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Research Article

ANTIBACTERIAL ACTIVITY OF *ADIANTUM LUNULATUM* BURM. F. TOWARDS BACTERIA IMPLICATED IN CUTANEOUS INFECTIONS

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Abstract

Adiantum lunulatum, a medicinal pteridophyte used as in treating various skin diseases including leprosy. In the present study, whole plant of *Adiantum lunulatum* 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. 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 50 mg/ml and minimum bactericidal concentration as 25 mg/ml towards *Pseudomonas aerogenosa.*

Keywords: *Adiantum lunulatum*; antibacterial activity; phytochemicals; *Adiantum philippense*

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 lunulatum* Burm. F. Adiantaceae, its synonym is *Adiantum philippense* L.[2]. The whole plant parts of *Adiantum lunulatum* are medicinal. Rhizome of the plant used to cure glandular swellings accompanied by fever. Juice of leaves used to treat dysentery, diseases of blood, ulcers, erysipelas, burning sensations etc. In Ayurveda the plant is recommended as a cure for epilepsy. The spores are said to be effective in the treatment of leprosy and other skin diseases[3]. Extensive use of antibiotic medicines in human being causes to develop drug-resistant bacteria. These drug-resistant bacteria remain as a major concern in hospital and community pathogens world-wide. Plants are known to have defence systems against phytopathogenic bacteria[4]. Present study is an attempt to evaluate antibacterial potential of the plant in various solvents extracts of increasing polarity towards bacteria involved in skin diseases.

MATERIALS AND METHODS

Preparation of Plant Extract

Fresh specimens of *Adiantum lunulatum* Burm. F. Adiantaceae Syn. *Adiantum philippense* L. were collected in the month of December from Pala, Kottayam District, Kerala, India. A voucher specimen (SS 1541) was deposited at the herbarium of St. Thomas College Palai. The air-dried roots of the plant material (100 g) was ground and utilised for preparing extracts. Soxhlet extracts of petroleum ether, acetone, methanol and water were made successively[5] with a yield of 0.66 %, 3.4 %, 6.4 % and 0.7 % respectively.

Microorganisms Used

The test organisms were collected from the culture collection of the institute of Microbial Technology (IMTECH), Chandigarh, India. These include *Staphylococcus aureus* sub sp *aureus* (MTCC 96), *Escherichia coli* (MTCC 443), *Pseudomomas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* sub sp *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°C for 24 hours and stored at 4°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°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 – 0.39 mg/ml) of the extract into sets of

test tubes with the culture media[8]. 50 µl of the bacterial broth culture was added into each of the test tubes. The bacterial cultures containing the plant extracts were incubated at 37°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 sub cultured 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

Petroleum ether extract did not show any antibacterial activity towards tested organisms. Acetone extract of *Adiantum* showed moderate level of inhibition towards *Staphylococcus aureus*, gram-positive bacteria. The plant showed lower level of inhibition towards *Escherichia coli* compared to the other bacterial strains. None of the water extracts showed antibacterial activity. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most sensitive organisms towards the methanol extract of the plant. Table 3 shows the results of antibacterial assays of pathogenic organisms towards standard antibiotics. The plant extracts did not show any antibacterial activity against *Escherichia coli*. No control discs exhibited antibacterial activity. The phytochemical evaluation of *Adiantum lunulatum* is shown in the Table 2.

Table 1: Antibacterial Activity of *Adiantum lunulatum*

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 lunulatum	Petroleum ether	-	-	-	-	-
	Acetone	+	+	-	-	+
	Methanol	+++	++	+	-	-
	Water	-	-	-	-	-

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

Table 2: Results of Phytochemical Evaluation of *Adiantum lunulatum*

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

Value = '+': Present '–': Absent

Table 3: Antibacterial Action of standard antibiotics

Name of Antibiotic (Con. 25 µg/Disc)	Zone diameter (in millimetre)		
	MTCC – 6164	MTCC – 96	MTCC – 741
Streptomycin	++++	+++	+++
Amoxylin	+++++	++++	-
Chloramphenicol	-	++++	-

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

DISCUSSION

Petroleum ether extract contains non-polar compounds dissolved in it and these compounds do not have antibacterial activity. 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 have antibacterial potential. Methanolic extract of *Adiantum lunulatum* showed maximum action against *Pseudomonas aeruginosa*, gram-negative bacteria. *Pseudomonas aeruginosa* is often encountered 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]. Water extract did not show any antibacterial activity. 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 ethno botanical importance of and *Adiantum lunulatum*[3].

CONCLUSION

Adiantum lunulatum was assessed 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. The plant exhibited 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 50 mg/ml and minimum bactericidal concentration as 25 mg/ml towards *Pseudomonas aerogenosa.*

REFERENCES

1. Singh L, Somarjit S, Kumar Singh S, Jadu E. Ethno botanical uses of some Pteridophytic species in Maipur. Indian Fern J 2001; 18: 14-17.
2. Easa PS. Biodiversity documentation for Kerala Part 5: Pteridophytes. Kerala Forest Research Institute Peechi Kerala. KRFRI Hand book No.17; 2003.
3. Nayar BK. Medicinal ferns of India. Bulletin No. 29. National Botanic Gardens Lucknow India; 1959.
4. Klein E, Smith DL, Laxminaraya R. Hospitalizations and Deaths Caused by Methicillin-Resistant *Staphylococcus aureus*, United States, 1999–2005. Emerg Infect Dis 2007; 13: 1840–1846. http://dx.doi.org /10.3201/eid1312.070629 PMid:18258033 PMCid:PMC2876761
5. Raghavendra MP, Satish S, Raveesha KA. *In vitro* evaluation of anti-bacterial spectrum and phytochemical analysis of *Acacia nilotica*. J Agr Sci 2006; 2: 77-88.
6. Valia RG, Valia AR. IADVL Textbook of Dermatology, Third Edition. Bhalani Publishing House Mumbai India; 2008. PMid:19171976
7. Bauer AW, Kirby MDK, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc diffusion method. Am J Clin Pathol 1966; 45: 493-496. PMid:5325707
8. Barry AL. Antimicrobic Susceptibility Tests: Principle and Practice, Lea and Febiger; Philadelphia; 1976.
9. Ratimi VO, Laughon BE, Barlet JS, Mosadomi HA. Activity of Nigerian chewing sticks extracts against *Bacterioides gingivalis* and *Bacterioides melaninogenicus*. Anti microb Agents Chemother 1988; 32: 598-600. http://dx.doi.org/10.1128/AAC.32.4.598
10. Harborne JB. Phytochemical methods. Chapman and Hall Ltd London; 1973.
11. Madigan MT, Martinko JM, Parker J. Brock Biology of Microorganisms 9th ed. Prentice Hall International Inc New Jersey; 2000.

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