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Exploring the Anti-bacterial Activity of *Cyperus rotundus* Extract against Multi-Drug Resistant *Acinetobacter baumannii* Isolates: A Potential Therapeutic Approach

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Abstract

The study investigates the antibacterial activity of *Cyperus rotundus* extract against multi-drug resistant *Acinetobacter baumannii*, indicating potential as an alternative treatment strategy. The extract demonstrates concentration-dependent inhibition zones, suggesting a dose-response relationship and highlighting its potential efficacy, among the 5 isolates tested, 2 of were well suppressed and inhibition activity was observed at 2.5mg of extract. Phytochemical analysis reveals bioactive compounds with antimicrobial properties, underscoring its therapeutic potential. Further research is needed to elucidate mechanisms of action and optimize dosage regimens for clinical use, contributing to efforts to combat antimicrobial resistance.

***Key words*:** *C. rotundus* extract, *A. baumannii*, MDR, Biofilm, Anti-bacterial activity

The rise of multi-drug resistant (MDR) bacterial strains poses a significant challenge to modern medicine, threatening the effectiveness of conventional antibiotics and necessitating the exploration of alternative treatment strategies [1]. Among these resistant pathogens, *Acinetobacter baumannii* stands out as a notorious nosocomial pathogen capable of causing severe infections, particularly in immunocompromised patients [2]. Compounding the issue, *A. baumannii* often exhibits the ability to form biofilms, further enhancing its resilience against antimicrobial agents and immune responses. In response to this pressing public health concern, researchers have turned to natural sources for novel antimicrobial compounds [4], [11].

One such source of interest is *Cyperus rotundus*, a widely distributed medicinal plant known for its diverse pharmacological properties [5-6]. Traditional medicinal practices have long recognized the therapeutic potential of *Cyperus rotundus* in treating various ailments, including microbial infections [7-8]. However, its efficacy against MDR pathogens, particularly *A. baumannii*, remains underexplored. In this study, we aimed to investigate the anti-bacterial activity of *Cyperus rotundus* extract against MDR *Acinetobacter baumannii* isolates, with a focus on biofilm-producing strains. By employing a well-established methodology, we assessed the inhibitory effects of the extract against a panel of clinically relevant *Acinetobacter baumannii* isolates obtained from diverse sources. Additionally, we conducted phytochemical analysis to elucidate the bioactive compounds present in the extract, shedding light on its potential mechanisms of action.

MATERIALS AND METHODS

Selection of microorganisms

Five MDR, biofilm producing potential isolates of *Acinetobacter baumannii* were procured from clinical laboratory from Coimbatore area. The procured isolates were confirmed with selective media.

Preparation of Cyperus rotundus extract

The dried *Cyperus rotundus* extract was prepared with maceration method as a stock solution of known concentration.

Preparation of inoculum

A. baumannii isolates were cultured overnight in nutrient broth at 37°C. The turbidity of the bacterial suspension was adjusted to match a 0.5 McFarland standard.

Anti-bacterial activity assay

In the anti-bacterial activity testing, various concentrations of *Cyperus rotundus* extract were prepared in methanol, and a standardized suspension of each *Acinetobacter baumannii* isolate was prepared. Wells were created on Mueller-Hinton agar plates, filled with specific concentrations of the *Cyperus rotundus* extract, with ampicillin (5mcg) as the positive control and methanol as the negative control. Each plate was inoculated with one *Cyperus rotundus* isolate using a sterile swab, followed by incubation at 37°C for 16-18 hours. After incubation, the diameter of the zone of inhibition around each well was measured using a calibrated ruler. Statistical analysis was then performed to determine the anti-bacterial activity of the *Cyperus rotundus* extract against the *Acinetobacter baumannii* isolates.

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RESULTS AND DISCUSSION

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development. Further, these tests may facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds [9]. The results of the study demonstrate the potential anti-bacterial activity of *Cyperus rotundus* extract against MDR *Acinetobacter baumannii* isolates. The isolates included in the study were obtained from various clinical sources such as blood, urine, and pus, indicating the widespread presence of MDR strains in clinical settings. Additionally, all isolates were found to produce biofilms.

