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Protein profiling of some lichens by using SDS-Page

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Abstract:

. This investigation elucidated the protein composition of four lichen species – *Lecanora argopholis, Diploschistes muscorum, Xanthoria soridiata,* and *Xanthoria alegan*s – employing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with Coomassie Brilliant Blue (CBB) staining. The results demonstrated species-specific protein bands with distinct molecular weights: Lecanora argopholis (35 kDa), *Diploschistes muscorum* (30 kDa), *Xanthoria soridiata* (24 kDa), and *Xanthoria alegans* (25 kDa and 40 kDa). These findings provide valuable insights into the protein diversity among lichen species, underscoring potential applications in ecological and biotechnological research.

Keywords: lichen

Keywords **: species, protein profiling, SDS-PAGE, molecular weight, species-specific markers.**

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I. INTRODUCTION

Lichens include a variety of biomolecules, including polysaccharides, phenolic, pigments, terpenes, hormones, aliphatic acids, secondary metabolites and phenolic compounds, according to data from the literature [1]. According to data, almost ten thousand secondary metabolites of lichens have been recognized [2]. For many years, natural substances produced from lichens, fungus and plants have served as an origin of anticancer substances for cancer therapies [3]. The useful substances derived from biological creatures are often cheap, abundant in nature and have few negative consequences when used. Usnic acid is a familiar natural product from therapeutic lichens that has potential as a cancer therapy [4]. Lichen proteins are thought to have special biochemical characteristics and have the potential to physiologically active molecules [5]. Recent developments in oxidative enzymes for the UVB protection, pulp industry, proteins and antibiotics with anti-prion action, as well as novel candidate molecules for pharmaceutical proteins from symbiotic cyanobacteria and antifreeze proteins for frozen foods have been discovered [6,7]. Keeping in view the uses of lichen in various industries and less study on protein content of lichen there is a need to extract and investigate protein contents from lichens that will serve as a base for further studies. Despite the uses of lichen in various industries, their protein content has not been studied extensively. We aim to establish An SDS-PAGE profile of lichen proteins to collect preliminary data and make the base for future studies on lichen protein profiling. To establish and optimize the protein extraction method from selected lichens To investigate protein contents in selected lichens. Identification of lichen proteins using SDS-PAGE.

II. MATERIAL AND METHOD

1. Sample collection

June and September is ideal for field collection. Lichens along with the substrate were collected, labelled, photographed and stored and transported to the department/lab in re-sealable polythene bags [8].

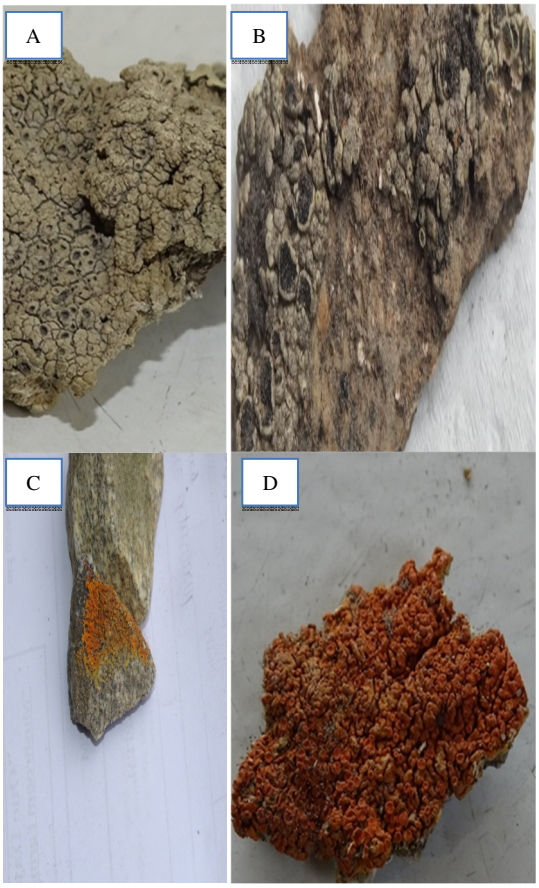

Fig. 1 *Diploschistes muscorum*, (B) *Lecanora argopholis*, (C) *Xanthoriasoridiata*, (D) *Xanthoria alegans*.

