SDS-PAGE Analysis of Protein Extracts From *Hybanthus enneaspermus* (L.) F. Muell: Regional Variations In Molecular Profiles

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ABSTRACT
Belonging to the Violaceae family, *Hybanthus enneaspermus* (L.) F. Muell is a petite perennial herbaceous plant found in tropical and subtropical regions worldwide, renowned for its medicinal applications. In this current investigation, the focus was on isolating proteins from this plant in four distinct regions (Marthandam, Petchiparai, Nagercoil, and Chothavilai) using SDS-PAGE. Proteins, being a class of biological macromolecules, present well-characterized physico-chemical properties compared to nucleic acids, simplifying their extraction and characterization. The research highlighted a higher protein concentration in the Marthandam region (2.63 mg/ml of the sample). SDS-PAGE analysis revealed diverse protein bands with varying molecular weights across the four regions, shedding light on the regional variations in the protein profile of *Hybanthus enneaspermus* (L.) F. Muell.

KEYWORDS: *Hybanthus enneaspermus* (L.) F. Muell, SDS Page, Proteins, Gel electrophoresis

INTRODUCTION

Proteins, essential macromolecules in living organisms, exhibit diverse functions, serving as structural components, enzymes catalyzing biochemical reactions, and regulators of various cellular processes**.** With an exceptionally large molecular weight, proteins qualify as macromolecules. These polymers are essentially chain-like structures created by linking numerous small amino acid units, often referred to as monomers, thus establishing amino acids as the fundamental "building blocks of proteins." In the majority of organisms, proteins constitute over half of the dry weight, emerging as the predominant intracellular macromolecules. The structure and functioning of living matter are heavily contingent on proteins, as they play a crucial role in every facet of the chemical and physical processes that characterize a cell's existence.

Plant protein characterization may be done using gel electrophoresis. For taxonomic investigations of plants, animals, microbes, and viroids, protein identification employing electrophoresis is important and helpful (Van den Berg, 2002; Arrel & Kalman, 2016). In the study on proteins and nucleic acids, polyacrylamide gel electrophoresis (PAGE) is a strong analytical method. Two-dimensional and one-dimensional gel electrophoresis are the two most common forms in use today. SDS-PAGE, the most used electrophoresis method for separating proteins, is a kind of one-dimensional PAGE and incorporates it. By clogging the pores of the gel, polysaccharides can hinder SDS PAGE. By interacting electrostatically with proteins, nucleic acids can bind them and stop a fluid gel run. Additionally, higher-molecular-weight nucleic acids might obstruct the acrylamide matrix's pores. The symmetrical charge distribution produced by SDS (Sodium dodecyl sulphate) may be disrupted by other substances, such as endogenous ions, nucleotide metabolites, and phospholipids, which are present in cell lysates and are frequently negatively charged (Wu *et al*., 2014). A successful protein extraction from plant tissues thus requires the removal of phenolics and other disruptive substances.

MATERIALS AND METHODS

1. Protein Extraction: (Ernst O & Zor T, 2010)

Prepare the buffer by combining 100 mM Tris-Cl (pH 6.8), 4% (w/v), SDS (sodium dodecyl sulfate), and 200 mM DTT (dithiothreitol). Store the SDS gel-loading buffer without DTT at room temperature and add DTT from a 1 M stock just before using the buffer.

To initiate the protein extraction process, first, obtain ice and use it to chill both the extraction buffer and the mortar and pestle. Clearly label two 1.5 ml microcentrifuge tubes for each plant tissue intended for protein isolation, specifying the name of the respective plant. Next, finely cut 1 g of fresh leaf from each plant using a razor blade and place it into a pre-

chilled mortar containing approximately 1 ml of cold extraction buffer. Grind the plant material thoroughly with a pestle, emphasizing the importance of achieving a smooth consistency. Maintain the cold temperature of the mortar, pestle, and plant tissue throughout the grinding process. Transfer 1 ml of the resulting slurry into a labelled 1.5 ml microfuge tube using a clean spatula, placing the tube on ice. Repeat this process for each plant tissue designated for protein isolation. Subsequently, spin the samples at top speed in a microfuge at 4 degrees Celsius for 15 minutes. Carefully transfer the liquid supernatant into a new microfuge tube, keeping it on ice until the Bradford Protein Assay is completed. Finally, freeze the samples once the assay is finished, ensuring proper preservation for subsequent analyses.

