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Antifungal Activity of the Crude Extracts and Extracted Phenols from Gametophytes and Sporophytes of Two Species of *Adiantum*

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ABSTRACT: The water extracts and extracted phenols from gametophytes and different parts of sporophytes of the two ferns, *Adiantum capillus-veneris* L. and *Adiantum lunulatum* Burm. f., used as folkloric medicines in India, China, Tibet, America, Philippines and Italy, were investigated for their antifungal activity against *Aspergillus niger* and *Rhizopus stolonifer*. Both crude extracts and extracted phenols of gametophytes and different parts of sporophytes of both fern species were found to be bioactive against the fungal strains. Antifungal activity was found to be higher in gametophytes than different parts of sporophytes. Among the different parts of sporophyte, immature pinnule possesses highest fungistatic property. *Adiantum capillus-veneris* was found a better antifungal agent than *Adiantum lunulatum*.

KEY WORDS: ***Adiantum capillus-veneris*, *Adiantum lunulatum*, *Aspergillus niger*, folkloric, fungistatic, *Rhizopus stolonifer*.**

INTRODUCTION

Maiden hair fern, *Adiantum capillus-veneris* L. and walking maiden hair fern, *Adiantum lunulatum* Burm. f. of the family Adiantaceae generally occur in the mountaineous regions throughout India; in plains they grow on rocks, inhabiting in shady places near swamps and on slopes of lower hills (Chandra, 2000). In traditional herbal medicinal system, *A. capillus-veneris* is used as expectorant, diuretic, febrifuge, as hair tonic, in chest diseases, in catarrhal infections, to treat hard tumours in spleen and it is anticancerous (Puri and Arora, 1961; Singh *et al*., 1989; Jain *et al*., 1992; Kumar *et al*., 2003). *A. lunulatum* is used as antidysenteric agent, effective against blood diseases, ulcers and erysipelas, in epileptic fits, in treatment of leprosy and other skin diseases, to cure bronchites and asthma, and as antidote in snake bites (Anand and Srivastava, 1994; Chatterjee and Pakrashi, 1994; Kaushik and Dhiman, 1995).

Antifungal and antibacterial activities of plant phenolics are well established (Taiz and Zeiger, 1998; Harbourne, 1994). However, antifungal activities of the crude extract and extracted phenols of *A. capillus-veneris* and *A. lunulatum* have not yet been studied. Antibacterial activity of the crude extracts of the sporophytic plant body (Guha *et al*., 2004; Parihar and Bohra, 2004) as well as the gametophyte of *A. capillus-veneris* is very recently reported (Guha *et al*., 2004). The purpose of this study was mainly to evaluate the bioactivity of different sporophytic plant parts and gametophytes of the two species of *Adiantum*, against the two fungal strains namely, *Aspergillus niger* and *Rhizopus stolonifer*.

MATERIALS AND METHODS

Different parts of the sporophytic plant body of *Adiantum capillus-veneris* and *Adiantum lunulatum* were collected from the experimental garden of Botany Department of Burdwan University and two sets of experiments were conducted. In one set, 100 mg fresh weight of each

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of rachis (RA), rhizome (RH), immature pinnule, i.e. just formed pinnule (IP), immature sporophyll, i.e. sporophylls with green sori (IS), mature pinnule, i.e. sporophylls from which spores were shedded off (MP) and mature sporophyll, i.e. sporophylls with brown sori (MS) from each species were taken and crude extracts were prepared according to the method of Guha *et al*. (2004). 100 mg of each plant parts was crushed with mortor and pestle and extracted in 80% concentration of boiled ethanol. The ethanolic mixture was centrifuged at 4000 rpm. for 10 min. The supernatant was taken and its total volume was made to 5 mL with ethanol (80% concentrated). 4 mL of distilled water was added to this alcoholic extract and kept at 40^{o}C to evaporate the alcohol. Thus the crude extract comes in water solution with a concentration of 2.5% volume / volume. In the second experimental set, total phenols from each 100 mg fresh tissue of different plant parts of the two species were extracted and estimated as per the methods of Bray and Thorp (1954). Since in plants, carbohydrates, amino acids and phenols, all come out from the tissue when extracted in 80% concentration of boiled ethanol, so it is assumed that the crude extract might contain these metabolites. As phenol content was estimated from 100 mg tissue and the crude extract was also made from 100 mg tissue, the amount of phenol content estimated was same in crude extract. Total amino acid content (Moore and Stein, 1948) and total carbohydrate content (Mc. Cready *et al.*, 1950) were estimated from each of the plant parts (Table 1).

