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ANTIMICROBIAL ACTIVITY OF METHANOLIC TUBER EXTRACT OF CYPERUS ROTUNDUS L.

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ABSTRACT

Antimicrobial efficiency of *Cyperus rotundus* L. a medicinal plant (Tuber extract) was examine using methanol as solvent and tested against human pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Candida tropicalis* and *Malassezia furfur* using agar well diffusion method. The methanolic tuber extract had shown zone of inhibition against all the test organisms at all concentration and zone of inhibition decreased in size with low concentration of tuber extract. It is revealed that the tuber extracts of *C. rotundus* showed maximum zone inhibition against *P. aeruginosa* (18.5±0.07 mm) and the minimum zone of inhibition against *E. coli* (15.5±0.07 mm) at 1000 µg/ml concentration.

KEYWORDS: Antimicrobial activity, *Cyperus rotundus* L. medicinal plants, Agar well diffusion method.

INTRODUCTION

Medicinal plants are the main source of drugs that are used from the ancient times as herbal remedies for health care, and prevention and cure of different diseases and ailments (Kaila, 2005). Totally, 77,000 plants (18.2% of total plant species) are used for medicinal purpose. Out of the these medicinal plants used worldwide, 3000 medicinal plants are internationally traded, only 900 species are cultivated and rest of the species are gathered from their natural habitats (Ramawat and Goayl, 2008). India with a wealth of 8000 medicinal plants, availability of high yielding cultivars, cultivation and processing technologies, has excellent potential to harness the economic power of medicinal plants. Medicinal plants have been used in developing countries for thousands of years. World Health organization (WHO) estimated

that 70-80% of the population living in India, Africa, and other developing nations depend on traditional healthcare systems for primary healthcare (Rajeswara *et al*., 2012).

Medicinal properties are due to active ingredients derived from plants can come from many different parts of a plant including leaves, roots, bark, fruit, seeds, flowers and tubers. Specifically tubers, corms and bulbs are various types of modified plant structures that are enlarged to store more active principles. They are used by plants to survive the long winters or dry months and provide energy and nutrients for re-growth during the next growing season (Sharma and Ramawat, 2016).

Tuberous plants have high calorific value and carbohydrate content along with medicinal properties due to the presence of bioactive molecules. The biological activities of tuberous plants range from analgesic, antimicrobial, hypolipidemic to hepatoprotective and anticancerous. Many tuberous medicinal plants are currently used in the preparation of stimulants, tonics, carminatives, expectorants and these are rich in dietary fibres, carotenoids, steroids, saponins, polyphenolics and anthocyanin (Sharma and Ramawat, 2016). Some of the tuberous plants are commonly used for the preparation of herbal formulations in India for healthcares include *Asparagus adscendens*, *Coleus forskohlii*, *Holostemma adakodien* and *Typhonium trilobatum*.

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. *Cyperus rotundus* L. is a field weed known in all the Southern States as nut grass. The plant produces rhizomes, tubers, basal bulbs and fibrous roots below ground, and rosettes of leaves, scapes, and umbels above ground. In the present study was to evaluate the antimicrobial activity of the methanolic tuber extract of *Cyperus rotundus* L. against human pathogenic organisms.

MATERIALS AND METHODS

Plant material

Fresh tubers of *Cyperus rotundus* was collected from NGM College campus, Pollachi and identified by PG and Research department of Botany, NGM College, Pollachi.

Sample Extraction

Healthy underground tubers of *Cyperus rotundus* was washed with tap water several times and then dried under shade. The air dried and powdered tubers were extracted in room temperature with methanol (500 ml) in a glass container for a week and then filtered (Whattman No.1 filter paper) and the filtrate was dried in room temperature and stored at 4°C for further use.

Microorganisms

Clinically isolated microorganisms were obtained from Department of Microbiology, PSG institute of Medical Sciences & Research, Peelamedu, Coimbatore. The bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Staphylococcus aureus*) were grown in the nutrient broth and maintained on Nutrient agar slants at 4^{o}C, while fungal strains (*Candida tropicalis and Malassezia furfur)* were grown in Potato Dextrose broth and maintained on Potato Dextrose Agar (PDA) slants at 4^{o}C.

Preparation of crude extract

The dried methanolic tuber extracts of *Cyperus rotundus* (10.0 mg) was dissolved in (1ml) DMSO. From the above 100µl contain 125, 250, 500 and 1000 µg concentration of samples were prepared.

