Antibacterial Evaluation of Gradient Extracts of Adiantum latifolium Lam. Towards Pathogenic Cutaneous Bacteria

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Abstract

Objective: The study focused to analyse antibacterial evaluation of gradient extracts of Adiantum latifolium, a healing pteridophyte towards bacteria involved in cutaneous infections. Methods: Whole plant of Adiantum latifolium was analyzed for its antibacterial nature and phytochemical components in different solvents extracts of accelerating polarity. Disc diffusion method was used to analyse antibacterial activity. Preliminary phytochemical evaluation was done using various spraying reagents. Minimum inhibitory concentration as well as minimum bactericidal concentration was determined towards Pseudomonas aeruginosa. Results: The plant primarily exhibited antibacterial activity in ethanol extract. Maximum intensity of activity was noticed towards Pseudomonas aeruginosa, a resistant strain towards amoxicillin and chloramphenicol. Petroleum ether and water extracts could not show any antibacterial activity towards the tested organisms. Flavonoids and phenols were found in different extracts. Occurrence of flavonoid and phenol in ethanol extract of the plant can be an explanation for its antibacterial activity. Ethanol extract of the plant showed minimum inhibitory concentration as 18.75mg/ml and minimum bactericidal concentration as 37.5mg/ml towards Pseudomonas aerogenosa. Conclusion: Adiantum latifolium showed antibacterial activity in ethanol extract especially towards Pseudomonas aeruginosa.

Keywords: Adiantum latifolium; Antibacterial activity; Phytochemical; Pteridophytes.

Introduction

Pteridophytes are primitive vascular plants, which flourish well in terrestrial habitat. Pteridophyte plants possess medicinal value [1]. Adiantum latifolium Lam., a common terrestrial herb, belongs to Adiantaceae; its synonym is Adiantum denticulate Sw. [2].

The whole plant parts of A. latifolium employed as medicine. A. latifolium used in Brazil to reduce different types of pain, but in Colombia the plant was used for the treatment of skin conditions in connection with inflammation and infection. Latin American traditional medicine utilised A. latifolium as anxiolytic, analgesic, and anti-inflammatory [3].

In France, large quantities of the plant used to prepare the famous “Sirop de capillare”. A. latifolium possessed antinociceptive and antiinflammatory activities executed through the inhibition of IL-1β production [4]. A new triterpenoid was isolated from the whole plant of A. lunulatum, another species also showed antibacterial activity [5]. Single ethanolic extract of leaves of A. latifolium showed activity against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa [6].

Widespread use of antibiotic medicines in human being caused to develop drug-resistant bacteria. These drug-resistant bacteria remained as a major problem in hospital and community pathogens [7]. Plant derived phytochemicals could be used as alternate therapeutic medicine. Present study aimed to evaluate antibacterial potential and phytochemical compounds of the plant in various solvents extracts of
increasing polarity towards some pathogenic bacteria involved in skin infection.

Materials and Methods

Preparation of Plant Extract

Fresh materials of *Adiantum latifolium* Lam. were collected in the month of January from Pala, Kottayam District, Kerala. A voucher specimen (TT 1561) was deposited at the herbarium of St. Thomas College Palai. The whole plants were shade dried for three weeks and ground to powder using mechanical grinder. The air-dried plant material (100g) was utilised for preparing extracts. Soxhlet extraction was successively done in petroleum ether, acetone, ethanol and water [8] yielded 0.56%, 2.5%, 3.3%, and 0.8% respectively.

Microorganisms Employed

The test organisms were procured from the culture collection of the institute of Microbial Technology (IMTECH), Chandigarh. These include *Klebsiella pneumoniae* subsp *pneumoniae* (MTCC-109), *Staphylococcus aureus* subsp *aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 741), *Serratia marcescens* (MTCC 6164) and *Escherichia coli* (MTCC 443). They were sub cultured on nutrient agar slants, further incubated at 37°C for 12 hours and kept at 4°C in the refrigerator to preserve the stock culture.

In Vitro Antibacterial Assay

Preliminary antibacterial activity was performed by disc diffusion method as explained by Bauer et al., [9]. Sterile liquid Mueller Hinton Agar media (pH 7.4 ± 2) was poured into sterile petridishes and after solidification, the bacteria (1 ml broth of approximately 10^5 CFU) were applied with a sterile needle under sterile conditions. Sterile discs were made using Whatman No. 4 Filter Paper, with 5-mm diameter were utilised for the study. The original solvents in which the extracts prepared were used as a control.

