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RESEARCH ARTICLE

# Antibacterial Evaluation of Gradient Extracts of *Stenochlaena palustris* (Burm.f) Bedd. Towards Bacteria Involved in Skin Diseases

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## Abstract

**Objective:** The work attempted to analyse the antibacterial character of gradient distillate of *Stenochlaena palustris,* a healing fern used to treat burn. The plant was evaluated for its antibacterial capability towards bacteria occupied in skin infections. **Methods:** Whole plant of *S. palustris* was examined for its antibacterial potential and phytochemical constituents in different solvents extracts of incrementing polarity. The typical disc diffusion method was done to inspect antibacterial activity. Basic phytochemcal assessment was accomplished by various standard spraying reagents and used to detect the same. Minimum inhibitory concentration and minimum bactericidal concentration were established towards *Pseudomonas aeruginosa.* **Results:** The plant could basically demonstrate antibacterial activity in acetone extract. *P. aeruginosa* was the most sensitive organism observed out of the tested bacteria. Water extracts did not provide antibacterial activity to the analysed bacteria. Phenols, flavonoids, polyphenols and sterols were track down in various extracts. Flavonoids, phenols, polyphenols and sterols found in acetone extract of the plant could be responsible for its antibacterial activity. Acetone extract of the plant yielded minimum inhibitory concentration as 12.5 mg/ml and minimum bactericidal concentration as 25.0 mg/ml towards *P. aeruginosa.* **Conclusion:** *S. palustris* manifested antibacterial activity in acetone extract notably towards *P. aeruginosa,* a bacterium frequently associated with nosocomial infection.

**Keywords:** *Stenochlaena palustris, Antibacterial activity, Pteridophytes, Disc diffusion, Phytochemicals.*

## Introduction

*Stenochlaena palustris* (Burm.f) Bedd. is a pteridophyte plant that comes under the group fern. It is a large, common, terrestrial fern, with green leaves and glabrous rhizome. The rhizome is prostrate as well as climbing in nature. Its common name in Malayalam is Tree Pana [1].

The plant can reach up to top of the tallest trees. *S. palustris* belongs to Blechnaceae, its synonym is *Polypodium palustre* Burm.f. [2]. Medicinal uses of the plant include its use as a cooling agent and in the treatment of burns and ulcers. The whole plant parts of *S. palustris* are utilized as medicine [1]. Ethnobotanical use of *S. palustris* reported from Northeast Thailand, where young leaves the plant were used as vegetables [3]. Fronds of the plant were used in skin diseases, throat and gastric ulcers [4]. Extensive use of antibiotic medicines in human being promoted to emerge drug-insusceptible bacteria. These types of drug-insusceptible bacteria stood as a bigger concern in hospital and community pathogens [5].

There is a newer trend to use natural remedies to tackle the problem. Hence, green plants and their phytochemicals can be utilised as a substitute medicine. Present study targeted to analyse the antibacterial potential and find out the major phytochemical compounds of the plant in various solvents extracts of accelerating polarity towards some pathogenic bacteria involved in skin infections.

## Materials and Methods

### Preparation of Plant Extract

Fresh specimens of *S. palustris* were collected in the month of December from Pala, Kottayam District, Kerala. A voucher specimen (TT 1571) was placed at the herbarium of St. Thomas College Palai. A habit of the plant is exhibited in Figure 1. The whole plants were shade dried for four weeks and ground to powder by employing a mechanical grinder. The air-dried plant material (100g) was used for preparing extracts. Soxhlet extractions were successively performed in petroleum ether, acetone, ethanol and water [8] provided 0.47%, 3.1%, 3.4%, and 0.7% yield respectively.


Figure 1: Habit of *Stenochlaena palustris* 1(A) fronds of the creeping fern 1(B) Fertile frond of the plant with sori on lower side.

