"TOTAL PROTEIN PROFILING OF *ALLAMANDA CATHARTICA* L. AND *THEVETIA PERUVIANA* USING POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS PAGE)"

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Plants are the backbone of life on Earth. The survival of human life on Earth depends on the continued use of wild fauna and flora. The plants provide the fundamental support systems upon which all life depends. Services provided by ecosystems include carbon sequestration, climate regulation, nutrient cycling and pollination. Plants provide us with many direct benefits such as food, medicine, clothes, shelter and the raw materials from which countless other products are made.

In plants various proteins are present. Proteins are found in all cells and carry out a variety of important cellular functions. Protein profiling, an emerging independent sub-specialty of proteomics, is poised to provide unprecedented insight into biological events. Quantitative evaluation of protein levels can be accomplished with protein profiling, which shows us unique expression patterns (diseased vs. healthy, treated vs. untreated, experimental vs. control) at the protein level when proteins from one cell type are compared with those of another cell type. Protein profiling is a potential tool for the early diagnosis of leukemias and other diseases [The importance of protein profiling in the diagnosis and treatment of hematologic malignancies; Sanlı-Mohamed, Taylan Turan, Hüseyin Atakan Ekiz, Yusuf Baran].

Protein expression profiling will make its mark in both high-brow and high-value research markets, including the elucidation of protein-protein interactions and signalling pathways, finding biomarkers for drug discovery and development, serum profiling to identify patient populations that respond to various treatments, and, eventually, medical diagnostics.

Genotypic analysis is used to profile the genetic variation among different populations of higher organisms especially plants. Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment.

Allamanda cathartica L. and *Thevetia peruviana(Pers.)Merr.* are ornamental plants. Both plants are medicinal and poisonous. In both plants all parts of the plant are poisonous but especially the seeds.

Objective of the Study

To analyse the genetic variability between *Allamanda cathartica* L. and *Thevetia peruviana* by analysing the total protein profiling using Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis (SDS PAGE)

Methods and Materials

Reagents for SDS Polyacrylamide gel electrophoresis (SDS PAGE)

Phosphate Buffered Saline (PBS), mortar and pestle, microcentrifuge, -20°C deepfreezer, PAGE gel electrophoresis apparatus, SDS PAGE gel loading dye, staining and destaining solutions

Sample Collection

Plant samples of *Allamanda cathartica* L. and *Thevetia peruviana* were collected from Alappuzha (Figure no. 1). Leaves, seed coat and seed of these samples were collected, properly labelled and stored at 4°C until used for total protein extraction for protein profiling.

1.TOTAL PROTEIN EXTRACTION FROM *ALLAMANDA CATHARTICA L. AND THEVETIA PERUVIANA*

For extraction of total proteins from *Allamanda cathartica* L. and *Thevetia peruviana,* leaves, seed coat and seed from each samples (100mg) were ground with pestle and mortar and resuspended in 1 ml Phosphate Buffered Saline (PBS) buffer. Samples were mixed thoroughly in buffer and kept for 1hr at 4°C. The homogenate samples were centrifuged for 13000 rpm for 10 min at room temperature and the supernatant was collected. The supernatant was transferred to a fresh 1.5 ml vial and stored at 4°C until use. This supernatant was used for SDS PAGE analysis to detect the variation and diversity among the samples.

SODIUM DODECYL SULPHATE POLYACYLAMIDE GEL ELECTROPHORESIS (SDS PAGE)

Preparation of stock solutions

1X running buffer (TGB Tris Glycine Buffer) was prepared by dissolving 3 g Tris base (25 mM), 14.4g of Glycine (190 mM) and 1g SDS (0.1 %) in 900 ml distilled water. The pH of the solution was adjusted to 8.3 and makeup to 1L using distilled water. Acrylamide/bisacrylamide solution (30%) was prepared by adding 14.6g of acrylamide powder and 0.4g of bisacrylamide powder in 50 ml distilled water and stored at 4°C until use. Also, 50 ml each of 1.5 M Tris pH 8.8 and 1 M Tris pH 6.8 were prepared for SDS gel preparation.

