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Research Article

Protein Profiling of Selected Medicinal Plants of Central Karakoram National Park (CKNP), Gilgit-Baltistan, Pakistan

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Abstract: Medicinal plants are the treasure of nature for human beings. Mountain communities inhabited buffer zones of Central Karakoram National Park (CKNP) are quite habitual in exploiting natural resources, especially for medicinal purposes. Three main potential medicinal plants from this area were selected based on their traditional applications and investigated further by protein profiling. Aerial parts of the selected plants namely; *Delphinium brunonianum* Royle, *Gentiana tianschanica* Ruprecht, Mém. and *Saussurea simpsoniana* (Fielding & Gardner) were used in our research study. Following the application of Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique, different protein bands were isolated and examined based on their diverse molecular weight (kD). Most of these proteins displayed low molecular weight ranged between 16 kD to 72 kD; while a few protein bands recorded higher molecular weight (72 kD). Total 16 types of protein bands were briefly examined through Liquid Chromatography-Mass Spectrometry LC-MS Analysis. This score showed the degree of significance of the identified proteins, while the taxonomy of viridiplantae score of > 44 indicated statistically significant match/ extensive homology at a confidence of $p < 0.05$. In this study, Rubisco was identified as a major protein while other protein types were identified (via LC/MS/MS) were very effective for diverse metabolic activities. To sum up, further research is required for the identified proteins in comparison with the data aerial parts and utilize it for advanced research, using X-ray crystallographic technique for the synthesis of these essential proteins.

Keywords: Central Karakoram National Park (CKNP); Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE); Liquid chromatography-mass spectrometry (LC-MS).

1. INTRODUCTION

Plants are a key element of our ecosystem and the main source of food. These natural resources are pertinent as a source of medicine for ages in modern medicines and drug development [1, 2]. Natural products obtained from these medicinal plant sources are highly significant for their traditional utilization to cure different ailments. Despite these features, some loopholes exist in the understanding of phytochemical and there is a requirement of investigation and exploration of targeted compounds using modern sophisticated techniques. Extraction of natural products, i.e. secondary metabolites, and their phytochemical investigation correlate with the traditional concept

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of medicinal plants and their therapeutic potential too [3].

Proteins play a vital role in every aspect of life including metabolic activities as catalysts, transporters, and messengers in handling systems of living cell like combinations, connections, cooperations, dimensions, alterations, guidelines, and confinement [4, 5]. Some potential medicinal plants belonging to Solanaceae family exhibited promising results in protein profiling reported previously [6].

Proteomics is an advanced technology and large-scale study to analyze proteins using a genome-wide scale. This field has grabbed more attention in functional genomics with the practice of genome sequencing and characterization of proteins using different analytical methods [6, 7, 8, 9, 10, 11]. Protein profiling is a developing subspecialty of proteomics that allows quantitative assessment of different levels of protein displaying extraordinary expression patterns by distinguishing a cell type from other. Evaluation of various bands of proteins via protein profiling is expanding exponentially day by day. With a comprehensive understanding of the sequences of proteins in the cell, it would be easier to search and predict effective techniques and alternatives to cure ailments like leukemia [11].

The main objectives of this research study were:

- Evaluation of protein profiling potency in the selected medicinal herbs
- Investigation of the application of these proteins in various metabolic activities

2. MATERIALS AND METHODS

2.1 Protein Profiling

Plants samples were collected from different valleys of CKNP dried well under the shade from three to four weeks. The selected medicinal plants for protein profiling were; *Delphinium brunonianum* Royle, *Gentiana tianschanica* Ruprecht, Mém. and *Saussurea simpsoniana* (Fielding & Gardner) respectively.

Before extraction, 10 gm of well-dried plant samples for each was species was ground well-using pestle and mortar. After proper grinding, buffers including Tris HCl, $MgCl_2$, Sucrose (18%), TCA (10 %) were used to run SDS-PAGE and protein profiling of certain plants by adjusting the PH 7.5, with the addition of Phenylmethane Sulfonyl Fluoride, or Phenyl methyl sulfonyl Fluoride (PMSF) for cell lysis. The total plant protein extracted from leaves and flowers in 15% Polyacrylamide Gel following the standard protocol of Laemmli.

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

Proteins were separated following the SDS-PAGE gel technique reported by Laemmli (1970) [12]. Each band on the gel was exercised and processed in the presence of trypsin following the liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify extracted proteins. A constant voltage of 200 Volt was used to isolated proteins from the extracts during the electrophoresis. After the electrophoretic separation, the gel was stained with Coomassie blue and examined using Quantity One software (Bio. Rad).

