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Phytochemical and Preliminary Antimicrobial Activity of *Cyperus Rotundus* linn. Extract Against *Micrococcus Luteus*

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ABSTRACT: The plant kingdom is recognized as the most valued source of medicine for humanity as herbal/ethno-medicines has fewer side effects as compared to allopathic medicine due to its complex nature of bioactive compounds present in the plant. In nature, one of the most tenacious, colony forming weed having widespread geographical distribution and being native to Indian sub-continent, the *Cyperus rotundus* (Linn.) has been widely used in several pharmacological fields including antibacterial infections. In the current study *C. rotundus* (Linn.), the leaves extract was qualitatively accessed for its phytochemical content. Different solvent used to prepare leaves extract was evaluated against Micrococcus luteus for its antimicrobial activities using disc diffusion method. Our result indicated the presence of phytochemical such as flavonoids, terpenoids etc. Crude leaf extracts prepared in acetone. methanol and ethanol exhibited antimicrobial activity. Interestingly, ratio of acetone and ethanol solvent exhibited enhanced antimicrobial activity against the tested microorganisms.

Keyword: Antimicrobial activity, Phytochemical, Cyperus rotundus, Medicinal plant, Drug resistance.

I. INTRODUCTION

Herbal medicines obtained from ethnomedicinal plants have been used by humans since ancient times. The use of medicinal plant has been well documented in various culture. Currently, the use of medicinal plants has gained importance not only in India, but all over the world (Abat et al., 2017; Farnsworth, 1990). One of the primary reasons for renewed interest in medicinal plant and subsequently identification of bioactive compounds is due to the emergence of drug-resistant organism across globe. There are multiple reasons which are responsible for the emergence of drug resistance microorganisms, some are due to indiscriminate usage of drugs, incomplete drug treatment regime of the infected individual and natural evolution of microorganism which plays crucial role in drug resistance (Vaou et al., 2021; Abdallah et al., 2023; Chaachouay et al., 2024)

Cyperaceae are the largest family consisting of 109 genera and approximately 5,500 species. Cyperus rotundus Linn., belonging to the family Cyperaceae, is commonly known as purple nutsedge or nutgrass or java grass. The sedge family is the third largest family of monocotyledonous plants (Ranjani et al., 2013). *C. rotundus* Linn. is Distributed throughout the plains of India, but now found in tropical, subtropical and temperate regions. Itis a perennial herb or weed with slender, scaly creeping rhizomes, bulbous at the base arising from the tubers about 1-3 cm long. The tubers are dark reddish brown in color and white inside, about 12 mm thick and vary from 10-35 mm long with a characteristic odor. The stems grow to about 25 cm tall and the leaves are linear, narrow, dark green and grooved on the upper surface, ranging in size from 5 to 12 mm wide to 50 cm long. Inflorescence are small, with 2-4 bracts, consisting of tiny bisexual flowers with a red-brown husk. The nut is three-angled, oblong ovate, yellow in color and black when ripe (Kamala et al., 2018; Ranjani et al., 2013; Uddin et al., 2006).

Plant description
Botanical name: Cyperus rotundus Linn
Hindi name: Nagarmotha roots, Nut grass roots, Mustak
Sanskrit: Bhadramusta, Granthi, Kachhda, Mustako, Suganthi-granthila
English: Cyperus roots, Cyperiol roots, Nut grass roots, Coco grass, Purple Nut Sedge, Red Nut Sedge

Classification:
Kingdom :Plantae
Phylum: Tracheophyta
Class: Liliopsida
Order: Poales

Family: Cyperaceae
Genus: Cyperus L.
Species: Cyperus rotundus L

Distribution:

It is widely distributed in Africa, Western Asia, Western Indian Ocean, Caucasus, Middle Asia, Eastern Asia, Europe, Pacific, North America and Southern America (Al-Snafi, 2016).

C. rotundus is regarded as one of the best Ayurvedic herb. The rhizomes are used as a folk medicine for the treatment of stomach and bowel disorders and inflammatory diseases. Clinical trials and research studies shows the beneficial use of the plant as anti-arthritic, antibacterial, anti-cancer, anti-diabetic, anti-malaria and anti-inflammatory (Kamala et al., 2018).

Micrococcus luteus is a Gram-positive, spherical bacterium commonly found in soil, water, and as part of the human skin microbiota. While generally considered a non-pathogenic or opportunistic organism, M. luteus can cause infections in immunocompromised individuals. M. luteus can colonize wounds and exacerbate infections, particularly in individuals with compromised skin integrity. In rare cases, M. luteus has been associated with bloodstream infections and endocarditis, especially in patients with indwelling medical devices such as catheters. Strains of M. luteus have been identified with resistance to common antibiotics, raising concerns about treatment challenges. (Zhuet al.,2021; Shi et al., 2023)

In this present study, we have extracted phytochemicals using different solvent from C.rotundusLand investigated the antimicrobial activity on the survival of M. luteususing disk diffusion method.

