone diameter and MIC/MBC value across the three replicates to ensure consistency and clarity. Data were statistically analyzed using one-way analysis of variance (ANOVA) to determine whether there were significant differences among inhibition zone diameters for the different bacterial strains and extract types. Post-hoc pairwise comparisons were performed using Tukey's HSD test. Differences were considered statistically significant at  $P < 0.05$ . Analyses were carried out using SPSS version 25. The variability in inhibition zones was minimal ( $\pm 1$ –2 mm), indicating reproducibility. Where applicable, bar graphs were generated to visualize differences in antimicrobial activity between extracts and bacterial strains (Figure 2).

**Table 1:** Ethanolic extract (inhibition zones in mm).

Organism	Rep 1	Rep 2	Rep 3	Mean $\pm$ SD
<i>Staphylococcus aureus</i>	17	16	17	16.7 $\pm$ 0.58
<i>Pseudomonas aeruginosa</i>	13	14	13	13.3 $\pm$ 0.58
<i>Klebsiella pneumoniae</i>	10	9	10	9.7 $\pm$ 0.58
<i>Escherichia coli</i>	8	8	7	7.7 $\pm$ 0.58

**Table 2:** Diethyl ether extract (inhibition zones in mm).

Organism	Rep 1	Rep 2	Rep 3	Mean $\pm$ SD
<i>Staphylococcus aureus</i>	15	14	15	14.7 $\pm$ 0.58
<i>Pseudomonas aeruginosa</i>	11	12	11	11.3 $\pm$ 0.58
<i>Klebsiella pneumoniae</i>	12	11	12	11.7 $\pm$ 0.58
<i>Escherichia coli</i>	10	10	9	9.7 $\pm$ 0.58
<i>Proteus mirabilis</i>	12	12	11	11.7 $\pm$ 0.58

**Figure 2:** Comparison of antibacterial activity (mean inhibition zones in mm  $\pm$  SD) of *Cymbopogon schoenanthus* ethanolic and diethyl ether leaf extracts against selected urinary tract infection (UTI)-causing bacterial strains. Data represent the average of three independent replicates. Error bars indicate standard deviation.

Across Tables 1–4, *S. aureus* consistently showed the largest inhibition zones and lowest MBC values, indicating strong susceptibility. *E. coli* had the lowest inhibition, while other Gram-negative bacteria, such as *K. pneumoniae* and *P. aeruginosa*, showed moderate responses. Diethyl ether extract exhibited slightly stronger activity against Gram-negative strains compared to the ethanolic extract.

## 3. Results

### 3.1. Agar well diffusion method

*In vitro* testing was conducted to evaluate the antimicrobial activity of ethanolic and diethyl

ether extracts of *C. schoenanthus* plants against selected bacterial pathogens, which are commonly associated with UTIs. Antimicrobial activity was assessed based on inhibition zone diameters (mm). Clear zones around the wells indicated susceptibility of bacteria to the extract. Ciprofloxacin served as a positive control and consistently produced inhibition zones, while DMSO showed no inhibition, confirming the validity of the assay.

Tables 1 and 2 present the inhibition zones (mm) recorded for each bacterial strain as mean  $\pm$  standard deviation (SD) for the ethanolic and diethyl ether extracts, respectively. Tables 3 and 4 show the MIC and MBC values for the ethanolic extract, while Tables 5 and 6 show the MIC and MBC values for the diethyl ether extract.

Five pathogens consisting of four Gram-negative bacteria (*K. pneumoniae*, *E. coli*, *P. aeruginosa*, and *P. mirabilis*) and one Gram-positive bacterium (*S. aureus*) were tested. All inhibition zone measurements were conducted in triplicate ( $n = 3$ ) and are presented as mean  $\pm$  standard deviation (SD).

For the ethanolic extract, *E. coli* gave the lowest inhibition zone of 8 mm, while *S. aureus* showed the highest inhibition zone of 17 mm (Figure 3). Similarly, for diethyl ether extract, *E. coli* gave the lowest inhibition zone of 10 mm, while *S. aureus* showed the highest inhibition zone of 15 mm (Figure 4).