### Bacterial Strains Employed

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

### *In Vitro* Antibacterial Assay

Disc diffusion experimental method as reported by Bauer *et al*. [7] was perfumed to evaluate antibacterial activity. Sterilized Mueller Hinton Agar media (pH 7.4 ± 2) was emptied into sterile petri-dishes and after solidification, the bacteria (1 ml broth of approximately $10^5$ CFU) were lawed with a sterile cotton plug needle under aseptic environment. Whatman No. 4 Filter Paper was used to prepare sterile discs of 5-mm diameter. The experimental control discs were prepared with the original solvents in which the extracts prepared. Phytochemicals were dissolved in the respective solvent to get a stock solution of concentration of 150 mg/ml.

Each sterile disc was impregnated with a volume of 10 µL of the extract to derive a mass of 1.5 mg/disc. The discs (including control) were placed in the medium after eliminating any trace of solvent from them, by keeping it in a hot air oven set at 40°C. The plates were placed in incubator kept at 37°C for 24 hours to see any inhibition zones. Experiments were conducted in more than three replicates and average inhibitory zone diameter was noted down.

### Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was performed using different amounts (400–0.78mg/ml) of the distillate dissolved in 10% dimethyl sulphoxide into an array of test tubes with the culture media [8]. Every test tube was mixed with 50 µl of the bacterial culture broth.

The cultures with plant extracts were kept in an incubator set at 37°C for 24 hours. Positive controls were also maintained and this consisted of growth medium, 10% dimethyl sulphoxide and each bacterium. The minimum inhibitory concentration was taken as the lowest concentration of the extracts that could not permit any visible growth as compared to that of the control tubes.

### Minimum Bactericidal Concentration (MBC)

Culture tubes from MIC studies, which didn't show 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 show a single colony on a nutrient agar plate after 24 hours incubation.

 

### Preliminary Detection of Phytochemicals

Silica gel Thin Layer Chromatographic separation (TLC) of the crude solvent extract was done in the appropriate solvent system. After that, the plates were sprayed with different spraying reagents as described by Harborne [12] and Stahl [13] to detect phytochemical components like alkaloids, phenolics, polyphenol, flavonoids and sterols.

## Results and Discussion

Gradient exaction of the phytochemicals from *S. palustris* aimed to separate nonpolar phytochemicals followed by polar compounds. If single exaction was done, it could yield a mixture of polar and non-polar compounds in a single solvent like water or ethanol. Gradient extraction would be helpful for separation of different phytochemicals based on their polarity, this would also help to isolate and characterize any compound of interest for further studies.

Water extracts of *S. palustris*, could not show antibacterial activity towards tested organisms. Acetone extract of *S. palustris* showed maximum level of inhibition towards *Pseudomonas aeruginosa* and moderate level of activity towards the rest of tested bacteria.

The plant showed lower level of inhibition towards *Klebsiella pneumoniae* and *Escherichia coli as* compared to the other bacterial strains in general. *Pseudomonas aeruginosa* showed higher level of antibacterial activity towards acetone extract of the plant (Table 1). No control discs could exhibit antibacterial activity. The phytochemical screening of *S. palustris* is reported in the Table 2. Another important observation was that the antibacterial potential of the plant exact would not be equated with the activity of standard antibiotics (Table 3).

**Table 1: Antibacterial activity of *S. palustris***

| 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 marcescens*** **(MTCC-97)** |
| *S. palustris* | Petroleum ether | 8.1 ± 0.45 | 7.3 ± 0.63 | - | - | 7.2 ± 0.44 |
| | Acetone | 15.1 ± 0.41 | 8.6 ± 0.58 | 6.1 ± 0.28 | 8.2 ± 0.38 | 8.3 ± 0.51 |
| | Ethanol | - | 6.2 ± 0.35 | - | - | 6.7 ± 0.35 |
| | Water | - | - - | - - | - | - |

Value = no obvious growth inhibition (-)

**Table 2: Results of phytochemical evaluation of *S. palustris***

| Name of plant | Plant extracts | Test for Flavonoids | Test For Alkaloids | Test for Phenols | Test for Sterols, steroid, phenol and polyphenol |
|---|---|---|---|---|---|
| *S. palustris* | Petroleum ether | + | - | + | - |
| | Acetone | + | - | + | + |
| | Ethanol | + | - | + | - |
| | Water | - | - | + | - |