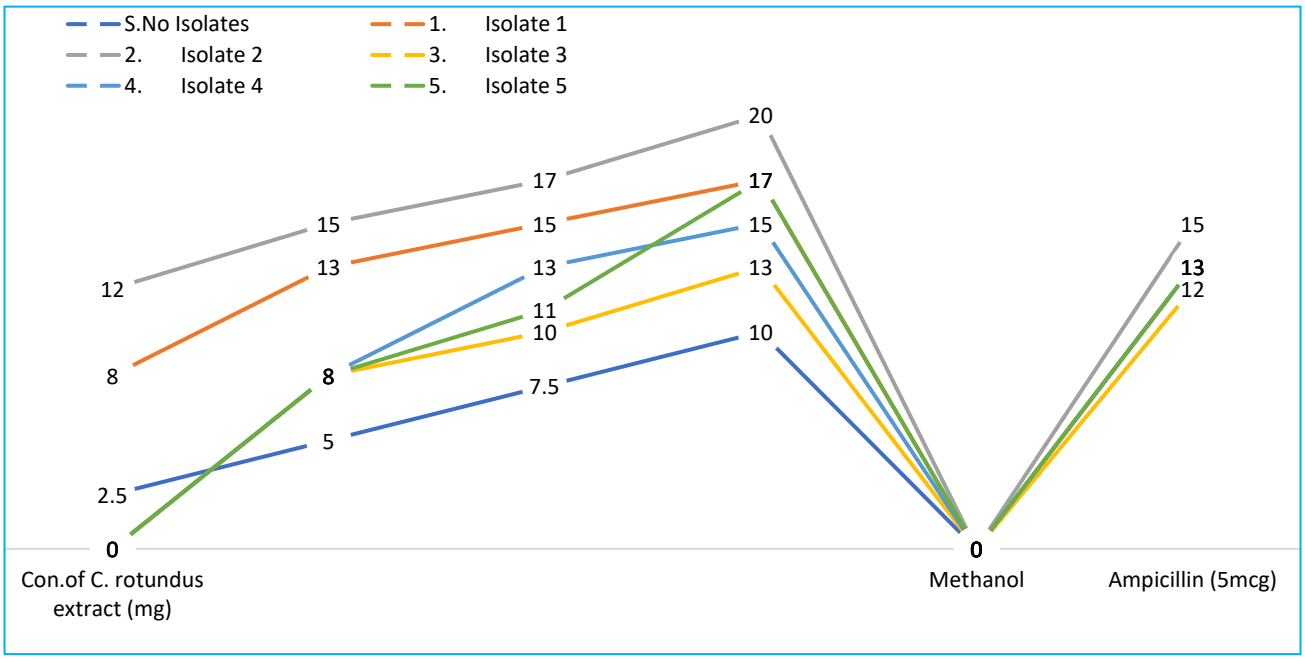

Fig 1 Anti-bacterial activities of the *Cyperus rotundus* extract

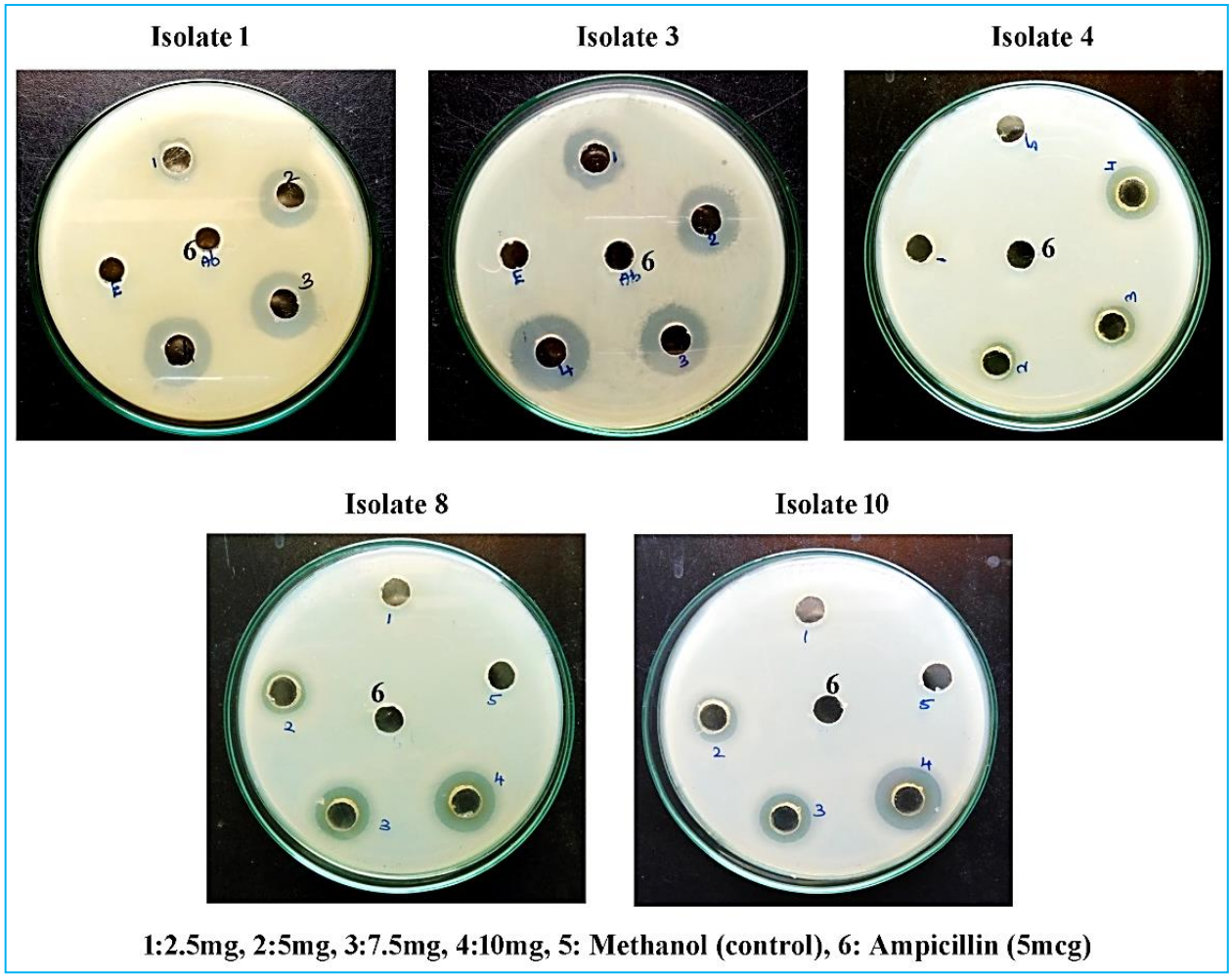

Fig 2 Anti-bacterial activities of the *Cyperus rotundus* extract

The observed potential anti-bacterial activity of *Cyperus rotundus* extract against multi-drug resistant (MDR) *Acinetobacter baumannii* isolates correlates with previous studies examining the phytochemical composition and antimicrobial effects of this botanical extract. El-Wakil *et al*. [9] conducted phytochemical screening and antimicrobial evaluation of *Cyperus rotundus*, revealing the presence of bioactive compounds with antimicrobial properties, which aligns with our findings. Additionally, Sabir *et al*. [10] isolated rotundine glycoside from *Cyperus rotundus* rhizomes and demonstrated its cytotoxicity and antimicrobial effects, further supporting the antimicrobial potential of this plant species. *Cyperus rotundus* as potential sources of antimicrobial agents, particularly in the context of combatting multidrug-resistant bacterial infections.

The phytochemical analysis of the *Cyperus rotundus* extract revealed the presence of bioactive compounds with alkaloids, carbohydrates, flavonoids, phenols, sterols, terpenoids, and quinones. The methanol extract exhibited varying degrees of inhibitory effects against the *Acinetobacter baumannii* isolates, with inhibition zones ranging from 8 mm to 20 mm at different concentrations (Fig 1-2). Moreover, the concentration-dependent response observed in our study is consistent with the concept of dose-response relationships documented in the literature. Gupta *et al*. [11] demonstrated enhanced biological activities in *Thymus vulgaris* essential oil following synthetic polyploidization, highlighting the significance of dosage optimization in maximizing therapeutic efficacy. Furthermore, Coșeriu *et al*. [12] investigated the