Gametophytes were raised and cultured in Moore's medium (Koch, 1973) at 24^{o}C ± 1^{o}C in 70% - 80% relative humidity and 16 h / 8 h light and darkness photoperiod with an intensity of 2000 lux in NK System Biotron growth chamber. Crude gametophytic extract and other biochemical analyses were made in the same way as mentioned above for sporophytes.

Antifungal activity was measured by taking *Aspergillus niger* and *Rhizopus stolonifer* as test materials. Antifungal assay was carried out in three distinct techniques.

Agar disc method: This was done following the methods of Alam (2004), where 20 mL of potato - dextrose - agar medium was poured in sterilized petri dishes along with 1 mL of plant extract and plated. 5 mm diameter cups were removed from the centre in which the same diameter mycelial discs were inoculated. Slight modification was made in this experiment. Here, at the time of mycelium disc inoculation, in order to avoid spores from falling haphazardly in the medium, sterilized filter papers with centres cut into circular patterns were taken. The diameters of the circular cuts were slightly greater than the cups made by the cork borer. After opening the petri dish, just before the mycelial disc inoculation, the filter paper was asceptically placed over the petri dish containing the medium. The filter paper was then removed and the petri dish was closed after the mycelial disc inoculation was complete. The petri dishes were incubated at 28^{o}C ± 1^{o}C for 14 days in dark. Colony growth was measured on the basis of linear dimensions as well as by mycelial dry weights from the methods of Medda (1991). For determining the dry matter weight, the mycelia were transferred to copper nets, which were then immersed in boiling distilled water to dissolve and remove the agar leaving only the killed mycelia in the net. The mycelia was oven - dried at 100^{o}C and dry weight was then determined. (Fig. 1 & Table 2 & 3)

Liquid culture method: This was done following the methods of Mukhopadhyay and Nandi (1997). Here single mycelial discs of each test strain were inoculated in 250 mL Erlenmeyer flask containing 100 mL of liquid medium (potato - dextrose) and 4 ml plant extract. The flasks were incubated at 28^{o}C ± 1^{o}C for 8 days. The mycelial biomass was measured by filtration through pre-weighed G-4 sintered glass filters. The filtrate was oven - dried and re-weighed. (Fig. 2 & Table 4)

Table 1. Total phenols, amino acids and soluble and insoluble carbohydrate content in the different parts of sporophytic plant body and gametophytes of *Adiantum capillus-veneris* and *Adiantum lunulatum*.

Sporophytic plant part / Gametophyte		*A. capillus-veneris*	*A. lunulatum*
		Phenol (µg/gm dry wt.) (mean ± SD)	
Sporophytic plant parts	IP	367.27 ± 21.53	333.87 ± 17.18
	IS	305.07 ± 15.81	270.80 ± 23.35
	MP	240.60 ± 19.76	216.63 ± 17.54
	MS	210.20 ± 13.73	186.20 ± 11.32
	RH	159.43 ± 18.89	130.93 ± 7.65
	RA	130.10 ± 11.63	104.90 ± 19.86
Gametophyte		434.81 ± 28.76	397.63 ± 29.35
F-value		26.34	
LSD at 5%		21.93	
		Amino acids (mg/gm dry wt.) (mean ± SD)	
Sporophytic plant parts	IP	0.994 ± 0.036	0.968 ± 0.058
	IS	0.934 ± 0.042	0.904 ± 0.043
	MP	0.879 ± 0.014	0.848 ± 0.025
	MS	0.811 ± 0.056	0.781 ± 0.089
	RH	0.748 ± 0.028	0.716 ± 0.077
	RA	0.644 ± 0.017	0.611 ± 0.028
Gametophyte		1.221 ± 0.099	1.060 ± 0.087
F-value		2.12	
LSD at 5%		0.032	
		Soluble carbohydrate (mg/gm dry wt.) (mean ± SD)	
Sporophytic plant parts	IP	1.326 ± 0.094	1.174 ± 0.160
	IS	1.168 ± 0.078	1.021 ± 0.091
	MP	1.0 ± 0.091	0.77 ± 0.037
	MS	0.804 ± 0.088	0.665 ± 0.053
Gametophyte		1.698 ± 0.190	1.403 ± 0.110
F-value		2.51	
LSD at 5%		0.076	
		Insoluble carbohydrate (mg/gm dry wt.) (mean ± SD)	
Sporophytic plant parts	IP	1.264 ± 0.140	1.136 ± 0.087
	IS	1.094 ± 0.086	0.972 ± 0.095
	MP	0.928 ± 0.075	0.669 ± 0.028
	MS	0.777 ± 0.069	0.526 ± 0.014
Gametophyte		1.534 ± 0.210	1.316 ± 0.017
F-value		3.13	
LSD at 5%		0.098	