2. Sample Preparation for protein extraction

The lichen thalli were washed more than once with 25ml per gram acetone and air dried. The lichen material was homogenized into a powder using liquid nitrogen in a Pestle and Mortar. Next, this powder was amended with 100mg of polyvinylpolypyrrolidone (PVPP), 4% ascorbic acid and 1ml of extraction solution (1:10 weight: volume) containing 5mM EDTA and 2mM phenylmethylsulfonyl fluoride (PMSF).
The samples were stirred at 180 rpm for 2 hours at 15^{0}C and centrifuged at 10,000 rpm for thirty minutes. The supernatant was collected in a 2ml Eppendorf tube and palette discarded. Supernatant was centrifuged again to make it clear. Trichloroacetic Acid (TCA) was used to precipitate the proteins in the resulting extracts at 12.5% final concentration. After that, the protein precipitates werethricerinsed in acetonethen placed in a minimum amount of 0.1Molar phosphate-buffered saline (PBS)pH 7.4or in sample buffer [9].

Fig. 2 *Grinding of lichen with liquid nitrogen*

3. SDS-PAGE

The acrylamide (14.2g) and bis-acrylamide (0.4g) were taken and dissolved in 50ml water and filtered and stored at 4^{0}C for future use. The Tris-base (12g), Glycine (57.6g) were taken and dissolved in 40ml of SDS with 4 liter deionizes water. The pH was adjustedat 8.6 andstored at 4^{0}C. The staining solution was prepared using Coomassie brilliant blue dye, 250g stock (2%, methanol 250ml, distilled water 500ml and glacial acetic acid 50ml.

The de-stain 1 was prepared using glacial acetic acid 50ml, methanol 250ml and distilled water up to 500ml. Methanol (25ml) and glacial acetic acid (35ml) were used with up to 500ml distilled water for the formation of de-stain 2. For the preparation of sample buffer, stacking gel buffer of volume 2.5 ml was initially dissolved in 4ml of SDS (10%), Glycerol (2ml), beta-mercaptoethanol (1ml) and 2 drops methylene blue were then added and the final volume was made up to 10ml by adding distilled water.
The mixture of resolving gel buffer contained 18.15g of Tris base dissolved deionized water. The pH was adjusted at 8.8. Distilled water was added to the remaining capacity to bring it upto 100ml. An amount of 6.00g of Tris base was dissolved in 85ml of distilled water and the total volume was made upto 100mlwith pH 6.8. The mixture of resolving gel contained 12.5ml of monomer stock, 7.5ml of resolving gel buffer, 200µl of ammonium persulfate, 0.3ml of SDS (10%), 15µl TEMED with distilled water up to 30ml. For making stacking gel, 1.33ml of monomer stock was added into 0.1ml of SDS (10%) and 2.5ml of stacking gel buffer. 50µL of ammonium persulfate (10%) and 10µL of TEMEDwere added before adding distilled water to make the final volume up to 10ml. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 12% acrylamide gels containing 0.2% bisacrylamide using the buffer system. The SDS-PAGE was run at 110/120V for 50–60 minutes and stained with Coomassie brilliant blue staining Gel was stained in Coomassie brilliant blue dye for 2 to 3 hours [9]. Two methods were applied for de-staining the gel. It was soaked in concentratedsolution for 2 hours or soaked usually overnight. Different sizes and patterns of protein bands were visualized after destaining the gel.SDS-PAGE bands were compared with the protein ladder that was simultaneously run along with sample.

This comparison provided an estimation of size of the proteins present in the lichen sample.

Fig .3*SDS-PAGE apparatus with running gel.*

III. RESULT

The SDS-PAGE was used to separate proteins according to their molecular size and no other physical features. There was information on how to extract proteins from lichen. For this purpose 0.1 Molar phosphate-buffered saline (PBS), pH 7.4 was used to produce protein extracts from the lichen *of Lecanora argopholis*. SDS-PAGE was used to differentiate proteins band according to their molecular weight.

