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Poly Acrylamide Gel Electrophoretic Studies on *Ampelopteris prolifera, Ophioglossum petiolatum* and *Marsilea minuta*

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The present study was aimed to produce a biochemical marker for the medicinally important pteridophytes viz., *Marsilea minuta* Linn, *Ophioglossum petiolatum* Hook. and *Ampelopteris prolifera* (Retz.) Copel. using SDS-PAGE. To produce protein marker for the selected pteridophytes SDS-PAGE was carried out following the method described by Anbalagan. A total of 18 bands with 10 varied Rf values were demonstrated in the SDS-PAGE gel system of pteridophytes. Among the three pteridophytes studied, *Marsilea minuta* displayed maximum number of protein bands (7) followed by *Ampelopteris prolifera* (6) and *Ophioglossum petiolatum* (5). The proteins with Rf vlaues 0.6 and 0.97 showed their unique occurrence in *Ampelopteris prolifera*. Similarly the proteins with Rf values 0.73 and 0.81 demonstrated their presence only in *Marsilea minuta*. *Ophioglossum petiolatum* failed to show its unqueness in the protein gel system of pteridophytes. These banding profiles can be used as biochemical and pharmacognostical marker to distinguish the medicinally important ferns from its adulterants in the pharmaceutical industries.

Introduction

Marsilea minuta Linn (Marsileaceae) is usually found near the ponds edges and channels and as a weed in wet rice field (Parrotta, 2001). The pharmacological and biological studies on *Marsilea minuta* clearly indicated the various medicinal values of an aquatic fern viz., anti-infertility (Bhardwaja and Garg, 1984), antibacterial (Parihar *et al.,* 2003), anxiolytic (Bhattamisra *et al.,* 2007), anticonvulsant and sedative (Chatterjee *et al.,* 1963), analgesic and anti-inflammatory (Bhattamisra *et al.,* 2009), antidepressant (Bhattamisra *et al.,* 2008), adaptogenic and antistress activity (Tiwari *et al.,* 2009), hypocholesterolemic (Gupta *et al.,* 2000) and hepatoprotective (Praneetha *et al.,* 2011) activities. Shirolkar *et al.,* (2014) studied the interspecific and intra specific variation between the *Marsilea minuta* Linn. and *Marsilea quadrifolia* Linn. using RAPD markers. Madhu *et al.,* (2014) studied the

pharmacognostical characters of *Marsilea minuta* Linn. Revathi and Cathrin Sara (2014) studied the phytochemical composition of *Marsilea minuta*. Thick paste of *Ophioglossum petiolatum* from fresh rhizomes and tubers were effective against hair fall. Lin et al. (2005) isolated homoflavonoids from *Ophioglossum petiolatum*. Shankar and Khare (1985) studied the phytochemical composition of *Ampelopteris prolifera* (Retz.) Copel. Mandal and Mondal (2012) studied the aminoacid profile of *A. prolifera*. Semwal *et al.,* (2013) evaluated the antioxidant properties of *A. prolifera*. Bharti and Pravesh (2012) studied the antibacterial potentials of *Ampelopteris prolifera*. But there is no report on the biochemical markers for *Ampelopteris prolifera, Ophioglossum petiolatum* and *Marsilea minuta*. Among the various analytical tools, electrophoresis is a relatively simple, rapid and highly sensitive tool to study the properties of proteins and nucleic acids. SDS-PAGE has often been employed to know the biochemical variation, inter and intra specific variation and evolutionary relationships among the plant species (Johnson *et al.*, 2005; Johnson, 2007; Sivaraman *et al.*, 2011; Revathy *et al.*, 2011; Narayani and Johnson, 2013 and Johnson, 2015). With this knowledge the present study was aimed to produce a biochemical marker for the medicinally important pteridophytes using SDS-PAGE.

Materials and Methods

For the electrophoresis studies, 500 mg young individual croziers (Young sporophytes) of *Ampelopteris prolifera* (Retz.)Copel (SAC – 327), *Ophioglossum petiolatum* (SAC – 307) and *Marsilea minuta* L (SAC – 315) were harvested from the natural habitat and established in the Botanic Garden of Department of Botany, St. Andrew's College, Gorakhpur- 273001, Uttar Pradesh, India. The croziers were ground on ice cold mortar and pestle with 0.1 M Tris buffer (pH 7.0). The resultant slurry was centrifuged at 10,000 rpm for 10 min at 4ºC in cooling centrifuge and the supernatant was stored at -70ºC before use. SDS-PAGE was carried out to obtained protein bands with 6% stacking and 10% separating gel (Anbalagan, 1999). After electrophoresis the gel was observed using a Vilber Loubermat gel documentation system and banding profiles of protein was compared by zymogram. After electrophoresis the gel was observed using a Vilber Loubermat gel documentation system and banding profiles of protein was compared by zymogram.