The F-value is significantly different at 0.05%

Suspension culture medium: This was done following the methods of Calam (1986). This method was same as liquid culture method, only here the flasks were incubated in a rotary shaker at 28^{o}C ± 1^{o}C and shaking at 150 rpm Dry weight was taken like that of liquid culture method. (Table 5)

For each experiment, control sets were maintained. Each of the experimental analysis was undertaken with replicates of ten along with the control sets and the values were considered with standard deviations (SD). The results were statistically analysed for LSD following two-factor analysis of variance method using Fisher's LSD procedure at P = 0.05 (Zar, 1974). Multiple comparisons among the means for the identification of the homogenous groups wherein the effect of a factor was significant ($p < 0.05$) were made using Turkey test ($p < 0.05$).

Table 2. Effect of crude extract and extracted phenols from the gametophytes and sporophytic plant parts of *Adiantum capillus-veneris* and *Adiantum lunulatum* on the area of the growth zones of *Aspergillus niger* and *Rhizopus stolonifer* in agardisc method.

Plant material		Area of mycelial growth in cm^2 (mean ± SD)			
		Aspergillus niger		*Rhizopus stolonifer*	
		Crude extract	Extracted phenol	Crude extract	Extracted phenol
A. capillus-veneris					
Control		63.64 ± 5.32	63.64 ± 5.32	56.26 ± 6.79	56.26 ± 6.79
Sporophytic plant parts	RA	51.55 ± 4.45	47.80 ± 7.56	46.59 ± 5.62	37.41 ± 7.32
	RH	46.59 ± 2.89	38.63 ± 4.67	39.61 ± 4.87	29.24 ± 5.35
	MS	38.50 ± 5.64	31.19 ± 5.55	33.20 ± 4.23	24.64 ± 4.82
	MP	34.23 ± 3.67	25.53 ± 3.67	27.35 ± 3.65	18.87 ± 3.56
	IS	30.20 ± 4.67	20.44 ± 4.94	20.84 ± 4.91	13.21 ± 2.13
	IP	22.91 ± 1.76	14.55 ± 2.45	18.10 ± 2.47	10.18 ± 1.55
Gametophyte		18.10 ± 3.42	9.08 ± 1.78	12.57 ± 1.25	6.60 ± 0.86
F-value		30.57		21.32	
LSD at 5%		4.31		2.72	
A. lunulatum					
Control		63.64 ± 5.32	63.64 ± 5.32	56.26 ± 6.79	56.26 ± 6.79
Sporophytic plant parts	RA	56.77 ± 6.38	51.55 ± 7.32	49.04 ± 7.82	40.62 ± 4.43
	RH	50.29 ± 4.63	43.03 ± 6.25	35.27 ± 5.54	33.24 ± 4.12
	MS	40.73 ± 5.82	34.23 ± 4.95	29.84 ± 5.76	27.54 ± 3.39
	MP	34.23 ± 4.87	27.35 ± 3.63	24.54 ± 4.64	21.82 ± 3.25
	IS	29.24 ± 3.62	21.43 ± 3.17	22.07 ± 3.88	15.91 ± 2.18
	IP	24.64 ± 2.18	15.21 ± 1.96	19.64 ± 2.73	11.95 ± 1.93
Gametophyte		20.44 ± 2.01	11.35 ± 1.35	14.53 ± 2.12	7.56 ± 0.87
F-value		41.73		25.72	
LSD at 5%		2.19		3.57	

The F-value is significantly different at 0.05%

RESULTS AND DISCUSSION

Phenols as antimicrobial agents are well established (Harbourne, 1994). In between the two plant materials in the present study, total phenol content of *A. capillus-veneris* was found to be more than that of *A. lunulatum* (Table 1). Irrespective of the two species, among the different parts of sporophytic plant body, total phenol content follow the order: immature pinnule (IP) > immature sporophyll (IS) > mature pinnule (MP) > mature sporophyll (MS) > rhizome (RH) > rachis (RA). In between the sporophyte and gametophyte, phenol content was more in the gametophyte of both species. Quantitative variation in phenol content in plant parts and between the two species has not yet been reported earlier.

