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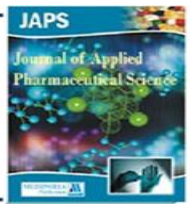

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Preliminary antibacterial evaluation of fronds of *Pteris quadriaurita* Retz. towards bacteria involved in dermatological diseases

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ABSTRACT

Pteris quadriaurita, a medicinally relevant pteridophyte used as an antihelmintic plant in traditional systems of medicine. In the present study fronds of *Pteris quadriaurita* was evaluated for its antibacterial potential and phytochemical contents in various solvent extracts of the plant in increasing polarity towards pathogenic bacterial species involved in skin diseases in human being. Antibacterial activity was evaluated by disc diffusion method. The results indicated that fronds of the plant showed antibacterial activity especially in methanolic extract. The mathanolic extract of the plant showed maximum activity towards *Pseodomonas aeruginosa*, a multi-drug resistant strain. Petroleum ether and water extracts did not show any antibacterial activity towards any of the tested organisms. The occurrence of flavonoid and terpenoids content in the plants may be one of the reasons for their antibacterial activity. Methanolic extract of the plant exhibited minimum inhibitory concentration as 25mg/ml and minimum bactericidal concentration as 50mg/ml towards *Pseudomonas aerogenosa*.

Key words: Antibacterial, *Pteris quadriaurita*, disc diffusion, Minimal inhibitory concentration (MIC).

INTRODUCTION

Pteridophytes are primitive vascular plants, which can adapt well in terrestrial habitat. With the introduction of Ethnobotany, scientists studied the relationship between people of primitive societies and their plants, many attempts had been made on the study of relationships of pteridophytes with man, and more particularly for their medicinal value. (Singh, 2001). A large number of bacteria are involved in various skin infections and plants contain phytochemicals to kill bacteria involved in skin diseases (Kar and Kumar, 2010). *Pteris quadriaurita* Retz. belongs to the family Pteridaceae. It is a common terrestrial herb growing in semi-shaded localities having completely dry soils in plains and Ghats at lower altitudes. The plant is common in all districts of Kerala (Easa, 2003) *Pteris quadriaurita* used as antihelmintic (Nayar, 1959). Decoction of fresh rhizome and fronds of *Pteris quadriaurita* are given in chronic disorders arising from obstructions of viscera and spleen. (Chopra et al., 1956). Widespread use of antimicrobial durgs continues to cause significant increase in resistant bacteria, particularly resistant gram-positive organisms. Currently, it has been emerging as one of the most important hospital and community pathogens in world-wide manner. The emergence of these resistant bacteria has caused a major concern and thus the urgent need for new antibacterial agents (Casellas et al., 1994). Present study is an attempt to evaluate antibacterial potential of the plant in various extracts of increasing polarity and to understand the phytochemial background of the extracts. The extracts were tested towards pathogenic bacteria involved in various dermatological diseases in human being.

METHODOLOGY

Preparation of plant extract

Fresh specimens were collected in the month of December from Pala, Kerala. A voucher specimen (TT 2053)) was deposited at the herbarium of Calicut University Herbarium (CALI). The air-dried fronds of the plant material (100g) was ground and utilised for preparing extracts. Soxhlet extracts of petroleum ether, acetone, ethanol and water were made successively (Raghavendra et al., 2006), with a yield of 0.68%, 3.7%, 4.9%, 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* (MTCC 96), *Escherichia coli* (MTCC 443), *Pseudomomas aeruginosa* (MTCC 741), *Micrococcus luteus* (MTCC 6164) and *Serratia marcescens* (MTCC 6164). All these bacteria are involved in various skin infections (Valia, 2008). 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

The disc diffusion method as illustrated by Bauer et al., (1966) was used to determine the growth inhibition of bacteria by plant extracts. 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 used in the study. The original solvent in which the extract prepared was used as a control. Test materials were dissolved in the respective solvent to obtain a stock solution of concentration of 100 mg/ml. 10 µL of the solution was loaded per disc to attain a concentration of 1 mg/disc. The discs (including control) were used after drying them in an incubator at 40°C to remove any trace of solvent. Discs were introduced onto the surface of the medium. The plates were 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 along with standard deviation.

