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RESEARCH PAPER

PRELIMINARY ANTIBACTERIAL AND PHYTOCHEMICAL EVALUATION OF *DRYOPTERIS COCHLEATA* (D.DON) C.CHR.

Toji Thomas*

Post Graduate and Research Department of Botany, St. Thomas College Palai under Mahatma Gandhi University,Kottayam, Kerala-India.

***For correspondence:** E mail:* **tojidr@yahoo.com**

Abstract: *ryopteris cochleata* Dryopteridaceae is one of the important plants of traditional systems of medicine. Fronds (leaves) of *Dryopteris cochleata* were evaluated for its antibacterial potential and phytochemical contents in various solvent extracts in increasing polarity towards pathogenic bacterial species. Antibacterial activity was tested by disc diffusion method. Petroleum ether, acetone, methanol and water extracts of *Dryopteris cochleata* were tested for antibacterial activity towards some pathogenic bacterial strains. Both acetone and methanol extracts exhibited antibacterial activity; maximum activity was shown by acetone extract compared to others. Antibacterial activity was confirmed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). MIC and MBC values of acetone extract of 12.5mg/ml and 25mg/ml were observed towards *Staphyococcus albus* while MIC and MBC values of 25mg/ml and 50mg/ml were observed towards *Pseudomonas aeruginosa*. Flavonoids, phenols, steroids were detected in acetone and methanol extracts. The study proves that *Dryopteris cochleata* possesses antibacterial principles, soluble in acetone, which hinder the growth and multiplication of some multidrug resistant bacterial strains.

Keywords: *Dryopteris cochleata*, yopteridaceae, Tibacterial, Dsc diffusion.

INTRODUCTION

Pteridophytes are lower group of plants with vascular anatomy. With the introduction of Ethnobotany, many attempts have been made on the study of relationships of pteridophytes with man, and more particularly for their medicinal value. *Dryopteris cochleata* (D.Don) C.Chr. belongs to the family Dryopteridaceae, a common, medium sized terrestrial herb found at higher altitudes in semi-exposed, well shaded localities in forest and grasslands. Its synonym is *Nephrodium cochleatum* D. Don. [1]. The leaves of the plant are used for the treatment of epilepsy [2, 3]. Pounded rhizomes are used in swellings and pain and have antifungal properties [4]. The whole plant is crushed in a bowl and their extract is given twice a day orally, in case of snake bite, besides a paste of the plant is also applied on the bite wound to prevent infection [5]. A small portion of the rhizome of the plant is powdered and taken with water twice a day in rheutmatism, epilepsy and leprosy [6]. Present study is an attempt to evaluate antibacterial potential of the plant in various extracts of increasing polarity and to understand the phytochemial background of the extracts. The extracts were tested towards pathogenic bacteria involved in various diseases in human beings.

MATERIALS AND METHODS

Preparation of plant extract

Fresh specimens were collected in the month of January from Vagamon Hills of Kerala State, India. A voucher specimen (TT 2077) was deposited at the herbarium of Calicut University Herbarium (CALI). The air-dried fronds (leaves) of the plant material (50g) was ground and utilised for preparing extracts. Extracts of petroleum

ether, acetone, methanol and water were made successively [7].

Microorganisms used

Test organisms were collected from the culture collection of the Institute of Microbial Technology (IMTECH) Chandigarh, these include *Staphylococcus aureus* subsp *aureus* (MTCC-96), *Escherichia coli* (MTCC-443), *Pseudomonas aeruginosa* (MTCC-741), *Serratia marcescens* (MTCC-97), *Klebsiella pneumoniae* subsp *pneumoniae* (MTCC-109), and *Micrococus luteus* (MTCC 6164). Some clinical isolates were also employed in the study, these included *Staphylococcus albus, Salmonella typhi, Salmonella paratyphi, Citrobacter freundii, Shigella sonnei* and *Shigella dysentriae.* The bacteria were subcultured on nutrient agar slants, incubated at 37^{o}C for 24 hours and stored at 4^{o}C in the refrigerator to maintain the stock culture; some of these bacteria are involved in various skin infections [8].

