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A Study on the Phytochemical and Antibacterial Properties of Three Selected Ferns

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ABSTRACT
It has been observed that, pteridophytes are not much infected by microbial pathogens, which may be due to the presence of various phytocompounds present in these group of plants. The present work deals with the Phytochemical and antibacterial analysis of Acrostichum aureum L., Pteris vittata L., Adiantum philippense L. belongs to Pteridaceae family. Phytochemical analysis revealed that the extract of the selected plants such as Acrostichum aureum L., Pteris vittata L., Adiantum philippense L. showed the presence of various phtocompounds. Antibacterial activity analysis of extracts of selected pteridophytes were done using both gram negative and gram positive bacteria such as Esherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus. A. aureum shows inhibition zone against all the four bacteria; Pteris vittata shows inhibition zone against E. coli and Staphylococcus aureus; Adiantum philippense showed inhibition zone against E. coli and Klebsiella pneumonia.

Key Words: *Acrostichum aureum L., Pteris vittata L., Adiantum philippense L. Phytochemicals, Antibacterial, Disc diffusion, Micrtitre plate*

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I. INTRODUCTION

A lot of studies are reported the medicinal properties of various plants, especially on angiosperms. But unfortunately limited amount of studies are done to explore the medicinal potentialities of pteridophytes. The pteridophytes which dominated the earth during carboniferous period are survived today by about 12,000 species comprising 305 genera. On the basis of preliminary check-list of pteridophytes (Dixit, 1984 [1]; Bir, 1992 [2]; Chandra, 2000 [3]), it has been reported that within present day political boundaries of India, about 191 pteridophyte genera comprising over 1250 species exist in India. Considering the rich diversity of Indian medicinal plants including pteridophytes, it is expected that, the screening of plant extract for antibacterial activity may be beneficial for humans and plants diseases. The synergistic interaction among crude extracts or the active compounds may be useful in the preparation of improved herbal or drug formulations. Traditionally people used pteridophytes as medicine and as antibacterial agents. In the present study an attempt has been made to evaluate the phytochemical and antibacterial properties of three selected pteridophytes namely *Acrostichum aureum* L., *Adiantum phillippense* L. and *Pteris vittata* L.

Acrostichum aureum L., known as Golden leather fern, Mangrove fern etc. occurs worldwide in mangrove swamps, salt marshes, canal margins, and low hammocks. It does not grow in areas where soil salinity exceeds 50 ppm, nor does it grow in arid coastlines (Medina *et al*., 1990 [4]). *Pteris vittata* originates from Asia and is widely naturalized throughout the tropics and subtropics. In its native habitat it is found growing in open sites on limestone, on walls, drains and in concrete. It is adapted to a variety of soils. *Pteris* can accumulate the heavy metal arsenic which attracted the attention of people interested in using plants to extract heavy metals from soils by phytoremediation or phytoextraction. *Adiantum philippense* L. syn. *Adiantum lunulatum* Burm.f. is an evergreen, perennial fern. Commonly known as walking Maidenhair fern and Black Maidenhair is found across south-east Asia. It inhabits tropical areas in Bangladesh, India, Thailand and Cambodia. *Adiantum philippense* grows in a creeping or semi erect position.

Shakoor *et al*., (2013)[5] studied the presence of different phytoconstituents in aqueous, methanolic, ethanolic and acetone extracts of 34 species of pteridophytes. Irudayaraj and Johnson (2011)[6] studied the pharmacognostical, morphological and physico-chemical characteristics of *Asplenium affine, Asplenium decrescens* and *Asplenium zenkeranum.* The studies revealed the presence of alkaloids, triterpenes, glycosides and flavonoids in *Asplenium* species. Pan *et al.*, (2011)[7] studied the phytochemical and pharmacological activities of various species of genus *Adiantum.* They revealed that the typical constituents of this genus are terpenoids and flavonoids. Among them, some exhibit strong bioactivities, especially analgesic, anti-

nociceptive, anti-implantation, and antimicrobial activities. Smitha and Vadivel (2019) [8] carried out the phytochemical screening on *Ceratopteris thalictroides.* And reported the presence of the secondary metabolites such as alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, anthroquinone, phenol, glycosides, catachin and terpenoids in extracts of *Ceratopteris thalictroides*.

