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Research Article

Antibacterial evaluation of *Cyperus rotundus* Linn. root extracts against respiratory tract pathogens

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Background: *Cyperus rotundus* has a long history of medicinal use in Indian Ayurvedic medicine and Chinese Traditional Medicine (CTM) including antipyretic, digestive system disorders, dysmenorrhoea and other maladies.

Objectives: The present study aimed to investigate antibacterial properties of *C. rotundus* root extracts against three gram-positive and two gram-negative bacteria causing respiratory tract infections.

Methodology: Dried plant materials were crushed and extracted in petroleum ether, acetone, methanol and aqueous by using Soxhlet apparatus. The agar well diffusion method was adopted to examine antibacterial activity of extracts against test organisms. Phytochemical analysis was also done for the plant extracts.

Results: Results showed that methanol extract was most active as comparison to other extract. The maximum inhibition was found against *H. influenzae* (18.4±0.07 mm) followed by *S. pyogenes* (17.3±0.13mm), *P. aeruginosa* (16.2±0.07 mm) and *S. pneumoniae* (15.5±0.15 mm) and minimum against *S. aureus* (15.3±0.05 mm) respectively. The phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, steroids, saponins and tannins in plant extracts.

Conclusion: The results signify traditional values of *C. rotundus* in treatment of respiratory diseases which might be accountable for its antimicrobial potential.

Key words: Antibacterial activity, agar well diffusion method, *Cyperus rotundus*, respiratory diseases

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1. Introduction

Respiratory diseases including allergies, asthma and chronic obstructive pulmonary disease (COPD) are chief public health concerns worldwide. The proportion of non-communicable disease deaths in 2008 due to respiratory diseases were 3.9%, with 4.2 million deaths reported due to asthma and COPD globally (WHO, 2010). The most regular causative organisms of these infections are *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* (Tillotson and Lerner, 1967; Chan-Yenug and Lam, 1986; Pepys et al, 1969; Sue et al, 2006).

Herbal therapy is a major component of Indian Ayurvedic medicine, traditional Chinese medicine, Native American medicine, Homeopathy and Naturopathy (Fetrow and Avila, 2000). Natural products, either as pure compounds or as standardized plant extracts, provide an excess of opportunities for novel drugs discovery. Various studies around the world have been initiated to develop scientific evidence-based rational herbal therapies. The advent of the pharmaceutical chemistry during the early twentieth century brought the ability to synthesize an

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enormous variety of medicinal drug molecules and allowed the treatment of previously incurable and or life threatening diseases (Kirtikar, 2001).

Cyperus rotundus Linn. (Cyperaceae) is a perennial weed grows in small clump, up to 140 cm height. It is known as Nagar motha in Hindi, Coco-grass, Java grass and Nut grass in English. *C. rotundus* has wide range of medicinal and pharmacological properties. The rhizomes exhibit astringent, diaphoretic, diuretic, analgesic, antispasmodic, aromatic, carminative, antitussive, emmenagogue, litholytic, sedative, stimulant, stomachic and vermifuge properties (Sivapalan, 2013).

In the present work, *C. rotundus* root extracts were screened for phytochemical constituents and for antibacterial properties against selected respiratory pathogens that usually cause infections in upper and lower respiratory tract region.

2. Materials and Methods

2.1 Sample Collection and Preparation

Plant material was collected from Kirtinagar, Srinagar, Uttarakhand and authenticated at Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar. Roots were chopped and washed in fresh running water and dried under shade at room temperature for 14-21 days. Finally crushed into small pieces by using pestle and mortar and powdered in an electric grinder.

2.2 Extraction procedure

Plant extracts were prepared by immersing 200 g of powdered plant material in 600 ml of four different solvents i.e. petroleum ether (PET), acetone (ACE), methanol (MeOH) and water (H_2O), loaded in Soxhlet assembly and extracted for 72 hr using the successive solvent extraction method (Ahmed et al, 1998). Plant extracts were filtered using Whatman No. 1 filter paper and crude extracts obtained by removing solvent *in vacuo* at 30°C. Residues were stored at 4 °C until further use. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/ml for agar well diffusion method.

2.3 Phytochemical analysis

The phytochemical analysis of the extract for tannins, saponins, flavonoids, glycosides, alkaloids, steroids and phenols was done using standard procedures with minor adjustments (Trease, 1989; Harborne, 1973).

2.4 Test Microorganisms

Five bacterial strains causing respiratory infections used in this study were *Haemophilus influenzae* MTCC 3826, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442.

Bacterial strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh.

2.5 Preparation of Inoculums

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hr at 37 °C.

2.5 Antibacterial testing

The antibacterial activity of different extracts was determined by agar well-diffusion method (Ahmed et al, 1998). 0.1 ml of 12-16 hr incubated cultures of bacterial species were mixed in molten Mueller Hinton Agar medium no. 173 (Hi media Pvt. Ltd., Mumbai, India) and poured in pre-sterilized petri plates. A cork borer (6 mm diameter) was used to punch wells in solidified medium and the wells were filled with extracts of 45 µl of 200 mg/ml final concentration of extracts. DMSO was used as negative control. The efficacy of extracts against bacteria was compared with the broad spectrum antibiotic erythromycin at 200 mg/mL (positive control). The plates were incubated at 37 °C for 24 hr in BOD incubator and the diameter of the zone of inhibition was measured in millimetre. Each sample was assayed in triplicate and the mean values were calculated. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimetre (mm) as observed from the clear zones surrounding the wells.

