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Research Article

ANTIMICROBIAL ACTIVITIES AND PHYTOCHEMICAL INVESTIGATION OF SOME NATIVE PTERIDOPHYTES

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ABSTRACT

The present investigation was carried out to screen the phytochemical and antimicrobial properties of three pteridophytes plants such as *Salvinia minima* Baker, *Thelypteris interrupta* (Wild.) K. Iwats and *Marsilea minuta* L. commonly found in Odisha, India. The dried and pulverized plant materials (whole) were extracted using two different solvent such as methanol and chloroform. The antimicrobial activity was demonstrated against eighteen bacterial pathogens such as *Staphylococcus aureus, S.citreus, Escherichia coli, Pseudomonas* sp., *Pseudomonas aeruginosa, Salmonella typhii, S. paratyphii* A, *S. paratyphii* B, *Chromobacter, Enterobacter, Citrobacter fruendii, Klebsiella, Vibrio cholera, Shigella sonnie, S. boydii, Providencia, Proteus mirabilis, P. vulgaris* and four fungal pathogens such as *Candida albicans, Aspergillus niger, A. flavus*, *Rhizopus* sp. Methanol extracts showed the presence of major phytoconstituents such as alkaloids, tannins, anthroquinone, steroids and terpenoids as compared to chloroform. Among the three pteridophytes, *T. interrupta* showed the presences of maximum phytoconstituents. The highest antibacterial activity (28mm) was observed in the methanol extract of *T. interrupta* against *S.citreus* whereas maximum antifungal activity (22mm) was observed against *A. flavus* in the methanol extract of *S.minima.*

Keywords: Antimicrobial activity, *Marsilea minuta* L., phytoconstituents, *Salvnia minima* Baker, *Thelypteris interrupta* (Wild.) K. Iwats

INTRODUCTION

Ferns and their allies are in a major division of the Plant Kingdom called *Pteridophyta* and they have been around for millions of years. There are over 250 different genera and 12,000 species of ferns reported all over the world [1]. It has been observed that pteridophytes are not infected by microbial pathogens which may be one of the important factors for the evolutionary success of pteridophytes and the fact that they survived for more than 350 million years [2]. As per folk medicine, the pteridophytes have been known for more than 2000 years and also been mentioned in ancient literature [3-8]. The Ayurvedic systems of medicine recommended the medicinal use of the pteridophytes. These are also used in the Unani system of medicine [9]. It showed bioactivity properties such as antimicrobial, anti-inflammatory, anti tussive, antitumor, etc. But a very little research has been carried out on the evaluation of bioactive properties of pteridophytes. Hence an attempt has been made to evaluate the phytochemical and antimicrobial properties of few native pteridophytes of Odisha such as *Salvnia minima* Baker, *Thelypteris interrupta* (Wild.) K.Iwats and *Marsilea minima* L.

MATERIALS AND METHODS

Collection of plant sample

Healthy and disease free plants of *Salvnia minima* Baker, *Thelypteris interrupta* (Wild.) K.Iwats and *Marsilea minima* L. were collected from Kanjia Lake, Nandankanan, Bhubaneswar and its adjoining areas in the month of May and identified through consulting the herbarium at CSIR-Institute of Minerals and Material Technology (RRL-B), Bhubaneswar following "The flora of Orissa", Volume III [10].

Table1: Pteridophytes selected for phytochemical and antimicrobial activity

Botanical name	Family	Vernacular name	Parts used
Salvinia minima Baker	Salviniaceae	Water spangles	Whole plant
Thelypteris interrupta (Wild.) K. Iwats	Thelypteridaceae	Willdenow's maiden or Hottentot fern	Whole plant
Marsilea minuta L.	Marsileaceae	Sushni saag	Whole plant

Extraction of plant extract

The collected samples were washed with distilled water properly for removing adhered soil and other particles and shade dried for about 7 to 10 days. The dried plant samples material was then pulverized into fine powder and stored. The dried and powered leaf materials (25 gm) were extracted successively with 250 ml of chloroform and methanol by using a Soxhlet extractor at low temperature. The extracts were concentrated and dried in a rotary evaporator and then placed in a desiccator and was allowed to dry completely. Once completely dry, the extracts were stored at 4^0 C for further tests.

Preliminary Phytochemical analysis

A qualitative phytochemical test to detect the presence of phytochemicals such as alkaloid, tannin, saponin, flavonoid, glycoside and phenol were carried out using standard procedures [11].

