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COMPARATIVE STUDY OF TOTAL PHENOLS AND TOTAL FREE AMINO ACIDS IN WILD AND CULTIVATED POPULATION OF *MARSILEA* SPECIES

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The present paper describes comparative study of total phenols and free amino acids in wild and cultivated population of two *Marsilea* species (*M.minuta* Linn and *M.rajasthanensis* Gupta). The significant variations have been reported in total phenols and total free amino acid contents between wild and cultivated population of the two *Marsilea* species.

Keywords : *Marsilea;* Total free amino acids; Total phenols; Wild and cultivated population.

Phytochemical studies on pteridophytes have been taken up extensively in later half of last century. Phytochemical aspects in pteridophytes and its importance in phylogeny of ferns was studied by a number of workers[1-3]. Phytochemical studies of *Marsilea* were initiated by extraction of marsilin, from *M.minuta* and *M.rajasthanensis*[4,5]. It was thought to be a macrocyclic ketone. Chakravarty and Debanath[6] reported an asymmetrical Hydroxy-ketone from leaves of *M.minuta*. Bhardwaja *et al*[7]. have analysed sporocarps of *M.diffusa* and reported five steroidal compounds. Sharma[8] carried out a detailed phyto-chemical investigation of some African, Australian and Indian species of *Marsilea* relating to pigments, ascorbic acid, amino acids, total phenols and total soluble proteins. Bhardwaja *et al*[9]. carried out phytochemical investigation and their relevance to phylogeny and intergeneric relationships in the family Marsileaceae. Wallace *et al.*[10] investigated polyphenolics of the family Marsileaceae and their possible phylogenetic utility. Bhardwaja and D'Souza[11] have studied phytochemical basis of land and water forms in *M.aegyptiaca*. Recently Raja Ram[12] revised phytochemical studies in *Marsilea* from different habitats.

For quantitative estimation of total phenols and total free amino acids in different organs (root, rhizome, petiole and leaflet) materials of both species *M.minuta* Linn and *M.rajasthanensis* Gupta was taken from Botanic Garden, Government College, Ajmer (cultivated population) and different localities of Rajasthan (wild population).

For Total phenols[13] : Reagents used were:

1. 30% sodium carbonate (Na_2CO_3) solution: (30 gm of Na_2CO_3 was dissolved in distilled water and made up to 100 ml) and 2. Folin's reagents: (Commercially available reagents was used after two times dilution with distilled water). 200 mg of dried material of each organ was homogenized in 10 ml of 80% alcohol. After centrifugation, residue was again extracted with 10 ml 80% alcohol. The supernatants were combined and made up to a specific volume and used as a source of total phenols. To suitable amount of sliquot was added 3 ml of Na_2CO_3 solution and 0.5 ml of Folin's reagent. In blank aliquot was replaced by an equal amount of distilled water. Test tubes were placed in boiling water for 1 minute and centrifuged to clear the turbidity. Optical density was recorded at 760 nm. Standard curve was prepared using caffeic acid.

For total free amino acids : Reagents used were : 1.1% Ninhydrin in 0.5M citrate buffer (pH 5.5) 2. Pure glycerol 3. 0.5M citrate buffer (pH 5.5). Ninhydrin reagents was prepared by mixing reagents 1,2,3 in the ratio of 5:12:2 respectively. 100 mg of dried material of each sample was homogenized in 10 ml of 80% ethyle alcohol. Chlorophyll pigments were removed by adding choloroform and water. The upper aqueous phase was used for amino acid estimation. 0.1ml of extract was taken and 5 ml of Ninhydrin reagents was added. Tubes were shaken vigorously. The tubes were placed in boiling water bath for 12 minutes. Optical density was recorded at 570 nm. Standard curve was prepared using glycine.

Observation of total phenols and total free amino acids in root, rhizome, petiole and leaflets, from both wild and cultivated populations were recorded (Table 1). Total phenols and total free amino acid contents in root, was found to be 2.10 mg/gdw and 0.85 mg/gdw respectively in wild population of *M.minuta* and 1.30 mg/gdw and 0.44 mg/gdw respectively in its cultivated population.Similarly, 3.40-mg/gdw total phenols and 1.50 mg/gdw total free amino acid content was recorded in wild population of *M.rajasthanensis* and 1.24 mg/gdw and 0.30 mg/gdw in its cultivated population.

In rhizome, maximum amount of total phenols 18.60 mg/gdw and 0.95 mg/gdw total free amino acid was recorded in wild population of *M.minuta* while 14.80mg/gdw total phenols and total free amino acid 2.40 mg/gdw in its cultivated population. Wild population of

