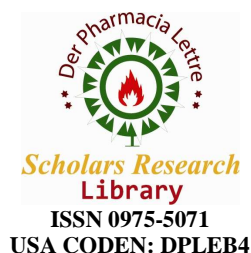




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Antimicrobial investigations on rhizomes of *Cyperus rotundus* Linn.

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ABSTRACT

Cyperus rotundus Linn. rhizomes extracts were evaluated against six important pathogenic microbes viz. *Staphylococcus epidermidis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans*. The powdered rhizome extracts were successively extracted with petroleum ether, chloroform, ethanol and water using Soxhlet apparatus. The antibacterial and antifungal activities were performed by both agar well diffusion and serial dilution methods. The ethanolic extract was found to exhibit highest activity against tested bacteria. However all extracts were ineffective against fungal strains. The inhibitory effect is very similar and comparable with that of standard drug.

Keywords: *Cyperus rotundus*, antibacterial, antifungal, minimum inhibitory concentration.

INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants are found in “Rigveda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge [1]. In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries [2]. There are 3,00,000 species of higher plants that occur in nature, only about 2 percent have been screened so far. Extract of plants from 157 families has been reported to be active against microorganisms [3]. Nowadays multiple drug resistance microorganisms have developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease [4]. Due to alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is

a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [5]. About 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced in the market are obtained from natural or semi synthetic resources. It has been reported that plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases [6]. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs [7]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [8]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [9]. Green plants represent a reservoir of effective chemotherapeutic agent and can provide valuable sources of natural pesticides [10]. Biopesticides have been suggested as an effective substitute for chemicals [11]. Reports are available on the use of several plant byproducts which possess antimicrobial properties, on several pathogenic bacteria and fungi [12]. The present study aimed at evaluating the *in vitro* antimicrobial activity of various extracts of rhizomes of *Cyperus rotundus*.

Cyperus rotundus Linn. (Family Cyperaceae), commonly known as ‘Nagarmotha’ is found throughout India. It is a pestiferous perennial weed with dark green glabrous culms, arising from underground tubers [13, 14]. A number of pharmacological and biological activities including antidiabetic, antidiarrhoeal, cytoprotective, antimutagenic, antioxidant, antimalarial, antiinflammatory, antipyretic and analgesic activities have been reported for this plant [15]. The rhizome part of *Cyperus rotundus* is one of the oldest known medicinal plants used for treatment of dysmenorrhoeal and menstrual irregularities [16]. The phytochemical investigation of *Cyperus rotundus* rhizome have revealed the presence of polyphenol, flavonol glycoside, alkaloid, saponins, sesquiterpenoids and essential oil [17].

MATERIALS AND METHODS

Plant material

The plant material was collected at its flowering stage from Hisar district, Haryana, July 2009 (29°10'12" N Latitude and 75°43'12" Longitude E). The plant was identified and authenticated by the Dr. H. B. Singh, Head, Raw Materials Herbarium & Museum, NISCAIR, New Delhi, Ref. No. NISCAIR/ RHMD/Consult/1491/89.

Preparation of extracts

The rhizomes were cleaned and dried under shade for 15 days. It was coarsely powdered and then exhaustively extracted successively with petroleum ether, chloroform, ethanol and water in a soxhlet apparatus. All extracts were respectively diluted in the concentration range of 1000µg/ml, 500µg/ml with DMSO.

