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Reserch Article

PROTEIN PROFILING OF *Bryophyllum Pinnatum* (LAM.) KURZ. LEAF

ABHISHEK SHARMA*[1], MEETA BHOT[1] AND NARESH CHANDRA[1]

[1]Department of Botany, Birla College, Kalyan 421304, Maharashtra, India. Email: * as220484@gmail.com

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ABSTRACT

Objective: *Bryophyllum pinnatum* (Lam.) Kurz. is a medicinal herb commonly used to treat ulcers, cough, diabetes and cancer. In this study, phosphate extraction buffer (pH) was used to extract proteins from the leaves of *Bryophyllum pinnatum*.

Method: On sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation, the results showed that most of the extracted proteins migrated in the range of 10 kDa to 30 kDa MW. Bands on the gel was then excised and digested with trypsin and subjected to liquid chromatography tandem mass spectrometry (LC/MS/MS) for protein identification.

Result: Proteinase K has been identified from the MS/MS data. The protein identified was Proteinase K, which is used commercially in digesting of unwanted proteins liked keratin.

Keywords: *Bryophyllum pinnatum* (Lam.) Kurz., Proteinase K, LC MS-MS

INTRODUCTION

Bryophyllum pinnatum (Lam.) Kurz., (Crassulaceae) Synonym: *Kalanchoe pinnata* (Lam.) Oken, *Bryophyllum calycinum* Salisb. It is commonly known as Zakham-e-hyat, Life plant, air or maternity plant, love plant, Canterbury bells, Cathedral bells, parnabija. It is a perennial herb growing widely and used in folkloric medicine in tropical Africa, tropical America, India, China and Australia, classified as a weed. The plant flourishes throughout the Southern part of Nigeria. This is the only *Kalanchoe* species found in South America, however, 200 other species are found in Africa, Madagascar, China and Java. A number of species are cultivated as ornamentals and are popular tropical house plants [1].

B. pinnatum is rich in alkaloids, triterpenes, glycosides, flavonoids, cardienolides, steroids, bufadienolides and lipids. The leaves contain a group of chemicals called bufadienolides which are very active. Bufadienolides like bryotoxin A, B, C which are very similar in structure and activity as two other cardiac glycosides, digoxin and digitoxin and possesses antibacterial, antitumor, cancer preventative and insecticidal actions [1].

Currently, the complete genome of a number of plant species has been sequenced [2-4]. However, the functions of many of the identified genes remain unclear. Thus, it is important to shift the focus towards the functional characterization of proteins that are encoded by the cellular genetic machinery [4-5]. The objective of this study is to develop a method for analysis and determination of the proteome of *B. pinnatum*, which has not been studied before. Extraction of proteins from the leaves of *B. pinnatum* revealed the complex proteome of the leaf that drive the biological processes of the plant.

MATERIALS AND METHODS

Samples

Leaves of *Bryophyllum pinnatum* (Lam.) Kurz. plants were collected from the Herbal Garden, Birla College, Kalyan, Maharashtra, India. After washing, the leaves were used for isolation of proteins.

Protein Extraction

20 g of fresh leaves were extracted in buffer comprising of 0.2 M phosphate buffer (pH 7.2), 150 mM sodium chloride (NaCl), 8 M urea, 1% (w/v) 3-(3-cholamidylpropyl)-dimethylammonio-1-propane sulfonate (CHAPS), 10% (v/v) glycerol and 2 mM ethylene diamine tetra acetic acid (EDTA). The mixture was then centrifuged (14, 000 rpm, 25 min, 18°C) and the supernatant was stored at 4°C. This supernatant was further used for gel electrophoresis (SDS-PAGE) for separation of proteins.

Sodium Dodecyl Sulfate -Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Gel electrophoresis (SDS-PAGE) was carried out according to the modified method of Laemmli (1970) [6]. Discontinuous gel of 6.0 cm x 8.4 cm thickness was used and it consisted of 10% resolving gel and 4% stacking gel. 20% (v/v) of buffer sample [0.5 M Tris-HCl, pH 6.8, 20% (v/v) glycerol, 10% (w/v) SDS, 0.05% (w/v) bromophenol blue, 5% (v/v) β-mercaptoethanol] was added in to the leaf extract. A constant voltage (200 volts) was maintained to separate the proteins in the leaf extract during electrophoresis. After the electrophoretic separation, the gel was stained with Coomassie blue.