Test materials were dissolved in the respective solvent to get a stock solution of concentration of 100 mg/ml. 10 μL of the solution was applied onto each disc to get a concentration of 1 mg/disc. The discs (including control) were inserted in the medium after drying them in an incubator at 40°C, aimed to remove any trace of solvent.

The plates were incubated at 37°C for 24 hours to observe any inhibition zones. Experiments were performed in more than three replicates and average inhibitory zone diameter was recorded.

Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was done by placing different quantities (300–0.59 mg/ml) of the extract into group of test tubes with the culture media [10]. 50 μl of the bacterial broth culture was poured into each test tube. The bacterial cultures with 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 lowest concentration of the extracts that could not permit any visible growth as compared to that of the control tubes was taken as the minimum inhibitory concentration.

Minimum Bactericidal Concentration (MBC)

Samples from the tubes in previous studies, which could not give any detectable visible growth after a period of incubation, were sub cultured onto a freshly prepared nutrient medium [11]. The lowest concentration of the extract that did not provide a single colony on the nutrient agar plate after 24 hours incubation time was taken as the minimum bactericidal concentration.

Preliminary Detection of Phytochemicals

The crude samples were subjected to phytochemical screening using different spraying reagents. Alkaloids, phenolics, Triterpenoids, flavonoids were detected after TLC separation using the method as described by Harborne [12] and Stahl [13].

Results and Discussion

Petroleum ether and water extracts could not show antibacterial activity towards tested organisms. Acetone extract of *A. latifolium* showed lower level of inhibition towards *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens*. The plant showed lower level of inhibition
Petroleum ether extract contained non-polar compounds dissolved in it, and these compounds did not have antibacterial activity. Medium polar compounds were soluble in acetone extract and these compounds showed moderate level of antibacterial activity, while ethanol extract contained polar compounds and they exhibited antibacterial potential. Ethanol extract of A. latifolium gave maximum action against Pseudomonas aeruginosa, gram-negative bacteria. Pseudomonas aeruginosa is often found in nosocomial infections and its infection is frequent in patients receiving treatment of severe burns or other traumatic skin damage and in patients suffering from cystic fibrosis. This pathogen occupies the lungs of patients and increasing mortality rate of individuals with the disease [14].

Water extract could not provide any antibacterial activity. Most of the polar compounds were eluted with methanolic extraction and there might be very few compounds left after ethanolic separation. Flavonoids and phenols found in various extracts of the plant. None of the extracts detected the occurrence of alkaloids. Occurrence of phenol and flavonoid in

Towards *Escherichia coli* compared to the other bacterial strains. *Pseudomonas aeruginosa* and *Escherichia coli* are the organisms which showed higher level of activity towards the ethanol extract of the plant (Table 1). No control discs exhibited antibacterial activity. The phytochemical evaluation of *A. latifolium* is reported in the Table 2. As compared with the activity of standard antibiotics, the plant extracts showed lower results (Table 3).

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<thead>
<tr>
<th>Table 1: Antibacterial Activity of <em>Adiantum latifolium</em>.</th>
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<td>Name of plant Extract used</td>
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Value = no obvious growth inhibition (-)

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<th>Table 2: Results of Phytochemical Evaluation of <em>Adiantum latifolium</em>.</th>
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<td>Name of plant</td>
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<td>A. latifolium</td>
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Value = ‘+’: Present ‘-’: Absent

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<th>Table 3: Antibacterial Action of standard antibiotics</th>
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<td>Name of Antibiotic (Con. 25 μg/Disc)</td>
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<td>Streptomycin</td>
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<td>Amoxicillin</td>
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<td>Chloramphenicol</td>
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Value = no obvious growth inhibition
ethanol extract of the plant; this could provide antibacterial activity. The present antibacterial analysis of the plant supported the ethnobotanical importance of *A. latifolium* [3]. Antibacterial activity was also reported for biological synthesis of silver nanoparticles using water extract of whole plant parts of *Adiantum capillus-veneris* L. against human pathogenic bacteria such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* [15].

**Conclusion**

*Adiantum latifolium* demonstrated antibacterial activity in ethanol extract as it was extracted in various solvent extracts of the plant in increasing polarity. The ethanol extract of the plant showed maximum level of activity towards *Pseudomonas aeruginosa*. Petroleum ether and water extracts could not give any antibacterial activity towards any of the examined organisms. The presence of flavonoids and phenols detected in various extracts. Minimum inhibitory concentration as 18.75mg/ml and minimum bactericidal concentration as 37.5mg/ml were observed towards *Pseudomonas aerogenosa* in ethanol extract of the plant.

**References**