Crude extracts from the gametophyte and different parts of the sporophyte of both species showed antifungal effect. The weight / volume ratio of the plant material taken for crude extracts and extracted phenols were same (2.5 volume / volume). Because the crude extract was made with 80% ethanol, the total phenols of the plant material were pooled in that extract. Like phenols, carbohydrates and amino acids are also present in the 80% ethanolic extract. While analyzing the amino acid and carbohydrate content, it was found that both metabolites were more in the gametophyte than in the sporophyte (Table 1). Moreover, the amount of amino acid and carbohydrate in different sporophytic parts follow the same order as that of the phenols. The content of soluble and insoluble carbohydrate and also of free amino acid were comparable

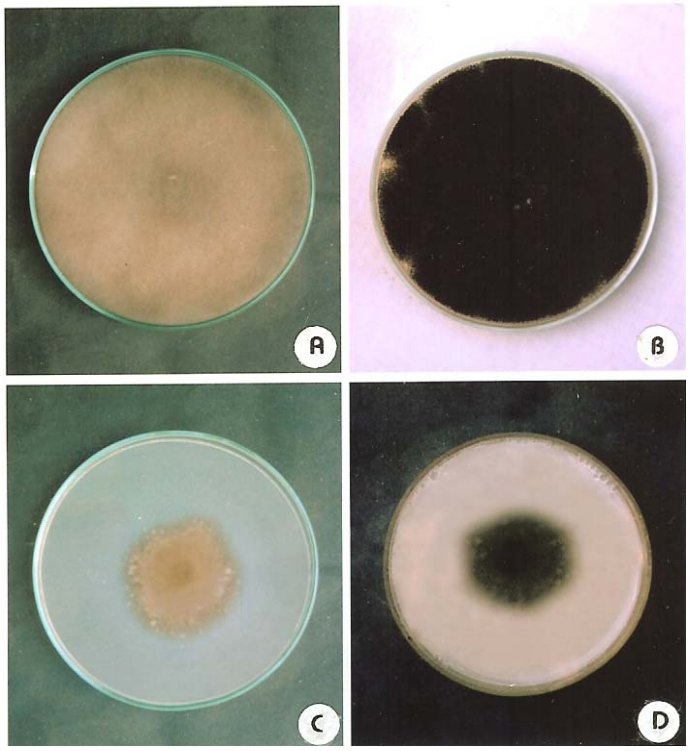

Fig. 1. Effect of plant extracts on mycelial growth in agar disc method. A: Control plate of *Rhizopus stolonifer*. B: Control plate of *Aspergillus niger*. C: Inhibition zone of *Rhizopus stolonifer* in medium supplemented with crude gametophytic extract of *Adiantum lunulatum*. D: Inhibition zone of *Aspergillus niger* in medium supplemented with extracted phenol from gametophytes of *Adiantum capillus-veneris.*

in gametophyte and immature pinnule. It may be that in the natural ecosystem, gametophytes, which remain active and viable for short duration in contrast to sporophytes, develop more resistance by producing higher quantities of primary and secondary metabolites for sustainance and survival.

Ferns are represented by two independent generations, the diploid sporophytic phase and haploid gametophytic phase. Available literature shows that, all works on biochemical analyses with ferns were done by taking sporophyte as test material (De. Britto *et al.*, 1992; Louis Jesudass *et al*., 2001; Henry Joseph *et al*., 2003). The present work emphasizes that like sporophytes, gametophytes are also good source-material for harvesting active principles in relatively shorter time in small spaces even in culture rooms. Gametophytes of the ferns may be grown in artificial medium containing few macro- and microelements without sugar and hormone (Koch, 1973) and materials may be harvested only after 45 days of growth.

In most plants, phenolics are derived from phenylalanine, a product of shikimic acid pathway, which in turn converts simple carbohydrate precursors derived from glycolysis and pentose phosphate pathway to aromatic amino acids (Taiz and Zeiger, 1998). As the metabolic rates of the plants are high in young stage (Hopkins *et al.*, 2001), this may be the reason for high level of amino acid and carbohydrate in this stage. During spore formation, many biochemical changes take place (Beri and Bir, 1995) as evidenced from the decrease in amino acid, carbohydrate and phenol content in the sporophyll compared to the pinnules. Moreover, in both species of *Adiantum*, all pinnules are transformed to sporophylls and mature pinnules are those sporophylls from which the spores already dispersed. Carbohydrate and amino acids have roles in controlling the rate of phenol synthesis, as these two are the precursors (Taiz and Zeiger,

Table 3. Effect of crude extract and extracted phenols from the gametophytes and sporophytic plant parts of *Adiantum capillus-veneris* and *Adiantum lunulatum* on the mycelial dry weight of *Aspergillus niger* and *Rhizopus stolonifer* in agardisc method.

