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IMPACT OF SEASONS ON SOME BIOCHEMICAL PARAMETERS IN THREE ADIANTOID FERNS

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SUMMARY

Chlorophylls, carbohydrates, carotenoids, total free amino acids, total free phenols, and total free proline were measured in different parts of *Adiantum capillus-veneris* L., *Adiantum incisum* Forssk. and *Adiantum lunulatum* Burm. f. in summer (April - June), rainy (July - October) and winter (November - February) seasons. Among the three species, *A. incisum* contains the highest amount of these metabolites. Significant differences of primary and secondary metabolites were observed in different parts of the same species. Seasonal impacts on the change of quantity of the metabolites were distinctly evident. Relative water content of the leaf tissue was more in winter in all the species, maximum being in *A. lunulatum*. Rainy season was found to be the best time for harvest of raw materials for herbal drug purpose as phenol content was found to be highest in this season.

***Key words: Adiantum capillus-veneris, Adiantum incisum, Adiantum lunulatum*, carotenoids, phenol, proline.**

INTRODUCTION

Phytochemical study of ferns has assumed an extraordinary importance due to its immense economic value (Anderson *et al.* 1979, Cui *et al.* 1990), especially for their vast medicinal importance (Rastogi and Mehrotra 1990, Chatterjee and Pakrashi 1994). *Adiantum capillus-veneris* L., *A. incisum* Forssk. and *A. lunulatum* Burm. f. have a wide range of medicinal values and are used in the treatment of many diseases (Singh *et al.* 1989, Kaushik and Dhiman 1995, Graham *et al.* 2000). Species of *Adiantum* were reported to have antibacterial and antifungal properties (Kombrink and Somssich 1995, Guha *et al.* 2004, Parihar and Bohra 2004). However, from available literature, it is evident that only few attempts have been made to identify the active constituents of these medicinally important ferns (Chatterjee and Pakrashi 1994).

Plant growth and development in relation to season have distinct impact on accumulation of secondary metabolites (Teiz and Zeiger 1998). Moreover, the synthesis of secondary metabolites depends primarily on the overall metabolic efficiency of the plants and also on the supply of primary constituents. Keeping in view of these two important criteria, an attempt has been made in the present investigation to evaluate the general metabolism of the test materials in different seasons and accumulation of active constituents in different parts. Since the pattern of growth, development and survival of the plants depend upon availability of water and also on prevailing temperature, some stress associated parameters such as relative water content and proline were also considered to evaluate the physiological status of the plant and correlation with accumulation of secondary metabolites, i.e. phenols. The study may help in determination of time of harvest of plant materials for extraction of active ingredients.

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MATERIALS AND METHODS

Plant parts (100 mg) of immature pinnule (IP) (just born pinnule without spores), immature sporophyll (IS) (young sporophyll with green sorus), mature sporophyll (MS) (sporophyll with brown sorus), mature pinnule (MP) (sporophyll from which spores were shed off), rhizome (RH) and rachis (RA) were collected from Shibpur, Howrah and from Fern house, Department of Botany, University of Burdwan in different seasons, summer (April – June), rainy (July – October) and winter (November – February) for phytochemical studies. Extraction and estimation of chlorophyll a, chlorophyll b and carotenoids were done as per the methods of Litchenthaler (1987). Soluble and insoluble carbohydrate were estimated following the methods of McCreedy *et al*. (1950). Total free amino acid was extracted and estimated by the method of Moore and Stein (1948). Total phenols from the tissue were estimated following the method of Bray and Thorp (1954). Proline content was measured following the method of Bates *et al*. (1973). Relative water content of the leaf tissue was measured according to the method of Barrs (1968). For each biochemical analysis 10 replicates were taken. All the experimental results were statistically analyzed for LSD following the two factor analysis of variance method using Fisher's LSD procedure at P = 0.05 (Zar 1974).

RESULTS AND DISCUSSION

Irrespective of the species, chlorophyll a and chlorophyll b contents did not show any significant decline, which pointed out that mature sporophylls are still functional for higher metabolic activity through photosynthesis (Table 1). The minor reduction of chlorophyll content in summer and winter than rainy season may be due to photoxidation of chlorophyll (Ramachandran *et al*. 1991). Like the different species of *Pteris* (Louis Jesudass *et al*. 1993), members of Dennstaedtiaceae (Gopalakrishnan *et al*. 1993), works of Vyas and Sharma (1988) and Rathore and Sharma (1991), the present workers also found the content of chlorophyll b more than chlorophyll a. Seasonal effect was distinctly evident with regard to production and

Table 1. Chlorophyll content (µg/g dw) in three adiantoid ferns in different seasons.

