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Efficacy of a *Cyperus rotundus* extract and antibiotics on bacteria isolated from urinary tract infections

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ABSTRACT

Urinary tract infection (UTI), an inflammatory condition resulting from pathogenic invasion of the urinary tract, remains a prevalent clinical concern. This study aimed at isolating common uropathogenic bacterial strains from urine samples collected from patients attending consultation clinics at the Al-Imam Sadeq and the Al-Hilla Teaching Hospitals (Iraq) between October and December 2024. The objectives were twofold: (i) to evaluate the antimicrobial susceptibility profiles of the isolated strains, and (ii) to assess the antibacterial efficacy of *Cyperus rotundus* extract (CRE). The findings demonstrated that CRE at a concentration of 1,000 µg/mL inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa*, alongside other locally isolated bacterial species. The minimum inhibitory concentration (MIC) of CRE was determined to be 62.5 µg/mL. Notably, *E. coli* and *P. aeruginosa* exhibited susceptibility to all tested antibiotics, whereas *Proteus mirabilis* showed resistance to all agents. Additional isolates, including *Staphylococcus* spp. and *Enterococcus faecalis*, displayed variable susceptibility patterns, with *E. faecalis* being sensitive to certain antibiotics and resistant to others.

1. Introduction

The emergence and proliferation of antibiotic-resistant bacteria have been significantly driven by the misuse of antimicrobial agents in developed countries. Among the most frequent bacterial pathogens affecting humans is the Gram-negative bacterium *Escherichia coli*,

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which is widely recognized as the principal etiological agent of urinary tract infections (UTIs). UTIs rank among the most prevalent health concerns globally, second only to respiratory tract infections, and encompass both hospital-acquired and community-acquired cases. Resistance to antimicrobial drugs is rising markedly, with recent studies reporting an increased incidence of antibiotic-resistant UTIs. This resistance complicates treatment, particularly in pregnant women, where therapeutic options are limited and the consequences may affect both maternal and foetal health[1].

Antibiotic resistance during pregnancy can lead to prolonged infections, elevated healthcare costs, and potential complications for the developing foetus and the mother. This underscores the critical need for judicious antibiotic use and the exploration of alternative therapeutic strategies[2]. Accordingly, the investigation of novel antimicrobial agents derived from natural sources is increasingly viewed as essential in order to address the multifaceted health and socioeconomic challenges posed by drug-resistant bacteria[3].

Research has increasingly focused on medicinal plants due to their notable antibacterial and bioactive properties. Unlike synthetic drugs, which are frequently misused and contribute to resistance, plant-based therapies offer a safer alternative for both human health and the environment[4]. *Cyperus rotundus*, also known as nut grass and belonging to the Cyperaceae family, is widely distributed in India and referred to locally as "Nagarmotha." *Cyperus rotundus* contains a rich array of bioactive compounds, including vitamin C, polyphenols, glycosides, flavonoids, tannins, alkaloids, saponins, terpenoids, essential oils, carbohydrates, and proteins[5].

Traditionally, *Cyperus rotundus* has been employed for its pharmacological properties, including its anti-inflammatory, anti-adiposity, antidiabetic, and neuroprotective effects. It has also exhibited potent antioxidant and free-radical scavenging activities in various *in vitro* assays. *In vivo* studies have further revealed its anxiolytic and cognitive-enhancing effects in murine models of anxiety and hypobaric hypoxia[6,7]. Given these therapeutic potentials, the present study was designed in order to isolate uropathogenic bacteria and evaluate the antimicrobial efficacy of the *Cyperus rotundus* extract (CRE).

2. Methodology

Bacterial isolates were obtained from urine samples of patients attending urology consultation clinics at the Al-Imam Sadeq and the Al-Hilla Teaching Hospitals (Iraq) between October and December 2024. All patients were clinically diagnosed with UTIs and referred for urine culture and antimicrobial susceptibility testing.

Rhizomes of *Cyperus rotundus* were collected from a medicinal plant source in Najaf, Iraq, and authenticated by a qualified botanist. The rhizomes were washed, dried, and ground into a fine powder. Ethanol (300 mL) was employed in order to macerate 100 mg of the powdered material at room temperature (RT) for 24 h so as to ensure a thorough extraction of the bioactive constituents. The resulting extract was cooled, filtered through sterile paper, and oven-dried at 40°C for 24 h.

The ethanolic CRE was tested against four bacterial species: two Gram-positive strains (*Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC BBA-335) and two Gram-negative strains (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). All strains were obtained from UTI cases and commercially sourced.