3. Results and Discussion

The results illustrate good antibacterial activity against selected respiratory tract pathogens. After extraction, yield of PET extract was 3.55 %, ACE extract 4.85 %, MeOH extract 5.6 % and H_2O extract 5.2 % respectively. The results for antibacterial activity are depicted in **Table 1**. MeOH extract was found most active against all test pathogens in comparison to other extracts. The maximum inhibition was found against *H. influenzae* (18.4±0.07 mm) followed by *S. pyogenes* (17.3±0.13 mm), *P. aeruginosa* (16.2±0.07 mm) and *S. pneumoniae* (15.5±0.15 mm) respectively. The lowest inhibition was noted against *S. aureus* (15.3±0.05 mm). *C. rotundus* crude extracts was found less active in comparison to positive control erythromycin.

The root extract of *C. rotundus* therefore had notable antibacterial activity against selected pathogens i.e. *H. influenzae*, *P. aeruginosa, S. aureus*, *S. pneumoniae* and *S. pyogenes*. In a similar study, an inhibitory effect of *C. rotundus* was observed against selected bacterial strains including *S. aureus, Salmonella enteritidis* and *Enterococcus faecalis* with total oligomers flavonoids and ethyl acetate extracts (Kilani et al, 2008). The decoction of *C. rotundus* tubers also showed antidiarrhoeal activity and effect on adherence of enteropathogenic *E. coli* and enteroinvasive *E. coli* and *Shigella flexneri* to Hep-2 cells (Daswani et al, 2011). Tambekar et al, (2009) also reported that MeOH extract of the rhizomes of *C. rotundus* showed considerable antibacterial potential against *S. aureus, K. pneumoniae, S. typhi, S. paratyphi, S. typhimurium, P. aeruginosa, E. aerogenes.*

Table 1 The percentage of potency of *Cyperus rotundus* extracts against respiratory tract pathogens

S. No.	Pathogens	Diameters of inhibition zone (mm)				
		PET	$CHCl_3$	MeOH	H_2O	Erythromycin
1.	*H. influenzae*	12.3±0.12	14.3±0.1	17.3±0.1	9.2±0.05	21.6 ±0.28
2.	*H. influenzae* (MTCC 3826)	11.2±0.1	13.7±0.07	18.4±0.07	10.4±0.16	21.3±0.76
3.	*P. aeruginosa*	9.5±0.07	10.2±0.1	15.5±0.15	11.2±0.07	16.3±0.57
4.	*P. aeruginosa* (MTCC 2474)	10.4±0.07	11.4±0.05	15.1±0.07	10.3±0.07	16.6±0.76
5.	*S. aureus*	9.4±0.12	11.3±0.07	15.3±0.05	9.2±0.1	29.3±1.04
6.	*S. aureus* (MTCC 1144)	10±0.15	10.4±0.07	14.4±0.13	11.1±0.05	30.3±0.76
7.	*S. pneumoniae*	10.1±0.13	9.5±0.1	16.2±0.07	12.5±0.1	20.0±0.50
8.	*S. pneumoniae* (MTCC 655)	9.4±0.07	10.7±0.07	15.1±0.08	11.3±0.07	19.3±0.57
9.	*S. pyogenes*	10.3±0.07	11.7±0.05	17.3±0.13	12.3±0.05	23.6±0.76
10.	*S. pyogenes* (MTCC 442)	10.4±0.11	10.7±0.05	16.1±0.03	13.1±0.02	25.6±0.28

Values are means of three replicates, Cork borer diameter: 6 mm

Table 2 The phytochemical screening of crude extracts of *Cyperus rotundus* root

S. No.	Phytoconstituents	Solvents			
		PET	ACE	MeOH	H_2O
1.	Alkaloids	-	+	+	+
2.	Flavonoids	-	+	+	+
3.	Glycosides	-	+	-	+
4.	Steroids	+	+	+	+
5.	Saponins	-	-	+	+
6.	Tannins	+	+	+	+

+ = Present, - = Absent

The phytochemical screening of *C. rotundus* extract has shown that plant contains alkaloids, flavonoids, glycosides, Steroids, Saponins and Tannins which are important and often pharmacologically active phytochemical constituents in plants (**Table 2**). Tambekar et al*,* (2009) had reported a variety of constituents i.e. alkaloids, flavonoids, glycosides, Steroids, Proteins, Phenolics and Tannins present in *C. rotundus*.

Various active phytoconstituents have been identified in *C. rotundus* i.e. α-cyperone, β-selinene, cyperene, patchoulenone, sugeonol, kobusone, pinene, cineole, sesquiterpenes, iso-cyperol, glycerol, linolenic, linolic, oleic, myristic, oleanolic acid and its glycosides, β-sitosterol and stearic acids (Chopra et al, 1956; Asolkar et al, 1992; Siebert et al, 2008).

Studies showed that *C. rotundus* can be used as herbal medicine to treat respiratory infections caused by tested pathogens as comparative to synthetic chemotherapeutic agents.

4. Conclusion

The investigation for antibacterial activity of *C. rotundus* root extracts revealed that the root extracts have broad spectrum activity against selected bacteria which provides a partial basis for its use in traditional medicines. The highest activity was exhibited by MeOH extract against test respiratory pathogenic microorganisms. By results, it can be concluded that *C. rotundus* may be helpful as an alternative source of medicine and new drug discovery.

Conflict of Interest declaration

The authors declare no conflict of interest

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