Season	Plant part	*A. capillus-veneris*		*A. incisum*		*A. lunulatum*	
		Chl-a	Chl-b	Chl-a	Chl-b	Chl-a	Chl-b
Summer	IP	9.55	11.55	10.80	11.40	7.65	11.20
	IS	9.35	11.25	10.60	10.85	7.25	10.90
	MP	8.80	10.95	10.45	10.50	6.75	10.45
	MS	7.90	10.85	9.95	10.3	6.45	10.15
Rainy	IP	9.95	11.95	10.91	11.55	7.75	11.40
	IS	9.45	11.75	10.75	11.3	7.35	11.10
	MP	9.10	11.10	10.55	10.95	6.6	10.55
	MS	8.10	10.6	10.10	10.45	6.55	10.30
Winter	IP	9.35	11.35	10.75	11.25	7.55	11.15
	IS	9.10	11.10	10.50	10.55	7.15	11.00
	MP	8.25	10.85	10.40	10.45	6.65	10.95
	MS	7.65	10.75	9.80	10.05	6.40	10.80
LSD at 5%		1.34		1.68		0.96	

IP = Immature pinnule, IS = Immature sporophyll, MP = Mature pinnule and MS = Mature sporophyll.

utilization of carbohydrate, both soluble and insoluble (Table 2). Higher productions of both soluble and insoluble carbohydrates were noted in pinnules than sporophylls. Louis Jesudass *et al*. (2001) observed higher amount of sugars in fronds compared to rhizomes, which were in conformity with the present study. Since, the chlorophyll content of mature sporophyll was comparable with that of immature pinnule, it appears that the primary metabolites, i.e. the carbohydrate in the present case, are utilized more effectively in mature sporophyll thereby showing the marked decline when compared in each season separately. In all the three species of *Adiantum*, carbohydrate content was less in the sporophylls than in the young pinnules as observed earlier by Vyas and Sharma (1988).

Total free amino acid content was also significantly low in mature sporophyll corroborating with the former observations (Table 3). But in this case also, free amino acid content was relatively high in rainy season irrespective of pinnule maturity status when compared with other two seasons i.e., summer and winter. Since the same trend was observed in the three species under seasonal condition, this might be due to their growth and development in the same habitat. Louis Jesudass *et al*. (2001) observed that no relationship can be drawn between total amino acid content and morphology of the fronds or habitat, eg. *Pteris multiaurita*, a shade fern and *Pteris linearis*, a sun fern are found to possess almost the same quantity of total free amino acid. On the other hand, Khare and Shankar (1987) reported maximum quantity of amino acids in aerial shoots followed by rhizome and synangia. Rathore and Sharma (1992) reported that the content of total free amino acid is maximum in exposed ferns and minimum in shaded ferns. In the present study, the content of proline was found to increase from immature to mature sporophyll in all the three species and maximum in the rhizome portion (Table 4). Rathore and Sharma (1988) reported that drought resistant ferns possess more concentration of proline than aquatic and moisture loving ferns. The imino acid proline is generally associated with drought resistance (Kaur *et al*. 1986, Rathore and Sharma 1988, Sharma *et al*. 1995). Proline acts as osmolyte giving

Table 2. Total carbohydrate content (mg/g dw) in three adiantoid ferns in different seasons.

Season	Plant part	*A. capillus-veneris*		*A. incisum*		*A. lunulatum*	
		Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
Summer	IP	1.312	1.166	1.354	1.211	1.282	1.147
	IS	1.142	1.013	1.175	1.085	1.103	0.987
	MP	0.937	0.673	0.951	0.704	0.894	0.649
	MS	0.732	0.567	0.764	0.608	0.701	0.541
Rainy	IP	1.48	1.267	1.542	1.343	1.362	1.193
	IS	1.3	1.108	1.433	1.185	1.176	1.027
	MP	1.16	1.008	1.312	1.077	0.952	0.765
	MS	1.002	0.932	1.191	0.995	0.767	0.573
Winter	IP	1.176	1.09	1.214	1.117	1.147	1.068
	IS	1.063	0.941	1.099	0.964	1.002	0.903
	MP	0.852	0.628	0.989	0.674	0.937	0.594
	MS	0.679	0.495	0.942	0.513	0.863	0.463
LSD at 5 %		0.057		0.082		0.074	

IP= Immature pinnule, IS= Immature sporophyll, MP= Mature pinnule and MS= Mature sporophyll.