Plant material		Mycelial dry wt. (mg) (mean ± SD)			
		Aspergillus niger		*Rhizopus stolonifer*	
		Crude ext.	Ext. phenol	Crude ext.	Ext. phenol
A. capillus-veneris					
Control		64.30 ± 6.72	64.30 ± 6.72	57.26 ± 5.88	57.26 ± 5.88
Sporophytic plant parts	RA	55.31 ± 4.23	52.70 ± 5.59	51.16 ± 5.31	50.26 ± 6.21
	RH	51.67 ± 3.56	48.82 ± 5.43	48.53 ± 5.17	45.75 ± 5.32
	MS	47.62 ± 3.12	45.26 ± 4.62	45.28 ± 5.02	40.28 ± 4.18
	MP	43.73 ± 4.97	39.24 ± 4.85	41.24 ± 4.19	33.76 ± 4.92
	IS	40.45 ± 5.66	36.53 ± 3.43	38.72 ± 4.83	30.13 ± 4.02
	IP	36.78 ± 2.14	30.81 ± 3.79	35.40 ± 3.67	26.54 ± 3.16
Gametophyte		28.39 ± 2.23	22.24 ± 3.15	26.36 ± 3.56	20.50 ± 2.82
F-value		5.63		1.56*	
LSD at 5%		5.62		1.79	
A. lunulatum					
Control		64.30 ± 6.72	64.30 ± 6.72	57.26 ± 5.88	57.26 ± 5.88
Sporophytic plant parts	RA	61.38 ± 7.32	56.91 ± 6.43	55.23 ± 6.82	53.21 ± 6.43
	RH	58.82 ± 6.65	52.82 ± 6.51	53.84 ± 6.13	49.95 ± 5.82
	MS	56.93 ± 6.31	47.33 ± 5.32	50.65 ± 5.71	45.33 ± 5.25
	MP	53.77 ± 5.93	43.71 ± 5.17	48.32 ± 5.06	41.46 ± 5.03
	IS	50.26 ± 4.87	40.27 ± 4.11	45.36 ± 4.89	37.89 ± 4.71
	IP	46.73 ± 4.63	36.54 ± 4.73	42.68 ± 4.24	33.22 ± 4.34
Gametophyte		40.30 ± 3.54	32.84 ± 4.05	36.51 ± 4.11	28.64 ± 3.53
F-value		13.35		16.83	
LSD at 5%		3.37		3.07	

The F-value is significantly different at 0.05%

∗ F-value is not significant at 0.05%

1998; Hopkins *et al*., 2001). Gametophytic tissue, being the forerunner of zygote development, has to remain metabolically much more active, which is evidenced from higher content of carbohydrate and amino acid. Production of higher content of phenols are therefore likely because of availability of precursors and for imparting resistance to the gametophytes against pathogens under natural habitats which may otherwise invade the delicate tissue.

The crude extracts of different parts of the sporophytic plant body as well as the gametophyte of both the plant materials were found to be bioactive against the two fungal strains. For studying the antifungal activity of the plant extracts, three different types of tests were performed to ensure the accuracy of the results. The aim of these three tests was to give a multidirectional approach towards the study in order to minimize the error, which could otherwise have occurred with single test. Of the two fungal strains, *R. stolonifer* is a plant pathogen, which damages fruits and vegetables during storage, shipping and marketing (Alexopoulos *et al*., 1996). *Aspergillus niger* is pathogenic to humans, causing a group of diseases called aspergilloses (Kwon-Chung and Bennett, 1992). It also produces mycotoxin in cereals, peanuts etc. which when consumed causes kidney and liver damages and may ultimately lead to haemorrhage of lungs and brain (Griffin, 1994).

Among the different parts of the sporophytic plant body, extracts of immature pinnule possess the highest fungicidal property. Like the crude extracts, extracted phenols from the gametophytes showed higher antifungal activity than that of the sporophyte. Gametophytes are

Table 4. Effect of crude extract and extracted phenols from the gametophytes and sporophytic plant parts of *Adiantum capillus-veneris* and *Adiantum lunulatum* on the mycelial dry weight of *Aspergillus niger* and *Rhizopus stolonifer* in liquid culture method.