2. SDS- PAGE (Laemmli, 1970)

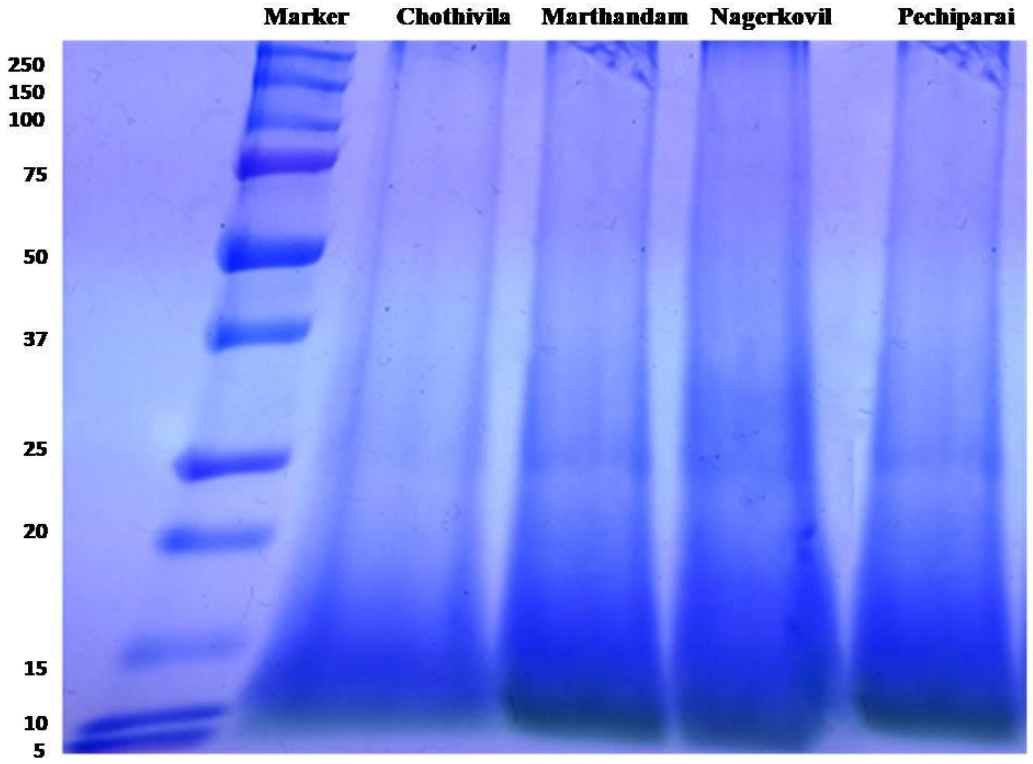
SDS-PAGE analysis was performed using the discontinuous buffer system (Laemmli, 1970) on mini gels (10 cm x 8 cm). Begin by preparing the gel with the appropriate acrylamide/bis-acrylamide ratio based on the target protein size range. After polymerization, mix protein samples with SDS sample buffer, denature them by heating, and load them into the gel wells. Run electrophoresis at a constant voltage until the dye front reaches the gel bottom. Disassemble the gel apparatus, stain with Coomassie Brilliant Blue, and destain for band visualization. Optionally, transfer proteins to a membrane for Western blotting. Block the membrane, incubate with primary and secondary antibodies, and detect bands using chemiluminescence. This process allows the separation and visualization of proteins.

RESULTS

Table 1: Amount of Proteins present in a sample

Sl. No.	Sample	Avg.OD at 595 nm	Concentration protein in mg/ ml of sample
1	Chothavilai	0.050	0.8
2	Marthandam	0.158	2.63
3	Nagercoil	0.065	1.08
4	Petchiparai	0.125	2.08

Fig 1: SDS-PAGE



Lane 1: Marker, Lane 2: Chothivilai, Lane 3: Marthandam, Lane 4: Nagercoil, Lane 5: Pechiparai

Table 2: Bands obtained from protein extracts of *Hybanthus enneaspermus* (L.) F. Muell

