

Study of Activity of Some Medicinal Ferns of Darjeeling

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Abstract: The present aim is to study of some medicinal ferns having antimicrobial activity and commonly found around the area of district Darjeeling of West Bengal, India. Common medicinal ferns from several areas of Darjeeling district are collected and tested against Gram +ve and Gram –ve bacteria for their antimicrobial activity. Collected plant materials are dried and the soluble extracts are made using organic solvent like ethanol . Antimicrobial activities are measured using agar cup diffusion method. Greater the area of inhibition zone indicates the presence of good potentiality of antimicrobial activity. Antimicrobial activity of three plant parts like rhizome, rachis and frond extracts of *Athyrium filix-femina*(L.)Roth., *Dicranopteris linearis*(Burm.f.)Underw., *Pleopeltis macromarpa* (Bory ex Willd.)Kaulf. are tested. Frond and rhizome extracts of these species show good antimicrobial activity than rachis. .

Key words- Antimicrobial, rachis, rhizome, frond, extract, gram(+ve) and gram(-)ve.

I. INTRODUCTION

In Darjeeling district of West Bengal, a large number of tribals are inhabiting and they use folk medicines to cure their ailments. They provide a good traditional knowledge regarding the use of folk medicine of Darjeeling district having a rich biodiversity. Present aim is to study and gain knowledge of medicinal ferns used by these tribals for treatment of their various ailments. For my present work three common ferns such as *Athyrium filix-femina*., *Dicranopteris linearis* and *Pleopeltis macromarpa*, are collected based on the ethnomedicinal knowledge of tribals of Darjeeling district and tested against the antimicrobial activities. It needs to study the various parameters like climatic condition, plant parts for use, processing of materials having therapeutic activity as well as their chemical nature . The voucher specimens are verified from the specimens present in the herbarium of Lloyd Botanic Garden of Darjeeling.

In ancient Indian medicine several ferns were used. A systematic survey of antimicrobial activity of ferns has been made by Banerjee & Sen (1980), Sen & Nandi (1981). They found that the fern extracts are effective against both gram +ve and gram –ve bacteria. Glands of superficial hairs on leaves and rhizome contain chemicals that are found to have antimicrobial activity.

Medicinal ferns of India are studied and listed by Nayer (1959), Josh (1997), Dhiman(1998), Singh et al(2001). Kirtikar et al(1935) have described 27 species of ferns having varied medicinal uses. Nayar(1959) recorded 29 medicinal ferns. May(1978) published a detailed review the uses of ferns and listed 105 medicinal ferns. The antimicrobial potential of some ferns has also been studied by Kumar and Kuushik(1999),Parihar and Bohar(2002&2003).

II. MATERIAL AND METHODS

Fresh specimens of ferns rhizome rachis and frond are collected from different places of Darjeeling district, W.B. The specimens are then dried at 40⁰ C in Hot-air-oven for 3-5 days. The dried specimens are powdered. Rhizome, rachis and frond extracts are made from powdered specimens in organic solvent ethanol. For each specimen and for each extraction 5 gm. powder is taken in three conical flasks(100ml) to which 20 ml solvent is poured respectively. The mouth of flasks are tightly plugged with non-absorbent cotton and sealed with grease to prevent evaporation. Then the flasks are placed in a shaker for about 24 hours at room temperature 37⁰ C. After shaking for 24 hours, the extracts are filtered using Whatman No-1 filter paper.

The filtered extracts are tested for antimicrobial activities against both gram +ve and gram –ve bacteria on nutrient agar plate by disc diffusion method (Baur et al 1966). The bacterium *E.coli* is taken as standard gram –ve specimen and the bacterium *Bacillus megaterium* is taken as standard gram +ve specimen for testing the antimicrobial activity.

In present experiment fresh bacterial culture solution having concentration 10⁶ cells/ml is taken and discs of 6mm in diameter are made on nutrient agar plate for diffusion assay. Sterile distilled water is used as control. After incubation for 24 hours at 37⁰ C, the diameter of inhibition zones are measured and analyzed. Three replicates are made for each set of experiment.

Protein is determined by the method of Lowry et al (1951)

III. RESULTS

The result of antibacterial activity of three selective ferns *Athyrium filix-femina*, *Dicranopteris linearis* and *Pleopeltis macromarpa*, are shown in table no.1,2and 3.The results show the well antimicrobial activity for both Gram(+)ve and gram(-)ve bacteria. The rhizome and frond extracts of these three ferns show good antibacterial activity. It indicates that the antibacterial substances present in rhizome and frond are in good amount. The best antimicrobial activity is found in frond and rhizome extracts of these species. Rachis extracts also show antimicrobial activity but in less amount than frond extracts.

