

Antibacterial activity of acetone extract of *Cyperus rotundus* Linn.

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Abstract

The whole parts of the plant *Cyperus rotundus* Linn. was extracted with acetone, vacuum dried and subjected to phytochemical screening. The acetone extract exhibited potent antibacterial activity against *Bacillus subtilis* than *Escherichia coli*.

Keywords: antibacterial activity, *Bacillus subtilis*, *Cyperus rotundus*, *Escherichia coli*, phytoconstituent

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from medicinal plants making them a rich source of drug compounds. Natural products play an important role in drug development in pharmaceutical industry as over fifty percent of all modern clinical drugs are of natural origin, (Barker *et al.*, 1995).

Medicinal plants are distributed world wide, but they are most abundant in tropical countries (Calixto *et al.*, 2000). It is estimated that today, plant materials are presently in or have provided the models for 50% allopathic drugs (Robbers *et al.*, 1996). A relatively small percentage of medicinal plants are used as food by both humans and other animal species. It is possible that even more might be found used for medicinal purposes in different traditions (Moreman, 1996). The World Health Organization (WHO) has estimated that as many as eighty percentage of the world population is dependent on traditional medicine for their primary health needs. The traditional system of medicine has a heritage of community acceptance and the experience and knowledge of local herbalists, who can utilize enormous diversity of plants, which is much sought after and patronized (CSIR, 2008).

The effect of plant extracts on pathogenic microbes have been studied by a very large number of researchers in different parts of the world (Recio, 1989; Digark *et al.*, 1999; Sahin *et al.*, 2003). Much work has been done on ethno-medicinal plants in India (Nair *et al.*, 2002). It has been suggested that aqueous and solvent extracts from plants are potential sources of antimicrobial agents (Reddy *et al.*, 2001). Although a number of plants with

antimicrobial activities have been identified, a great number still remains to be identified (Dimayuga, 1998).

Cyperus rotundus is the plant of the Cyperaceae family, consisting of stems that are tuberous at the base rising from creeping. Underground root-stock, is about 10-25 cm tall. Leaves are as long as the stems and are dark green in colour. Flowers are in rather small inflorescence umbel type, simple or compound, 2 to 6 cm long with rather long spikes with 3-8 spikelets. The spikelet is brown, flat, slender, 10-25 cm long with 10 to 25 florets per spikelet. Its uses in modern ayurvedic medicines are primarily for treating fevers and digestive tract disorder. It is also known as an emenagogue (treats delayed menstruation) and is useful for dysmenorrheal (painful menstruation). This paper reports the results of qualitative screening of phytochemical compounds in the acetone extract of *Cyperus rotundus* and its activity against the pathogenic bacterial strains *Bacillus subtilis* and *Escherichia coli*.

MATERIALS AND METHODS

Collection of Plant Materials

The plant *Cyperus rotundus* Linn was collected from the village Moovanallur near Mannargudi, Tamil Nadu, South India.

Preparation of Plant Extract

The parts of the plants are collected and air dried for five days and then ground to powder using wooden mortar and pestle. Ten gm of the powdered sample was subjected to soxhlet extraction for 18 hrs using double distilled water and 100ml acetone. The extracts were evaporated to dryness under reduced pressure at 40°C and weighed (Ibrahim *et al.*, 1984; Kale *et al.*, 1989). The different concentrations (100µg/ml, 200µg/ml, 300µg/ml) of acetone extracts were used for further studies.

Phytochemical screening

Qualitative analysis of secondary metabolites were done by making use of standard methods (Trease and Evans, 1971).

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Bacterial Strains

Bacterial strains were collected from Sri Gowri Biotech Research Academy, Thanjavur. The subcultures were prepared from the stock culture using nutrient broth medium. Then the subculture is used for the antibacterial study.