Test microorganisms

The following bacterial and fungal strains were used in the study, *Staphylococcus epidermidis* MTCC-3615, *Bacillus cereus* MTCC-430, *Pseudomonas aeruginosa* MTCC-424, *Escherichia coli* MTCC-443, *Aspergillus niger* MTCC-1344 and *Candida albicans* MTCC-227

Antibacterial assay

The antibacterial activity of extracts was studied by agar well diffusion method [18] with slight modification. Molten nutrient agar (25 ml) was poured into presterilized petriplates and allowed to solidify at room temperature. Potato dextrose agar was used for fungal cultures. The plates were then seeded with 0.1ml (10^5 - 10^6 cells/ml) of overnight bacterial culture. Subsequently 8 mm wide wells were bored within these agar plates using a sterile cork borer. The wells were aseptically filled with 100µl of various extracts and labeled accordingly. The plates were incubated over night at 37°C for bacterial cultures and at 20-22°C for 5 days for fungal cultures. Microbial growth was determined by measuring the diameter of zone of inhibition. For each strain, a negative control was maintained where DMSO without extract was used. Standard antibiotic like gentamycin and amphotericin were also maintained as positive control (Table 1). The above experiment was carried out three times and mean values are presented herewith.

Determination of minimum inhibitory concentration (MIC)

The MIC of active extracts were determined by tube dilution method. Successive tubes filled with 15 ml nutrient broth containing 1000µg/ml, 500µg/ml, 250µg/ml up to 31.75µg/ml respective concentrations of extracts were inoculated with 100µl of the bacterial suspension containing 10^8 CFU/ml of respective test organisms. The tubes were incubated at 37°C in an incubator and observed for change in turbidity after 24 h. A tube containing nutrient broth without extract was taken as control. The least extract concentration which inhibited the growth of the test organisms was taken as MIC.

RESULTS

The data reported in Table 1 presents the antimicrobial activity of various extracts of *Cyperus rotundus*. The results showed inhibition of growth of some of the tested microorganisms with various degrees. The ethanolic extract was found to be most effective antimicrobial agent as compared to other extracts. *B. cereus* was the most susceptible gram-positive bacteria followed by *S. epidermidis* whereas *E.coli* was the most resistant gram-negative bacteria against all extracts. None of the extracts exhibited antifungal activity against *Aspergillus niger* and *Candida albicans* strains. The MIC values of the active extracts are reported in Table 2.

Table No 1: Antimicrobial activity of various extracts of *Cyperus rotundus* rhizomes

Sample	Dose µg/ml	Zone of inhibition (mm)					
		<i>S. epidermidis</i>	<i>B. cereus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albicans</i>
Petroleum ether extract	500	–	8	–	–	–	–
	1000	–	9.6	–	–	–	–
Chloroform extract	500	–	–	–	–	–	–
	1000	–	–	–	–	–	–
Ethanolic extract	500	8.2	7.7	9.9	–	–	–
	1000	10.2	10.1	12.2	–	–	–
Aqueous extract	500	–	–	–	–	–	–
	1000	–	–	–	–	–	–
Gentamycin	20	17.8	18.3	16.9	18.3	–	–
Amphotericin	20	–	–	–	–	17.7	19.1

Data are mean of 3 values

Table 2: MIC values of active extracts of *Cyperus rotundus*

Sample	Microorganism	MIC value
Petroleum ether extract	<i>B. cereus</i>	250
Ethanollic extract	<i>S. epidermidis</i>	250
Ethanollic extract	<i>B. cereus</i>	250
Ethanollic extract	<i>P. aeruginosa</i>	125

DISCUSSION

The antibacterial activities of medicinal plants are attributed due to the presence of flavonoids, tannins and steroidal alkaloids [19, 20, 21]. This study suggests that the rhizome extract of *Cyperus rotundus* have a board spectrum of antibacterial activity, although the degree of susceptibility could differ between different organisms. The antibacterial activity found in this present study may be attributed to the presence of secondary metabolites of various chemical types present in the plant material either individually or in combination. Our results indicates the potential usefulness of *Cyperus rotundus* in the treatment of various pathogenic diseases as it may help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of bacterial diseases. The discovery of a potent remedy from plant origin will be a great advancement in microbial infection therapies. Antibacterial agents currently available in the market are limited due to their toxicity, low effectiveness and prove costly in case of prolonged treatment. Therefore, there is need to develop new antibacterial agents which can satisfy the present demand.

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