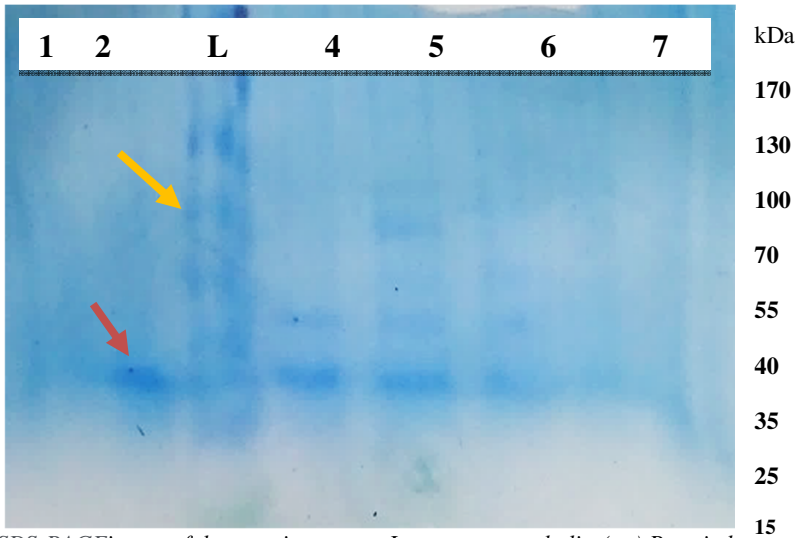

Fig .4 *(→) shows SDS-PAGEimage of the protein extracts Lecanora argopholis. (→) Protein ladder PageRuler™ Prestained Protein Ladder, 10 to 180 kDa.*

One less distinct band measuring of 35 kDa in *Lacanora argopholis* according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis(SDS-PAGE). The molecular weight of band was about 35 kDa with CBB staining.

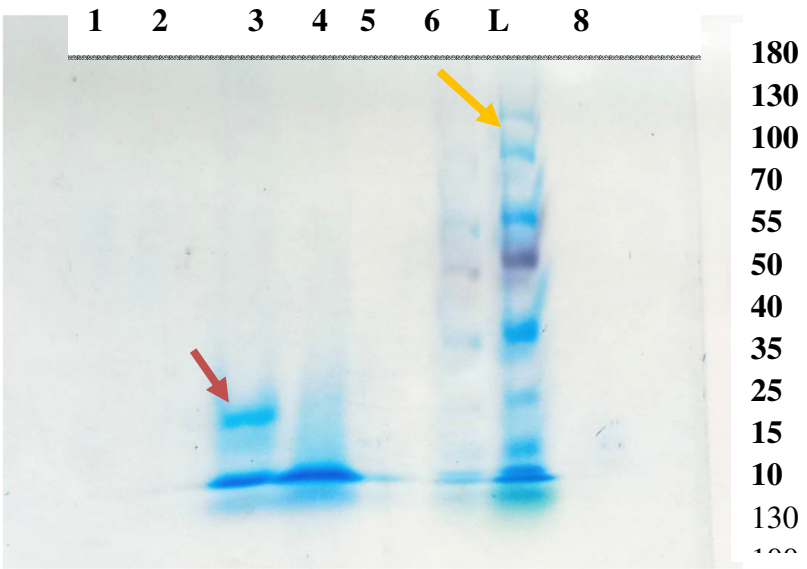

Fig. 5 *(→) shows SDS-PAGE image of the protein extracts Xanthoria soridiata. (→) Protein ladder PageRuler™ Prestained Protein Ladder, 10 to 180 kDa.*

One distinct band measuring of 24 kDa in *Xanthoria soridiata* according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight of band was about 24 kDa with CBB staining.