**Table 3:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Cymbopogon schoenanthus* ethanolic extract.

Organism	Concentration (mg/ml)	Zone of inhibition (200 mg/mL)
<i>Staphylococcus aureus</i> ATCC 25923	200	16.7 $\pm$ 0.58
<i>Pseudomonas aeruginosa</i> ATCC 27853	200	13.3 $\pm$ 0.58
<i>Klebsiella pneumoniae</i> ATCC 53657	200	9.7 $\pm$ 0.58
<i>Escherichia coli</i> ATCC 25922	200	7.7 $\pm$ 0.58

**Table 4:** Antibacterial activity (mm) and minimum bactericidal concentration (MBC; mg/mL) of *Cymbopogon schoenanthus* ethanolic extract against five bacterial test strains.

Organisms	Concentrations (mg/ml)			MBC (mg/ml)
	200	100	50	
<i>Escherichia coli</i> ATCC 25922	8	7	0	50
<i>Proteus mirabilis</i> ATCC 12453	13	10	8	50
<i>Klebsiella pneumonia</i> ATCC 53657	10	8	7	50
<i>Pseudomonas aeruginosa</i> ATCC 27853	13	11	8	50
<i>Staphylococcus aureus</i> ATCC 25923	17	14	10	6.25

**Table 5:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Cymbopogon schoenanthus* diethyl ether extract.

Organism	Concentration (mg/ml)	Zone of inhibition (200 mg/mL)
<i>Staphylococcus aureus</i> ATCC 25923	200	14.7 $\pm$ 0.58
<i>Proteus mirabilis</i> ATCC 12453	200	11.7 $\pm$ 0.58
<i>Klebsiella pneumonia</i> ATCC 53657	200	11.7 $\pm$ 0.58
<i>Pseudomonas aeruginosa</i> ATCC 27853	200	11.3 $\pm$ 0.58
<i>Escherichia coli</i> ATCC 25922	200	9.7 $\pm$ 0.58

**Table 6:** Antibacterial activity (mm) and minimum bactericidal concentration (MBC; mg/mL) of *Cymbopogon schoenanthus* diethyl ether extract against five bacterial test strains.

Organism	Concentrations (mg/ml)			MBC (mg/ml)
	200	100	50	
<i>Escherichia coli</i> ATCC 25922	10	8	0	25
<i>Proteus mirabilis</i> ATCC 12453	12	11	0	50
<i>Klebsiella pneumoniae</i> ATCC 53657	12	8	0	50
<i>Pseudomonas aeruginosa</i> ATCC 27853	11	9	8	50
<i>Staphylococcus aureus</i> ATCC 25923	15	13	10	<b>6.25</b>

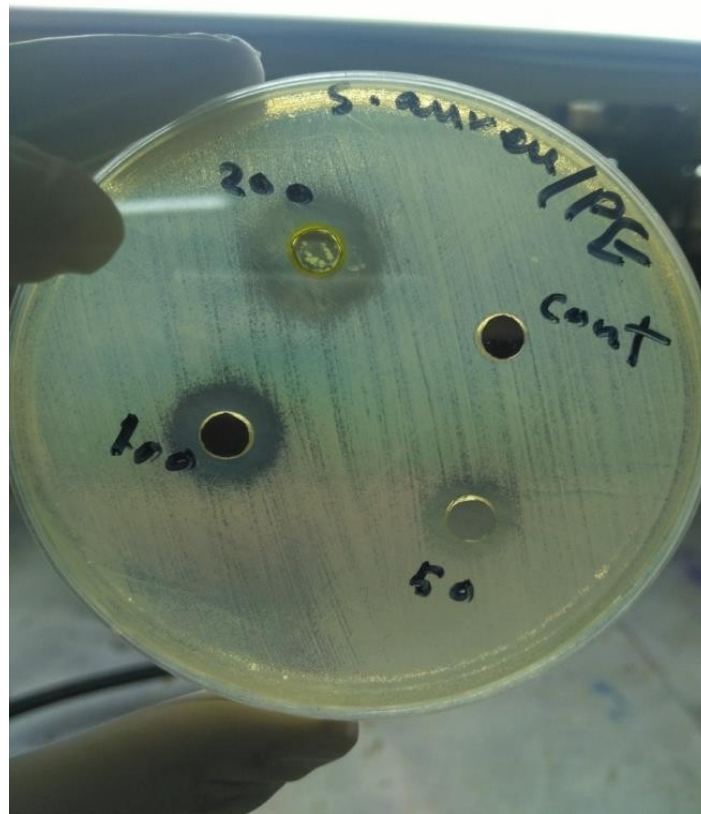
**Figure 3:** Antibacterial activity of *C. schoenanthus* ethanolic extract against *S. aureus*.