Gopalakrishnan, *et al.,* (1993) [9] studied the phytochemical, antimicrobial and antioxidant activities on *Pteris cretica* L. extracts. Their analysis showed significant antioxidant activities as well as antimicrobial activities against *Bacillus subtilis, Staphylococcus aureus, Clostridium sporogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans* and *Aspergillus oryzae.* Gracelin, *et al.,* (2012) [10], Gracelin, *et al*., (2013)[11], Saleem (2016)[12] and Shakoor *et al.,* (20143[13] carried out the phytochemical and antimicrobial analyses on pteridophytes. Their study revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, and reducing sugars. The antimicrobial analysis showed significant resistance in *Citrobacter freundii* and *Escherichia coli. Staphylococcus aureus,* and *Klebsiella pneumoniae* were also sensitive to the plant extracts. The antibacterial potentials of ferns, underlines their application as a biocontrol herbal remedy for various infectious diseases in plants.

II. MATERIALS AND METHODS

The present study was conducted to analyze the phytochemical and antimicrobial characteristics of the three ferns namely *Acrostichum aureum* L., *Pteris vittata* L., and *Adiantum philippense* L. belonging to the family Pteridaceae. The plants were collected from various places. *Acrostichum aureum* L. from Mulavukad (Ernakulam), *Pteris vittata* L. from Karumalloor village (Ernakulam) and *Adiantum philippense* L. from Madathumpady village (Thrissur). The fronds of the plants were washed under running tap water, rinsed with distilled water, air dried for 1 hour and shade dried. It was ground into powder using a blender and stored in room temperature. The ethanolic and aqueous extracts of the samples prepared were used for phytochemical and antibacterial analyses. The phytochemical estimations were carried out according to the method of Daniel (1991) [14] and Evans (2000) [15].

2.1 Estimation of Alkaloids

The total alkaloid contents in the samples were measured using 1, 10-phenanthroline method described by Singh *et al.* (2004) [14] with slight modifications. 100mg drug powder was extracted in 10ml 80% ethanol. This was filtered through muslin cloth and centrifuged at 5000rpm for 10 min. Supernatant obtained was used for the further estimation of total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M $FeCl_3$ in 0.5M HCl and 1ml of 0.05M of 1, 10- phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2 0 C. The absorbance of red coloured complex was measured at 510nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of colchicines (0.1mg/mL, 10mg dissolved in 10ml ethanol and diluted to 100mL with distilled water).

2.2 Estimation of Flavonoids

Total flavonoid content was measured by taking 1ml aliquot of extract and added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask 0.3ml 5% Sodium nitrite was added. After 5 minutes 0.3 ml 10% Aluminium Chloride was added. After 6 minutes, 2ml 1M Sodium hydroxide was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. The O.D value obtained was plotted in the standard graph to obtain the concentration of flavonoid in the sample.

2.3 Estimation of Tannin

Pipetted out 0.1, 0.2, 0.3, 0.4, and 0.5 ml of the working standard solution into a series of test tubes. 0.1ml of the sample was added to the test tube. To all the test tubes, distilled water was added to make up to 7ml. 1ml of Potassium Ferricyanide and 1ml of $FeCl_3$ was added and mixed well. The absorbance was measured spectrophotometrically at 700nm.

2.4. Estimation of Phenol

0.1, 0.2, 0.3, 0.4, and 0.5 ml of the working standard solution was pipetted out into a series of test tubes. 0.125ml of the unknown sample was added to the test tube. To all the test tubes, including blank, distilled water was added to make up to 3.5ml. 0.125ml of Folin's reagent was added to all the test tubes. The test tubes were then incubated at room temperature for 6 minutes. 1.25ml of 7% Sodium Carbonate was added in all the test tubes. The test tubes were again incubated at room temperature for 90 minutes. The absorbance was measured spectrophotometrically at 760nm.

