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Antimicrobial activity in roots extract of *Cyperus rotundus l.* on pathogenic organisms

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Abstract

The increasing prevalence of drug-resistant pathogens has gained the attention of pharmaceutical and scientific communities towards potential antimicrobial agents from plant derived sources. The present research work has been undertaken to study the antimicrobial activity of the chloroform extract of Cyperus rotundus L. against some human pathogens like *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium Staphylococcus aureus, Shigella flexneri, Streptococcus pneamoniae, Klebsiella pneumonia* and fungi *Aspergillus niger* by using agar well diffusion method. Inhibition zones ranged between 4.l2 ± 0.22 - 16.24 ± 0.11 mm. Roots extract inhibited the growth of all tested microorganisms with large zones of inhibition .The standard antibiotics chloramphenicol and miconuzole nitrate were found to have zone of inhibitions 10.05 ±0.12-24.12 ±0.20 mm at the concentration of 30 ug/ml. In contrast, the inhibition zone of chloroform (negative control) was almost zero for all the tested microorganisms. The spectrum activity of chloroform extract of this plant could be a possible source to obtain new and effective herbal medicines to treat various infectious diseases.

Keywords: Antimicrobial activity, *Cyperus rotundus* L.,*chloroform* extract, human pathogens, zones of inhibition

Introduction

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine [1].

Since antiquity, many plant species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides. saponins. flavonoids, steroids, tannins, alkaloids and terpeonoids which are utilized to combat the disease-causing pathogens [2-4]. With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs [5]. Antibiotics are indisputably one of the most important therapeutic discoveries of the 2oth century that had effectiveness against serious bacterial infections [6]. Despite the huge number of antimicrobial agents for various purposes that already exist the search for new drugs is a continuous task since the target microorganisms often develop new genetic variants which subsequently become resistant to available antimicrobial agents [7-8]. The world's attention is now increasing directed toward plant sources for developing antimicrobial drugs, since natural products are considered safer than synthetic ones [9-10], According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs [11]. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [12]. There are several published reports describing the antimicrobial activity of various crude plant extracts [13-14]. It is estimated that there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological activities [15].

The different herbal plant extracts are traditionally has been used as anticancer antioxidant, antiulcer, analgesic and antidiabetic [16], and they also having the antiparasitic, antifungal, antibacterial, antimalarial activity, analgesic and anti-inflammatory aclivity [17], Different species of *Cyper rotundus* are used as a folk medicine for the treatment of various ailments such as skin diseases, intestinal parasites and warts. It has been reported that *Cyper rotundus* possesses antidiarrhoeal and antidysenteric activity [18]. *Cyper rotundus* L. belongs to the family Cyperaceae. Perennial herbs, 30 - 50 cm tall; stolons wiry ending in ellipsoid tuber; stem trigonous. Leaves: sheaths glabrous; blade linear, cariaceous, long-acuminate. Umbles compound; involucral bract 4-5 spreading; spikes ovoid, with 8-10 spikelets. [19]. The present research was set up to determine the antimicrobial activity in roots extract of Cyperus rotundus against some pathogenic bacteria and fungi.

Methodology

Chemicals and Plant collection

The following ingredients were used for the preparation of nutrient agar media and Potato dextrose media: Agar, Peptone, Sodium chloride. Beef extract, Potato, dextrose water. All other chemicals and analytical reagents were purchased from Hi-media, India, unless stated otherwise. Mature plants of *Cyperus rotundus,* used for this study was collected from Field area of yavatmal district (M.S.) India.

Preparation of the plant extract

The fresh plants were collected from Field area of yavatmal district (M.S.) India and identified with the help of flora and well-known taxonomist. The roots were washed for 2-3 times with tap water and finally with distilled water. Further air dried in shade for ten days and then dried in an oven at 60°C for one to two days, and finally milled to obtained a coarse powder (Sieve no.80). About 100 grams of powdered material was extracted by maceration in chloroform (400 mL) for 14 days with frequent agitation [20-22]. The mixture was filtered through clean muslin cloth followed by double filtration with Whatman No.l filter paper and the filtrate was concentrated by rotary evaporation under vacuum (vacuum pressure: 500 N/m^2) al 40^0C until a volume of about 15 mL waste reached. Next the concentrate was poured into glass Petri-dishes and brought to dryness in an oven at 60°C The obtained paste like mass was then stored in paraffin. Sealed petri-dishes in a dark cabinet. The extracts were reconstituted by dissolving in chloroform 10 the required concentrations. The reconstituted extracts were maintained at 2-8°C.