In *Athyrium filix-femina*, highest activity for Gram(-)ve bacteria is found in rhizome extract. Rachis extract is less effective. A good antimicrobial activity against Gram (-)ve bacteria is also found in frond extract. All these extract are less effective for Gram(+)ve bacteria.

In *Dicranopteris linearis* highest activity for both gram(-)ve and gram (+)ve bacteria is found in rhizome extract. Rachis extract is less effective. Frond extract also shows a good effective for both gram (-)ve and gram (+)ve bacteria.

In *Pleopeltis macromarpa* the highest activity for both gram(+)ve and gram(-)ve bacteria is found in frond extract. Rachis extracts are less effective. The rhizome extract also shows good activity against both gram(+)ve and gram (-)ve bacteria.

Table-1.Antibacterial activity of *Athyrium filix-femina*

Name of the test organism.	Zone of inhibition					
	Rhizome	Rachis	Frond	Control(mm)		
<i>Escherichia coli</i>	10.0mm	8.2mm	9.7mm	A	M	E
				6.3	6.2	6.2
<i>Bacillus megaterium</i>	8.8mm	7.8mm	8.5mm	6.4	6.3	6.3

Table-2. Antibacterial activity of *Dicranopteris linearis*

Name of the test organism	Zone of inhibition					
	Rhizome	Rachis	Frond	Control(mm)		
<i>Escherichia coli</i>	10.6mm	9.6mm	10.4mm	A	M	E
				6.3	6.2	6.2
<i>Bacillus megaterium</i>	10.5mm	9.0mm	10.2mm	6.4	6.3	6.3

Table-3.Antibacterial activity *Pleopeltis macromarpa*.

Name of the test organism	Zone of inhibition					
	Rhizome	Rachis	Frond	Control(mm)		
<i>Escherichia coli</i>	10.8mm	10.2mm	11.5mm	A	M	E
				6.3	6.2	6.2
<i>Bacillus megaterium</i>	10.4mm	10.0mm	11.2mm	6.4	6.3	6.3

Table-4: Study of p^H value range of extracted samples:

Name of the specimens	P ^H value of the extracts		
	Rhizome	Rachis	FronD
<i>Athyrium filix-femina</i>	7.0	6.8	6.8
<i>Dicranopteris linearis</i>	7.2	7.2	7.0
<i>Pleopeltis macromarpa</i>	7.2	7.0	7.0

Table-5: Study of protein value of extracted samples

Name of the specimens	Protein value of the extracts(mg/ml)		
	Rhizome	Rachis	FronD
<i>Athyrium filix-femina</i>	0.066	0.0048	0.0046
<i>Dicranopteris linearis</i>	0.054	0.0034	0.0032
<i>Pleopeltis macromarpa.</i>	0.060	0.0038	0.0037

IV. DISCUSSION

The rhizome, leaf blade and rachis of ferns are covered by glands densely. These epidermal glands(Manikam,2002) contain substances like phenolic compounds, glycosides, flavonoids. and alkaloids(Alcaraz et al,2000,Cushnie and Lamb,2005, Yusuf,1994). These substances are largely responsible for the antimicrobial activity and are being soluble in organic solvents easily extracted in methanol, ethanol and acetone but less soluble in water(Adedapo et al,2009, Banerjee and sen1980)

The present results show the good antimicrobial activity of three species indicating the presence of good amount substances like phenolic compounds, glycosides, flavonoids and alkaloids. These observations are good agreement with the findings of Sen and Nandi(1951), Banerjee and Sen(1980), Natarajan et al(2005). The antimicrobial activities of the ferns are also in agreement with the common usage of ferns in folk medicine for bacterial infection such as infection of throat, boil, ulcer and in wound healing(Banerjee and Sen 1980), tumour(Creasey 1969),dermatophytes(Davvamani et al 2005).

The antibiotic spectra of three ferns cover both gram positive and gram negative bacteria. These observations provide support that the ferns produce a variety of antimicrobial substances. It is necessary to keep in mind that the factors like climatic condition, nature of plant parts, age of plant at the time of collection etc are also responsible for the enhancement of the activity of the antimicrobial substances and it needs to be studied more in details.

The amount of protein present in the extracts show little variation though antimicrobial activities show wide variation among the specimens. It indicates that proteins may have little effect on antimicrobial activities.

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