2.5. AntiBacterial Analysis

For the antibacterial analysis, the ethanol extracts of the *Acrostichum aureum* L., *Adiantum philippens* L. *and Pteris vittata* L. were used as the test solution. The antibacterial study was carried out against the bacteria such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa.*

2.5.1. Disc Diffusion Method

The disc diffusion method was used to determine the growth inhibition of bacteria by plant extracts. Nutrient agar was prepared and 25 ml of each was poured into sterile petridishes. Then the bacterial inoculum (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*) were uniformly swabbed in each plate using a sterile cotton swab. Sterile discs prepared using Whatman No.1 Filter Paper, of 5-mm diameter were used in the study. The original solvent in which the extract prepared was used as a control. Test samples were added to the disc and placed over the agar plates. The plates were incubated at 35-37^{o}C for 18-48 hours, a period sufficient for the growth. After incubation, the diameter of inhibitory zones formed around each disc were measured in cm and recorded.

2.5.2. Microtiter Plate Method

Microtiter plates were prepared under aseptic conditions. A sterile 96 well plate was used for the study. A volume of 10µl, 20µl, 30µl, 40µl, 50µl of test material was pipetted into the wells. 100µl of nutrient broth was added to each well. Finally, 100 µl of microbial suspension (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus*) was added to each well. Control dilutions of test material were also kept. Plate was wrapped loosely with cling film to ensure that organism did not become dehydrated. The plates were incubated at 37^{0}C for 24 hours and OD reading was taken at 600nm wavelength after sufficient incubation.

III. RESULTS AND DISCUSSION

3.1. Phytochemical Analysis

Alkaloid content was recorded very high in the ethanolic extract of plant samples. In *Acrostichum aureum* L. it was reported 160µg/ml; in *Pteris vittata* L. 150µg/ml; and *Adiantum philippense* L. 130µg/ml. Flavonoid content was recorded to be very high in *Pteris vittata* L. of 105µg/ml while in *Acrostichum aureum* L. and *Adiantum philippense* L.81.5µg/ml and 82.6µg/ml respectively. Tannin contents of the ethanolic extracts were estimated as 12.1µg/ml for *Acrostichum aureum* L.; 44µg/ml for *Pteris vittata* L. and 35µg/ml for *Adiantum philippense* L. Phenol content was recorded to be 64µg/ml in *Acrostichum aureum* L., 32µg/ml in *Pteris vittata* L., and 37µg/ml in *Adiantum philippense* L. (Table 1).

Table 1: Phytochemical analysis of plant extracts

Sl. No	Phytocompoud	Name of the plant		
		A. Aureum	*P. vittata*	*A. philippense*
1	Alkaloids	160 µg/ml	150 µg/ml	130 µg/ml
2	Flavonoids	81.5 µg/ml	105 µg/ml	82.6 µg/ml
3	Tannins	12.1 µg/ml	44 µg/ml	35 µg/ml
4	Phenols	64 µg/ml	32 µg/ml	37 µg/ml

3.2. Antibacterial Analysis

Antibacterial activity was tested by using disc diffusion method and microtiter plate method. Antibacterial activity was done against four bacteria: - *E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

3.2.1. Disc Diffusion Method

For checking the antibacterial activity of the ethanolic plant extract, the discs are dipped in ethanol, it used as negative control and antibiotic Tetracyclin was considered as the positive control. *Acrostichum aureum* L. shows antibacterial activity against all the above mentioned bacteria. *Adiantum philippense* L. shows antibacterial activity against *Klebsiella pneumoniae* and *E. coli*. *Pteris vittata* L. shows antibacterial activity against *Staphylococcus aureus* and *E. coli* (Table 2).

Table 2. Antibacterial Analysis by Disc Diffusion Method				
Plant	Zone of inhibition (cm)			
	Escherichia coli	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
A.aureum	0.5	0.6	1.5	0.8
P. vittata	1.0	0	0	0.6
A.phillippense	0.6	0	0.9	0
Tetracycline	2.5	2.0	1.4	2.5

3.2.2. 2icrotiter Plate Method

Microtiter plate method was done to calculate the percentage of inhibition against the four selected bacteria in 5 different concentrations (10µl, 20µl, 30µl ,40µl and 50µl) of ethanolic extracts of *Acrostichum aureum* L., *Adiantum phillippense* L. and *Pteris vittata* L. (Table 3-6).