2.3. Mascot Protein Identification

The Tandem mass spectrometry (MS/MS) data ob-tained from mass spectrometry analysis was used to identify proteins using the Mascot Protein Search Database (MSDB) engine. The selected taxonomy and enzyme were viridiplantae (green plants) and *Arabidopsis,* respectively. The protein functions and their characteristics were obtained from SwissProt and NCBI [12].

3. RESULTS AND DISCUSSION

3.1 SDS PAGE of Medicinal Plants

In the present research, Figure 1 revealed that a total of sixteen protein bands were expressed at the range of 25 to 116 kDa (molecular weight). Protein bands and their molecular weight was recorded for *Delphinium brunonianum* highlighted the bands (B) with orange color B_1: 116 kDa, B_2: 116 kDa, B_3: 60 kDa and B_4: 60 kDa, and B_5: 45 kDa). Following the same procedure for *Gentiana tianschanic*a six protein bands were observed in L1 and L2, labelled as; B6 and B7: 55 kDa; B8: 116 kDa; B9: 66 kDa,

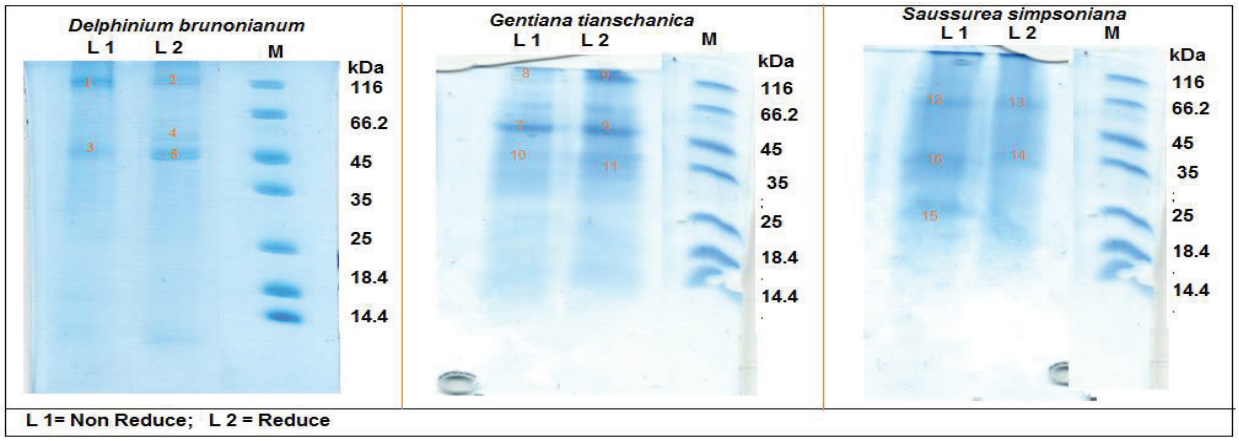

Fig.1. Protein profiles of leaves of three medicinal plants

B_{10} 60kDa, and B_{11} 35 kDa. Whereas five protein bands (B_{12}: 65 kDa, B_{13}: 65 kDa; B_{14}: 45 kDa; B_{15}: 25 kDa, and B_{16}: 45 kDa were noted in *Saussurea simpsoniana*. Protein bands 1, 2, 8, 7, 10, 12, 15, and 16 did not reduce while the remaining bands were reduced in the given figure. Overall, the highest number of protein bands were found in *Gentiana tianschanica* while *Delphinium brunonianum* and *Saussurea simpsoniana* exhibited five protein bands each. The SDS-PAGE is considered a reliable technique to express the molecular mass and occurrence of significant proteins relatively. [14].

3.2. Identification of Differentially Expressed Proteins

To obtain assorted variety in hereditary, an explanation of genetic diversity is incredibly significant for the successful support, assessment, and exploitation of germplasm, as it is a chief source to exploit with enhanced novel varieties in breeding programs [15]. Proteins have been utilized as markers to assess breeding by producing diverse species [16]. The current study specified proteins that were present in the leaf extracts of three medicinal plants collected from the mountains. The SDS-PAGE displayed different protein bands with different molecular weights in kD obtained on the gel. The diverse bands were labeled with numbers in corresponds to the protein bands in Figure 2. Protein profiling results show that most of the proteins in each species have a low molecular weight ranging from 16 kD to 72 kD, while few protein bands were examined in the higher molecular weight. Yadav et al. (2015) studied 14 protein bands that were gained which were more classified under three distinct zones A, B, and C liable on their reducing molecular weights. A standard medium-range protein molecular weight marker of identified molecular weight (14-95 kDa) was used laterally with samples. Low molecular weight proteins which are stress proteins, may purpose to sustain osmotic homeostasis under stress and are accountable for the fabrication of organic antioxidants [17, 18].

