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ANTIBACTERIAL ACTIVITY OF RHIZOME EXTRACTS OF FOUR PTERIDOPHYTES FROM SOUTHERN ASSAM, NORTH EAST INDIA

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ABSTRACT

Increasing resistance to antibiotics is a growing problem in both developed and developing countries. Thus, it is important that constant research should be undertaken to produce newer and newer antibacterial substances. A good success in this direction has been achieved using extracts of plant parts against micro organisms. The aim of the present study is to characterize the rhizome of four ferns viz. *Drynaria quercifolia*, *Leptochilus pteropus*, (Syn. *Microsorium pteropus*) *Microsorium punctatum* and *Tectaria impressa* (Syn. *Tectaria variolosa*) for their antibacterial property against potentially pathogenic microorganisms. Extract was prepared in four different solvents such as water, methanol, acetone, and petroleum ether. The extract of each fern was tested against *Bacillus subtilis* (MTCC NO. 736), *Escherichia coli* (MTCC NO. 1610), *Pseudomonas aeruginosa* (MTCC NO. 3541), *Staphylococcus aureus* (MTCC NO. 3160), and *Proteus vulgaris* (MTCC NO. 1771) by disc diffusion assay. The result revealed that out of the four ferns, three ferns viz. *Drynaria quercifolia*, *Microsorium pteropus*, *Tectaria variolosa* have antibacterial property in their rhizome extract against all the tested micro-organisms.

KEYWORDS

Ferns, Rhizome extract, Micro-organisms and Antibacterial activity.

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INTRODUCTION

The newly developing branch of antibacterial resistance research is focused on use of natural products mainly from plants for production of new antimicrobials. Nature has served as a rich source of medicine since thousands of years and plants have proved to have high degree of medicinal potential. Herbal medicine based on traditional uses has been the basis of treatment for many ailments. The use of plants as complementary and alternative medicine

has increased dramatically in the last 20-25 years. Active compounds produced during secondary metabolism are usually responsible for the biological properties of some plant species and are used throughout the globe for various purposes, including treatment of infectious diseases and other such as cancer, Alzheimer's disease¹. Extracts of medicinal plants are rich source of unique phytochemicals.

The medicinal values of ferns have been known to many for more than 2000 years. The Greek botanist Theophrastus (372-287 B.C) mentioned medicinal value of ferns in his book *Historia Plantarum*. Medicinal ferns of India are studied and listed by Nayer² Dhiman³, Singh *et al*⁴. Kirtikar and Basu⁵ have described 27 species of ferns having varied medicinal uses. Nayar² recorded 29 medicinal ferns. May⁶ published a detailed review the uses of ferns and listed 105 medicinal ferns.

Screening of antimicrobial activity of ferns has been made by Banerjee and Sen⁷, Sen and Nandi⁸. They reported that the fern extracts are effective against both Gram positive and Gram negative organisms. Glands of superficial hairs on leaves and rhizome contain chemicals that are found to have antimicrobial activity. The antimicrobial potential of some ferns has also been studied by Kumar and Kaushik⁹, Parihar and Bohar^{10,11}. Rhizome, the stem of the fern plant may creep along or under the ground or even up a tree (a creeping rhizome). It may grow into a short or tall trunk (a vertical rhizome), or it may be a solid mass that gives rise to a tuft of fronds (an erect rhizome). Very often, the rhizome grows underground. The antimicrobial substances present in rhizome of pteridophytes generally belong to four important group viz. alkaloids, terpenoids, flavonoids and others¹². About 600 plant derived alkaloids have been analyzed by different workers for their bio medicinal properties and many of them have become important *drug leads* or actual drugs in pharmaceutical industry¹³.

In this study four ethno-medicinally imperative ferns of Southern Assam have been assessed for their antibacterial property of rhizome extract.

These fern have been claimed for some ethno medicinal value. The leaf paste of *Microsorium punctatum* is applied on cuts and wounds. Its rhizome is crushed and mixed with seeds of *Piper nigrum* to cure cough and cold¹⁴. The rhizome's paste of *Drynaria quercifolia* mixed with molasses is taken during cardiac problem. The paste of rhizome is also useful in blood coagulation and prevents dislocation of fractured bones¹⁴. The rhizome is as well known to have antibacterial property and anti-inflammatory¹⁵. In Barak Valley of Southern Assam, it is commonly called as *Uphatkarul* and is used to treat tuberculosis and throat infections¹⁶. The plant of *Microsorium pteropus* is crushed along with leaves of bringel and applied locally on cuts and wounds of dogs for haemostatic and antiseptic action¹⁷.

EXPERIMENTAL

MATERIAL AND METHODS

Collection of plant materials

Fresh plant material was collected from Southern Assam for a period of 5 months i.e., December, 2012 to April, 2013. The collected plants were cleaned under tap water and were allowed to shade dry.

Preparation of plant extract

The shade dried plants were then grinded to powder. Five g of each plant was dipped in 20 ml of four different solvents i.e., water, methanol, acetone, and petroleum ether for 72 hours. After 72 hours the extract was filtered through what man filter paper No.1. The extract was then left in the room temperature so that the solvent could evaporate. But in case of water, the extract was kept in an oven at 50°C for water evaporation. The dried plant extract was then stored in a refrigerator at 4°C.