II. MATERIAL AND METHODS

Plant Preparation:

The whole aerial part of the plant *C. rotundus* (L.)were collected from the field area Located to: 5PVM+F23, VIP Rd, Banarsi, Barauda-1, Chhattisgarh 492015, India. The plant was identified according to their morphology and structure. The Leaves were separated, weighed and washed with tap water followed by distilled water to remove dust and other organic contents. After washing the excess water was removed and spread them on the filter paper. The plant samples were shade dried at room temperature to prevent direct sunlight and degradation of active compounds. The dried sample were grinded using grinding machine (Remi). The powdered sample was stored in airtight containers and stored at room temperature/4°C for further use.

Preparation of extracts:

The extraction process was performed by maceration, briefly the sample material was placed inside a clean, airtight container. Then the extraction solvent (menstruum) is poured on the top of the sample (1:10), mixed gently and kept at room temperature for 3-4 days. The sample was agitated gently to ensure maximum surface adsorption. The powdered plant material was extracted in different solvents. viz., Aqueous, Acetone, Ethyl acetate, chloroform, Ethanol, Methanol, Acetone: ethanol (1:1), Acetone: ethanol (7:3). The mixture was filtered through Whatman No.1 filter paper and the clear filtrate was stored till further use.

Media preparation and microorganism culturing:

Nutrient Agar Medium (NAM) and Nutrient Borth were used to maintain. M. luteus culture at 37 °C. For antimicrobial susceptibility assay Mueller Hinton Agar was used, which was prepared and sterilized at 121°C for 15 min.

Qualitative analysis of phytochemical:

Phytochemical analysis of all extracts was done by following (Harbone,1989, and Trease and Evans,2009) to detect the presence of major phytoconstituents.

(a) Test for proteins:
Xanthoproteic test: 0.5 ml of plant extract was taken into the test tube, then 2-3 drops of Nitric acid were added. Yellowish color shows the presence of protein.
(b) Test fir saponins:
Foam test: 0.5 ml of plant extract were mixed with 0.5 ml Distilled water. After vigorously shaking the formation of stable persistent froth indicates the presence of saponins.
(c) Test for flavonoids:

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Alkaline reagent Test: 0.5 ml of plant extract was taken into the test tube. Then 1-2 drops of 10N NaOH were added. Yellow color appears. Then diluted HCl (10 times) were added drop by drop the disappearance of yellow color indicates the presence of flavonoids.

(d) Test for terpenoids:

Salkowski test: 0.5 ml of plant extract was mixed with 2 ml of chloroform and 3 ml concentrated sulphuric acid were added carefully. Formation of reddish-brown color in interphase indicates the presence of terpenoids.

(e) Test for tannins:

Ferric chloride test: 0.5 ml of plant extract were mixed with 5 ml of Distilled H_2O. Then boil the solution gently and 1-2 drops of $FeCl_3$ were added. Deep blue-black color indicates the presence of tannins.

Assessment of antibacterial activity:

The antibacterial activity was carried out with aqueous extract, acetone extract, ethyl acetate extract, chloroform extract, ethanol extract, methanol extract, acetone: ethanol (1:1) and acetone: ethanol (7:3) of C. rotundus (L.). The antibacterial activity of plant extracts was determined by disk diffusion method. The filtrate extract was used for primary screening before testing by crude extract. Each extract was tested against M. luteus. The concentration of each crude extract used for antibacterial activity was 8µl and the concentration of each pure extract used for antibacterial activity was 20 mg/ml, 40 mg/ml,60 mg/ml, 80 mg/ml and 100 mg/ml and a parallel control (2.5% DMSO) was also maintained.

The extracts were loaded into 6 mm diameter sterile discs at the above-mentioned concentrations. 100µl culture was spread on Muller Hinton Agar (Hi-Media) plate using a sterile cotton swab. The discs were placed in the plate using sterile forceps. The plates were incubated at 37˚C for 24h. The effects were compared with azithromycin, amoxicillin, ciprofloxacin and gentamicin at concentration of 0.2 mg/ml the plates were observed for the zone of inhibition after 24h.

III. RESULT AND DISCUSSION

In the present study traditional medicinal plant *C. rotundus* Linn. were selected to evaluate the bioactivities on the basis of their medicinal importance. Leaves were extracted sequentially by maceration process with different solvents viz., aqueous, acetone, ethyl acetate, chloroform, ethanol, methanol, acetone: ethanol (1:1) and acetone: ethanol (7:3) extract based on their polarity index

Preliminary phytochemical analysis of leaf extracts of *C. rotundus* Linn. As shown in Table (1), revealed that flavonoids were found in all extract. In aqueous extract, protein, saponins, flavonoids and tannins were present while terpenoids were absent**.** In acetone extract protein, flavonoids, terpenoids and tannins were present while saponins were absent. In ethyl acetate extract only flavonoids and terpenoids were present while proteins, saponins and flavonoids were absent.