The proteins recognized in the current study were further elaborated, detected as several bands, comprising ribulose bisphosphate carboxylase large chain (Rubisco), fructose-bisphosphate aldolase, ATP synthase beta subunit. Each band showed that after comprehensive investigation certain multiple bands, including ribulose bisphosphate carboxylase large chain (Rubisco), ATP synthase subunit beta, chloroplast, ATP synthase subunit beta-2, mitochondrial, Ferredoxin, and V. type proton ATPase Sub. Unit B2Os were prominent. The seeds are the main part of the plants where maximum protein storage is reported while the leaves have a low amount of proteins. The identified Rubisco, ATP synthase subunit beta, chloroplast, and ATP synthase subunit beta-2, mitochondrial displayed numerous migrations in SDS-PAGE. Some recent reports conducted to assess protein profiling and application of medicinal herbs efficacy are described in Table 1. According to results, there is still a need of unleashing the hidden arenas in this field by exploring diverse extraction, isolation techniques, characterization of diverse protein bands and that could assist in drug discovery and development [19, 20, 21].

Table 1. List of proteins identified from the leaves of medicinal plants in comparison with *A. thaliana* database [19, 20, 21]

Band no.	Accession	Protein Name	Coverage	Peptide %	Score Hit	MW (kD)	pI Value
B_1 = Viridiplan *D. brunonianum*	O03042	Ribulose bisphosphate carboxylase large chain	35%	17%	209	53	6.3
	P19366	ATP synthase subunit beta, chloroplastic	61%	25	199	55	6.3
	P83484	ATP synthase subunit beta 2, mitochondrial	39%	14	119	59.7	6.62
	Q91ZS1	V.type proton ATPase Sub. Unit B2Os	63%	19	79.45	54.3	5.15
B_2 = Viridiplan *B. brunonianum*	O03042	Ribulose bisphosphate carboxylase large chain	40%	16	326.67	52.9	6.29
	Q9ZNZ7	Ferredoxin-dependent glutamate synthase, chloroplastic	10%	12	75.37	176.6	6.32
	P22954	Probable mediator of RNA polymerase II transcription	30%	15	71.03	71.3	5.12
	Q9C7X7	Heat shock 70 kDa protein 18OS Arabidopsis Thaliana	26%	13	59.97	68.3	9.38
B_3 = Viridiplan, *B. brunonianum*	O03042	Ribulose bisphosphate carboxylase large chain	35%	15	247	52.9	6.29
	P19366	ATP synthase subunit beta, chloroplastic	61%	24	219.16	53.9	5.5
	P50318	Phosphoglycerate kinase 2, choloroplast	35%	13	119.13	49.9	8.27
	P83483	ATP synthase subunit beta-1, mitochondrial	39%	15	112.66	59.6	6.62
B_4 Viridiplan, *B. brunonianum*	O03042	Ribulose bisphosphate carboxylase large chain	38%	18	341.64	52.9	6.29
	P19366	ATP Synthase subunit beta, Chloroplastic	58%	26	256.78	53.9	5.5
	Q9C5A9	ATP synthase subunit beta-3, chloroplastic	40%	15	110.86	59.8	6.49
	P83484	ATP synthase subunit beta-2, mitochondrial	40%	15	110.86	59.7	6.62
B_5 Viridiplan *D. brunonianum*	O03042	Ribulose bisphosphate carboxylase large chain	47%	25	744.05	52.9	6.29
	P19366	ATP synthase subunit beta, chloroplastic	57%	26	360.15	53.9	5.5
	Q8W4E2	V-type proton ATPase subunit B3	60%	18	169.71	54.3	5.1
B_6 = Viridiplan, *G. tianschanica*	O03042	Ribulose bisphosphate carboxylase large chain	37%	17	525.01	52.9	6.29
	Q9ZNZ7	Ferredoxin-dependent glutamate synthase, chloroplastic	12%	14	138.23	176.6	6.32
	P19366	ATP synthase subunit beta, chloroplastic	45%	17	109.4	53.9	5.5
	O03042	Ribulose bisphosphate carboxylase large chain	41%	17	417.18	52.9	6.29

