Antimicrobial and Antifungal Activities of Local Edible Fern *Stenochlaena Palustris* (Burm. F.) Bedd

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Summary

This current research was conducted to study the uninvestigated (or less investigated) medicinal ferns found in Malaysia in the search for more effective and non-toxic pharmacological natural compounds. Methanol of extracts from winged bean *Stenochlaena palustris* root, stem and leaf extracts were tested for their antimicrobial activity against 15 microbial species, including 10 bacterial pathogens, one yeasts, and four molds using the disk diffusion assay technique. The leaf extract was found to be most effective against all of the tested organisms, followed by the stem and root extracts. The minimum inhibitory concentrations (MICs) of the leaf extracts determined by the broth dilution method ranged from 50 to 12.5 mg/mL. The preliminary results of present investigation appear to indicate that *S. palustris* of Malaysian Edible fern have higher potential antimicrobial properties.

Key words: antimicrobial activity, antifungal activity, fern, *Stenochlaena palustris*

Introduction

Infectious diseases and their control become serious problem in the medical field. Antibiotics usually suggested for the treatment of infectious diseases, have never been pleasing because of their toxic effect and exert a negative impact on the consumer. As an alternative strategy to prevent infectious diseases, natural compound of plants are being tested for their antimicrobial activity and serve as template for new and more effective antimicrobial agents. This research attempted to study uninvestigated (or less investigated) medicinal plants and ferns found in Malaysia in the search for potential, more effective and non-toxic pharmacological natural compounds.

The pteridophytes which constitute ferns and ferns allies, have been known to man for more than 2000 years, and also been mentioned in ancient literature (1, 2). It has been observed that pteridophytes are not infected by microbial pathogens, which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years. This information's encouraged us to further investigate other tropical fern found in Malaysia for antimicrobial activity.

Stenochlaena palustris (Burm.) Bedd. (Pteridaceae) is a fern trailing over the ground or scrambling high up trees. It is endemic to a large part of tropical areas from southern and northern India through Malaysia to Polynesia and Australia (3). The tender leaves of *S. palustris* are used as a contraceptive by the local people in the central district province of Papua New Guinea (PNG) and in the Nicobar Islands (4, 5). A search for alkaloid-containing plants in New Guinea found the leaves of *S. palustris* to be alkaloid-negative (6). No other chemical studies on this species have been reported. Therefore, the current study was conducted to study the antimicrobial activity of methanolic extract of *S. palustris* against bacterial and fungal strains.

Materials and methods

Materials: The root, stem and leaf of *S. palustris* were collected from Seberang Jaya, Penang, Malaysia, in Mac 2009.

Extraction of plant material: Fern sample was extracted by cold percolation in methanol (consecutively three times) at room temperature for 24 h the resultant extracts were filtered and concentrated to dryness under reduced pressure below 40 ◦C in rotary evaporator.

Antimicrobial activity: Antibacterial and antifungal activities of the fern extracts were investigated by the disk diffusion method (7, 8). The MHA plates, containing an inoculum size of 10^6 colony-forming units (CFU)/mL of bacteria or $2x10^5$ CFU/mL yeast cells or molds spores on SDA and PDA plates, respectively, were spread on the solid plates with an L-shaped glass rod. Then disks (6.0-mm diam.) impregnated with 25 µL of each extract at a concentration of 100.0 mg/mL were placed on the inoculated plates. Similarly, each plate carried a blank disk by adding every solvent control alone in the center, and antibiotic disks (6.0-mm diam.) of 30 µg/mL Chloramphenicol (for bacteria), and 30 µg/mL Miconazole nitrate (for fungi) were also used as positive controls. All of the plates were incubated at 37^{o}C for 18 hours for bacteria and at 28^{o}C for 48 hours for fungi. The zones of growth inhibition around the disks were measured after 18 hours of in incubation at 37^{o}C for bacteria and 48 hours for fungi at 28^{o}C, respectively.

MIC was determined by both broth dilution methods. For broth dilution tests, 0.1mL of standardized suspension of bacteria (10^6CFU/mL) and fungal cell or spores ($5x10^5$CFU/mL) was added to each tube (containing fractions of three extracts at a final concentration of 0 to 20.0mg/mL) and incubated at 37^{o}C for bacteria for 18 hours or at 28^{o}C for fungi for 48 hours. MICs were taken as the average of the lowest concentration showing no growth of the organism and the highest concentration showing visible growth by macroscopic evaluation. Each assay was performed in triplicate.