## 4. Discussion

The well-established and consistent occurrence of urinary infections has always been considered to be related to microbiological etiology. Nowadays, isolates from many parts of the world are multidrug-resistant (MDR), therefore, there is an urgent need to look for and test alternative herbal drugs. This study aimed to assess the antimicrobial activity of natural substances extracted from *C. schoenanthus* against UTI pathogens. Given the growing resistance and potential side effects associated with conventional antimicrobial agents,

natural plant-derived compounds are increasingly being investigated for their therapeutic potential. Previous studies have demonstrated the efficacy of various herbal extracts, including *Cymbopogon* species, against a range of pathogenic microorganisms.

The antimicrobial activity of ethanolic and diethyl ether leaf extracts of *C. schoenanthus* was studied against five standard bacteria, one Gram-positive bacterium (*S. aureus*), and four Gram-negative bacteria (*P. aeruginosa*, *P. mirabilis*, *E. coli*, and *K. pneumoniae*).



**Figure 4:** Antibacterial activity of *C. schoenanthus* diethyl ether extract against *S. aureus*.

High antibacterial activity was shown against *S. aureus* from both extracts of *C. schoenanthus*, while lesser activity was demonstrated against *E. coli*, *P. mirabilis*, *K. pneumoniae*, and *P. aeruginosa*. These findings are consistent with what has been found in other studies [1, 16, 28, 29]. The observed antimicrobial activity may be attributed to various phytochemicals present in *C. schoenanthus*, including essential oils (e.g., citronellal, geraniol), flavonoids, phenols, and terpenes, which have been previously associated with antibacterial and anti-inflammatory properties [19, 29]. While this study focused on *C. schoenanthus*, other species in the *Cymbopogon* genus, such as *C. citratus*, *C. nardus*, and *C. winterianus*, have also demonstrated similar or even stronger antimicrobial effects, depending on their phytochemical profile and extraction method [17, 28]. Future comparative studies could better elucidate the most effective species and compounds.

The comparison between ethanolic and diethyl ether extracts showed that the ethanolic extract had the strongest effect against *S. aureus* (MBC: 6.25 mg/mL) and showed moderate activity against *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, and *E. coli* (MBC: 50 mg/mL). In contrast, the diethyl ether extract demonstrated enhanced activity against *E. coli* (MBC: 25 mg/mL) and similar activity to the ethanolic extract against the remaining strains, including *P. mirabilis*, *K. pneumoniae*, *P. aeruginosa* (MBC: 50 mg/mL), and *S. aureus* (MBC: 6.25 mg/mL; Tables 5 & 6).

In summary, the MBC values indicate that both extracts exhibit strong bactericidal properties against *S. aureus* (6.25 mg/mL). Diethyl ether extract demonstrated a stronger effect against *E. coli* (25 mg/mL) compared to the ethanolic extract (50 mg/mL), possibly due to differences in solubility and phytochemical content. For the remaining bacteria, both extracts required 50 mg/mL to

achieve bactericidal activity, indicating comparable efficacy.

