

In vitro evaluation of antibacterial activity of *Acrostichum aureum* Linn.

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Acrostichum aureum Linn., a medicinal pteridophyte is used as an anthelmintic and styptic in traditional systems of medicine. In the present study, fronds of *A. aureum* were evaluated for their 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 beings. Antibacterial activity was evaluated by disc diffusion method. The results indicated that the fronds of the plant showed antibacterial activity especially in methanol and acetone extracts. The methanol extract showed maximum activity towards *Pseudomonas aeruginosa*, a resistant strain towards Amoxicillin 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 were observed in various extracts. The high flavonoid and phenol content in the plants 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 *P. aeruginosa*.

Keywords: Antibacterial, *Acrostichum aureum*, Fern, *Mayur-sikha*, Medicinal, Phytochemicals, *Pseudomonas aeruginosa*, Pteridophyte.

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Introduction

Pteridophytes are primitive vascular plants, which can adapt well in terrestrial habitat. With the introduction of ethnobotany many attempts have been made on the study of relationships of plants particularly for medicinal value of pteridophytes¹. *Acrostichum aureum* Linn., belongs to the family Pteridaceae, a large terrestrial plant observed in flooded areas during rainy seasons and at high tides, its association with mangroves is common in Kerala². Whole plant is used as an antihelmintic and styptic,

also used as a worm remedy and as an astringent in haemorrhage³. Widespread use of antibiotics caused significant increase in antibiotic resistance of bacteria⁴. Currently, these multidrug resistant bacteria have been emerging as one of the most important hospital and community pathogens worldwide. The emergence of these resistant bacteria has caused a major concern and thus the urgent need for new antibacterial agents⁵. Present study is an attempt to evaluate antibacterial potential of this plant in various extracts of increasing polarity and to understand the phytochemical background of the extracts. The extracts were tested towards pathogenic bacteria involved in various diseases in human beings.

Materials and Methods

Preparation of plant extract

Fresh specimens of *A. aureum* were collected in the month of December from Kottayam District of Kerala State, India (Plate 1). A voucher specimen (TT 2052) was deposited at the herbarium of Calicut University Herbarium (CALI). The air-dried fronds (leaves) of the plant material (100 g) were ground and utilised for preparing extracts. Soxhlet extracts of petroleum ether, acetone, ethanol and water were made successively⁶ with yield of 0.58, 4.7, 5.4 and 0.9%, 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), *Pseudomonas aeruginosa* (MTCC 741), *Micrococcus luteus* (MTCC 6164) and *Serratia marcescens* (MTCC 6164). All these bacteria are involved in various skin infections⁷. The bacteria were sub-cultured on nutrient agar slants, incubated at 37°C for 24 h and stored at 4°C in the refrigerator to maintain the stock culture.

In vitro antibacterial assay

The disc diffusion method as illustrated by Bauer *et al*⁸ 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

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Plate 1—*Acrostichum aureum* Linn.

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. 20 μ L 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°C for 24 h 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 (128-0.125 mg/ml) of the extract into sets of test tubes with the culture media⁹. 50 μ l 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 used in the MIC assays, which did not show any visible growth after a period of incubation were sub-cultured onto a freshly prepared nutrient medium¹⁰. 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 h 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 Harborne¹¹.

Results

Methanolic extract of *A. aureum* showed maximum activity against *P. aeruginosa*, Gram negative bacteria. While the acetone and methanolic extracts showed moderate level of inhibition towards *E. coli*, Gram negative bacteria and lower level of inhibition towards *Serratia marcescens* when compared to the other bacterial strains. None of the water extracts showed any antibacterial activity. *P. aeruginosa* and *S. aureus* are the most sensitive organisms. The plants

Table 1—Antibacterial activity of *Acrostichum aureum* fronds

Extract used	Zone diameter (mm)				
	<i>Pseudomonas aeruginosa</i> (MTCC-741)	<i>Staphylococcus aureus</i> (MTCC-96)	<i>Escherichia coli</i> (MTCC-443)	<i>Micrococcus luteus</i> (MTCC-6164)	<i>Serratia marcescens</i> (MTCC-97)
Petroleum ether	-	-	-	-	-
Methanol	-	-	10 ± 0.12	-	7.6 ± 0.58
Acetone	12.3 ± 0.23	9.7 ± 0.48	10.6 ± 0.14	-	7 ± 0.32
Water	-	-	-	-	-

Value = Mean ± Standard deviation; -=No inhibition; control disc no inhibition

did not show any antibacterial activity against *M. luteus*. No control discs exhibited antibacterial activity (Table 1).

The phytochemical evaluation of *A. aureum* is shown in the Table 2. The presence of flavonoids and phenols observed as general feature the plant extracts. Test for sterols, steroids, phenol and poly phenols exhibited positive result in all cases except the methanolic and water extracts. The plant extracts showed negative results with alkaloids. Flavonoid and phenol content observed in methanol extract of the plant; it might be one of the reasons for its antibacterial activity. Table 3 shows the results of antibacterial assays of pathogenic organisms towards standard antibiotics. Methanolic extract showed stronger inhibition towards *P. aeruginosa* when compared to standard antibiotics like Amoxicillin and Chloramphenicol.

Discussion

The present investigation validated the antibacterial property of the leaves towards one of the clinically important multidrug resistant stains, *P. aeruginosa* which 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 disease¹². The present antibacterial analysis of the plant confirms the ethnobotanical importance of it³. The plant possesses antibacterial principles, soluble in methanol, which hinder the growth and multiplication of some multi-drug resistant bacterial strains. In view of the analysis, the fronds of *A. aureum* can be recommended as source for isolating and characterizing new antibacterial drugs for modern medicine. Further investigations are necessary to isolate and purify antibacterial principles from active acetone extract of the plant and may be later used as a

Table 2—Phytochemical constituents of various extracts of *Acrostichum aureum*

Extracts	Flavonoids	Alkaloids	Phenols	Sterols, steroid, phenol and polyphenol
Petroleum ether	+	-	+	+
Acetone	+	-	+	+
Methanol	+	-	+	-
Water	+	-	+	-

Value + = Present, - = Absent

Table 3—Antibacterial activity of standard antibiotics

Name of Antibiotic (Conc. 25 µg/Disc)	Zone diameter (mm)		
	<i>Micrococcus luteus</i> (MTCC-6164)	<i>Staphylococcus aureus</i> (MTCC-96)	<i>Pseudomonas aeruginosa</i> (MTCC-741)
Streptomycin	25 ± 0.23	18 ± 0.56	19 ± 0.42
Amoxylin	35 ± 0.48	31 ± 0.52	-
Chloramphenicol	-	25 ± 0.49	-

potential phytomedicine instead of synthetic antibiotics especially towards multidrug resistant pathogenic bacterial species.

Conclusion

A. aureum showed antibacterial activity especially in methanol and acetone extracts. The methanolic extract of the plant showed maximum activity towards *Pseudomonas aeruginosa*. Petroleum ether and water extracts did not show any antibacterial activity towards any of the tested organisms. Flavonoids and phenols were detected in active extracts.

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