Results and discussion

The antimicrobial activity data of methanol extract of *S. palustris* are shown in Tables 1 and 2. All the extracts were found to be active in at least one of the microbial strains tested. In general, both bacteria and fungi were found to be susceptible to the test agents than fungi. The preliminary disk diffusion assay (Table 1) of *S. palustris* extracts of root, stem and leaf showed that the leaf extract had the most distinct effect on most of the tested microorganisms, followed by the stem and root extracts. However, two commercial antibiotics were more effective than any of the extract tested. The minimum inhibitory concentration (MIC) values of methanolic extract of *S. palustris* leaf was evaluated and summarized in Table 2. The determination of MIC of root extract was carried out in this study since the extract showed the best activity in the preliminary antimicrobial screening assay. The methanolic extracts of *S. palustris* root gave low MIC values against *Enterobacter aerogenes,Salmonella typhi* and *Azospirilium lipoferum* with concentration of 12.5 mg/ml. However, the *Klebsiella pneumoniae* showed a highest MIC value of 50.0 mg/ml when tested with root extract of *S. palustris*. The MIC values of the extracts seem to be relatively higher. However, being crude extracts, the overall antimicrobial activity screening results are only indicative of the potential of this fern extracts for the medicinal purposes.

The preliminary results of present investigation appear to indicate that *S. palustris* of Malaysian Edible fern especially lower groups (pteridophytes) have higher potential antimicrobial properties. Further studies aimed at the isolation and identification of active substances from the methanol extracts of *S. palustris* may disclose othe rcompounds with better value for antimicrobial agents.

References

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Table 1: Antimicrobial activity (zone of inhibition)[a] of *Stenochlaena palustris*

Microorganisms	Zone of inhibition (mm)[b]				
	Leaves	**Stems**	**Roots**	**C**	**M**
BACTERIA					
Gram-positive					
Staphylococcus aureus	14	13	15	24	ND
Bacillus subtilis	10	9	18	22	ND
Micrococcus sp	9	16	8	26	ND
Gram-negative					
Enterobacter aerogenes	16	10	13	25	ND
E. coli	18	14	15	22	ND
Proteus mirabilis	17	24	21	22	ND
Klebsiella pneumoniae	16	17	14	25	ND
Salmonella typhi	–	9	9	21	ND
Azospirilium lipoferum	14	18	20	26	ND
Azobacter	18	12	17	23	ND
FUNGUS					
Penizillium chrysogenum	14	13	9	ND	21
Rhizopus stolonifer	–	–	–	ND	20
Aspergillus niger	11	15	16	ND	24
Fusarium sp.	12	8	10	ND	22
Saccharomyces cerevisiae	15	11	16	ND	21

[a]Disc diffusion technique
[b]The values (average of triplicate) are diameters of zone of inhibition at 100mg/mL of crude extract, 30µg/mL of Chloramphenicol and 30µg/mL of Miconazole nitrate
ND : Not Determined
C : Chloramphenicol
M : Miconazole nitrate

Table 2: Determination of MIC values of root extracts of *Stenochlaena palustris*

Bacteria	**Concentration (mg/ml)**										
	100.000	50.000	25.000	12.500	6.250	3.125	1.563	0.781	0.391	0.195	0.098
Gram-positive											
Staphylococcus aureus	−	−	−	+	+	+	+	+	+	+	+
Bacillus subtilis	−	−	−	+	+	+	+	+	+	+	+
Micrococcus sp	−	−	−	+	+	+	+	+	+	+	+
Gram-negative											
Enterobacter aerogenes	−	−	−	−	+	+	+	+	+	+	+
E. coli	−	−	−	−	+	+	+	+	+	+	+
Proteus mirabilis	−	−	−	+	+	+	+	+	+	+	+
Klebsiella pneumoniae	−	−	+	+	+	+	+	+	+	+	+
Salmonella typhi	−	−	−	−	+	+	+	+	+	+	+
Azospirilium lipoferum	−	−	−	−	+	+	+	+	+	+	+
Azobacter	−	−	−	+	+	+	+	+	+	+	+

− : Indicates absence of growth

\+ : Indicates presence of growth