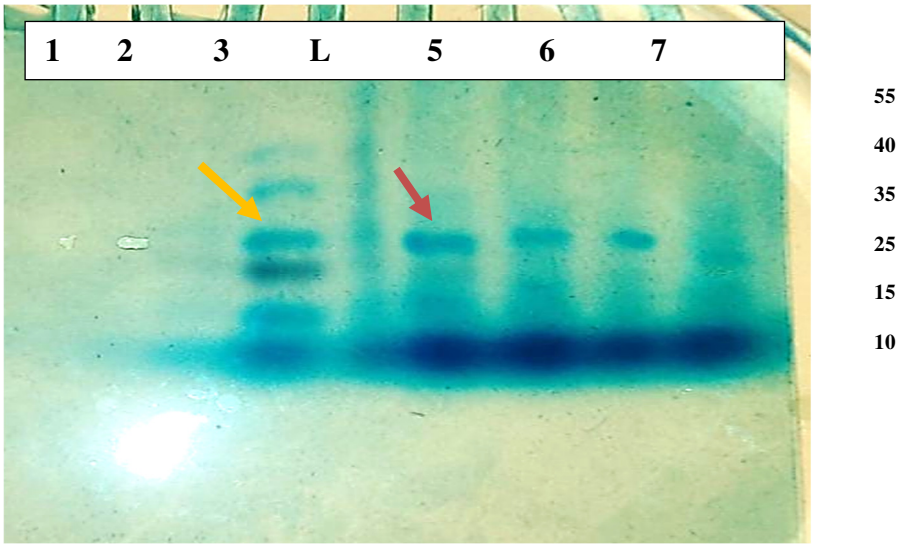

Fig .6 *(→) shows SDS-PAGE image of the protein extracts Diploschistes muscorum. (→) Protein ladder PageRuler™ Prestained Protein Ladder, 10 to 180 kDa.*

One distinct band measuring of 30 kDa in*Diploschistes muscorum*according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight of band was about 30kDa with CBB staining.

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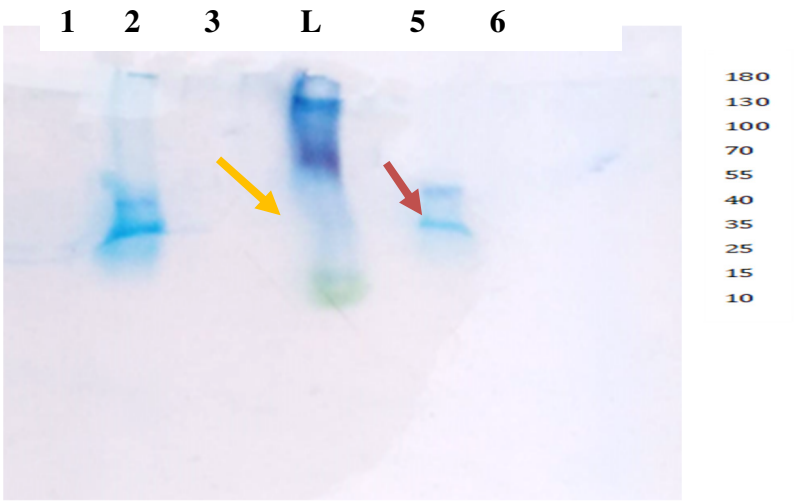

Fig.7 (→) *shows SDS-PAGE image of the protein extracts Xanthoria alegans. (→) Protein ladder PageRuler™ Prestained Protein Ladder, 10 to 180 kDa.*

Two protein bands measuring between 25kDa and 40kDa in *Xanthoria alegans* according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight of band was about 25kDa and 40kDa with CBB staining.

Diploschistes muscorum	30kDa	
Lecanora argopholis	35kDa	
Xanthoria soridiata	24kDa	
Xanthoria alegans	25kDa	40kDa

Table 1: *Protein profiling of lichens according to SDS-PAGE analysis.*

IV. DISCUSSION

The protein bands observed in our study revealed the presence of protein bands in selected lichens species. We analyzed 4 species of lichens using SDS-PAGE with CBB staining and detected different protein bands with different molecular weight in each sample.

The result of our study indicate the presence of different protein bands of different molecular weight such as *Lecanora argopholis* showed protein band with molecular weight 35kDa according to SDS-PAGE, *Diploschistes muscorum* showed protein band of 30kDa and *Xanthoria soridiata* exhibited band of 24kDa, while *Xanthoria alegans* exhibited two protein bands at 25kda and 40kDa.