Stacking and Resolving gel composition for 15% SDS-PAGE gel

Resolving Gel (1mm plate) 15%	
Water	1.76 ml
30% Acrylamide/Bisacrylamide solution	2.14 ml
1.5M Tris pH	1.4 ml
10% SDS	54 µl
10% APS (freshly prepared)	54 µl
TEMED	5.4 µl

Stacking gel (1mm plate) 8%	
Water	1.82 ml
30% Acrylamide/Bisacrylamide solution	454 µl
1M Tris pH	334 µl
10% SDS	27 µl
10% APS (Freshly prepared)	27 µl
TEMED	2.7 µl

Preparation of slab gels for SDS PAGE

Glass plates were cleaned with 70% ethanol and fixed by using seal gasket and clips, resolving gel solution (15%) was poured into the space between a set of glass plates (upto 2cm from the top) and layered with a small amount of methanol (200-300 µl) to prevent gel surface from air and promote fixation. Ethanol was removed after 10-20 minutes when the gel was fixed. Stacking gel was put on the resolving gel in the remaining space and comb was inserted into the stacking gel. After polymerization, the combs, plastic clips and rubber gasket were removed and the gel was processed for loading samples

Sample loading and electrophoresis

Gel plates were placed in the electrophoresis apparatus carefully to prevent bubbles formation at the bottom of the gel plates. Running buffer was added to the lower tray and then the upper tray was filled with the same buffer. About 10 µl each of the samples (supernatant) and SDS PAGE loading dye was taken in a fresh vial, mixed thoroughly for 30-60 seconds and boiled for about 10 minutes inorder to denature and nuetralise the

charge in the proteins in the sample. After the denaturation, the samples were chilled on ice and then loaded at the bottom of each well using a micropipette. Close the lid of the apparatus tightly and connect the power supply at 100 V until the dye front reached the bottom of the gel plate

Visualization of proteins in the gels

To visualize the protein in the gel, the gel was placed in 40% distilled water, 10% acetic acid, and 50% methanol solution containing 0.25% Coomassie Brilliant Blue R-250 (staining solution) and incubated for 1 hr at room temperature with continuous shaking. The gel was then transfered to a mixture of 40% distilled water, 10% acetic acid,and 50% methanol (detaining solution) and incubated room temperature with continuous shaking until the excess dye has been removed. As a result, the proteins in the gel can be seen as deep blue bands (Figure no. 2).

Data analysis

Electrophoregrams for each sample were scored and the presence (1) or absence (0) of each band was noted (Table no. 1). Presence and absence of bands were entered in a binary data matrix. Based on electrophoresis band spectra, Jaccard's similarity index (JSI) was calculated by the formula:

$S=W/(A+B-W)$

Where W is the number of bands of common mobility, A is the number of bands in type A and B is the number of bands in type B. The similarity matrix generated (Table no. 2) was used to identify the genetic diversity among the different isolates.

Results

In the present study Plant samples of *Allamanda cathartica* L. and *Thevetia peruviana* were collected from Alappuzha district in Kerala (Figure no. 1).

Sample Collection

Figure no. 1: Plant samples collected from Alappuzha for the study. A-.*Thevetia peruviana* plant, B-*Allamanda cathartica* L. plant, Seed and seed coat of *Thevetia peruviana.*

TOTAL PROTEIN EXTRACTION FROM *ALLAMANDA CATHARTICA L. AND THEVETIA PERUVIANA*

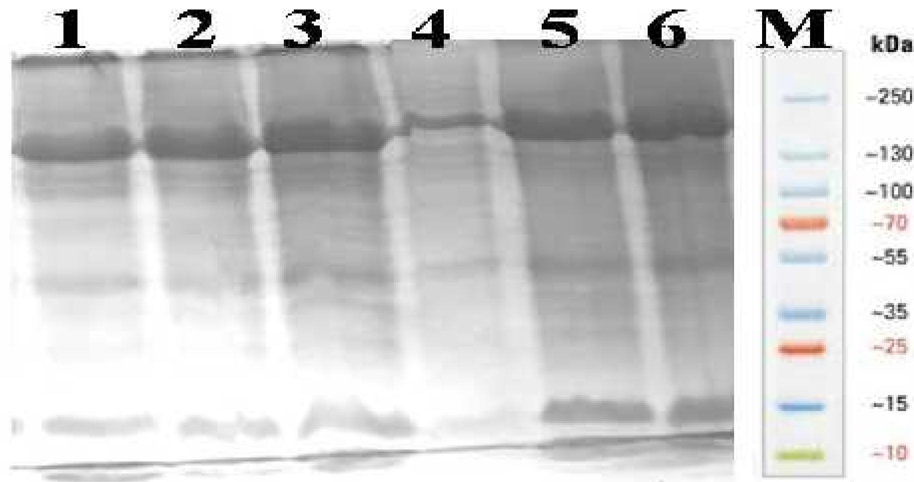

Figure no. 2: Electrophorogram of 15% polyacrylamide gel banding pattern showing diversity of total proteins in *Thevetia peruviana* and *Allamanda cathartica* L. 1-leaf proteins of *Thevetia peruviana*, 2- seed proteins of *Thevetia peruviana*, 3- seed coat proteinsof *Thevetia peruviana*, 4- leaf proteins of *Allamanda cathartica*, 5- seed proteins of *Allamanda cathartica*, 6- seed coat proteins of *Allamanda cathartica,* M-Prestained protein marker (Thermo scientific)