Minimum inhibitory concentration (MIC)

The MIC of the extracts was performed by incorporating various amounts (200 – 0.39 mg/ml dissolved in sterile distilled water) of the extract into sets of test tubes with the culture media (Barry, 1976). 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^{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 used in the MIC assays, which did not show any visible growth after a period of incubation were subcultured onto a freshly prepared nutrient medium (Ratimi et al., 1988). 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 (1973).

RESULTS AND DISCUSSION

Methanolic extract of *Pteris quadriaurita* showed maximum activity against *Pseudomonas aeruginosa*, gram-negative bacteria. While the acetone and methanolic extracts of *Pteris quadriaurita* showed moderate level of inhibition towards *Escherichia coli*, gram-negative bacteria. The plant showed lower level of inhibition towards *Serratia marcescens.* None of the water extracts showed any antibacterial activity. *Pseudomonas aeruginosa* and *staphylococcus aureus* are the most sensitive organisms. The plants did not show any antibacterial activity against M*icrococcus lutens*. No control discs exhibited antibacterial activity. The results of phytochemical evaluation of *Pteris quadriaurita* is shown in the Table 2.

Table 1: Antibacterial activities of *Pteris quadriaurita.*

Name of plant Extract used		Zone diameter (in millimeter)				
		Pseudomonas aeruginosa **(MTCC-741)**	**Staphylococcus aureus (MTCC-96)**	***Escherichia coli*** **(MTCC-443)**	***Micrococcus luteus*** **(MTCC-6164)**	**Serratia marcesens (MTCC-97)**
P. quadriaurita	Pet. ether	-	-	-	-	-
	Acetone	-	-	9 ± 0.13	-	8.6 ± 0.53
	Methanol	13.3 ± 0.22	10.7 ± 0.43	10.6 ± 0.12	-	7 ± 0.31
	Water	-	-	–	-	-

Value = Mean ± Standard deviation; No inhibition; control discs no inhibition

Table 2: Results of Phytochemical evaluation of *Pteris quadriaurita*

Name of plant	Plant extracts	Test for Flavonoids	Test For Alkaloids	Test for Phenols	Test for Terpenes
Pteris quadri aurita	Petroleum ether	+	-	-	+
	Acetone	+	-	+	+
	Methanol	+	-	+	-
	Water	-	-	+	-

Value = '+' : Present '–' : Absent

Phenols were detected in all the extracts except petroleum ether extracts of *Pteris quadriaurita*. The plant extracts showed negative results with alkaloids. Flavonoids and phenols were observed in methanol extract of the plant. Presence of flavonoids and phenols might be one of the reasons for its antibacterial activity. The present antibacterial analysis of the plants confirms

the ethnobotanical importance of and *Pteris quadriaurita* by Chopra et al., (1992). Methanolic extract *Pteris quadriaurita* showed stronger inhibition towards *Pseudomonas aeruginosa*. Antibacterial activity observed at its maximum in methanol extract towards *Pseudomonas aeruginosa* and therefore methanol extract was selected for detailed antibacterial evaluation tests like MIC and MBC. MIC and MBC values of 25mg/ml and 50mg/ml were observed towards *Pseudomonas aeruginosa*. The present investigation supported the antibacterial property of the fronds towards tested pathogens involved in various diseases. *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 (Madigan et al., 2000). Further investigations are necessary to isolate and purify antibacterial principles from active methanol extract of the plant and may be later used as a potential phtomedicine instead of synthetic antibiotics especially towards multidrug resistant pathogenic bacterial species.

CONCLUSION

Antibacterial activity of fronds of *Pteris quadriaurita* was tested towards bacteria involved in various diseases in human being. Methanol extract was found to be effective against *Pseudomonas aeruginosa*. Phytochemical analysis of active extracts indicated the presence of flavonoids and phenols. Alkaloids were not detected in any of the extracts. Active methanol extract of the plant exhibited minimum bactericidal concentration (MBC) of 50 mg/ml towards *Pseudomonas aeruginosa*. In view of the analysis, the fronds can be used as source for isolating and characterizing new antibacterial drugs.

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