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ANTIBACTERIAL ACTIVITY OF FRONDS OF *ADIANTUM CAUDATUM* L. TOWARDS BACTERIA INVOLVED IN CUTANEOUS DISEASES

Toji Thomas*

Post Graduate and Research Department of Botany,St. Thomas College Palai, Arunapuram P.O.Pala, Kerala-686574, India.

ABSTRACT

Adiantum caudatum, a medicinal pteridophyte, fronds of *Adiantum caudatum* evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity towards bacterial species involved in skin diseases in human beings. Antibacterial activity was evaluated by disc diffusion method. The results indicated that the plant exhibited antibacterial activity in methanol extract. The methanol extract of the plant showed maximum level of activity towards *Pseodomonas aeruginosa*, a resistant strain towards amoxycilin and chloramphenicol. *Pseudomonas aeruginosa* is often encountered in nosocomial infections and in people suffering from cystic fibrosis and its infection is common in-patient receiving treatment of severe burns. Polar compounds are extracted during methnolic extraction and these are responsible for antibacterial activity. Petroleum ether and water extracts did not show any antibacterial activity towards any of the tested organisms. The presence of flavonoids and phenols observed in various extracts. Flavonoid and phenol content in methanol extract of the plant may be one of the reasons for their antibacterial activity. Methanolic extract of the plant exhibited minimum inhibitory concentration as 50mg/ml and minimum bactericidal concentration as 25mg/ml towards *Pseudomonas aerogenosa.* The plant showed lower level of inhibition towards *Escherichia coli* compared to the other bacterial strains..

Keywords: *Adiantum caudatum;* Antibacterial activity*; Cutaneous diseases;* Phytochemicals.

INTRODUCTION

Pteridophytes are ancient vascular plants, which grow well in terrestrial habitat. Pteridophyte plants have medicinal value [1]. The plant selected for investigation is *Adiantum caudatum* L. Adiantaceae. The plant is a small terrestrial herb. The plant grows in shaded localities in soil cuttings, slopes, or in crevices of rocks in forest. The plant is not common [2]. The whole plant parts of *Adiantum caudatum* are medicinal. In Ayurveda the fronds (leaves) used to cure cough and fever [3]. Widespread use of antibiotic medicines in human being helps to develop drug-resistant bacteria. These drug-resistant bacteria stand as a major problem in hospital and community pathogens world-wide. Plants are known to have defence systems against phytopathogenic bacteria [4]. Present study aims to evaluate antibacterial potential of the plant in various solvents extracts of increasing polarity towards bacteria involved in skin diseases.

MARERIALS AND METHODS

Preparation of Plant Extract

Fresh specimens of *Adiantum caudatum* L. were collected in the month of January from Vellikulam, Kottayam District, Kerala. A voucher specimen (SS 1542) was deposited at the herbarium of St. Thomas College Palai. The air-dried fronds of the plant with sori of the plant material (50g) was ground and utilised for preparing extracts. Soxhlet extracts of petroleum ether, acetone, methanol and water were made successively [5] with a yield of 0.56%, 3.1%, 5.4%, and 0.8% respectively.

Microorganisms Used

The test organisms were collected from the culture collection of the institute of Microbial Technology (IMTECH), Chandigarh. These include *Staphylococcus aureus* subsp *aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Pseudomomas aeruginosa* (MTCC 741), *Klebsiella*

Corresponding Author:- **Toji Thomas** Email: tojidr@yahoo.com

pneumoniae subsp *pneumoniae* (MTCC-109) and *Serratia marcescens* (MTCC 6164). All these bacteria are involved in various skin infections [6]. The bacteria were sub cultured on nutrient agar slants, incubated at 37^{o}C for 24 hours and stored at 4^{o}C in the refrigerator to maintain the stock culture.

***In Vitro* Antibacterial Assay**

Preliminary antibacterial activity was tested by disc diffusion method as illustrated by Bauer et al [7] Sterile liquid Mueller Hinton Agar media (pH 7.4±2) was poured into sterile petridish and after solidification, the bacteria (1 ml broth of approximately 10^5 CFU) were swabbed with a sterile needle under aseptic conditions. Sterile discs prepared using Whatman No. 4 Filter Paper, of 5-mm diameter were employed in the study.

The original solvents in which the extracts prepared were used as a control. Test materials were dissolved in the respective solvent to obtain a stock solution of concentration of 100 mg/ml. 20 µL of the solution was loaded per disc to attain a concentration of 1 mg/disc. The discs (including control) were placed after drying them in an incubator at 40°C to remove any trace of solvent. The plates incubated at 37^{o}C for 24 hours to obtain inhibition zones. Experiments were conducted in more than three replicates and average inhibitory zone diameter was determined.