Antimicrobial Assay

Antimicrobial activity of different concentration of methanol extracts were assayed separately using agar well diffusion method (Azoro, 2002). The petriplates were sterilized using autoclave at 121^{0}C for 15-20 minutes. Freshly prepared nutrient agar medium and PDA medium was poured into sterilized petriplates and allowed to cool. Different bacterial and fungal species were inoculated onto the Nutrient and Potato Dextrose broth and incubated at room temperature. Nutrient agar (NA) and PDA plates were swabbed (sterile cotton swabs) with 12 hours-old broth culture of bacteria and fungi respectively. Using the sterile cork borer, the well (8 mm) was made into the each petriplate. 100 µl of different concentration of methanolic tuber extracts of *Cyperus rotundus* were added to the well. The DMSO (acts as a negative control) and ampicillin (10 mg/ml) (served as positive controls) was added into the separate wells by using sterile micropipettes. Then the plates were incubated at 37^{o}C for all the bacterial strains while that of fungal strains were incubated at 28^{o}C for 48 h. The

antimicrobial activities of the extracts were assessed based on the measurement of the inhibition zone formed around the well.

Data analysis

Results were averaged, and given as mean ± standard deviation, calculated by using the Microsoft excels.

RESULT AND DISCUSSION

The use of antimicrobial agents is critical to the successful treatment of infectious diseases. Although there are numerous classes of drugs that are routinely used to treat infections in humans, pathogenic microorganisms are constantly developing resistance to these drugs (Al-Bari *et al*., 2006). The results for antimicrobial activity of tuber extract under study against human pathogenic bacteria and fungi are shown in Figure 2. The diameter of zone of inhibition was increased with the increase in concentration of tuber extract. The methanolic tuber extract had shown zone of inhibition against all the test organisms at all concentration and zone of inhibition decreased in size with low concentration of tuber extract. Early study Shinde *et al.* (2012) also reported that 70% ethanolic extract of *C. rotundus* displayed concentration dependent antimicrobial activity.

For methanol extract the zone of inhibition of bacterial and fungal growth was increasing in a dose dependent manner (Table-1). The highest zone of inhibition (18.5±0.07 mm) was observed in *P. aeruginosa* at 1000 µg concentration. The lowest zone of inhibition (15.5±0.07mm) was observed in *E. coli* at 1000 µg concentration. All the tested organisms were responded well and the diameters of zone of inhibition for these microbial cultures were above 15 mm at 1000 µg dose. The highest zone of inhibition (16 ± 0.14 mm) was observed in *P. aeruginosa* and *S. aureus* at 500 µg concentration. The lowest zone of inhibition (13 mm) was observed in *E. coli* at 500 µg concentration. All the tested organisms were responded well and the diameter of zone of inhibition for these microbial cultures was above 13 mm at 500 µg dose. The highest zone of inhibition (15 mm) was observed in S. *aureus* and the lowest zone of inhibition (10.5 mm) was observed in *E. coli* at 250 µg concentration. The highest zone of inhibition (13 mm) was observed in *M. furfur* and the lowest zone of inhibition (9.5 mm) was observed in *E. coli* at 100 µg concentration. All the tested microorganisms were showed lesser activity than antibiotic ampicilin.

The methanol extracts of *C. rotundus* leaves was subjected to antibacterial assay on *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. The maximum antimicrobial activities were observed at concentration of 100% of extract than at 10%. The extract was found effective against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Escherichia coli* and was found ineffective against *Staphylococcus aureus* (Wangila, 2017). In the present study also proofed that the tuber extract were found effective against all strains tested at all the concentration.

Methonolic tuber extracts of *C.rotundus* were tested for antimicrobial activity against 4 strains of pathogenic bacteria and 2 strains of fungal cultures were tested. Ampicillin was used as standard antibiotic to compare the inhibitory effect of the *C.rotundus* extract. Methanolic tuber extracts *C.rotundus* was showed remarkable activity against gram-positive bacteria and less activity against gram negative bacteria. Similar result was reported in *Cyperus* oil extracted from *C.rotundus* (Nima *et al.*, 2008).

Vijisaral and Subramanian (2013) had reported that the ethanol extracts of *C.rotundus* leaves had broad-Spectrum of activity against both bacteria and fungi. Similar results obtained in methanolic tuber extract. The ethanolic tubers extract of *Dioscorea deltoidea* showed significant activity of 19±1 mm, 17±1 mm and 15±1 mm against *S. aureous, P. aeruginosa* and *E. coli* respectively (Subhash *et al*., 2013). The significant activity was also resulted in this methanol extract of *C.rotundus* tubers.

Alcoholic tuber extract of *Amorphophallus konkanensis* and *Amorphophallus bulbifer* showed less zone of inhibition in gram negative bacteria (Chidanand *et al*., 2015). In the current study showed potent inhibitory zone against gram negative bacteria.

The antimicrobial activity was carried out in methanol extract of *Pueraria tuberose* tuber against human pathogenic bacteria and fungi. *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus* and *C. tropicalis* showed the inhibitory zone of 10mm, 0 mm, 9.1 mm, 0 mm and 0 mm respectively at 50mg/disc concentration (Aruna *et al*., 2015). In the current study methanolic tuber extract showed the remarkable inhibitory zone against *E. coli* (15.5 mm), *K. pneumonia* (17.5 mm), *P. aeruginosa* (18.5 mm), *S. aureus* (18 mm) and *C. tropicalis* at 1mg concentration.