Value = '+': Present '–' : Absent

**Table 3: Antibacterial Action of standard antibiotics**

| Name of Antibiotic (Con. 25µg/Disc) | Zone diameter (in millimetre) | | |
|---|---|---|---|
| | MTCC – 6164 | MTCC – 96 | MTCC – 741 |
| Streptomycin | 25.5± 0.26 | 19.6± 0.46 | 20.4 ± 0.37 |
| Amoxicillin | - | 24.4± 0.36 | - |
| Chloramphenicol | - | 22.5± 0.34 | - |

Value = no obvious growth inhibition

The exaction was started with non-polar solvent, petroleum ether, the extract contained non-polar compounds and these compounds did not possess antibacterial activity. Medium polar compounds were dissolved in acetone extract and they

 

exhibited maximum level of antibacterial activity as compared to others. While, ethanol extract occupied with polar compounds and they exhibited comparatively lower level of antibacterial potential. Acetone extract of *S. palustris* provided maximum antibacterial activity towards *Pseudomonas aeruginosa*, a gram-negative bacterium. Acetone extract of the plant showed minimum inhibitory concentration of 12.5mg/ml and minimum bactericidal concentration of 25mg/ml towards *P. aeruginosa. P. aeruginosa* is common in nosocomial infections and its infection is more or less common in-patients receiving treatment of severe burns or other traumatic skin damage and in patients suffering from cystic fibrosis.

This pathogen can infect lungs of patients and increase mortality rate [12]. Almost all polar compounds were removed with ethanol extraction and there might be very few compounds left after ethanolic extraction. This might be one of the reasons for decreased activity of water extract. Flavonoids were found in various extracts of the plant. None of the extracts showed the occurrence of alkaloids. The present antibacterial analysis of the plant supports the ethnobotanical importance of and *S. palustris* [3,4].

## Conclusion

Antibacterial activity was demonstrated in in ethanol extract of *S. palustris*. It was exhibited as gradient exaction was performed. The acetone (a medium polar solvent) extract of the plant showed maximum level of activity towards *P. aeruginosa. W*ater extracts of the plant could not provide antibacterial activity towards the tested organisms. Phytochemicals like flavonoids, phenols, Sterols, steroid, and poly phenol were mainly detected in acetone extract of the plant. Acetone extract, the potent extract exhibited a Minimum inhibitory concentration of 12.5mg/ml and minimum bactericidal concentration of 25mg/ml towards *P. aeruginosa.*

## References


1 Nayar BK. Medicinal Ferns of India Bulletin No.29. National Botanical Gardens Lucknow; 1959.

2 Easa PS. Biodiversity documentation for Kerala Part 5: Pteridophytes. Kerala Forest Research Institute Peechi Kerala; 2003.

3 Suksri S, Premcharoen S, Thawatphan C, Sangthongprow S. Ethnobotany in Bung Khong Long non-hunting area, Northeast Thailand. Kasetsart J (Nat Sci) 2005; 39: 519-533.

4 Singh HB. Economically viable Pteridophytes of India. In: Chandra S and Srivastava (eds.), Pterigology in the New Millennium. Kluwer Academic Publishers, Netherlands; 2003.

5 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.

6 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.

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.

8 Barry AL. Antimicrobic Susceptibility Tests: Principle and Practice, Lea and Febiger Philadelphia; 1976.

9 Ratimi VO, Laughon BE, Barlet JS, Mosadomi HA. Activities of Nigerian chewing sticks extracts against *Bacterioides gingivalis* and *Bacterioides melaninogenicus*. Antimicrob Agents Chemother 1988; 32:598-600.

10 Harborne JB. Phytochemical methods. Chapman and Hall Ltd London; 1973.

11 Stahl E. Thin layer chromatography, a laboratory handbook, Springer International, New York; 1961.

12 Madigan MT, Martinko JM, Parker J. Brock Biology of Microorganisms 9th ed., Prentice Hall International Inc New Jersey; 2000.


 