Minimum inhibitory Concentration (MIC)

The MIC of the extracts was performed by incorporating various amounts (400–0.39mg/ml) of the extract into sets of test tubes with the culture media [8]. 50µl of the bacterial broth culture was added into each of the test tubes. The bacterial cultures containing the plant extracts were incubated at 37^{o}C for 24 hours. Test tube containing only the growth medium and each of the organisms was also incubated under the same conditions as positive controls. The minimum inhibitory concentration was expressed as the lowest concentration of the extracts that did not permit any visible growth when compared to that of the control tubes.

Minimum Bactericidal Concentration (MBC)

Samples from the tubes in previous studies, which did not show any visible growth after a period of incubation, were subcultured onto a freshly prepared nutrient medium [9]. The minimum bactericidal concentration was taken as the lowest concentration of the extract that did not yield a single colony on the nutrient agar plate after 24 hours incubation period.

Preliminary Detection of Phytochemicals

The crude samples were subjected to phytochemical screening for the presence of alkaloid, phenolics, Triterpenoids, flavonoids using the method of Harborne [10].

RESULTS

Water extracts did not show any antibacterial activity towards tested organisms the same condition was observed with petroleum ether extracts. Acetone extract of *Adiantum* showed moderate level of inhibition towards *Staphylococcus aureus*, the plant showed lower level of inhibition towards *Escherichia coli* compared to the other bacterial strains (Table 1). *Pseudomonas aeruginosa* and S*erratia marcesens* are the most sensitive organisms towards the methanol extract of the plant.

The plant extracts did not show any antibacterial activity against *Escherichia coli*. No control discs exhibited antibacterial activity. The phytochemical evaluation of *Adiantum caudatum* is shown in the Table 2. Table 3 shows the results of antibacterial assays of pathogenic organisms towards standard antibiotics. Amoxylin and chloramphenicol were not acting against *Pseudomonas aeruginosa.* Chloramphenicol was not effective towards *Klebsiella pneumoniae.*

DISCUSSION

Petroleum ether extract contained non-polar compounds dissolved in it, and these compounds did not have antibacterial activity. Likewise, water extract contained highly polar compounds and these compounds also showed lowest level of antibacterial activity.

Table 1. Antibacterial Activity of fronds of *Adiantum caudatum*

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 marcesens* (MTCC-97)**
Adiantum caudatum	Petroleum ether	-	-	-	-	-
	Acetone	+	+	-	-	+
	Methanol	+++	++	+	-	++
	Water	-	-	-	-	-

Value= no obvious growth inhibition (-); zone of inhibition with diameter 7mm-10.99mm (+); 11mm-14.99mm as (++); 15-21mm (+++); 22-31mm (++++)32-41mm (+++++)

Table 2. Results of Phytochemical Evaluation of fronds of *Adiantum caudatum*

Name of plant	Plant extracts	Test for Flavonoids	Test For Alkaloids	Test for Phenols	Test for Sterols, steroid, phenol and poly phenol
Adiantum caudatum	Petroleum ether	+	-	+	+
	Acetone	+	-	+	-
	Methanol	+	-	+	-
	Water	+	-	+	-

Value = '+' : Present '–' : Absent

Table 3. Antibacterial Action of standard antibiotics

Name of Antibiotic (Con. 25µg/Disc)	Zone diameter (in millimetre)		
	MTCC – 109	MTCC – 96	MTCC – 741
Streptomycin	++++	+++	+++
Amoxylin	+++++	++++	-
Chloramphenicol	-	++++	-

Value= no obvious growth inhibition (-); zone of inhibition with diameter 7mm-10.99mm (+); 11mm-14.99mm as (++); 15-21mm (+++); 22-31mm (++++)32-41mm (+++++)

Medium polar compounds are soluble in acetone extract and these compounds have moderate level of antibacterial activity, while methanol extract contains polar compounds and they possessed antibacterial potential. Methanolic extract of *Adiantum caudatum* showed maximum action against *Pseudomonas aeruginosa*, gram-negative bacteria. *Pseudomonas aeruginosa* is often observed in nosocomial infections and its infection is common in-patients receiving treatment of severe burns or other traumatic skin damage and in people suffering from cystic fibrosis. This pathogen colonises the lungs of patients and increasing mortality rate of individuals with the disease [11]. Most of the polar compounds are eluted with methnolic extraction and there may be few compounds left after methanolic extraction. The presence of flavonoids and phenols observed as general feature the plant extracts. None of the extracts showed the presence of alkaloids. Flavonoid and phenol content observed in methanol extract of the plant; it might be one of the reasons for its antibacterial activity. The present antibacterial analysis of the plant supports the ethnobotanical importance of and *Adiantum caudatum* [3].

CONCLUSION

Adiantum caudatum was evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity towards bacterial species involved in skin diseases. The plant showed antibacterial activity in methanol extract. The methanol extract of the plant showed maximum level of activity towards *Pseodomonas aeruginosa.* Petroleum ether and water extracts did not show any antibacterial activity towards any of the tested organisms. The presence of flavonoids and phenols observed in various extracts. Methanolic extract of the plant exhibited minimum inhibitory concentration as 50mg/ml and minimum bactericidal concentration as 25mg/ml towards *Pseudomonas aerogenosa.*

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