Antimicrobial Screening

Test pathogens

Various bacterial pathogenic strains such as *Staphylococcus aureus, S.citreus, Escherichia coli, Pseudomonas sps., Pseudomonas aeruginosa, Salmonella typhii, S. paratyphii* A , *S. paratyphii* B, *Chromobacter, Enterobacter, Citrobacter fruendii, Klebsiella, Vibrio cholera, Shigella sonnie, S. boydii, Providencia, Proteus mirabilis, P. vulgaris* and fungal pathogens such as *Candida albicans, Aspergillus niger, A. flavus* and *Rhizopus* species were collected from Kalinga Institute of Medical Science (KIMS), Bhubaneswar and maintained in Nutrient agar slant.

Antimicrobial assay

Preliminary screening of the extracts was carried out by disc diffusion method [12]. Freshly grown liquid culture of the test pathogens was seeded over the nutrient agar plates with a sterile swab. Sterile filter paper discs were soaked with different

concentration of extracts of individual solvents and were placed on the plates at equidistance. Then the plates were incubated at 37°C for 18-24 hrs. Clearance zone formed around the discs indicates a positive antimicrobial activity and were measured. Each experiment was carried out in triplicates. The mean ± SD of the inhibition zone was taken for evaluating the antibacterial activity of the extracts.

RESULTS

The preliminary phytochemical screenings of *Salvnia minima* Baker, *Thelypteris interrupta* (Wild.) K.Iwats and *Marsilea minuta* L. revealed the presence of various secondary bioactive compounds as shown in Table 2.

The crude extracts of *Salvinia minima* Baker showed diverse phyto-profiles with reference to the solvents of the other plant extracts. The methanol extracts of *S. minima* demonstrated maximum occurrence of phytoconstituents (6/11), followed chloroform (2/11) (Table 2). Anthroquinone and protein was present in both the tested extracts of *Salvinia minima* Baker. The crude extracts of *Thelypteris interrupta* illustrated the presence various metabolites with reference to the solvents of the plant extracts. The methanol extracts of *T.interrupta* demonstrated maximum occurrence of phytoconstituents (8/11), followed by chloroform (4/11) (Table 2). Presence of phytochemicals such as steroids and terpenoids, saponins, anthroquinone and protein was found in both the tested extracts of the plant. The crude extracts of *Marsilea minuta* demonstrated different metabolites presence with reference to the solvents of the plant. Similar to *Salvinia minima* and *Thelypteris interrupta*, the methanol extracts of *Marsilea minuta* also showed the presence of maximum bioactive compounds as compared to chloroform.

Table2: Phytoprofiles of crude methanol and chloroform extracts of pteridophytes

PHYTOCHEMICAL	METHANOL			CHLOROFORM		
	S. minima	*T. interrupta*	*M. Minuta*	*S. minima*	*T. interrupta*	*M. minuta*
Alkaloid	+	+	+	-	-	-
Saponins	-	+	-	-	+	-
Glycosides	-	+	+	-	-	-
Steroids & Terpenoids	+	+	+	-	+	-
Carbohydrate	+	+	+	-	-	-
Flavonoids	-	-	-	-	-	-
Tanins	+	+	+	-	-	-
Phenols	-	+	-	-	-	-
Phylobatanins	-	-	-	-	-	-
Anthroquinone	+	+	+	+	+	+
Protein	+	+	+	+	+	+
Total	6	8	7	2	4	2

The present study also focused on the antimicrobial activity of *Salvinia minima*, *Thelypteris interrupta* and *Marsilea minuta* extracts against 18 Gram-positive and Gram-negative bacteria and 4 fungal strains using the disc diffusion method as shown in table 3 and Figure 1-2.

In case of *S. minima*, the methanol extracts showed zone of inhibition against many bacterial pathogens as compared to chloroform extracts. The highest zone of inhibition (20mm) was against *Proteus mirabilis* in chloroform extracts. The antifungal activity was maximum in the methanol extract against *Aspergillus flavus* (22mm) as shown in table 3.

Tabale3: Antimicrobial activity in methanol and chloroform extract of *S. minima*

Organisms	Methanol	Chloroform
S. aureus	16	-
S. citreus	16	-
E. coli	-	-
Pseudomonas sp.	-	-
P. aeruginosa	14	-
S. typhii	-	10
S. paratyphii A	-	-
S. paratyphii B	-	-
Chromobacter sp.	15	-
Enterobacter sp.	-	-
C. fruendii	-	-
V. cholera	-	-
Klebsiella sp.	-	-
S. sonnei	-	-
S. boydii	-	-
Providencia sp.	-	-
P. mirabilis	-	20
P. vulgaris	-	-
C. albicans	12	12
A. niger	21	-
A. flavus	22	-
Rhizopus sp.	-	-

The chloroform extract of *Thelypteris interrupta*, inhibits growth of maximum bacterial pathogens as compared to methanol extract. The highest zone of inhibition (28mm) was against *Staphylococcus citreus* in methanol extracts. Expect *Candida albicans,* all other fungal species were resistant to both the extracts.