ROI 1: Lane 1 Marker							
Band No.	Band Name	Rf	Band Area (Pixel)	Mol. Weight (bp)	Band Volume	Portion of Bands (%)	Cal. Band Volume (ng)
1	Band 1	0.01	834	250.00	59,145	3.72	N/A
2	Band 2	0.05	1,946	150.00	134,721	8.48	N/A
3	Band 3	0.11	2,502	100.00	172,901	10.88	N/A
4	Band 4	0.16	1,529	75.00	125,330	7.89	N/A
5	Band 5	0.30	1,668	50.00	141,295	8.89	N/A
6	Band 6	0.41	1,529	37.00	107,298	6.75	N/A
7	Band 7	0.59	1,807	25.00	129,991	8.18	N/A
8	Band 8	0.70	2,224	20.00	155,783	9.80	N/A
9	Band 9	0.86	1,946	15.00	177,198	11.15	N/A
10	Band 10	0.95	1,668	10.00	205,056	12.90	N/A
11	Band 11	0.98	1,529	5.00	180,336	11.35	N/A
ROI 1: Lane2 Chothavilai							
Band No.	Band Name	Rf	Band Area (Pixel)	Mol. Weight (bp)	Band Volume	Portion of Bands (%)	Cal. Band Volume (ng)
1	Band 1	0.58	756	25.67	47,331	11.75	N/A
2	Band 2	0.90	1,080	13.32	151,426	37.58	N/A
3	Band 3	0.94	1,512	10.60	204,219	50.68	N/A
ROI 1: Lane3 Marthandam							
Band No.	Band Name	Rf	Band Area (Pixel)	Mol. Weight (bp)	Band Volume	Portion of Bands (%)	Cal. Band Volume (ng)
1	Band 1	0.58	890	25.56	71,513	17.06	N/A
2	Band 2	0.92	2,136	11.63	347,581	82.94	N/A
ROI 1: Lane4 Nagercoil							
Band No.	Band Name	Rf	Band Area (Pixel)	Mol. Weight (bp)	Band Volume	Portion of Bands (%)	Cal. Band Volume (ng)
1	Band 1	0.60	910	24.89	81,939	41.67	N/A
2	Band 2	0.94	728	10.90	114,682	58.33	N/A
ROI 1: Lane 5 Petchiparai							
Band No.	Band Name	Rf	Band Area (Pixel)	Mol. Weight (bp)	Band Volume	Portion of Bands (%)	Cal. Band Volume (ng)
1	Band 1	0.58	1,089	25.67	83,540	14.94	N/A
2	Band 2	0.67	990	21.18	84,549	15.12	N/A
3	Band 3	0.91	2,376	12.77	391,069	69.94	N/A

Analysis of Table 1 reveals notable variations in protein content across different regions. Marthandam region exhibited the highest protein concentration at 2.63 mg/ml, followed by Petchiparai region with 2.08 mg/ml and Nagercoil region with 1.08 mg/ml, while the lowest protein content was observed in Chothavilai region. Further insights from Table 2 highlight distinctive protein band patterns in each region. Chothavilai region displayed three bands with molecular weights of 25.67, 13.32, and 10.60, Marthandam region exhibited two bands at 25.56 and 11.23, Nagercoil region displayed three bands with molecular weights of 24.89 and 10.90, and Petchiparai region exhibited three bands with molecular weights of 25.67, 21.18, and 12.77, providing valuable information about the protein profile variations in these geographical locations.

DISCUSSION

Constituting over half of the dry weight in the majority of organisms, proteins stand as the predominant intracellular macromolecule. Their pivotal role in the structure and functioning of biological matter is undeniable, influencing every facet of the chemical and physical processes defining the existence of a cell. Proteins are intricately linked to each stage of the cell's life, contributing significantly to its overall functionality and activities. Various workers estimated the amount of protein in various medicinal plants such as *Citrullus colocynthis* (Meena, 2021); *Bacopa monnieri* (Mohapatra & Rath, 2005); *Berberis lyceum*, *Foenicum vulgare* and *Justicia adhatoda* (Uma *et al*., 2006). Variation in protein content is also considered as an important biochemical event during growth and differentiation of cells (Audichya, 1999). The present study showed amount of proteins in leaf samples of *Hybanthus enneaspermus* in four different regions such as Marthandam, Petchiparai, Nagercoil and Chothavilai. Meena, 2021 found out that leaf showed higher protein in *Citrullus colocynthis*. Vijayvergia & Kumar (2007) also reported maximum protein in leaf of *Nerium indicum* as compared to other intact plant parts. Of the four study areas, more amount of proteins were found in samples of Marthandam region followed by Petchipari, Nagercoil and Chothavilai region.

