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Research Article

ANTIBACTERIAL AND PHYTOCHEMICAL EVALUATION OF LEAF EXTRACT OF *Microsorum punctatum* (l.) Copel. TOWARDS BACTERIA INVOLVED IN CUTANEOUS DISEASES

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ABSTRACT

The work aimed to verify the antibacterial and phytochemical nature of the leaves of *Microsorum punctatum.* The leaves of the plant were especially evaluated for its antibacterial capability with respect to the bacteria involved in skin infections. Leaves of *M. punctatum* were checked for antibacterial potential and plant chemical constituents, especially in petroleum ether, acetone, ethanol and water in the manner of rising polarity. Antibacterial activity was confirmed by the standard disc diffusion experiment. Phytochemicals after being separated in silica gel Thin Layer Chromatography were identified by employing various standard spraying reagents. *Pseudomonas aeruginosa* was the most sensitive organism with respect to the extracts and therefore, its activity was further confirmed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) experiments. The phytochemicals obtained in acetone extracts of leaves the plant were active in terms of its antibacterial capability. No antibacterial activity was recorded for phytochemicals extracted in petroleum ether and water extracts. Detection of phenols, flavonoids, polyphenols and sterols in various extracts was noted in the experimentation. Presence of flavonoids, phenols, sterols and polyphenols in the ethanol extract of the plant accounted for its antibacterial activity. MIC and MBC values of 25 mg/ml and 50 mg/ml respectively were noted towards *P. aeruginosa*. Phytochemicals present in leaves of *M. punctatum* capable enough to react with the bacterium found in nosocomial infection.

Keywords: *Microsorum punctatum*, pteridophytes, fern, phytochemicals, disc diffusion, antibacterial activity

INTRODUCTION

Microsorum punctatum (L.) Copel. is a member of Pteridophyte that belongs to the group fern. The plant is a common, medium sized epiphytic herb, found in semi exposed as well as shaded localities. This species is also cultivated as an ornamental plant. *M. punctatum* belongs to Polypodiaceae, its synonym is *Acrostichum punctatum L.*[1]. Medicinal uses of the plant include its use as purgative, diuretic and wound healing[2]. Leaves of the plant were utilized as medicine[3,4]. Wide spread application of antibiotics in human being helped to develop new drug-resistant bacteria. These types of drug-resistant bacteria are a larger problem today[5]. Hence, people search for alternative natural source of medicine to overcome the problem. Normally, human beings depend on plants-based phytochemicals that can be employed as a substitute medicine. Present study aimed to evaluate the antibacterial capability and to detect the major phytochemical compounds present in the plant, especially in various solvents extracts of increasing polarity towards some pathogenic bacteria observed in skin infections.

MATERIALS AND METHODS

Preparation of Plant Extract

Healthy specimens of *M. punctatum* were obtained in the month of January from Pala, Kottayam District, Kerala. A voucher specimen (TT 1572) was preserved and deposited at the herbarium of St. Thomas College Palai. Leaves of the plant were shade dried for three weeks and ground to fine powder by utilising a mechanical grinder. The air-dried plant material (100g) was used for preparing extracts. Soxhlet extractions or distillations were successively performed in petroleum ether, acetone, ethanol and water[6] provided 0.37%, 3.4%, 4.1%, and 0.8% yield respectively.

Bacterial Strains Used

The bacterial strains were procured from the culture collection of Institute of Microbial Technology (IMTECH), Chandigarh. These consist of *Staphylococcus aureus* subsp *aureus* (MTCC 96), *Klebsiella pneumoniae* subsp *pneumoniae* (MTCC-109), *Serratia marcescens* (MTCC 6164), *Pseudomomas aeruginosa* (MTCC 741) and *Escherichia coli* (MTCC 443). The bacteria were later sub cultured on nutrient agar slants, further incubated at 37°C for 12 hours and kept at 4°C in the refrigerator to maintain the stock culture for future use.

In Vitro Antibacterial Assay

The standard procedure of disc diffusion method as explained by Bauer *et al*. [7] was conducted to detect antibacterial activity. Petri-dishes were sterilised before use and they were poured with sterile liquid Mueller Hinton Agar media (pH 7.4 ± 2). They were allowed to solidify and the bacterial broth culture (1 ml broth of approximately 10^5 CFU) was spread on the medium with a sterile cotton plug needle in aseptic environment. Whatman No. 4 Filter Paper was utilised to prepare sterile discs of 5-mm diameter. The experimental control discs were also made with the original solvents in which the extracts prepared. Phytochemicals were then dissolved in the respective solvent to get a stock solution with an accumulation of 150 mg/ml. Each disc was added with 10 µL of the sample to receive an accumulation of about 1.5 mg/disc. All the discs, including control were placed onto the medium only after removing any trace of solvent from them. It was done by

keeping it in a hot air oven set at 40°C until all the solvent removed from the disc. The finished plates were inserted in incubator, kept at 37°C for 24 hours to detect any inhibition zones. More than three replicates of the experiments were conducted, and average inhibitory zone diameter was recorded.

Minimum inhibitory Concentration (MIC)

The MIC of the extracts was done by placing various amounts (400–0.78mg/ml) of the extract prepared in sterile distilled water into an array of test tubes with the culture media[8]. To each test tube 50 µl of the bacterial broth suspension culture was added[9]. The cultures with plant extracts were kept in an incubator set at 37°C for 24 hours. Positive controls were also prepared, and this consisted of growth medium and each bacterium. The minimum inhibitory concentration was noticed as the lowest concentration of the extracts that could not permit any visible growth as compared to that of the control tubes.