Plant material		Mycelial dry wt. (mg) (mean ± SD)			
		Aspergillus niger		*Rhizopus stolonifer*	
		Crude ext.	Ext. phenol	Crude ext.	Ext. phenol
A. capillus-veneris					
Control		144.50 ± 22.23	144.50 ± 22.23	121.32 ± 30.06	121.32 ± 30.06
Sporophytic plant parts	RA	130.26 ± 18.76	100.67 ± 19.54	108.34 ± 20.63	92.84 ± 19.69
	RH	117.18 ± 16.67	92.64 ± 13.45	100.25 ± 14.84	84.35 ± 9.75
	MS	109.65 ± 13.32	81.85 ± 12.86	96.62 ± 10.78	76.53 ± 6.76
	MP	104.54 ± 11.19	73.91 ± 8.65	93.43 ± 8.12	68.46 ± 6.67
	IS	95.43 ± 12.21	57.83 ± 6.43	89.61 ± 6.34	60.81 ± 5.93
	IP	85.47 ± 8.76	42.62 ± 4.83	86.46 ± 5.59	51.72 ± 5.13
Gametophyte		73.49 ± 9.95	31.68 ± 2.13	67.57 ± 6.71	36.57 ± 3.65
F-value		3.56		2.95	
LSD at 5%		9.32		9.83	
A. lunulatum					
Control		144.50 ± 22.23	144.5 ± 22.23	121.32 ± 30.06	121.32 ± 30.06
Sporophytic plant parts	RA	139.14 ± 19.88	123.11 ± 17.61	115.60 ± 18.77	103.64 ± 12.23
	RH	134.05 ± 21.63	110.43 ± 15.18	109.82 ± 13.35	92.53 ± 8.33
	MS	130.53 ± 18.54	99.34 ± 11.93	102.57 ± 11.19	83.81 ± 7.61
	MP	126.86 ± 16.45	88.62 ± 9.80	97.65 ± 10.65	71.72 ± 6.29
	IS	120.52 ± 14.89	80.55 ± 8.76	93.81 ± 7.63	65.46 ± 5.96
	IP	114.81 ± 15.57	73.26 ± 6.83	88.53 ± 6.77	59.8 ± 5.78
Gametophyte		100.57 ± 11.17	67.47 ± 7.17	80.84 ± 9.32	50.35 ± 5.55
F-value		2.11		10.68	
LSD at 5%		22.34		8.76	

The F-value is significantly different at 0.05%

therefore appearing to be stronger antifungal source than the sporophytes. The area of the fungal colony in disc method in medium containing extracted phenols from gametophyte of *A. capillus-veneris* was 6.6 ± 0.86 cm^2 in *R. stolonifer* plates and 9.08 ± 1.78 cm^2 in *A. niger* plates (Table 2). In case of *A. lunulatum* it was 7.56 ± 0.87 cm^2 in *R. stolonifer* plates and 11.35 ± 1.35 cm^2 in *A. niger* plates. Area of the fungal colony (Fig. 1) as well as dry weight of the mycelial discs (Table 3) showed the indication of the activity of crude extracts and extracted phenols of both species of *Adiantum*. In case of liquid culture method, though the horizontal growth of the mycelium was not much affected by the plant extracts or extracted phenols, the cultures showed positive response when vertical growth (depth) (Figs. 2A-2D), and dry weight (Table 4) was checked.

In suspension culture method, the mycelial discs of the controlled cultures were small in size (Diameter of *R. stolonifer* was 1.52 ± 0.043 mm and of *A. niger* was 1.61 ± 0.032 mm) than that of the cultures in medium with plant extracts or extracted phenols (Diameter of *R. stolonifer* was 6.66 ± 0.71 mm and of *A. niger* was 5.03 ± 0.84 mm in extracted phenols) (Figs. 2E-2H). Similar type of result was obtained by Lavermicocca (2003) while studying antifungal activity of phenyllactic acid against 19 fungal strains. Secondary metabolites of the plants particularly phenols, give defence against fungal infection by disrupting the membrane as well as by preventing cell wall synthesis (Maher *et al.*, 1994; Taiz and Zeiger, 1998; Kucuk and Kivanc, 2003). The plant extracts or extracted phenols may be responsible for antagonizing the synthesis of fungal cell wall synthesizing material for which the cell volume increased and the