Gel electrophoresis proves to be a valuable tool for the characterization of plant proteins. This technique is used to study protein diversity in variety of plants such as *Triticum aestivum* (Stoddard and Marshall, 2005); *Oryza sativa* (Nozu *et al.,* 2006) etc. Distinct protein banding patterns were observed in *Hybanthus enneaspermus* (L.) F. Muell with Chothavilai region displaying three bands at molecular weights of 25.67, 13.32, and 10.60, Marthandam region exhibiting two bands at 25.56 and 11.23, Nagercoil region showing three bands with molecular weights of 24.89 and 10.90, and Petchiparai region featuring three bands at 25.67, 21.18, and 12.77, indicating regional variations. Additionally, variations in the intensity of protein bands were noted across samples from different regions, further emphasizing the diversity in the protein profiles of *Hybanthus enneaspermus* (L.) F. Muell

SUMMARY AND CONCLUSION

The SDS-PAGE analysis of protein extracts from different regions of *Hybanthus enneaspermus* revealed significant regional variations in molecular profiles. The investigation identified distinct protein banding patterns, with the Chothavilai region displaying three bands at molecular weights of 25.67, 13.32, and 10.60, Marthandam region exhibiting two bands at 25.56 and 11.23, Nagercoil region showing three bands with molecular weights of 24.89 and 10.90, and Petchiparai region featuring three bands at 25.67, 21.18, and 12.77. These findings indicate a diverse protein composition across different geographical locations. Moreover, variations in the intensity of protein bands were observed, underscoring the complexity and uniqueness of protein profiles in each region. The study sheds light on the regional heterogeneity of protein expression in *Hybanthus enneaspermus*, contributing valuable insights for further research into the plant's molecular characteristics and potential applications in diverse fields.

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REFERENCES

1. Arrel, SM & Kalman, F 2016, 'Estimation of protein concentration at high sensitivity using SDS-capillary gel electrophoresis-laser induced fluorescence detection with 3-(2-furoyl) quinoline-2-carboxyaldehyde protein labelling', Electrophoresis, 37(22): 2913-2921.
2. Audichiya, M 1999, 'Regeneration studies on oil containing seed through tissue culture', Ph.D. Thesis, University of Rajasthan, Jaipur.
3. Ernst O & Zor T 2010, 'Linearization of the bardford Protein assay'.*J.Vis Exp*.1 (38):1918.
4. Laemmli 1970, 'Cleavage of structural proteins during the assembly of the head of bacteriophage T4', Nature, 22: 680.
5. Meena, MC 2021, 'Estimation of proteins profile by gel electrophoresis (SDS-PAGE) in *Citrullus colocynthis* (Linn.) schrad, *in vivo* and *in vitro.* Vol. 12(4) pp. 01-06.
6. Mohapatra, HP & Rath, SP 2005, '*In vitro* studies of *Bacopa monnieri*- an important medicinal plant with reference to its biochemical variations', Indian J. Exp. Biol., 43(4): 373-376.
7. Nozu, Y, Tsuquita, A & Kamijo, K 2006, 'Proteomic analysis of rice, stem and root tissues during growth course', Proteomics, 6(12): 3665-70.
8. Stoddard, FL & Marshall, DR 2005, 'Variability in grain protein in Australian hexaploidy wheats', Aust. J. Agri. Res., 41(2): 277-288.
9. Uma, V, Kanoogo, N & Tikku 2006, 'Extraction and purification of various organic compounds in selected medicinal plants from Rajasthan', in: Proc. of International Conference Bot. Expo., 25-27 March, Jaipur, 294-296.
10. Van den Berg, C 2002. 'A phylogenetic analysis *Laelinae* (Orchidaceae) based on a sequence data from internal transcribed spacers (ITS) of nuclear ribosomal DNA', Lindleyana, 15: 96-144.
11. Vijayvergia, R & Kumar, J 2007, 'Quantification of primary metabolites of *Nerium indicum* Mill.' Asian Journal of Experimental Science, 21: 123-128.