Stained protein bands migrated in the range of 10 kDa to 30 kDa MW from SDS-PAGE gel were excised and transferred to eppendoff. The excised bands were stored in distilled water and its characterization and identification was carried out by using LC-MS-MS at National Institute of Plant genomic Research (NIPGR), New Delhi.

RESULTS AND DISCUSSION

Figure 1 shows the protein profiles of leaf extracts of *Bryophyllum pinnatum* (Lam.) Kurz. The protein profiles of *Bryophyllum pinnatum* (Lam.) Kurz. indicate that most of the proteins isolated from *B. pinnatum* were of a low molecular weight ranging from 10 kDa to 30 kDa. Protein bands were not detected in the higher molecular weight region.

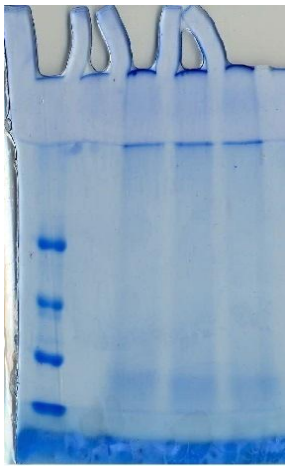
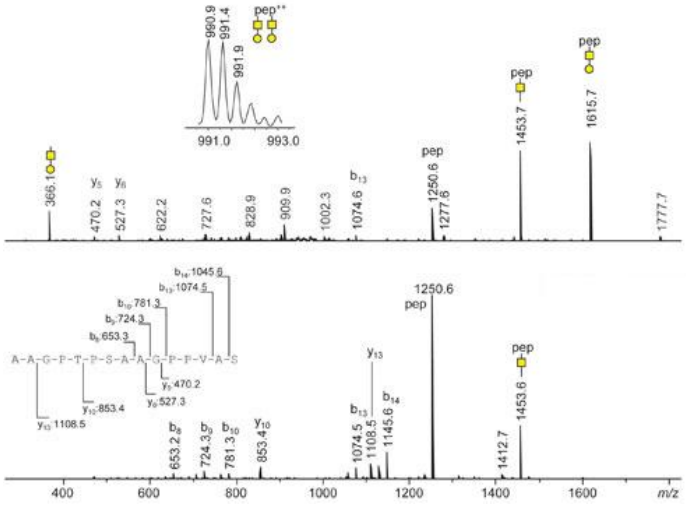


Fig 1: Protein profiles of phosphate buffer extracts of *Bryophyllum pinnatum* (Lam.) Kurz.

From LC MS-MS technique, peptide was **AAQTNAPWGLAR** with mass of 28,647 dalton (Figure 2).



c) **AAQTNAPWGLAR**

Fig. 2: Identification of Proteinase K (a) the MS scan; (b) the MS-MS spectrum of 28,647 molecular ion; (c) the amino acid sequence derived from the MS-MS spectra.

In the present study, a phosphate extraction buffer made up of a mixture of salt, chaotropic agent and detergent was used to extract proteins from *B. pinnatum*. The buffer was used mainly to isolate total cellular protein including the stringent membrane proteins. Nevertheless, due to the high salt content of the buffer, separation of proteins by 2D-gel was not possible, therefore we used SDS-PAGE method for protein separation. Although SDS-PAGE is limited in resolution of protein bands when compared with 2D-gel, it was compensated for by the subsequent LC/MS/MS analysis, whereby multiple proteins in a band could be specifically analyzed.

The phosphate extraction buffer consisted of a chaotropic agent, CHAPS, EDTA, glycerol and sodium chloride. CHAPS is a zwitterionic detergent that was used to improve the solubility of proteins [7,8], an effect that was further enhanced by the addition of NaCl, a chaotropic agent (urea), and glycerol [7]. EDTA acts as metalloprotease inhibitor by chelating Ca^{2+} ions that are required in cell-cell adhesion [8-9].

The medicinal property of *B. pinnatum* is undeniable. Nevertheless, there is no information on the protein profile of the plant as all the research done to date on the plant has concentrated on its small molecules. The detection of Proteinase K from leaves of *B. pinnatum* first time to be isolated by SDS PAGE method.

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