Table 3: MTP analysis of Antibacterial Activity using *Escherichia coli*						
Conc. Extract (µl)	*Acrostichum aureum* L.		*Adiantum philippense* L.		*Pteris vittata* L.	
	Optical Density	Percentage of inhibition (%)	Optical Density	Percentage of inhibition (%)	Optical Density	Percentage of inhibition (%)
Control	0.644	0	0.644	0	0.644	0
10	0.60	7.0	0.63	2.0	0.50	22.0
20	0.57	11.0	0.612	5.0	0.44	32.0
30	0.55	14.5	0.51	13.0	0.39	39.0
40	0.53	18.0	0.49	24.0	0.30	53.0
50	0.5	22.0	0.47	27.0	0.27	58.0

Table 4: MTP analysis of Antibacterial Activity *Klebsiella pneumonia*						
Conc. of Extract (µl)	*Acrostichum aureum* L.		*Adiantum philippense* L.		*Pteris vittata* L.	
	Optical Density	Percentage of inhibition (%)	Optical Density	Percentage of inhibition (%)	Optical Density	Percentage of inhibition (%)
Control	0.703	0	0.703	0	0.703	0
10	0.64	9.0	0.65	7.5	0.64	9.0
20	0.5	29.0	0.6	14.0	0.62	12.0
30	0.41	41.0	0.57	19.0	0.59	16.0
40	0.3	57.0	0.5	28.0	0.54	23.0
50	0.2	71.0	0.47	33.0	0.5	28.0

Table 5: MTP analysis of Antibacterial Activity
Pseudomonas aeruginosa

Conc. of Extract (µl)	*Acrostichum aureum* L.		*Adiantum philippense* L.		*Pteris vittata* L.	
	Optical Density	Percentage of inhibition (%)	Optical Density	Percentage of inhibition (%)	Optical Density	Percentage of inhibition (%)
Control	1.077	0	1.077	0	1.077	0
10	1.04	3.0	1.03	3.0	1.01	6.0
20	1.00	7.0	1.01	6.0	0.98	9.0
30	0.97	10.0	0.98	9.0	0.94	12.0
40	0.84	22.0	0.93	13.0	0.87	19.0
50	0.78	27.5	0.89	17.0	0.84	22.0

Table 6: MTP analysis of Antibacterial Activity
Staphylococcus aureus

Conc. of Extract (µl)	*Acrostichum aureum* L.		*Adiantum philippense* L.		*Pteris vittata* L.	
	Optical Density	Percentage of inhibition (%)	Optical Density	Percentage of inhibition (%)	Optical Density	Percentage of inhibition (%)
Control	0.696	0	0.696	0	0.696	0
10	0.6	14.0	0.65	6.0	0.623	10.0
20	0.55	21.0	0.6	13.0	0.60	14.0
30	0.45	35.0	0.58	16.0	0.58	17.0
40	0.38	45.0	0.54	22.0	0.54	22.0
50	0.28	59.0	0.50	28.0	0.49	29.5

IV. SUMMARY AND CONCLUSION

Nature is a unique source of phytochemical with high diversity and many of them possessing interesting biological activities with significant medicinal properties. Though, lot of studies focusing on the medicinal properties of angiosperm plants are reported, limited amount of studies have been done to explore the medicinal potentialities of the pteridophytes. The pteridophytes constitute a significant part of the plant diversity of nature. They form a dominant component of many plant communities. The ferns used for the present study includes *Acrostichum aureum* L., *Pteris vittata* L., and *Adiantum philippense* L. which belongs to Pteridaceae family. Phytochemical studies showed the presence of secondary metabolites in *Acrostichum aureum, Adiantum philippense* and *Pteris vittata*. The antibacterial study was carried out against the bacteria *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus. Acrostichum aureum* shows antibacterial activity against all the selected bacteria. *Pteris vittata* shows antibacterial activity against *Escherichia coli* and *Staphylococcus aureus. Adiantum philippense* shows antibacterial activity against *Escherichia coli* and *Klebsiella pneumoniae.*

The present study reveals the phytochemical and antibacterial potentials of the selected Pteridacean members as a potential antimicrobial agent. Further investigations are necessary to explore the complete potential of the selected plants in the field of medicine and industry.

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