antibacterial effects of essential oils against multidrug-resistant Pseudomonas aeruginosa clinical isolates and emphasized the importance of understanding genetic variations and resistance mechanisms in bacterial susceptibility to natural products. Notably, the extract demonstrated comparable or even superior activity compared to the control of Ampicillin, in all cases. The observed differences in susceptibility among the isolates may be attributed to variations in their genetic makeup and resistance mechanisms. Moreover, the concentration-dependent response suggests a dose-response relationship, with higher concentrations of the extract resulting in increased inhibition of bacterial growth.

The therapeutic potential of *Cyperus rotundus* extract as an alternative or adjunctive treatment for MDR *Acinetobacter baumannii* infections aligns with the broader context of exploring natural products as antimicrobial agents. Hashim [13] assessed the antimicrobial potential of commercial herbal products, underscoring the importance of investigating traditional remedies for combating microbial resistance. Further studies are warranted to elucidate the underlying mechanisms of action, optimize dosage regimens, and evaluate the efficacy of the extract in clinical settings. Additionally, the identification and isolation of the bioactive compounds responsible for the observed anti-bacterial activity are essential for the development of novel antimicrobial agents.

CONCLUSION

In conclusion, our findings support the growing body of evidence regarding the antimicrobial properties of *Cyperus rotundus* extract and its potential utility in addressing the challenges posed by MDR bacterial infections. Further research is warranted to elucidate the underlying mechanisms of action, optimize dosage regimens, and evaluate the clinical efficacy of this botanical extract.

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