Inocula were prepared from ten single colonies grown on Mueller-Hinton agar (MHA) for 24 h. Six serial dilutions of CRE (1,000, 500, 250, 125, 62.5, and 31.25 μg/mL) were tested using the broth microdilution method in 96-well microtiter plates. Colonies were suspended in 2 mL of sterile water, and the optical density at 600 nm (OD_{600}) was measured using a Biotech 800ST plate reader (Biotech, USA), yielding a final OD_{600} of 0.236 (equivalent to the McFarland standard 0.67). The bacterial suspension (1×10^6 CFU/mL) was diluted 100-fold, and 50 μL was inoculated into each well, excluding blank controls.

The half-maximal inhibitory concentration (IC_{50}) and minimum inhibitory concentration (MIC) values were determined in triplicate for both positive

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Table 1. Antibiotic susceptibility for the herein assessed Gram-positive and Gram-negative bacteria. Abbreviations used: I, intermediate (susceptibility); R, resistant; S, sensitive.

Antibiotic	***Staphylococcus aureus***	***Enterococcus faecalis***	***Streptococcus* spp.**
Cefoxitin screen	+	-	-
Benzylpenicillin	R	-	-
Ampicillin	-	R	R
Oxacillin	R	-	-
Imipenem	-	-	-
Gentamicin	-	R	S
Streptomycin	-	R	R
Gentamicin	S		-
Ciprofloxacin	R	S	S
Moxifloxacin	R	-	-
Inducible clindamycin resistance	-	-	-
Erythromycin	R	R	R
Clindamycin	S	-	-
Teicoplanin	S	R	R
Vancomycin	S	R	R
Tetracycline	R	R	R
Tigecycline	S	S	S
Fusidic acid	-	-	-
Rifampicin	S	-	-
Trimethoprim	R	-	-
Antibiotic	***Proteus mirabilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
Ticarcillin	R	S	S
Ticarcillin and clavulanic acid	R	S	S
Piperacillin / tazobactam	R	S	S
Ceftazidime	R	S	S
Cefepime	R	S	S
Aztreonam	R	-	-
Imipenem	R	S	R
Meropenem	R	S	R
Amikacin	R	S	S
Gentamicin	R	S	S
Tobramycin	R	S	S
Ciprofloxacin	R	S	S
Pefloxacin	-	-	-
Minocycline	R	-	-
Colistin	-	S	I
Rifampicin	-	-	-
Trimethoprim / sulfamethoxazole	R	-	-

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and negative controls. Each 96-well plate had a final volume of 225 μL per well. Prior to adding the CRE stock solution (100 mg/mL), 100 μL of broth medium was dispensed. MIC was defined as the lowest concentration at which no visible bacterial growth was observed following overnight incubation.

3. Results and Discussion

Bacterial strains isolated from UTI patients included *P. aeruginosa* (ATCC PAO1 and ATCC 27853), *S. pneumoniae* (ATCC BAA-334), and *E. coli* (ATCC 25922). The CRE at 1,000 μg/mL inhibited the growth of *E. coli* and *P. aeruginosa*, as well as other locally isolated strains. The MIC of CRE was determined to be 62.5 μg/mL, effectively suppressing the growth of *S. pneumoniae* and *Staphylococcus* spp. Previous studies corroborate these findings, reporting high antimicrobial activity of CRE against *S. aureus* and *Bacillus subtilis* (31 and 30 mm inhibition zones, respectively), and moderate activity against *Aspergillus niger* and *Candida albicans* (20 and 26 mm)[8]. Inhibition zones of 19 mm and 20 mm were observed against *E. coli* and *P. aeruginosa*, respectively. Additionally, aqueous leaf extracts and ethanolic rhizome-tuber extracts demonstrated antibacterial activity against *S. aureus* and *E. coli*, consistent with prior research[9]. However, the *Cyperus* oil showed no inhibitory effect on bacteria or fungi, including *C. albicans*.

Antibiotic susceptibility testing (Table 1) revealed that *Enterococcus faecalis* was sensitive to ciprofloxacin and tigecycline, but resistant to other agents. *Streptococcus* spp. were susceptible only to gentamicin, ciprofloxacin, and tigecycline. These results align with earlier studies identifying ciprofloxacin as the most effective agent against *E. coli*, which exhibited universal susceptibility[10]. In contrast, *Proteus mirabilis* was resistant to all tested antibiotics, likely due to indiscriminate drug use or the presence of resistance-conferring genes. Further investigation is warranted in order to explore the clinical applicability of CRE in UTI management.

4. Conclusion

This study demonstrated that CRE possesses notable antibacterial activity against both Gram-positive and Gram-negative bacteria, with distinct MIC values. The findings support the potential of CRE as a natural therapeutic agent for UTIs, particularly in the context of rising antibiotic resistance.

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Conflicts of interest

None exist.

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