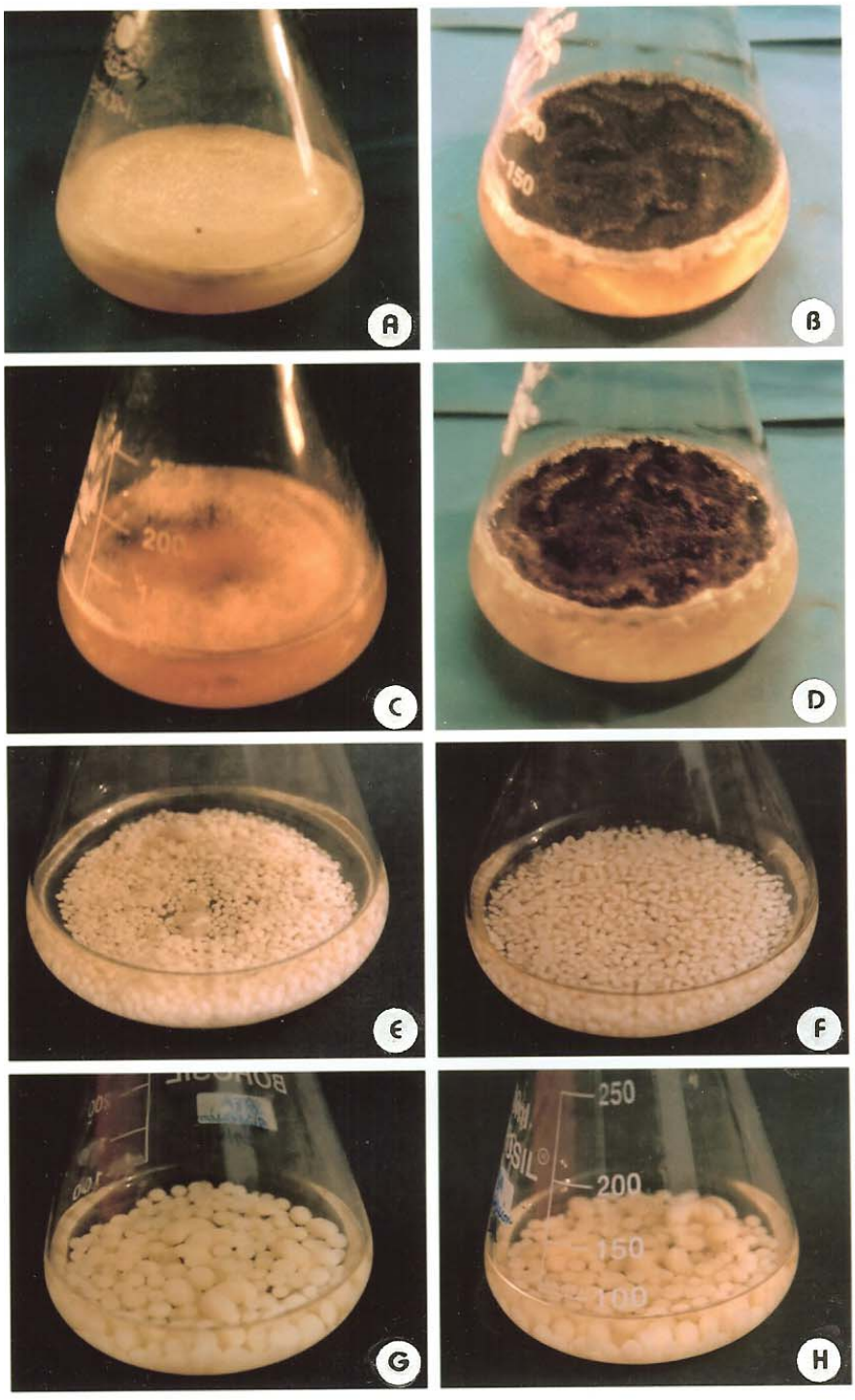

Fig. 2. Effect of plant extracts on mycelial growth in liquid culture method (A-D) and suspension culture method (E-H). A: Control cultures of *Rhizopus stolonifer*. B: Control cultures of *Aspergillus niger*. C: Culture of *Rhizopus stolonifer* in liquid medium supplemented with extracted phenol from gametophytes of *Adiantum capillus-veneris.* D: Culture of *Aspergillus niger* in liquid medium supplemented with crude gametophytic extract of *Adiantum lunulatum*. E: Control cultures of *Rhizopus stolonifer*. F: Control cultures of *Aspergillus niger*. G: Culture of *Rhizopus stolonifer* in liquid medium supplemented with crude gametophytic extract of *Adiantum capillus-veneris*. H: Culture of *Aspergillus niger* in liquid medium supplemented with crude gametophytic extract of *Adiantum lunulatum.*

Table 5. Effect of crude extract and extracted phenols from the gametophytes and sporophytic plant parts of *Adiantum capillus-veneris* and *Adiantum lunulatum* on the mycelial dry weight of *Aspergillus niger* and *Rhizopus stolonifer* in suspension culture method.