Test microorganisms and growth media

Pure cultures of all experimental bacteria; *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium,*

Staphylococcus aureus, Shigeilla flexneri, Streptococcus pneumonia, Klebsiella pneumonia and fungi *Aspergillus niger* were obtained from the microbial type Culture Collection and Gene Bank Institute of Microbial Technology (IMTECH), Chandigarh. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by sub culturing regularly on the same medium and stored at 4^0C before use in experiments.

Determination of the antimicrobial activity

Agar well-diffusion method was followed to determine the antimicrobial activity [23-25]. Nutrient agar (gm/1: beef extract, 3g; peptone. 5g; sodium chloride, 5g; agar, 20g) and Potato Dextrose Agar (39 gm/l) plates were swabbed (sterile cotton swabs) with 24h old-broth culture (10^6-10^8 bacteria CFU ml-^{1}) of respective bacteria and fungi. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of plant extract was prepared at a concentration of 100 mg/mL About 100 ml of plant extracts was added with sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract.30ug/ml chloramphenicol, and 30ug/ml miconazole nitrate were also used at positive controls for bacteria and fungi, respectively. The plates were incubated at 37^0C for 24h for bacteria pathogens and 37°C for 48h fungal pathogens. The diameter of the inhibition zone (mm) around each well was measured and express as antimicrobial activity. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Statistical analysis

The results of the experiment are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple pair wise comparison tests to assess the statistical significance.

Results and Discussions

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growlh of microorganism [11, 26], these compounds have significant therapeutic application against human pathogens including bacteria. Fungi or virus.

Table No. 1 : Cyperus rotundus Root the show Zone of Inhibition (mm)

Microorganisms	**Zone of Inhibition (mm)**		
	Chloroform	**Chloramphenicol**	**Miconazole nitrate**
Staphylococcus aureus	10.46 ± 0.14	12.12 ± 0.14	ND
Escherichia coli	13.12.± 0.18	20.10.± 0.22	ND
Aspergillus niger	16.24 ± 0.11	ND	24.12 ± 0.20
Salmonella typhimurium	8.06 ± 0.28	10.05 ± 0.12	ND
Shigella flexneri	4.12.± 0.22	12.10 ± 0.16	ND
Streptococcus pneumoniae	8.20 ± 0.16	14.16 ± 0.10	ND
Klebsilla pneumoniae	6.02 ± 0.22	10.18 ± 0.32	ND
Pseudomonas aeruginosa	10.22 ± 0.12	14.10 ± 0.26	ND

ND: Not determined. The inhibition zone diameter was taken as an average value of triplicate plates for each microorganism at 100 uL of 100 mg/ml crude extract, 30 ug/ml of chloramphemcol and 30 ug/ml of miconazole nitrate.

Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [27-28]. Therefore, medicinal plants are finding their way into pharmaceuticals, neutralceuticals and food Supplements

In the present investigation, the inhibitory effect of *Cyperus rotuntus* roots m chloroform extract was evaluated against both fungal and bacterial strains. The antimicrobial activity was determined by using agar well diffusion method and the results as summarized in Table 1.Methanolic extract (100.00 mg/ml) of the fruits displayed good antibacterial activity against

Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Shigella flexneri, Streptococcus pneumonia, Klebsiella pneumoniae and fungi *Aspergillus niger*.chloroform extract inhibited the growth of all tested microorganisms with large zones of inhibition ranged from 4.12 ± 0.22 - 16.24 ± 0.11 mm.The standard antibiotics chloramphenicol and miconazole nitrate were found to have zone of inhibitions 10.05 ± 0.12-24-12 ±0.20 mm at the concentration of 30 ug/ml In contrast, the inhibition zone of chloroform l (negative control) was almost zero for all The tested microorganisms. The large inhibition zones exhibited by the extract against *Aspergillus niger* justificd the plant use in the treatment of fungal infections.

Conclusion

Bacterial and fungal infections can be treated with the *Cyperusrotundus*,since it exhibited favourable antibacterial and antifungal activities. On the basis of the present study, further phytochemical and pharmacological studies will be needed to isolate the bioactive compound(s) and investigate the antimicrobial activities against a wider range of pathogenic microorgnisms.

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Conflicts of interest: The authors stated that no conflicts of interest.

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