Table 1: Antimicrobial activity of methanolic extract of *C. rotundus* tuber.

Organism	Zone of inhibition in mm					
	1000 µg	500 µg	250 µg	125 µg	Ampicillin 25µl (10 mg/ml)	DMSO
E. coli	15.5±0.07	13±0.00	10.5±0.07	9.5±0.21	20.00	0
P. aeruginosa	18.5±0.07	16±0.14	10.5±0.21	10±0	19±1.4	0
K. pneumonia	17.5±0.21	14.5±0.07	12±0.00	11±0.28	18±0.70	0
S. aureus	18±0.14	16 ±0.14	15±0.00	11.5±0.21	19±0.70	0
C. tropicalis	17.5±0.07	14.50±0.21	14±0.14	11.5±0.07	18±0.35	0
M. furfur	17±0	15.00±0.14	14±0.14	13±0.28	17.9±0.14	0

Values are expressed as mean ± Standard Deviation (SD).

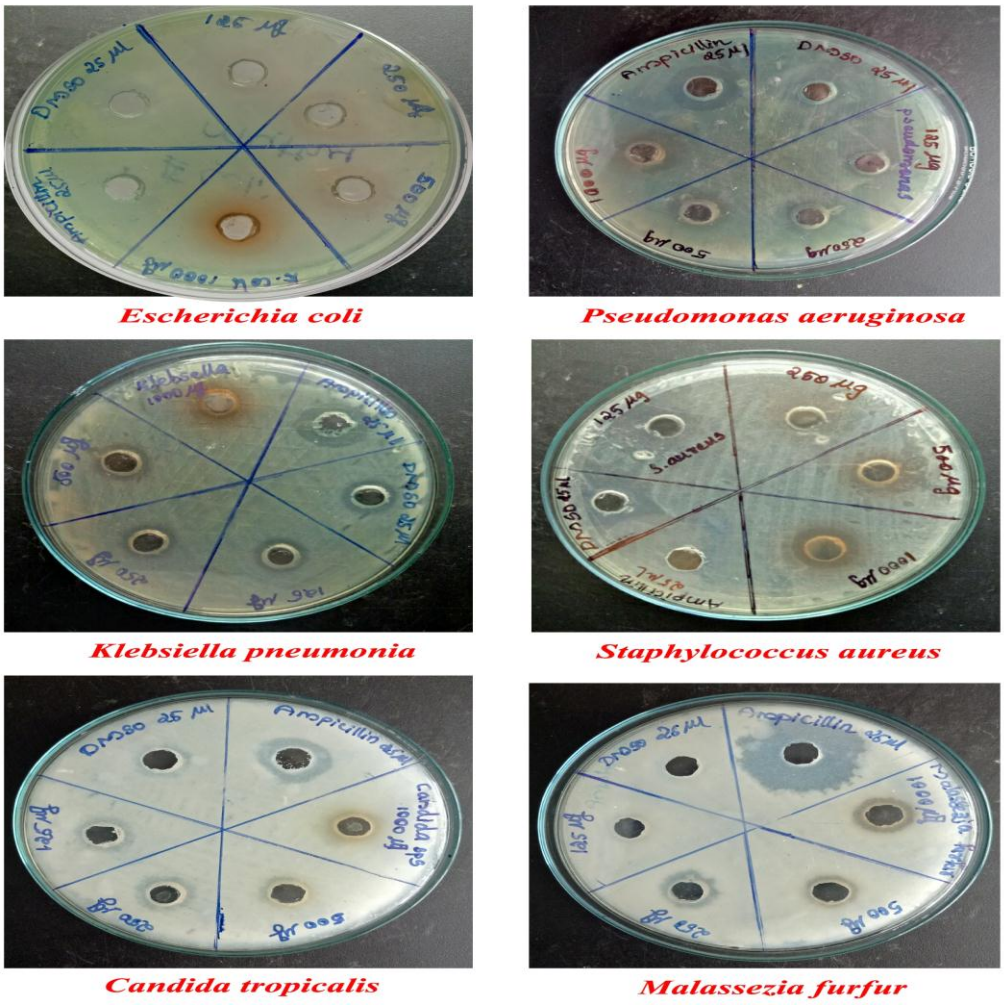

Figure 2: Antimicrobial activity of methanolic extract of *C. rotundus* tuber.

CONCLUSION

Based on my results it may concluded that the methanolic extract of *Cyperus rotundus* tuber exhibited effective antimicrobial activity against all tested organisms and had maximum zone of inhibition. The varying range of inhibition of antimicrobial test organisms due to intrinsic

tolerance of microorganisms and also plant extracts have more bioactive compounds which are responsible for the bactericidal and fungicidal activities. Therefore, it has been suggested that the tuber extracts from the study plant might be used as alternative antimicrobial natural substances and also play a great role in the discovery of new drugs.

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