Plant material		Mycelial dry wt. (mg) (mean ± SD)			
		Aspergillus niger		*Rhizopus stolonifer*	
		Crude ext.	Ext. phenol	Crude ext.	Ext. phenol
A. capillus-veneris					
Control		123.00 ± 20.76	123.00 ± 20.76	117.32 ± 18.18	117.32 ± 18.18
Sporophytic plant parts	RA	116.51 ± 17.64	106.55 ± 13.43	102.58 ± 12.39	100.57 ± 11.56
	RH	101.37 ± 15.16	89.41 ± 11.24	93.63 ± 10.29	86.83 ± 10.75
	MS	90.42 ± 11.34	77.53 ± 9.87	82.05 ± 10.34	72.52 ± 9.74
	MP	86.56 ± 9.76	70.82 ± 8.16	71.87 ± 9.56	63.51 ± 8.23
	IS	81.82 ± 8.76	62.87 ± 7.79	65.48 ± 8.96	57.67 ± 7.19
	IP	72.50 ± 7.64	55.43 ± 6.64	60.66 ± 8.67	50.48 ± 6.48
Gametophyte		60.63 ± 7.18	42.61 ± 5.75	48.72 ± 6.89	36.83 ± 4.65
F-value		18.76		21.75	
LSD at 5%		7.42		3.56	
A. lunulatum					
Control		123.00 ± 20.76	123.00 ± 20.76	117.32 ± 18.18	117.32 ± 18.18
Sporophytic plant parts	RA	118.56 ± 19.87	110.47 ± 17.64	111.16 ± 21.13	111.32 ± 16.57
	RH	113.48 ± 15.43	101.61 ± 14.32	104.54 ± 17.65	100.54 ± 10.17
	MS	108.12 ± 13.27	92.33 ± 11.46	99.81 ± 12.11	91.36 ± 11.11
	MP	101.54 ± 12.93	83.45 ± 10.75	93.73 ± 10.03	81.81 ± 9.83
	IS	93.85 ± 10.70	72.82 ± 9.64	86.45 ± 8.75	70.47 ± 7.63
	IP	87.61 ± 9.84	60.56 ± 7.59	81.52 ± 7.87	60.53 ± 6.66
Gametophyte		80.83 ± 10.34	51.44 ± 5.42	67.87 ± 6.58	45.55 ± 5.37
F-value		26.79		7.65	
LSD at 5%		5.89		9.82	

The F-value is significantly different at 0.05%

mycelial discs were bigger than the controlled ones. In untreated controls, mycelial dry weight was also much higher than those of the cultures in media with plant extracts or extracted phenols (Table 5).

The adiantoid ferns comprise many species with biological activities, which are used in nutrition or as alternate medicines (Rastogi and Mehrotra, 1989; Chatterjee and Pakrashi, 1994). But their exact quantification, clinical investigation and dosage have not yet been standardized. Nowadays, several antimicrobial drugs are available though, particularly, the antimycotic drugs present have limited use by a number of factors such as low potency, poor solubility, emergence of resistance strains and drug toxicity (Bisignano *et al.*, 1999). Therefore, the search for discovery of new antimicrobial agents is necessary to stimulate the research of new chemotherapeutic agent in medicinal plants. Nevertheless, further studies are needed including *in vitro* and *in vivo* investigations, especially in gametophytes with change of ploidy level that might alter the synthesis of secondary metabolites in desirable direction. Alternatively, under laboratory or greenhouse condition osmotic stress or enrichment of media may be provided artificially to the growing gametophytes to enhance the production of secondary metabolites especially the phenols. Under *in vitro* system such as in callus or suspension cultures these methods are well established in higher plants (Dixon, 1987; Choudhury and Gupta, 2001).

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兩種鐵線蕨屬植物的粗抽取物和抽取的 phenols 之抗真菌活性

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摘　　要

蕨類植物鐵線蕨 (*Adiantum capillus-veneris*) 和半月鐵線蕨 (*Adiantum lunulatum* Burm. f.) 的配子體和孢子體之水抽取物與抽出之 phenols，在印度、中國、西藏、美洲、菲律賓和義大利被當作民俗藥物。本篇以此為材料，研究其對黑曲霉 (*Aspergillus niger*) 和葡枝根霉 (*Rhizopus stolonifer*) 的抗菌活性。發現兩者都有抗真菌活性，配子體之抽取物要比孢子體各部位的抽取物之抗菌活性大，在孢子體中則以未成熟的小孢子葉 (pinnule) 具有最大的抑真菌性能。比較兩種鐵線蕨植物，*Adiantum capillus-veneris* 為較佳的抗真菌劑。

關鍵詞：鐵線蕨、半月鐵線蕨、黑曲霉、民俗的、抑真菌的、葡枝根霉。

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