Bacterial strains

The test organisms were collected from the culture collection of the Institute of Microbial Technology (IMTECH) Chandigarh. These includes *Bacillus subtilis* (MTCC NO. 736), *Escherichia coli* (MTCC NO. 1610), *Pseudomonas aeruginosa* (MTCC NO. 3541), *Staphylococcus aureus* (MTCC NO. 3160), *Proteus vulgaris* (MTCC NO. 1771), The bacteria

were sub-cultured on nutrient agar (HIMEDIA M0010100G) plates and incubated at 37 °C for 24 hours and stored at 4 °C in the refrigerator to maintain stock culture.

Maintenance of bacterial strains

Organisms were maintained at -80 °C and -20 °C refrigerators in a glycerol stock.

The Inoculum

The inoculum for antibacterial assay was prepared by inoculating freshly grown bacterial culture in normal saline solution and the turbidity was matched with 0.5 McFarland Standard.

The medium

Mueller Hinton agar medium was employed for disc diffusion antibacterial testing. Dehydrated medium supplied by Sisco Research Laboratories (SRL) Pvt. Ltd., Mumbai was used. The medium contained per litre, beef infusion from 300g beef, acid hydrolysate of casein 17.5g, starch 1.5g, and agar 17g. The medium was prepared by dissolving the dehydrated mixture of ingredients in distilled water. After boiling, pH was adjusted to 7.4 and sterilized by autoclaving at 121°C for 15 minutes. The medium was poured in Petri plates.

In vitro antibacterial assay

The MuellerHinton agar plates were seeded with bacterial inoculums aided with sterile swab and was allowed to dry for 30 minutes. Then over the plates sterile filter paper discs were placed, which were impregnated with 10µL of each plant extract (500mg/500µl of solvent). For negative control discs impregnated with each four different solvents was used. The plates were then incubated at 37 °C for 24 hours. The inhibition zone was measured from 6mm to 14mm.

RESULTS AND DISCUSSION

The rhizome produces roots and new fronds. Since the rhizome mostly remains in contact of soil or other substratum, it has to developed defensive mechanism against potentially pathogenic bacterial flora. The extracts of rhizome of the ferns therefore more often than not contain phytochemicals for their protection. The results obtained in the present

experiment indicated that rhizome extract of three out of four ferns contain antibacterial substances.

The extracts obtained from ferns have effective antibacterial activities against gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), gram negative bacteria (*E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*), as well as fungi^{8,18,19}. Antimicrobial activities of rhizome of different ferns have been studied recently against Gram +ve and Gram -ve bacteria²⁰. The antimicrobial activity of the extracts of three plant parts viz. rhizome, rachis and frond of *Thelypteris interruptus* (Willd) H, *Gleichenia microphylla* R.Br, and *Microsorium pteropus* (Bl.) Copel. have been studied and found that frond and rhizome extracts of all these three species show good antimicrobial activity²⁰. The study of antimicrobial activities of rhizome, rachis and frond extracts of *Athyrium filix-femina* (L.) Roth, *Dicranopteris linearis* (Burm.f.) Underw, *Pleopeltis macromarpa* (Bory ex Willd.) Kaulf was done. Frond and rhizome extracts of these species also show good antimicrobial activity than rachis²¹. In the present experiment, rhizome extract of *Microsorium punctatum* with all solvents failed to produce any inhibition zone against the test organisms. However, in case of other ferns the rhizome extracts showed good antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. Against *Staphylococcus aureus* good inhibition zones were obtained using methanol extract of rhizome of *Microsorium pteropus* (9 mm) and *Tectaria variolosa* (8 mm) respectively. These findings are in line with the observations of Sen and Nandi (1951), Banerjee and Sen (1980), Pal (2013 a, b) and others. It has been stated that the epidermal glands of ferns contain substances like phenolic compounds, glycosides, flavonoids and alkaloids²². It is suggested that such substances are responsible for the antimicrobial activity of the 3 species of pteridophytes studied in course of the present experiment.

Table No.1: The inhibition zones of the ferns against the tested organisms have been tabulated and are measured in mm

S.No	Ferns	Solvent	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Proteus vulgaris</i>
1	<i>Microsorium punctatum</i>	Petroleum ether	0	0	0	0	0
		Acetone	0	0	0	0	0
		Methanol	0	0	0	0	0
		Water	0	0	0	0	0
2	<i>Drynaria quercifolia,</i>	Petroleum ether	0	0	6	0	0
		Acetone	0	0	8	0	0
		Methanol	7	9	0	0	0
		Water	0	0	0	0	0
3	<i>Microsorium pteropus,</i>	Petroleum ether	0	0	0	0	0
		Acetone	0	7	7	0	0
		Methanol	7	0	8	9	0
		Water	0	0	0	0	0
4	<i>Tectaria variolosa</i>	Petroleum ether	0	0	0	0	0
		Acetone	0	9	8	0	0
		Methanol	7	7	0	8	0
		Water	0	0	0	0	0

CONCLUSION

The results obtained in the present experiment indicates that rhizome extract of three out of four ferns contain antibacterial substances. The antimicrobial activity of the extracts of three ferns viz. *Drynaria quercifolia*, *Microsorium pteropus*, *Tectaria variolosa* have been studied and found that rhizome extracts of all these three species show good antimicrobial activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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