Table 3. Total amino acid content (mg/g dw) in three adiantoid ferns in different seasons.

Plant	*A. capillus-veneris*			*A. incisum*			*A. lunulatum*		
part	S	R	W	S	R	W	S	R	W
IP	0.975	1.118	0.889	0.999	1.143	0.913	0.954	1.098	0.852
IS	0.929	1.062	0.809	0.957	1.102	0.847	0.902	1.023	0.788
MP	0.881	1.015	0.74	0.908	1.052	0.764	0.852	0.988	0.704
MS	0.816	0.954	0.663	0.847	0.988	0.703	0.778	0.922	0.643
RH	0.772	0.876	0.596	0.787	0.913	0.619	0.744	0.842	0.561
RA	0.667	0.744	0.52	0.699	0.767	0.552	0.637	0.703	0.493
LSD at 5 %		0.024			0.015			0.041	

S = Summer, R = Rainy, W = Winter, IP = Immature pinnule, IS = Immature sporophyll, MP = Mature pinnule, MS = Mature sporophyll, RH = rhizome and RA = rachis.

Table 4. Total proline content (mg/g dw) in three adiantoid ferns in different seasons.

Plant	*A. capillus-veneris*			*A. incisum*			*A. lunulatum*		
part	S	R	W	S	R	W	S	R	W
IP	7.15	5.6	8	8.35	7.25	10.6	7.85	6.5	8.65
IS	7.5	6.05	8.55	8.7	7.55	10.65	8	7.1	9.2
MP	8.15	7.05	9.55	9.8	8.55	10.8	8.75	7.85	10.5
MS	8.9	7.5	10.5	10.5	9.15	10.85	9.2	8.1	10.55
RH	9.15	8.05	10.55	10.55	9.65	10.95	9.85	8.75	10.6
RA	7.85	6.5	9.1	9.15	8	10.7	8.45	7.5	9.6
LSD at 5%		0.56			0.84			0.73	

S= Summer, R= Rainy, W= Winter, IP= Immature pinnule, IS= Immature sporophyll, MP= Mature pinnule, MS= Mature sporophyll, RH= rhizome and RA = rachis.

stress relief to plants (Hopkins *et al*. 2001). Along with age, there was an increase in proline content in the three species in three seasons and was also found to be higher in summer and winter, indicating their drought avoiding metabolic adjustment. Such age induced proline accumulation as reported in the present study is also befitting with the survival mechanism of the ferns under stress condition. Higher amount of proline content in rhizome than that of frond has also been reported by Henry Joseph *et al*. (2003) working with Dryopteridaceae.

Irrespective of seasons, the protective pigment carotenoids maintained almost identical level in fronds and sporophylls of different maturity status (Table 5), thereby maintaining the photosynthetic ability of the plants (Hopkins *et al*. 2001) and also might give further resistance through production of abscissic acid (Cutler and Kronchko 1999).

In the present investigation, irrespective of the three species of *Adiantum*, RWC was highest in winter and least in rainy season (Table 6). Osmoregulation may also

Table 5. Total carotenoid content (mg/g dw) in three adiantoid ferns in different seasons

Plant	*A. capillus-veneris*			*A. incisum*			*A. lunulatum*		
Part	**S**	**R**	**W**	**S**	**R**	**W**	**S**	**R**	**W**
IP	6.2	6.45	6.7	7.1	7.25	7.5	6.65	6.75	7.2
IS	6.01	6.35	6.6	7	7.1	7.3	6.5	6.6	7.05
MP	6.1	6.4	6.65	7.05	7.15	7.45	6.55	6.65	7.1
MS	6	6.3	6.5	6.75	6.9	7.25	6.4	6.5	6.9
LSD at 5 %		0.45			0.67			0.32	

S= Summer, R= Rainy, W= Winter, IP= Immature pinnule, IS= Immature sporophyll, MP= Mature pinnule and MS= Mature sporophyll.

Table 6. Relative water content (%) in three adiantoid ferns in different seasons.

Plant	*A. capillus-veneris*			*A. incisum*			*A. lunulatum*		
Part	**S**	**R**	**W**	**S**	**R**	**W**	**S**	**R**	**W**
MP	40.62	31.36	51.08	46.42	39.86	55.32	50.63	42.36	61.65
IS	22.36	14.32	32.36	28.61	21.86	35.36	33.42	24.83	39.32
MS	31.32	22.36	43.46	35.65	30.32	43.56	41.36	33.56	50.65
IP	12.31	6.36	21.36	20.63	13.32	27.42	25.36	16.36	30.36
LSD at 5%		0.97			1.14			1.78	

S= Summer, R= Rainy, W= Winter, IP= Immature pinnule, IS= Immature sporophyll, MP= Mature pinnule and MS= Mature sporophyll.

play an important role in helping partial wilt to remain turgor once the water supply recovers (Hopkins *et al*. 2001). In higher plants RWC increases with increasing age (Choudhury and Gupta 1996). Species of *Adiantum* behaved in the similar fashion.