The research employed a Na3PO4 solution to achieve the greatest protein amountin the lichen sample. Therefore, it was determined that a PBS system and components that effectively reduce the influence of phenolics and their derivatives in the extract should be included in a perfect extraction solution. High-quality protein bands should be able to separate using the approach.PAAG.Several polymers, like polyvinylpyrrolidone and PVPP that are water insoluble, can be used to extract phenolic chemicals. The attempt to use PVP-40 in the lichen protein extraction process was unsuccessful due to the formation of an excessively thick and insoluble precipitate during TCA precipitation. However, the use of PVPP did not affect its properties in the presence of TCA ,allowing for its centrifugation out of the protein extract. Ascorbic acid contributes to the reduction of quinones, leading to their conversion into phenols. When 1% PVPP was combined with an appropriate amount of

ascorbic acid, it resulted in colorless extracts with improved resolution on the PAAG, surpassing the outcomes achieved when these components were used separately [9] (Kondratiuk *et al*. 2015).

Comparing our result to previous study, the enzyme's obvious purity was validated by SDS-PAGE analysis, which revealed a single polypeptide chain structure with a molecular weight of 19 kDa. This molecular weight roughly resembles the measured molecular weights of 18,092 and 18,628 in S. g*riseus*, albeit being slightly lower than subtilisins, which have molecular weights between 22,700 and 27,400 proteases of serine [10].

After denaturation using SDS-PAGE, several protein bands were observed. These included a prominent band of approximately 37 kDa in size, as well as two less distinct bands measuring 35 and 32 kDa. No bands were detected indicating proteins larger than 116 kDa, which was the largest protein standard used in the experiment.The zymogram analysis and SDS-PAGE results together show that the enzyme is probably an oligomeric protein made up of six monomers. This is noteworthy because most laccases are typically monomeric proteins, with fungal laccases ranging from 60 to 70 kDa and plant laccases having a molecular mass around 100 kDa [11].

The researchers provided evidence that two lichens from the Peltigerales order possess two distinct types of laccase enzymes. One of these enzymes stands out due to its exceptionally high molecular weight (MW). Through the utilization of SDS-PAGE electrophoresis, the authors verified that the "large" Sc1 and Pa1 laccases are proteins consisting of two identical units. Previous research primarily indicated that fungal laccases are single-unit proteins with MW ranging from 60 to 80 kDa. Nevertheless, a recent investigation reported the laccase found in the lichen Peltigera malacea as a protein comprising four similar units with a MW of 340 kDa [12] (Laufer *et al*., 2006). Certain researchers have also observed fungal laccases composed of multiple subunits, such as in ascomycetes like Podospora anserine, which has a tetramer MW of 390 kDa and a subunit MW of 80 kDa [13] .

To better understand the impact of altitude, we conducted a comparative study between lichens collected at 1800 m and 750 m. We deliberately chose a larger difference in elevation to uncover any subtle changes that might be missed when considering smaller intervals. By employing SDS-gels and conducting densitometric scans, we were able to demonstrate that the protein bands with molecular weights of 29 kDa, 48 kDa, and 64 kDa showed a higher concentration at 750 m compared to 1800 m. Therefore, we can observe a decrease in the protein content of Pseudevernia furfuracea var. ceratea as altitude increases [14.

In conclusion, our study highlights the importance of carefully selecting conditions to achieve maximum efficiency in protein extraction for each specific lichen species, particularly with regard to the quantity of pH, molarity of the buffer solution and ascorbic acid.

V. CONCLUSION

This study successfully optimized the extraction and profiling of proteins from lichens, revealing distinct expression patterns through SDS-PAGE analysis. The presence of unique protein bands underscores the biochemical richness of lichens and their potential as an alternative protein source. These findings open new avenues for utilizing lichen-derived proteins in nutritional, pharmaceutical, and industrial applications, particularly for those seeking sustainable, plant-based alternatives.

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VII. REFRENCES

PAGE LAYOUT

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