Angiopteris evecta (Forst.) Hoffm: a potential source for antibacterial activity

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Introduction

The continuous increase in resistance to pathogenic microbes and shortage of new antimicrobial agents are a serious problem in the treatment of infectious diseases. Several studies have been done to reveal the antimicrobial agent on higher plants. However, very little attention has been given to the lower plants, especially the pteridophytes. Being a tropical island, Sri Lankan flora also has a substantial contribution to the pteridophytes. This paper presents the investigation of the antimicrobial activity of *Angiopteris evecta* (Forst.) Hoffm which was selected through an ethnomedicinal survey conducted in two selected areas in Sri Lanka namely Mahiyanganaya and Bulathsinghala. *A. evecta* is a terrestrial fern with well developed subterranean rhizome and pinnately compound large fronds.

Methodology

Considering the richness of bio diversity of pteridophytes, a field survey was conducted from January 2011 to April 2011 to explore ethnomedicinally important pteridophyte floras used by the local healers, local people and tribal people in Mahiyanganaya and Bulathsinghala areas. Structured questionnaire survey method was conducted to collect the information. To obtain more detailed information of ethnomedicinal usage, informants were accompanied to the field to collect plant material as much as possible. The collected plants were photographed and pressed to show the interviewees to collect information. Data on plant species, families, vernacular names, parts used, traditional use and modality of use were recorded. Interviews were conducted as personal and groups with 165 people including 38 local healers, 105 local people and 22 tribal people. All the specimens were collected in duplicate forms and the samples were authenticated by the national herbarium Peradeniya in Kandy, Sri Lanka. Voucher specimens were deposited in the herbarium.

Based on the ethnomedicinal survey, *A. evecta* was selected for further investigation of the antibacterial activity. Freeze dried samples of fronds, roots and rhizome were used for solvent extraction. Each sample was mixed with the solvents in the ratio of 1:10 (v/w) and follows the serial exhaustive extraction method (Das, K *et al*., 2010). Extraction procedure was repeated for complete extraction of the bio active compounds. The filtrate was concentrated *in vacuo* at 35^0C using rotary evaporator. *In vitro* antibacterial activity of crude extracts was determined by standard disk diffusion method (Bauer, A. W *et al*., 1959). Pre identified bacterial cultures of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Streptococcus fecalis* were collected from the Department of plant sciences, University of Colombo and grown on nutrient broth at 37 ^{0}C for 24 h. The cultures were adjusted to the concentration of 1×10^8 -1×10^9 CFU/ml by using spectrophotometer at 600 nm.

10 µl of 100 mg/ml the stock solution of extract was aseptically introduced to sterilized filter paper disks of 5 mm in diameter. The disks were kept for few minutes to allow them to absorb the solution and were placed on the surface of the inoculated MHA plates with different bacterial strains. Standard antibiotic disks (25 µg/disks of cloxacilin) were used as the positive control. A filter paper disk impregnated with 10 µl respective pure solvent was used as a negative control. The plates were incubated at 37 ^{0}C overnight. The experiment was performed in triplicate and the antibacterial activity of the extract compound was expressed the mean of diameter of zone of inhibition (in mm).

Results and Discussion

A total of 35 plant species belonging to 17 families were collected through the survey. Among these plant species, 33 have been identified at the National herbarium Peradeniya in Kandy, Sri Lanka and 2 species were unable to be identified. Result of the survey demonstrates that, 27 species have ethnomedicinal usage to cure 13 diseases including wounds, constipation, hemorrhoids, diabetics, growing cartilage, dysentery, muscle pain etc. More than 10 species are used to cure wounds, fractured bones and boils. Figure 1 shows ethnomedicinal usage of 35 pteridophyte species and it indicates *A. evecta* is used to cure maximum number of diseases than the other pteridophytes species. Therefore *A. evecta* was selected to investigate the antibacterial activity.

Table 1. Inhibitory zone diameter (mm) of fronds, roots and rhizome of *A. evecta* on different bacterial strains

Plant part	Test Organism	Positive control	Chloroform	Diclhoro Methane	Acetone	Ethanol	Methanol
Fronds	*S.aureus*	10.25±0.21	0.00	10.02± 0.28	8.09± 0.35	12.05±0.35	9.91± 0.46
	E. coli	18.00±0.11	0.00	10.94± 0.54	7.88± 0.60	9.07± 0.75	8.08± 0.38
	K. pneumonia	11.05±0.17	0.00	0.00	0.00	0.00	0.00
	S. fecalis	16.43±0.31	0.00	0.00	8.62± 0.72	0.00	0.00
Root	*S.aureus*	10.25±0.21	0.00	0.00	8.47± 0.32	0.00	8.16± 0.85
	E. coli	18.00±0.11	0.00	10.34± 0.37	14.11± 0.54	14.15± 0.61	8.88± 0.91
	K. pneumonia	11.05±0.17	0.00	0.00	0.00	0.00	0.00
	S. fecalis	16.43±0.31	0.00	0.00	0.00	0.00	0.00
Rhizome	*S.aureus*	10.25±0.21	0.00	13.39± 0.59	12.26± 0.32	8.51± 0.32	7.30± 0.58
	E. coli	18.00±0.11	9.31± 0.66	7.82± 0.34	10.05± 0.60	7.82± 0.13	0.00
	K. pneumonia	11.05±0.17	0.00	0.00	7.69± 0.20	0.00	0.00
	S. fecalis	16.43±0.31	0.00	0.00	0.00	0.00	0.00