Minimum Bactericidal Concentration (MBC)

Culture tubes from MIC studies, which didn't give any visible growth after a period of incubation, were again sub-cultured onto a freshly prepared nutrient medium[10]. The minimum bactericidal concentration was observed as the lowest concentration of the extract that did not produce a single colony on a nutrient agar plate after 24 hours incubation.

Preliminary Detection of Phytochemicals

Silica gel Thin Layer Chromatography (TLC) was performed to separate various phytochemicals in the crude solvent extract of the plant. This was done in appropriate solvent system. Then, the plates were sprayed with different spraying reagents as pointed out by Harborne[11] and Stahl[12] to detect phytochemical components like alkaloids, phenolics, flavonoids, Triterpenoids, and sterols.

RESULTS AND DISCUSSION

Phytochemicals from the leaves of *M. punctatum* were extracted by gradient separation protocol and therefore, this technique helped to separate nonpolar phytochemicals came before polar compounds. If we perform single exaction in a solvent, consequently, it can yield a mixture of polar and non-polar compounds in solvents like ethanol or methanol. Gradient separation may be advantageous to separate different phytochemicals depending on their polarity. Phytochemicals separated in water distillate of *M. punctatum*, did not provide any antibacterial capability with respect to experimented organisms. Phytochemicals eluted in ethanol distillate of *M. punctatum* gave the greatest antibacterial action towards *Pseudomonas aeruginosa.* Moderate grade of action was established towards the rest of experimented bacteria. The ethanol extract of the plant showed insensitivity to *Klebsiella pneumonia* in relation to other bacteria. *Pseudomonas aeruginosa* could show the highest performance in terms of its antibacterial activity with respect to ethanol extract of the plant (Table 1). No control discs could give positive results of antibacterial action. Results of the phytochemical experimentation of *M. punctatum* are shown in the Table 2. It was evident from the experiments that the antibacterial power of leaves of the plant distillate could not be matched with the efficiency of standard antibiotics (Table 3).

Table 1: Antibacterial Action of *M. punctatum*

Name of plant Extract used		Zone diameter (in millimetre)				
		Pseudomonas aeruginosa (MTCC-741)	*Staphylococcus aureus* (MTCC-96)	*Klebsiella pneumoniae* (MTCC-109)	*Escherichia coli* (MTCC-443)	*Serratia marcescens* (MTCC-97)
M. punctatum	Petroleum ether	8.4 ± 0.45	7.4 ± 0.58	-	-	7.5 ± 0.44
	Acetone	-	7.2 ± 0.53	-	-	7.8 ± 0.41
	Ethanol	14.3 ± 0.35	6.1 ± 0.55	-	7.6 ± 0.39	6.4 ± 0.45
	Water	-	-	-	-	-

Value = no obvious growth inhibition (-)

Table 2: Results of Phytochemical Evaluation of *M. punctatum*

Name of plant	Plant extracts	Test for Flavonoids	Test for Alkaloids	Test for Phenols	Test for Sterols, steroid, phenol and poly phenol
M. punctatum	Petroleum ether	+	-	-	+
	Acetone	-	-	+	+
	Ethanol	+	-	+	+
	Water	-	-	+	-

Value = '+' : Present '–' : Absent

Table 3: Antibacterial Action of standard antibiotics

Name of Antibiotic (Con. 25µg/Disc)	Zone diameter (in millimetre)		
	MTCC – 6164	MTCC – 96	MTCC – 741
Streptomycin	25.6± 0.28	19.7± 0.46	21.4 ± 0.36
Amoxicillin	-	24.5± 0.45	-
Chloramphenicol	-	23.5± 0.35	-

Value = no obvious growth inhibition

The exaction technique initiated with non-polar solvent like petroleum ether. The extract contained non-polar molecules and these chemicals provided moderate level of antibacterial activity with respect to ethanol distillate. Medium polar chemicals were dissolved in acetone distillate and they showed more or less similar intensity of antibacterial activity as petroleum ether extract; while, ethanol distillate contained mostly polar compounds and they gave better results of antibacterial action. Ethanol distillate of leaves of *M. punctatum* provided good antibacterial action to *Pseudomonas aeruginosa*, a gram-negative bacterium. Ethanol distillate of leaves of the plant showed Minimum Inhibitory Concentration (MIC) of 25 mg/ml and Minimum Bactericidal Concentration (MBC) of 50 mg/ml against *P. aeruginosa*. *P. aeruginosa* is invariably detected in nosocomial bacterial contaminations and its infection is almost frequent in-patients receiving medication of serious burns or other dreadful skin injury and in patients affected with cystic fibrosis. This pathogen can spread lungs of diseased individuals and accelerate the death rate[13]. Almost entire polar phytochemical compounds were carried away with ethanol distillate and there might be very less phytochemicals compounds left after ethanolic distillation. This would be one of the reasons for decline in antibacterial action of water extract. Flavonoids were found in various extracts of leaves of the plant. Not any of the extracts gave positive indication of alkaloids. The present antibacterial analysis of the plant reinforces the medicinal and ethnobotanical usefulness of M. *punctatum* [2-4].

CONCLUSION

Ethanol distillate of leaves of *M. punctatum* exhibited antibacterial action. Antibacterial activity detected as gradient exaction was done. The ethanol distillate of leaves the plant gave better result of action against *P. aeruginosa. W*ater extracts of the plant did not provide antibacterial action against the tested bacteria. Phytochemicals like flavonoids, phenols, Sterols, steroids, phenol and poly phenol were detected in ethanol extract of the plant. The effective ethanol distillate demonstrated a minimum inhibitory concentration of 25 mg/ml and minimum bactericidal concentration of 50 mg/ml with respect to *P. aeruginosa.*

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