Band no.	Accession	Protein Name	Coverage	Peptide %	Score Hit	MW (kD)	pI Value
B_7 = Arabidopsis *B. tianschanica*	P19366	ATP synthase subunit beta, chloroplastic	60%	26	217	53.9	5.5
	P25696	Bifunctional enolase 2/transcriptional activator	35%	15	158.88	47.7	5.77
	P83484	ATP synthase subunit beta-2, mitochondrial	39%	15	155.89	59.7	6.62
	O03042	Ribulose bisphosphate carboxylase large chain	34%	15	473.45	52.9	6.29
	Q9LK36	Adenosylhomocysteinase 2 OS= Arabidopsis thaliana	40%	15	168.54	53.1	5.74
B_8 = Arabidopsis *G. tianschanica*	P19366	ATP synthasis subunit beta, choloroplast	57%	22	163.08	53.9	5.5
	P25696	Bifunctional enolase 2/transcriptional activator	39%	14	157.13	47.7	5.77
	O03042	Ribulose bisphosphate carboxylase large chain	37%	16	139.76	52.9	6.29
B_9 = Arabidopsis *G. tianschanica*	P19366	ATP synthasis subunit beta, choloroplast	43%	17	117.14	53.9	5.5
	P83483	ATP synthasis subunit beta-1, mitochondria	35%	12	104.27	59.6	6.62
	O03042	Ribulose bisphosphate carboxylase large chain	32%	12	100.81	52.9	6.29
B_{10} = Arabidopsis *G. tianschanica*	P19366	ATP synthasis subunit beta, choloroplast	48%	16	76.76	53.9	5.5
	P46077	14-3-3-like protein GF 14 phi OS=Arabidopsis thaliana	28%	7	55.53	30.2	4.87
	O03042	Ribulose bisphosphate carboxylase large chain	35%	17	209.24	53.9	6.39
B_{11} = Arabidopsis *G. tianschanica*	P19366	ATP synthasis subunit beta-1, choloroplast	61%	25	199.34	53	5.5
	P83484	ATP synthasis subunit beta-2, mitochondria	39%	14	119.98	59.7	6.62
	Q9C5A9	ATP synthasis subunit beta-3, mitochondria	35%	13	93.84	59.8	6.49
B_{12} Arabidopsis *S. simpsoniana*	P83483	ATP synthasis subunit beta-1, mitochondria	35%	13	93.84	59.6	6.62
	P19366	ATP synthasis subunit beta, choloroplast	50%	19	89.88	53.9	5.5
	P19366	ATP synthasis subunit beta, choloroplast	45%	15	66.08	53.9	5.5
B_{13} Arabidopsis *S. simpsoniana*	P46077	14-3-3like protein GF 14 phi OS=Arabidopsis thaliana	31%	8	52.08	30.2	4.87
	P42643	14-3-3like protein GF 14phi OS=Arabidopsis thaliana	31%	8	48.22	29.9	4.81
	P19366	ATP synthasis subunit beta, choloroplast	28%	10	36.35	53.9	5.5
B_{14} Arabidopsis *S. simpsoniana*	Q682T9	Calmodulin-5 OS= Arabidopsis thaliana	36%	4	27.78	16.8	4.27