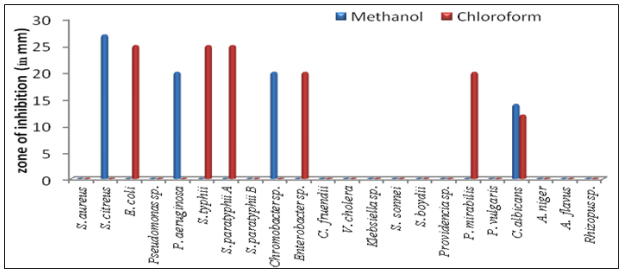

Figure 1: Antimicrobial Activity of *Thelypteris interrupta* (Wild.) K.Iwats

Unlike *T. interrupta*, *Marsilea minuta* also showed the similar results i.e chloroform extracts inhibit growth of maximum pathogens as compared to methanol extract. The highest zone of inhibition (25mm) was against *Staphylococcus citreus* in methanol extracts. The methanol extract of *M. minuta* inhibits the growth of *Candida albicans* and *Aspergillus flavus* whereas in case of chloroform extract it only inhibits the growth of *Candida albicans*.

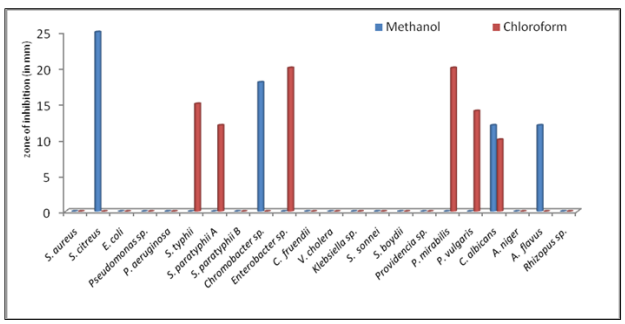

Figure 2: Antimicrobial Activity of *Marsilea minuta*

The MIC values for *S. minima* were 7mg/ml and 6mg/ml against *S.aureus* and *A.flavus* respectively. The MIC values were 5mg/ml and 6mg/ml against *Staphylococcus citreus* and *C. albicans* respectively in case of *T. interrupta* whereas 8mg/ml and 5mg/ml against *Staphylococcus citreus* and *C. albicans* respectively in case of *Marsilea minuta.*

DISCUSSION

The present study has screened the phytochemical properties of three pteridophytes such as *S. minima, T. interrupta, M. Mint* with two different solvent extracts (methanol and chloroform). From the results it can be concluded that the methanol extracts of all the three plants showed the presence of maximum phytoconstituents as compared to chloroform, which may be due the fact that methanol was comparatively more polar in nature. Among the three ferns, *T. interrupta* showed the presence of maximum phytoconstituents which might be due to the physiological property of individual taxa. Thus the preliminary phytochemical screening may be useful and lead to the detection of bioactive principles and drug discovery.

It has been observed that the antimicrobial activity was fairly well distributed among pteridophytes. The selected fern species examined exhibited antimicrobial properties against various test pathogens used. Our observations are in good agreement with the findings of other Sen & Nandi, Mickell and Maruzzella [13-15]. These are, however, in sharp contradiction to the findings of Dhar et al., Bhakuni et al. and Dhawan et al., who did not find any antimicrobial activity in the 42 species of pteridophytes examined by them. *T. interrupta* inhibits the growth of maximum pathogens which might be due to the presence of maximum phytoconstituents [16-18]. Hence further research is recommended for the isolation of active compounds as well as screening of various bioactive properties such as antioxidant, antitumor, antibiotics, etc. of the potent taxa.

CONCLUSIONS

Antimicrobial activity of plant extract (methanol and chloroform solvent) of three different pteridophytes such as *Salvinia minima*, *Thelypteris interrupta* and *Marsilea minuta* commonly found in Odisha, India was observed against eighteen bacterial pathogens and four fungal. Our study concluded that among the three pteridophytes, *T. interrupta* inhibits the growth of maximum pathogens which might be due to the presence of maximum phytoconstituents. Phytochemical analysis was also done which shows the phytochemical constitutes such as anthroquinone, tannins, steroids and terpenoids are present in plants and which may possesses pharmaceutical and medicinal value As these ptreidophytes are abundantly present as weed and also having antimicrobial importance hence further extensive studies should be carried out for new drug production.

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