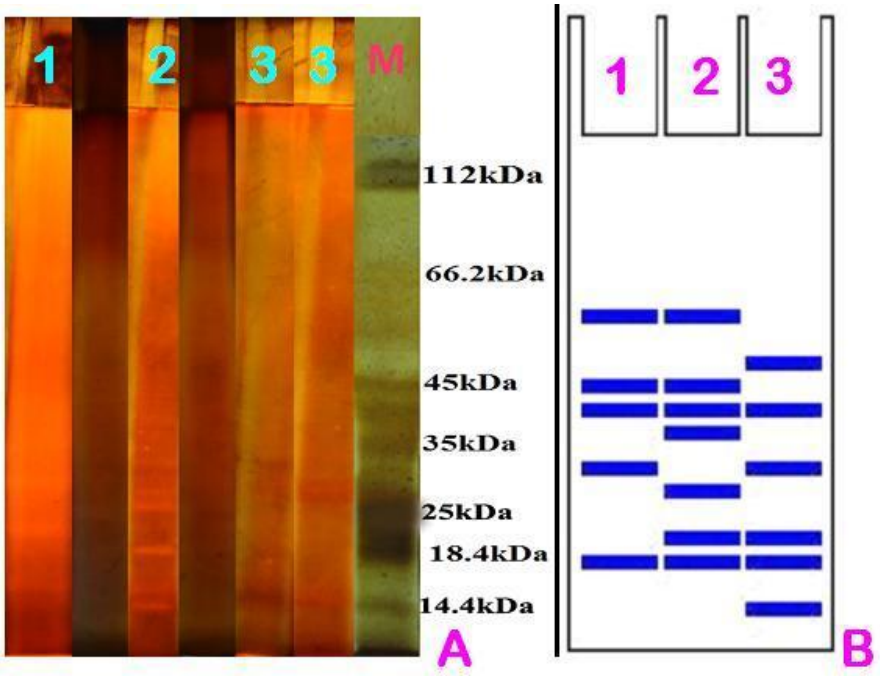
Results and Discussion

The relative positions of the protein bands of the studied ferns viz., *Ampelopteris prolifera, Ophioglossum petiolatum* and *Marsilea minuta* were collected from natural habitat of Gorakhpur India were revealed by SDS-PAGE. Multiple regions (5) of activity were observed from protein electrophoretic system of pteridophytes. A total of 18 bands with 10 varied Rf values were demonstrated in the SDS-PAGE gel system of pteridophytes (Table 1; Fig. 1). Among the three pteridophytes studied, *Marsilea minuta* displayed maximum number of protein bands (7) followed by *Ampelopteris prolifera* (6) and *Ophioglossum petiolatum* (5). The observed protein profile clearly demonstrated the simialrity and variation among the pteridophytes and confirmed the role of protein profile in similarity and variation among the studied pteridophytes. Each region expressed different proteins which act as representative of the expression of a particular gene in the studied pteridophytes.

Table.1 Protein Profile of *Ampelopteris prolifera, Ophioglossum petiolatum* and *Marsilea minuta*

MW-Rf	Positions	*Ophioglossum petiolatum*	*Marsilea minuta*	*Ampelopteris prolifera*
0.56	$PP6^1$	*	*	
0.6	$PP6^2$			*
0.65	$PP7^1$	*	*	
0.69	$PP7^2$	*	*	*
0.73	$PP8^1$		*	
0.79	$PP8^2$	*		*
0.81	$PP9^1$		*	
0.87	$PP9^2$		*	*
0.9	$PP10^1$	*	*	*
0.97	$PP10^2$			*

Fig.1 SDS – PAGE Profile and Zymogram of Adiantum from Gorakhpur
A: 1 - Ampelopteris Prolifera; 2 - Marsilea Minuta; 3 - Ophioglossum Petiolatum
B: 1- Ophioglossum Petiolatum; 2 - Marsilea Minuta; 3 - Ampelopteris Prolifera



Based on the occurrence of proteins in the pteridophytes gel system, the protein profiles were classified into ten regions. The first five regions have failed to show the presence of proteins in the gel system of pteridophytes. The proteins with Rf vlaues 0.6 and 0.97 showed their unique occurrence in *Ampelopteris prolifera*. Similarly the proteins with Rf values 0.73 and 0.81 demonstrated their presence only in *Marsilea minuta*. The *Ophioglossum petiolatum* failed to show its unqueness in

the protein gel system of pteridophytes. The protein with Rf value 0.69 and 0.9 showed their common existence in all the three studied pteridophytes. The proteins with Rf value 0.56 and 0.65 illustrated their jointly presence in the terestrial fern *Ophioglossum petiolatum* and aquatic fern *Marsilea minuta*. The protein with Rf value 0 0.87 dispalyed its occurrence in aquatic fern *Marsilea minuta* and terestrial fern *Ampelopteris prolifera*. The protein with Rf value 0.79 showed its common existence in terestrial ferns *Ophioglossum petiolatum* and *Ampelopteris prolifera*. Similarly Johnson *et al.*, (2005), Johnson (2007), Sivaraman et al. (2011), Revathy et al. (2011), Narayani and Johnson (2013) and Johnson (2015) used the protein profiles as a tool to distinguish the ferns. The results of the present study directly supplemented with the previous observation. These banding profiles can be used as biochemical and pharmacognostical marker to distinguish the medicinally important ferns from its adulterants in the pharmaceutical industries.

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