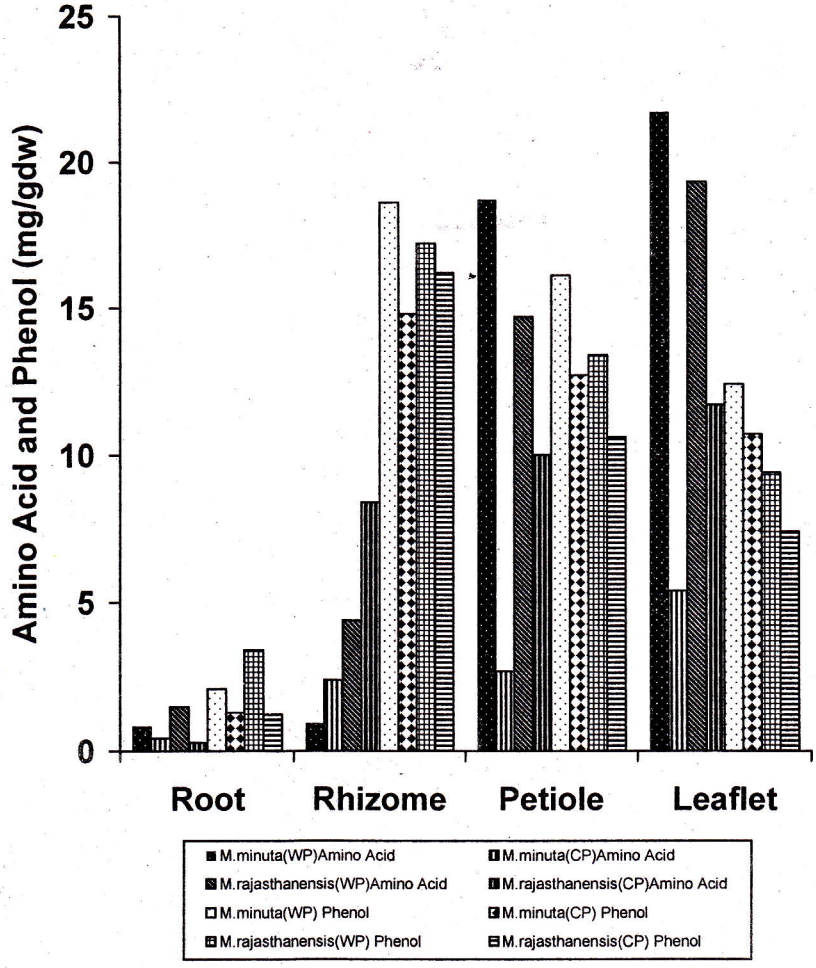

Fig. 1

Table 1. Total phenols and total free amino acids (mg/gdw)in different organs of wild (wp) and cultivated population (cp) of *Marsilea* species.

S.No.	Organ		*M.minuta*		*M.rajasthanensis*	
			W.P.	*C.P.*	*W.P.*	*C.P.*
1.	Root	Phenols	2.10	1.30	3.40	1.24
		Amino Acids	0.85	0.44	1.5	0.30
2.	Rhizome	Phenols	18.60	14.80	17.20	16.20
		Amino Acids	0.95	2.40	4.40	8.40
3.	Petiole	Phenols	16.10	12.70	13.40	10.60
		Amino Acids	18.70	2.70	14.70	10.00
4.	Leaflet	Phenols	12.40	10.70	9.40	7.40
		Amino Acids	21.70	5.40	19.30	11.70
5.	Average	Phenols	12.30	9.87	10.85	8.86
		Amino Acids	10.55	2.51	9.97	7.60

M.rajasthanensis contained 17.20 mg/gdw total phenols and 4.40 mg/gdw total free amino acids while its cultivated population showed minimum total phenols 16.20 mg/gdw and 8.40 mg/gdw total free amino acids contents.

In petiole, higher total phenols contents 16.10 mg/gdw and total free amino acid 18.70 mg/gdw was recorded in wild population of *M.minuta*, as compared to 12.70 mg/gdw total phenols and 2.70 mg/gdw total free amino acids found in its cultivated population. Similarly, 13.40 mg/gdw total phenols and 14.70 mg/gdw total free amino acids was noted in wild populations of *M.rajasthanensis* while in its cultivated population 10.60 mg/gdw total phenols and 10.00 mg/gdw total free amino acids was recorded.

In leaflets total phenol content 12.40 mg/gdw and total free amino acids 21.70 mg/gdw was recorded in wild population of *M.minuta* while 10.70 mg/gdw total phenols and 5.40 mg/gdw total free amino acids was found in its cultivated population. Likewise leaflet of wild population of *M.rajasthanensis* were observed to contain 9.40 mg/gdw total phenols and 19.30 mg/gdw total free amino acids while in its cultivated population 7.40 mg/gdw phenols and 11.70 mg/gdw total free amino acids was found (Tabel 1 and Fig. 1).

Observations reveal that both total phenols and total free amino acids for entire plant were recorded to be higher in their wild population as compared to their respective cultivated population . However in rhizome a higher total free amino acid content was recorded in cultivated population as compared to wild population. Average maximum phenol contents 12.30 mg/gdw was observed in wild population of *M.minuta* while lesser amount 9.87 mg/gdw was recorded in its cultivated population. Maximum total free amino acids 10.55 mg/gdw was recorded in wild population of *M.minuta* and minimum in cultivated population 2.51 mg/gdw .Similarly wild population of *M.rajasthanensis* showed total average of phenols 10.85 mg/gdw and 8.86 mg/gdw for cultivated population. Total free amino acid was found 9.97 mg/gdw in wild population while this was 7.60 mg/gdw in its cultivated population. Organwise comparision has revealed that wild population of both the species posses maximum phenols contents in their rhizome followed by petiole and leaflets while wild population of both the species reveals that maximum total free amino acid were recorded in leaflet followed by petiole and rhizome. Both total phenols and total free amino acids were minimum in roots.

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