Band no.	Accession	Protein Name	Coverage	Peptide %	Score Hit	MW (kD)	pI Value
	P0DH96	Calmodulin-4 OS= Arabidopsis thaliana	36%	4	27.78	16.9	4.27
	O03042	Ribulose bisphosphate carboxylase large chain	37%	15	106.67	52.9	6.29
B $_{15}$ = Arabidopsis *S. simpsoniana*	Q9C5A9	ATP synthasis subunit beta-3, mitochondria	35%	13	94.35	59.8	6.49
	P83483	ATP synthasis subunit beta-1, mitochondria	35%	13	94.35	59.6	6.62
B_{15} = Viridiplan *S. simpsoniana*	S8DTS7	ATP synthasis subunit beta (Fragment), OS= Genlisea aurea	50%	18	152.26	59.3	6.37
	B9HJ80	ATP synthasis subunit beta, OS= Populus trichocarpa	44%	16	144.31	59.9	6.32
	S8DTS7	ATP synthasis subunit beta (Fragment), OS= Genlisea aurea	49%	17	133.83	57	6.67
	P19366	ATP synthasis subunit beta, choloroplast OS=Arabidopsis thaliana	34%	10	48.73	53.9	5.5
B $_{15}$ = Arabidopsis *S. simpsoniana*	P46077	14-3-3-like protein GF 14 phi OS=Arabidopsis thaliana	33%	8	48.42	30.2	4.87
	P42643	14-3-3-like protein GF 14 chi OS=Arabidopsis thaliana	33%	8	48.23	29.9	4.81
	A0A68V2W8	Coffea canephora DH200=94 genomic scaffold	8%	3	103.83	37	5.07
B $_{16}$ = Viridiplan *S. simpsoniana*	Q68V46	Beta-1,3-glucanase OS=Olea europaea GN=glu	11%	2	99.28	37	9.38
	A0A0D2QB48	Gossypiumraimondii chromosome 9	4%	1	90.68	37.2	4.75
	P19366	ATP synthasis subunit beta, choloroplast	34%	10	48.73	53.9	5.5
B_{16} = Arabidopsis *S. simpsoniana*	P46077	14-3-3-like protein GF 14 phi OS=Arabidopsis thaliana	33%	8	48.42	30.2	4.87
	P42643	14-3-3-like protein GF 14 chi OS=Arabidopsis thaliana	33%	8	48.23	29.9	4.81

Although multiple relocations of a protein are usually be elucidated by the animation and nature of its post-translation modification, however, Rubisco was observed in diverse molecular forms. In terms of protein expression in medicinal plants, they were categorized based on molecular weight. Results showed the highest molecular weight in ferredoxin-dependent glutamate synthase followed chloroplast (the probable mediator of RNA polymerase II transcription) while the least molecular weight was assessed in calmodulin-5 OS. There were no conflicting results found regarding the current species, but comparable results were reported in *Artemisia vulgaris* [22], *Glycine max* (L.) Merr [23], and *Plumbago zeylanica* L, respectively [20]. Subunits of ATP synthase, i.e. alpha and beta subunit correlate energy metabolism and other function [24, 25]. Dhabhai and Batra (2011)

identified protein bands possessing molecular weights 19.5, 28.5, and 42.3 kD expressing similar patterns of protein in callus, and *in vivo* leaves with differences in their intensity. Some other metabolic activities including Calvin cycle pathways were considered significant in heat stress response in pea due to the presence of capable proteins enlisting 86 kD protein [26, 27, 28].

4. CONCLUSION

From this research study, we conclude that all plants have diverse groups of amino acid sequences. Most of the identified proteins were reported from seeds, which are considered a key source of proteins. Moreover, some other plants' vegetative parts especially leave also possessed a variety of amino acids. Rubisco, as a major plant protein, showed multiple migrations on SDS-PAGE. Laconically, different plant species from different families exhibited almost 16 different types of protein. They expressed bands of different molecular weights on SDS PAGE later analyzed further via LC-MS/MS. Some other storages for these diverse proteins were chloroplast, mitochondria, cell wall, cell membrane, and cytoplasm. LC-MS/MS clarified accurate sequence coverage and percentage of the amino acid found in proteins. The score showed the degree of significance of the protein identified while the taxonomy of viridiplantae with a score of > 44 indicated a statistically significant match or extensive homology with $p < 0.05$. A detailed list of diverse protein bands identified from the leaf extract of the *Gentiana tianschanica* that corresponding *Delphinium brunonianum* and *Saussurea simpsoniana* protein bands. These plants have compatibility against cancer and anti-oxidant. Protein profiling is one of the emerging fields in the recent era. Significant results obtained from medicinal plants could be used in the therapeutic study. To conclude, these medicinal plants with the least attention in advance research were common traditionally due to their significant effects in curing ailments like cancer and anti-inflammatory. Advance research would authenticate both folk wisdom and modern investigations in identifying prominent compounds extracted from their plants to formulate drugs and remedies.

Hence, the main objective of selecting these three potential medicinal plants for protein profiling was their diverse medicinal applications and indigenous knowledge. These identified proteins through LC/MS/MS analy¬sis are well-recognized enzymes and effective in varied metabolic activities; for instance, Beta-1,3-glucanase possess intestinal immune function along with anti-cancer and anti-oxidant properties; Calmodulin-4 & 5 assist growth and development activities in plants in addition to biotic and abiotic stresses, and Beta-1,3-glucanase regulates immunization, while, Coffea canephora peptides have antimicrobial (fungus strain *Fusarium solani*).

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