***In vitro* antibacterial assay**

The disc diffusion method [9] was used to determine the growth inhibition of bacteria by plant extracts. Sterile liquid Mueller Hinton Agar media (pH 7.4 ± 2) was transferred into sterile petridish and after solidification; the bacteria (1 ml broth of approximately 10^{5}CFU) were swabbed with a sterile swab under aseptic conditions. Commercially available blank sterile discs (Hi Media Laboratories Pvt. Ltd, Bombay) of 6 mm diameter were used in the study. Original solvent in which the extract prepared was used as a control. Test materials were dissolved in the respective solvent to obtain a stock solution of concentration of 100 mg/ml. 10 µL of the solution was loaded per disc to attain a concentration of 1 mg/disc. The discs (including control) were used after drying them in an incubator at 50°C to remove any trace of solvent. Discs including controls were also prepared in the same way as those with extracts. Discs were introduced onto the surface of the medium. The plates were incubated overnight at appropriate incubation temperatures. Microbial growth inhibition was determined by measuring the diameter of zone. The tests were conducted in more than three replicates and average inhibitory zone diameter was determined along with standard deviation.

Minimum inhibitory concentration (MIC)

The MIC of the extracts was done by incorporating various amounts (200 – 0.39 mg/ml) of the extract (dissolved in 10%DMSO) into sets of test tubes with the culture media. 50 µl of the bacterial broth culture was added into each of the test tubes. The bacterial cultures with the plant extracts were incubated at 37^{o}C for 24 hours. Test tube containing the growth medium and 10% DMSO plus each of the organisms was also incubated under the same conditions as positive controls. The minimum inhibitory concentration was expressed as the lowest concentration of the extracts that did not allow any visible growth when match up to that of the control tubes [10].

Minimum bactericidal concentration (MBC)

Samples from the tubes used in the MIC assays, which did not exhibit any visible growth after a period of incubation were subcultured onto a freshly prepared nutrient medium. The minimum bactericidal concentration was taken as the lowest concentration of the extract that did not give a single colony on the nutrient agar plate after 24 hours of incubation period [11].

Preliminary detection of phytochemicals

The crude samples were subjected to phytochemical screening for the detection of alkaloid, phenolics, steroids, flavonoids using the method of Harborne [12].

RESULTS AND DISCUSSION

Antibacterial activity and phytochemical assessment of *Dryopteris cochleata* were conducted using petroleum ether, acetone methanol and water as extracting solvents in the gradation of increasing polarity. *Dryopteris cochleata* showed broad range of antibacterial activity against Five bacterial species including gram-positive and gram-negative. The results are reported in the Table 1 and 2. The acetone extract of *Dryopteris cochleata* exhibited maximum activity compared to others. The plant did not show antibacterial activity towards *Serratia marcescens, Salmonella typhi, Salmonella paratyphi, Citrobacter freundii, Klebsiella pneumoniae, Shigella sonnei* and *Shigella dysentriae.* Acetone extract *showed* maximum antibacterial activity towards *Pseudomonas aeruginosa and Staphylococcus albus.* The present investigation clarified the antibacterial property of the leaves towards one of the clinically important multi-drug resistant stains, *Pseudomonas aeruginosa. Pseudomonas aeruginosa* is often encountered in nosocomial infections and its infection is common in patients receiving treatment of severe burns or other traumatic skin damage and in people suffering from cystic fibrosis. This pathogen colonises the lungs of patients and increasing mortality rate of individuals with the disease [13]. *Pseudomonas aeruginosa* exhibited sensitivity towards petroleum ether extract of *Dryopteris cochleata.* Water extracts did not show antibacterial activity towards any of the tested organisms. Methanol extract exhibited its maximum activity towards *Staphylococcus aureus* and *Pseudomonas aeruginosa*.Antibacterial activity observed at its maximum in acetone extract towards *Pseudomonas aeruginosa* and *Staphylococcus albus* and therefore acetone extract was selected for detailed antibacterial evaluation tests like MIC and MBC. MIC and MBC values of 12.5mg/ml and 25mg/ml were observed towards *Staphylococcus albus* while MIC and MBC values of 25mg/ml and 50mg/ml were observed towards *Pseudomonas aeruginosa*. The present investigation supported the antibacterial property of the rhizome towards tested pathogens involved in various diseases. The phytochemical evaluation of *Dryopteris cochleata* showed that phenolics steroids and flavonoids were present in active acetone extract. Petroleum ether extract showed the presence of flavonoids. Methanol extract showed the occurrence of flavonoids and phenolics. None of the extracts showed the occurrence of alkaloids. The presence of flavonoids, steroids and phenolics in acetone extract might be responsible for its maximum antibacterial activity. Another interesting observation is that medium polar extract (acetone) performed well towards the tested organisms. The present result supported the ethnobotanical role of the plant in controlling pathogens involved in wound infections reported by [5] in addition to its antifungal activity [4]. In view of the analysis, the leaves can be recommended as source for isolating and characterizing new antibacterial drugs for modern medicine. Further investigations are necessary to isolate and purify antibacterial principles from active acetone extract of the plant and may be later used as a potential phytomedicine instead of synthetic antibiotics especially towards multidrug resistant pathogenic bacterial species.