As adiantoid ferns under investigation are variously reported in literature for their medicinal properties (Singh *et al*. 1989, Kaushik and Dhiman 1995, Kumar *et al*. 2003), their conservation and successful multiplication in natural habitat is very much essential. Content of phenol and their antimicrobial and antifungal properties have been reported in *Adiantum capillus-veneris* (Guha *et al*. 2003, 2004) both in gametophyte and sporophytic plant parts. However no seasonal impact as evidenced from this study on change in phenol content of the pinnules at different growth phases was available in literature. The phenol content was found to be maximum in immature pinnules irrespective of seasons (Table 7). The highest content being noted in *A. incisum*. Among the different growth stages of the frond, phenol content was minimum in mature sporophyll though in this case also, the content was comparatively high in rainy season in comparison to summer and winter in the three species. The phenol content in the adiantoid ferns followed the trend as that of the primary metabolites, i.e. of carbohydrate and amino acids. Most of the plant phenolics are derived from phenyl-alanine, a product of shikimic acid pathway (Taiz and Zeiger 1998). Therefore, plants rich in amino acid content (phenyl-alanine) also contain greater quantity of phenol (Guha *et al*. 2004). Moreover, shikimic acid is derived from erythrose-4-phosphate (a product of pentose phosphate pathway), and also from phosphoenolpyruvate (a product of glycolysis) (Taiz and Zeiger 1998). As chlorophyll content of *A. incisum* was higher than the other two species, so it may be the reason for high carbohydrate content and subsequently, the amino acid and the phenol content. Shankar and Khare (1985,

Table 7. Total phenol content (µg/g dw) in three adiantoid ferns in different seasons.

Plant	*A. capillus-veneris*			*A. incisum*			*A. lunulatum*		
Part	**S**	**R**	**W**	**S**	**R**	**W**	**S**	**R**	**W**
IP	359.5	402.3	340	399.4	442.4	363	328.3	369.3	304
IS	292	366.6	256.6	317.8	396.3	297.3	262.1	337.5	212.8
MP	220.5	320	181.3	253	357.5	212.5	199.4	299	151.5
MS	189	278.4	163.2	212.5	317.4	186.5	169.3	252.3	137
RH	133.6	229.7	115	158.5	259	147.2	102.4	193.1	97.3
RW	111	181	98.3	137.9	213.6	117.8	88	152.6	74.1
LSD at 5%		4.35			3.33			4.19	

S= Summer, R= Rainy, W= Winter, IP= Immature pinnule, IS= Immature sporophyll, MP= Mature pinnule, MS= Mature sporophyll, RH= rhizome and RA= rachis.

1986) estimated the quantitative values of phenolics in *Ampelopleris prolifera*, *Diplazium esculentum*, *Adiantum caudatum* and *Cheilanthes farinosa*. Vyas *et al*. (1989) studied the total phenolic constituents in six species of Rajasthan pteridophytes and observed that *Hypodematium crenatum* and *Tectaria macrodonata* possess sufficient amount of total phenols in their soft, green, well-laminated leaves. Similar results were noted by Kaur *et al*. (1986) in three species of Rajasthan pteridophytes, Varma (1992) in *Asplenium* species and Yadav (1992) in *Ophioglossum* species. The present study with adiantoid ferns are in agreement with the observations of Rathore and Sharma (1988), because immature pinnules contained the highest amount of phenols when compared to mature sporophylls. In present case however, the content of phenol was relatively low both in winter and summer, i.e. when the plants are under partially stressed condition. These results are not in agreement with Louis Jesudass *et al*. (1993) and De. Britto *et al*. (1992, 1994). These may be due to overall low metabolic status of the species in these two seasons.

From the practical view point the investigation on this fern species is important in determining the time of harvest of the raw material for extraction of phenolics. The immature pinnules having high phenolic content may be carefully harvested during rainy season without disturbing the natural population. Since gametophytes are also prospective source of isolation of active principles, care should be taken to conserve the sporophyte under natural habitat at least during summer and winter months of growth.

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