Assay for antibacterial testing

Antibacterial activity of the plant extract was tested using disc diffusion method (Bauer *et al.*, 1996). The disc was prepared by using Whatmann No.1 filter paper. Then, the filter paper discs were sterilized and soaked in different concentrations of plant extract. The bacterial cultures were diluted with sterile water and mixed thoroughly to get a clear homogenous suspensions. Each suspension was uniformly spread on solidified agar medium. Sterile filter paper discs were soaked in different concentrations of plant extract. Then the filter paper discs were allowed to dry and immersed again in different concentrations of plant extract. The filter paper discs were spread carefully over the cultures and incubated at 37°C for 24 hrs. Paper discs treated with distilled water alone served as control. The antibacterial activity was recorded by measuring the width of the clear zone around each discs.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of acetone extract showed the presence of flavonoids, glycosides, tannins, alkaloids, carbohydrates, oils and fats, lignins, and saponins Table.1.

Table 1. Phytochemical screening of acetone extract of *Cyperus rotundus* Linn.

S. No.	Test	Result
1	Alkaloids	+
2	Carbohydrates	+
3	Sterols	-
4	Fixed oil and fats	+
5	Tannins	+
6	Proteins	-
7	Flavonoids	+
8	Lignins	+
9	Saponins	+
10	Glycosides	+

Note: + indicates the presence of a compound and - indicates the absence of a Compound

Antibacterial activity of acetone extract of *Cyperus rotundus* was evaluated against the pathogenic microbes *Bacillus subtilis* and *E.coli*, using disc diffusion method. Preliminary screening of plant extract was carried out using acetone extracts in three concentrations (100, 200, 300µg/ml). Considerable antibacterial activity was detected in acetone extract. The concentration of 300µg/ml was found to be more potent, when compared to the other two concentrations (100, 200µg/ml), as it effectively inhibited all the tested organisms. The acetone plant extract showed higher antibacterial activity against *Bacillus subtilis* with the zone of inhibition 15.7mm and a lesser antibacterial activity was detected against *E.Coli* with a zone of inhibition of 13.5 mm (Table.2). Two mg/ml of Streptomycin showed wider zones of inhibition of both *B.subtilis* and *E.coli*

Table 2. Antibacterial activity of acetone extract of *Cyperus rotundus* Linn.

Name of the pathogen	Reference Drug (Streptomycin)	Aqueous extract (Zone of Inhibition (mm))			Acetone Extract (Zone of inhibition (mm))		
		100 µg/ml	200 µg/ml	300 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml
		<i>Bacillus subtilis</i>	17.6	4.2	5.5	8.0	9.2
<i>E.Coli</i>	15.8	3.4	4.6	6.2	7.5	9.5	13.5

The results are the mean values of triplicate tests obtained after 24 h of incubation at 37°C.

which could be comparable to the effect of 300µg/ml of the acetone extract of *C. rotundus*.

The results indicated a great potency of the acetone extract as antimicrobial agent against both the gram positive (*B. subtilis*) and the gram negative organisms (*E.Coli*). However the aqueous extract was less potent against *B. subtilis* and *E.Coli*. It has been reported that certain plant secondary metabolites like tannins, flavonoids and steroids show antibacterial activity (Lutete *et al.*, 1999). So it is inferred that antibacterial property of the plant extract might be due to the presence of these metabolites as they have been found to be present in whole plant extract. However the chief constituent of *C. rotundus* is cyperone a complex mixture of alkaloids and diterpenoids.

Similar to the present study, earlier many plant extracts were tried for their antibacterial activity against pathogenic bacteria (Ahmed *et al.*, 1998; Kudi *et al.*, 1999). Shahidi (2004) reported the use of acetone extract of *C. rotundus* in the Iranian native medicine against *Pseudomonas aeruginosa*, *E.Coli*, *Micrococcus leteus* and *B. subtilis*.

From the above results it is evident that the extracts of *Cyperus rotundus* Linn might be a potent antibacterial

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agent and can be studied further to develop a potent antibacterial drug.

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