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Table: 1 Phytochemical Screening of leaf extract of *C. rotundus* (L.), where Solvent type: Aqu. – Aqueous, Ace. – Acetone, Et. A.- Ethyl acetate, Chl.- Chloroform, Eth. – Ethanol, Met. Methanol

Abbr.	Solvent System	Phytochemicals				
		Protein	Saponins	Flavonoids	Terpenoids	Tannins
L1	Aqu.	++	++	+++	-	++
L2	Ace.	+++	-	++	+++	+
L4	Et. A.	-	-	++	++	-
L6	Chl.	-	-	+	-	+
L8	Eth.	+++	+	++	+++	++
L10	Met.	++	+++	+	-	++
L11	Ace. /Eth. (1:1)	+	+++	+	-	++
L12	Ace. /Eth. (7:3)	+	+++	+	-	++

In chloroform extract only flavonoids and tannins were present while proteins, saponins and terpenoids were absent. In ethanolic extract all the phytochemical were present i.e. proteins, saponins, flavonoids, terpenoids and tannins. In methanolic extract except terpenoids all phytochemical were present i.e. proteins, saponins, flavonoids and tannins. In acetone; ethanol (1:1) extract all phytochemical were present except terpenoids i.e. proteins, saponins, flavonoids and tannins. In acetone: ethanol (7:3) all phytochemical were present except terpenoids i.e. proteins, saponins, flavonoids and tannins.

Antibacterial Activity of leaf filtrate extract of *Cyperus rotundus* Linn.:

Acetone and methanol extracts displayed antibacterial activity against M. luteus (7mm) respectively, however acetone: ethanol ratio (1:1 and 7:3) exhibited enhanced antibacterial activity (8mm). Ethanol extract showed moderate activity against M. luteus Bacteria. While other extract i.e. aqueous extract (L1), ethyl acetate extract (L4), chloroform (L6) extract displayed negligible activity against *M. luteus* (data not shown). (Fig 2 c). Extraction solvent was used as control (Fig 2 b).

Amoxicillin, Azithromycin, Ciprofloxacin and Gentamicin (0.2 mg/ml each) was used to test the drug sensitivity of M. luteus. In our experiments, only Ciprofloxacin and Gentamicin exhibited zone of inhibition against M. luteus (7 mm and 8 mm) (Fig 2 a)

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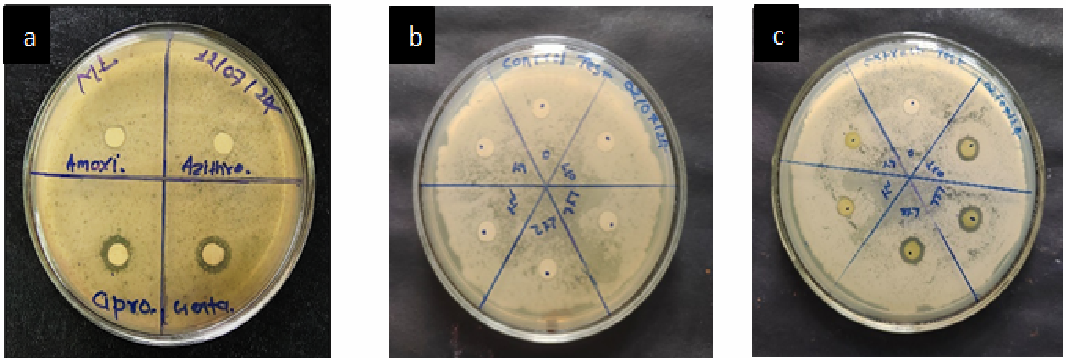

Fig:2 Antibacterial activity of leaf filtrate extract of *C. rotundus* (L.) against M. luteus (a) antibiotic control, (b) Extraction Solvent control (L2, L10, L11, L2), (c) extract (L2, L10, L11, L2), (0 represent DMSO control)

IV. DISCUSSION

Medicinal plants have always contributed extensively to the development of modern medicine, and plays important role in drug discovery. The vast genetic diversity available in plants presents a possibility for the production of novel medicine. However, only a small fraction of the plant kingdom has yet been analyzed for their possible medicinal uses

The present study exploring the phytochemical analysis of leaves of *C. rotundus* L. demonstrated the presence of flavonoids in all solvents used. While tannins, saponins and proteins were undetectable in ethyl acetate extract. Terpenoids were detectable only in acetone, ethyl acetate and ethanol extract respectively. Results from the preliminary antibacterial activity of *C. rotundus* (L.) indicated that the acetone, ethanol, methanol, acetone: ethanol (1:1) and acetone: ethanol (7:3) extract have antibacterial activity against *M. luteus.*

Our study has provided preliminary insight encompassing the antimicrobial activity of *C. rotundus* (L.) against M. luteus, and may help in future to formulate effective drug treatment regime.

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