

STUDY OF SOLVENT EXTRACTS OF SOME SELECTED FERNS FOR ANTIMICROBIAL ACTIVITY

Samir Kumar Pal*

P.G Department of Botany,
Darjeeling Govt. College Darjeeling, 734101,W.B., India

ABSTRACT ■ The present study was aimed to study the antimicrobial activity of different solvent extracts of some selected ferns commonly found around the area of district Darjeeling of West Bengal, India against Gram positive and Gram negative bacteria. Collected plant materials were dried and the soluble extracts were made using different solvents like distilled water, methanol, ethanol and acetone. Antimicrobial activities were measured by disc diffusion method. In *Cyclosorus interruptus* (Willd) H.I acetone extract was suitable than other solvent for Gram negative and ethanol extract for Gram positive bacteria. In *Gleichenia microphylla* R.Br acetone extract for Gram positive and methanol extract for Gram negative bacteria showed better antimicrobial activity. In *Microsorium pteropus*(Bl.) Copel acetone extract showed good activity for both Gram positive and Gram negative bacteria. In *Athyrium filix-femina* (L.) Roth had good antimicrobial activity in acetone extract for both Gram negative and Gram positive bacteria.

Key words: Ferns, solvent, extract, antimicrobial, Gram positive, Gram negative.

INTRODUCTION

The medicinal value of ferns has been known to many for more than 2000 years (Theophrastus ,C.A 372-287 B.C). The Greek botanist Theophrastus (C.A 372-287 B.C) has referred to the medicinal value of ferns in his book *Historia Plantarum*. In ancient Indian medicine, several ferns were used 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). A systematic survey of antimicrobial activity of ferns has been made by Banerjee and Sen (1980), Sen and Nandi (1981). They found that the fern extracts were effective against both Gram positive and

Gram negative bacteria. Glands of superficial hairs on leaves and rhizome contain chemicals that were found to have antimicrobial activity (Manickam et al 2002). 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).

The antimicrobial substances present in fronds of fern are needed to be extracted in

* E-mail: msasish@yahoo.co.in

pure form before the study of their effects. Most of these substances are complex organic secondary metabolites of plant and soluble in organic solvent. For this it is better to select suitable solvent for extraction of these antimicrobial substances.

METHODS

Four fern leaves namely *Cyclosorus interruptus*, *Gleichenia microphylla*, *Microsorium pteropus* and *Athyrium filix-femina* were collected from different places of Darjeeling district, West Bengal, India. The specimens are then dried at 40°C in Hot-air-oven for 3-5 days. The dried specimens are powdered. Leaf extracts were made from powdered specimens in sterile distilled water and in different organic solvents like methanol, ethanol and acetone. Five gram powder of each was taken in four conical flasks (100 ml) to which 20 ml of respective solvent were poured. The mouth of flasks were tightly plugged with non-absorbent cotton and sealed with grease to prevent evaporation. Then the flasks were placed in a shaker for about 24 hours at room temperature (37°C). After shaking, the extracts were filtered using Whatman No-1 filter paper.

The filtered extracts were tested for antimicrobial activities against both Gram positive and Gram negative bacteria on nutrient agar plate by disc diffusion method (Baur et al 1966). The cups were made on nutrient agar. The solvent extracts were poured into the cups and after incubation period the inhibition zones were measured. *Escherichia coli* was taken as standard Gram negative bacteria and *Bacillus megaterium* was taken as standard Gram positive bacteria for testing the antimicrobial activity.

In present experiment fresh bacterial culture solution having concentration 10^6 cells/ml was taken and discs of 6 mm in diameter are made on nutrient agar plate for diffusion assay. Sterile distilled water was used as control. After incubation for 24 hours at 37°C, the diameter of inhibition zones were measured and analyzed. Three replicates were made for each set of experiment.

RESULTS AND DISCUSSION

The result of antibacterial activity of four selective ferns *Cyclosorus interruptus*, *Gleichenia microphylla*, *Microsorium pteropus*, *Athyrium filix-femina* were shown in Table no. I, II, III and IV respectively. The results show the well antimicrobial activity for both Gram positive and Gram negative bacteria. The water soluble extracts of these four materials show less antibacterial activity. It indicates that the antibacterial substances present with the fern are not soluble in the water. Three organic solvent ethanol, methanol, acetone were used for preparation of crude extracts. All these organic solvents show the effect of solubility of antibacterial substances to some extent significantly and their effect of solubility also variable depending upon the material used for preparation of crude extracts. In *Cyclosorus interruptus*, highest activity against Gram negative bacteria was found in acetone extract. Methanol and ethanol extract were less effective. Highest activity against Gram positive bacteria was found in ethanol extract. Acetone and methanol were less effective for Gram positive bacteria. In *Gleichenia microphylla* highest activity for Gram positive bacteria was found in acetone and methanol extract. Ethanol extract was less effective. High activity for Gram negative bacteria was found in methanol extract. Acetone extract was also effective for Gram negative bacteria.

In *Microsorium pteropus* the high activity for Gram positive bacteria was found in acetone extracts. Ethanol and methanol extracts were less effective for Gram positive bacteria. The highest antimicrobial activity for Gram negative bacteria was also found in acetone extract but methanol and ethanol extracts were less effective for Gram negative bacteria. In *Athyrium filix-femina* the antibacterial activity for both Gram positive and Gram negative bacteria acetone extract was suitable. Ethanol extract also shows a good activity against Gram positive bacteria.

The leaf blade and rachis of ferns are covered by glands densely. These epidermal glands (Manickam, 2002) contain substances like phenolic compounds, glycosides, flavonoids and alkaloids (Alcazar 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 Sen 1980).

The present results showed the good antimicrobial activity of four 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 (Davamani et al 2005).

The antibiotic spectra of four 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.

Table I. Antibacterial activity of the leaf extracts of *Cyclosorus interruptus*

Name of the test organism	Zone of inhibition						
	Water extracts	Ethanol extracts	Methanol extracts	Acetone e	Control (mm)		
<i>Escherichia coli</i>	12mm	17mm	17mm	20mm	A	M	E
					6.4	6.4	6.4
<i>Bacillus megaterium</i>	10mm	22mm	15mm	12mm	6.5	6.4	6.5

Table II. Antibacterial activity of the leaf extracts of *Gleichenia microphylla*

Name of the test organism	Zone of inhibition						
	Water extracts	Ethanol Extracts	Methanol extracts	Acetone e	Control (mm)		
<i>Escherichia coli</i>	10mm	13mm	17mm	16mm	A	M	E
					6.5	6.5	6.6
<i>Bacillus megaterium</i>	8mm	13mm	10mm	16 mm	6.5	6.5	6.6

Table III. Antibacterial activity of the leaf extracts of *Microsorium pteropus*

Name of the test organism	Zone of inhibition						
	Water extracts	Ethanol Extracts	Methanol extracts	Acetone e	Control (mm)		
<i>Escherichia coli</i>	8mm	15mm	14mm	16mm	A	M	E
					6.5	6.4	6.5
<i>Bacillus megaterium</i>	7.5mm	13mm	15mm	18mm	6.5	6.4	6.5

Table IV. Antibacterial activity of the leaf extracts of *Athyrium filix-femina*

Name of the test organism	Zone of inhibition						
	Water Extracts	Ethanol Extracts	Methanol extracts	Acetone e	Control (mm)		
<i>Escherichia coli</i>	7mm	15mm	12mm	18mm	A	M	E
					6.5	6.4	6.5
<i>Bacillus megaterium</i>	6mms	18mm	14mm	20mm	6.5	6.4	6.5

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