Band no.	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
1	1	1	1	1	1	1
2	1	1	1	1	1	1
3	1	1	1	1	1	1
4	1	1	1	1	1	1
5	1	1	1	1	1	1
6	1	1	1	0	1	1
7	1	1	1	0	1	0
8	1	1	1	1	1	1

Table no. 1: **Data matrix created using SDS PAGE banding pattern in *Thevetia peruviana* and *Allamanda cathartica* L.** 1-leaf proteins of *Thevetia peruviana*, 2- seed proteins of *Thevetia peruviana*, 3- seed coat proteinsof *Thevetia peruviana*, 4- leaf proteins of *Allamanda cathartica*, 5- seed proteins of *Allamanda cathartica*, 6- seed coat proteins of *Allamanda cathartica.*A total of 8 bands were obtained on SDS PAGE of total protein analysis of *Thevetia peruviana* and *Allamanda cathartica* L., among which bands 6 and 7 were absent in sample 4, but present in all other samples. Also band 7 is absent in sample 6 but present in all others indicating the diversity of samples 4 and 6 with others. Therefore, as a preliminary analysis, we can conclude that *Allamanda cathartica* L total proteins differ with that of *Thevetia peruviana*.

matrix file input						
	sample 1	sample 2	sample 3	sample 4	sample 5	sample 6
sample 1	1	1	1	0.75	1	0.75
sample 2	*	1	1	0.75	1	0.75
sample 3	*	*	1	0.75	1	0.75
sample 4	*	*	*	1	0.75	0.714
sample 5	*	*	*	*	1	0.75
sample 6	*	*	*	*	*	1

Table no. 2**: Jaccard's similarity co-efficients of the SDS PAGE bands for *Thevetia peruviana* and *Allamanda cathartica* L.** 1-leaf proteins of *Thevetia peruviana*, 2- seed proteins of *Thevetia peruviana*, 3- seed coat proteinsof *Thevetia peruviana*, 4- leaf proteins of *Allamanda cathartica*, 5- seed proteins of *Allamanda cathartica*, 6- seed coat proteins of *Allamanda cathartica.*Jaccard's similarity matrix was prepared based on SDS PAGE data of *Thevetia peruviana* and *Allamanda cathartica* L. and Jaccard's similarity co-efficients of the bands for *Thevetia peruviana* and *Allamanda cathartica* L were calculated (Table no. 2). A maximum similarity value of 100% was observed between samples 1, 2 and 3 followed by 75% between samples 4 and 1, 4 and 2, and 4 and 3, followed by 71.4% between samples 4 and 6. An average similarity value observed across the each samples was 70%.

Conclusion

Genotypic analysis is used to profile the genetic variation among different populations of higher organisms especially plants. Two plant species *Thevetia peruviana* and *Allamanda cathartica* L. were used in this study to analyze the genetic diversity between these species. Samples of *Thevetia peruviana* and *Allamanda cathartica* L. were collected from Alappuzha alappuzha district of Kerala. Sodium Dodecyl Sulphate Polyacrylamide Agarose Gel Electrophoresis analysis was used to study the genetic variation among these species

SDS-PAGE results showed that there is not much diversity observed between the three samples used for the study. Although some variations were noted in the density of some common bands especially band no. 6 and 7 in leaves and seed coat of *Allamanda cathartica* L., most of the bands were similar. From a total of 8 bands obtained on SDS PAGE of total protein analysis of these samples, bands 6 and 7 were absent in sample 4, but present in all other samples. Also band 7 is absent in sample 6 but present in all others indicating the diversity of samples 4 and 6 with others. Based on the Jaccard's similarity matrix created using the SDS bandng pattern, it was identified that Thevetia *peruviana* and *Allamanda cathartica* L samples were genetically similar with an average of 70% similarity value revealing that very low genetic distance at protein level reflecting the similarity of genes responsible for the total proteins. Further investigation is necessary to identify much variations and diversity among these two species

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