CONCLUSION

Antibacterial activity of leaves of *Dryopteris cochleata* was tested towards bacteria involved in various diseases in human beings. Acetone extract and methanol extracts were found to be effective against *Staphylococcus albus* and *Pseudomonas aeruginosa.* Antibacterial activity was demonstrated towards five pathogens, while seven showed resistance. Phytochemical analysis of active extracts indicated the presence of flavonoids, triterpenoids and phenols. Alkaloids were not detected in any of the extracts. Active

ethanol extract of the plant exhibited minimum bactericidal concentration (MBC) of 25 mg/ml towards *Staphylococcus albus* and 50mg/ml towards *Pseudomonas aeruginosa*. In view of the analysis, the leaves can be recommended as source for isolating and characterizing new antibacterial drugs for modern medicine as an alternative to synthetic antibiotics.

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Table 1: Antibacterial activity of *Dryopteris cochleata* leaves towards pathogenicstrains of bacteria

Extract used	pecies											
	S.al	S.au	M.lu	S.ma	S.ty	S.pt	C.fr	K.pn	S.so	S.dy	E.co	P.ae
PE	-	-	-	-	-	-	-	-	-	-	-	+
AE	++	-	+	-	-	-	-	-	-	-	+	++
ME	-	+	-	-	-	-	-	-	-	-		+
WA	-	-	-	-	-	-	-	-	-	-	-	-

Value= no obvious growth inhibition (-); zone of inhibition with an average diameter 7mm-10.99mm (+); 11mm-14.99mm (++) and zone of inhibition with diameter 15-21mm (+++) Abbreviations: PE - Petroleum ether Extract; AE - Acetone Extract; ME - Methanol Extract WE - Water Extract. S.al - *Staphylococcus albus*; S.au - *Staphylococcus aureus*; M.lu – *Micrococcus luteus*; S.ma - *Serratia marcescens*; S.ty - *Salmonella typhi*; S.pt - *Salmonella paratyphi*; C.fe - *Citrobacter freundii*; K.pn - *Klebsiella pneumoniae*; S.so - *Shigella sonnei*; S.dy - *Shigella dysentriae*; E. co - *Escherichia coli*; P.ae - *Pseudomonas aeruginosa*;

Table 2: Phytochemicals detected in various extracts of *Dryopteris cochleata*.

Extract used	Phytochemicals detected in various extracts (values: + present; - absent)			
	Flavonoids	Alkaloids	Steroids	Phenolics
Petroleum Ether	+	-	-	-
Acetone	+	-	+	+
Methanol	+	-	-	+
Water	-	-	-	+

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