In vitro antibacterial activity of crude extracts was determined by standard disk diffusion method. The mean diameter of zone of inhibition of fronds, roots and rhizome of *A. evecta* on different bacterial strains are listed in Table 1. The results were statistically analyzed and F test results showed there was no significant difference among plant parts in inhibiting the bacteria growth but there was a significant difference among bacteria strains and the crude extracts made with different solvents. Dichloromethane, acetone, methanol and ethanol crude extracts showed significantly higher effect on inhibiting growth of *Escherichia coli* and *Staphylococcus aureus* than the chloroform extract used for test. Also *Escherichia coli* and *Staphylococcus aureus* showed more sensitivity than *Klebsiella pneumonia* and *Streptococcus fecalis* towards all extract tested except chloroform extract. Altogether these results revealed the crude extracts to be a good source of potential antimicrobial compounds.

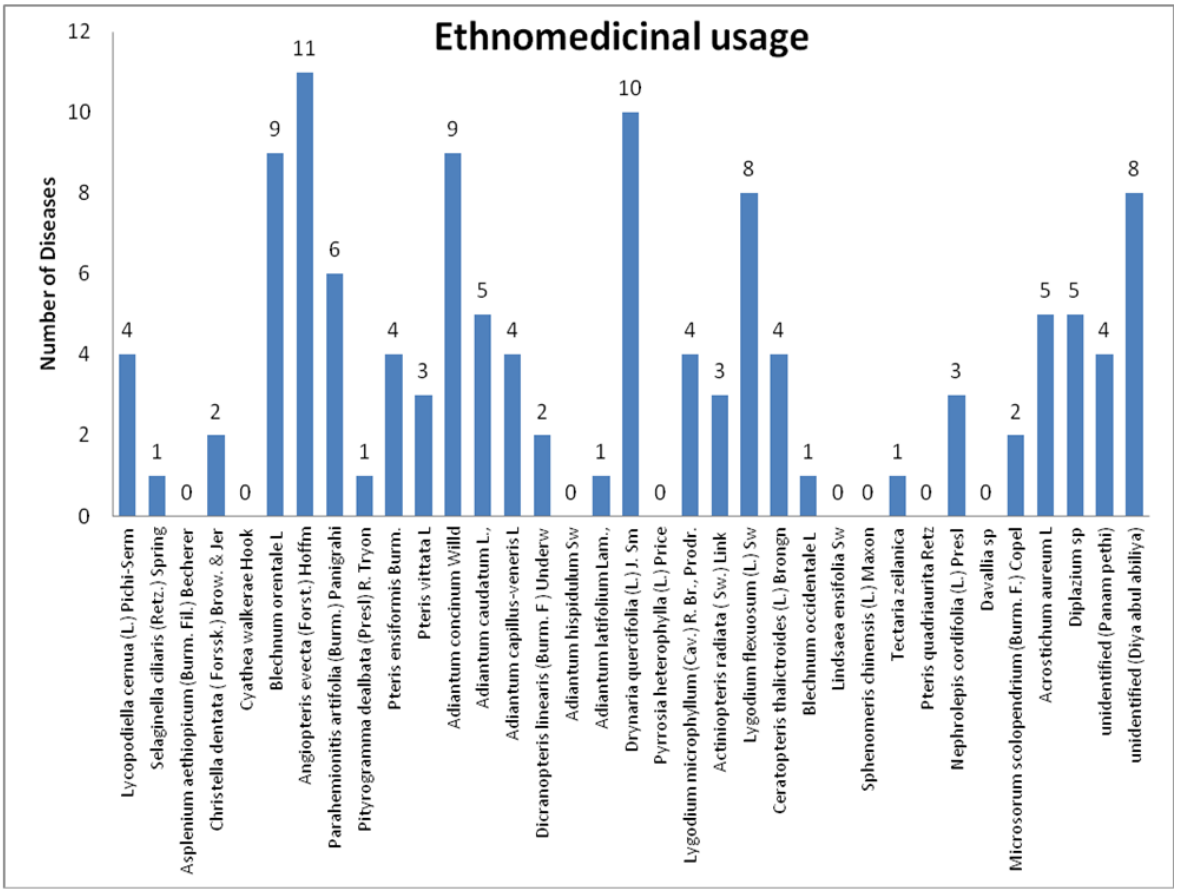

Figure 1: Ethnomedicinal usage vs number of diseases

References

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