The bactericidal efficacy increased with increasing extract concentration at all concentrations utilized in this study (200, 100, and 50 mg/mL). This indicates the inhibition zones on plates containing extract with a low dilution factor were larger. This is also mentioned by Esimone *et al.*, who found that the extract of *C. schoenanthus* inhibited the growth of several bacteria at varying concentrations [30]. Hashim *et al.* reported that the reason for the variation in the results may be attributed to the differences in methods of plant extraction, geographical conditions, harvest time, and differences in the source of organisms [1].

This study is limited by its use of a small number of standard bacterial strains rather than clinical isolates, which may differ in resistance profiles. Additionally, no phytochemical screening or chemical analysis (e.g., Gas Chromatography–Mass Spectrometry [GC-MS] and High-Performance Liquid Chromatography [HPLC]) was performed to confirm the active constituents. The absence of cytotoxicity testing also limits conclusions regarding the therapeutic safety of the extracts. Color interference precluded visual MIC reads; future work will use a resazurin (AlamarBlue) microdilution or OD<sub>600</sub> spectrophotometric readout to obtain MICs in colored extracts. Furthermore, the study utilized only five ATCC reference uropathogenic strains. The absence of MDR clinical isolates limits the direct clinical relevance of the findings for UTI therapy.

## 5. Conclusion

Traditional medicine often relies on various plant species for treating a wide range of diseases, and *C. schoenanthus* is among those plants. This particular species, which is currently traded in the

Sudan market, is believed to possess medicinal properties and value. As a consequence, in this study, the *C. schoenanthus* ethanolic and diethyl ether leaf extracts revealed antibacterial activity against selected bacterial pathogens, which commonly cause UTI. This study demonstrated that both ethanolic and diethyl ether extracts of *C. schoenanthus* exhibited bactericidal activity against common UTI-causing pathogens. Diethyl ether extract showed stronger activity against *E. coli*, while both extracts were highly effective against *S. aureus*. Given its *in-vitro* activity against reference strains, *C. schoenanthus* extracts may serve as complementary agents to enhance the action of conventional antibiotics.

## Recommendations

Further in-depth studies, including large sample sizes and a broader range of bacterial strains—particularly MDR isolates—are recommended to enhance clinical relevance. Moreover, evaluation of the possible toxicity and safety of the plant extracts should be conducted using *in vitro* cytotoxicity assays (e.g., MTT assay on mammalian cell lines) and *in vivo* animal studies. In addition, advanced phytochemical analyses such as GC-MS, Liquid Chromatography–Mass Spectrometry (LC-MS), and HPLC are necessary to identify and quantify the active constituents responsible for the antibacterial effect and to better understand their mechanisms of action.

## Declarations

## Acknowledgments

None.

## Ethical Considerations

All laboratory procedures were conducted in accordance with Biosafety Level 2 (BSL-2) guidelines and institutional biosafety protocols approved by the Microbiology Laboratory, National Ribat University.

## Competing Interest

None.

## Availability of Data and Materials

The datasets used in the present study are available from the corresponding author upon reasonable request.

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## Abbreviations and Symbols

UTI: Urinary tract infection  
 CFU: Colony-forming unit  
 MBC: Minimum bactericidal concentration  
 MIC: Minimum inhibitory concentration  
 CLSI: Clinical and Laboratory Standards Institute  
 DMSO: Dimethyl sulfoxide  
 NA: Nutrient agar  
 MHA: Mueller Hinton Agar  
 ATCC: American Type Culture Collection  
 SD: Standard deviation  
 ANOVA: Analysis of variance  
 SPSS: Statistical Package for the Social Sciences  
 GC-MS: Gas chromatography–Mass spectrometry  
 LC-MS: Liquid chromatography–Mass spectrometry

HPLC: High-performance liquid chromatography

BSL-2: Biosafety level 2

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

MDR: Multidrug-resistant

DNAse: Deoxyribonuclease

## AI Use Disclosure

The author declares that no generative artificial intelligence (AI) tools were used in the writing, editing, data analysis, or any other part of the preparation of this manuscript.

## Author Contributions

Concept or design of the work: SSAA; Acquisition, analysis, or interpretation of data: SSAA; Drafting the work or reviewing it critically: SSAA; Final approval; Accountability for all aspects of the work: SSAA.

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