RESEARCH ARTICLE

Validation of *Solanum nigrum* Proteins as Snake Antivenoms

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Traditionally, medicinal plants such as *Solanum nigrum* have been used as antidotes for the treatment of venomous snakebites. Combining these plants with antiserum is hypothesized to yield better results than the use of either treatment alone. Therefore, this study aims to validate the use of *Solanum nigrum* as antivenom by extracting and identifying the proteins of this plant. The sequences of 358 *Solanum nigrum* proteins were submitted to PSI-Blast to search for homologous snake proteins. The resulting 101 snake proteins were subjected to multiple sequence alignments with the 358 *Solanum nigrum* proteins. A phylogenetic tree analysis resulted in 79 nodes in 26 clades, three of which contained snake proteins clustered with similar Solanum nigrum proteins. Homology modeling and structural comparison showed that three clustered snake proteins-5Z2G, 5THP, and 6IMF-were similar to proteins from *Solanum nigrum*. The snake protein 5Z2G shared a similarity of 18.75% with two *Solanum nigrum* proteins, whereas 5THP shared only 3.85% similarity. Of the three identified snake proteins, 6IMF B, with a similarity of 1.96%, was the least similar to *Solanum nigrum* proteins. The method used in the current study validates the use of several *Solanum nigrum* proteins as agents to reduce the toxicity of venomous snakebites, specifically those of the snake *Naja atra*.

Key Words: *Solanum nigrum; Proteins; Antivenom; Snake; Structural comparison; Homology modeling*

INTRODUCTION

Venomous snakebites are associated with the acute onset of medical emergencies that involve breathing difficulties, bleeding disorders, severe paralysis, kidney failure, and severe local tissue destruction. Every year, they cause around 137,880 deaths and numerous permanent disabilities worldwide [1]. Snake venom, secreted by venom glands, is composed of amino acids, nucleic acids, carbohydrates, lipids, proteins, and peptides [2]. The proteins in the venom can be used for drug discovery and the design of possible templates [3]. Snakes use their venom to immobilize and digest their prey as a defence and survival mechanism [4]. The toxicity caused by the bite of a venomous snake is treated by neutralization, which can be accomplished by using its endogenous proteins or remedies composed of certain medicinal plants. For many years, valuable medicinal plants have been used as immediate treatment of snake or scorpion bites [5]. For instance, at a low dose (0.17 mg), Mimosa pudica root significantly reduces the lethality caused by the venom of two snakes, namely Russell's viper and the saw-scaled viper [6].

Plants in the Solanaceae family were found to be the most frequently used for snakebite remedies [7]. Solanine, a glycoalkaloid, is a bioactive compound extracted from the berries of *Solanum nigrum*, which is used as a food as well as a medicinal plant [8,9]. Solanine is found in all parts of the plants in the Solanaceae family, including *Solanum melongena, Solanum tuberosum*, and *Solanum lycopersicum*. These plants are replete with secondary metabolites that have practical value; for example, steroidal alkaloids from *Solanum campaniforme* neutralize the myotoxicity and skin necrosis induced by the crude venom of *Bothrops pauloensis* [10,11]. Similarly, a glycoprotein from *Solanum nigrum* was observed to exert anti-inflammatory activity that decreased the number of viable HT-29 cells (human colon cancer cell line) and MCF-7 cells (breast cancer cell line) in a dose-dependent manner. This protein is also considered a natural anticancer agent [12,13].

Solanum nigrum is used as a snakebite treatment for its ability to neutralize venom enzymes and induce anti-inflammatory activity [12-14]. Some tribes applied the leaf juice of *Solanum nigrum* locally on wounds caused by snakebites, or they mixed a fruit paste of *Solanum nigrum* with the leaves of *Heteropogon contortus* [15,16]. Hypothetically, combining the compounds isolated from these plants with antiserum may produce better outcomes of venom toxin neutralization compared with either treatment alone. A crucial step in this direction is the validation of *Solanum nigrum* proteins as snake antivenoms through protein identification and homology modeling to compare them with snake venom proteins. Therefore, in the current study, *Solanum nigrum* proteins were identified and compared with snake proteins to assess their similarity and identify potential antidotes.

MATERIALS AND METHODS

Plant material

Samples *of Solanum nigrum* were collected from Al Jubail (27.123450, 49.537920), Saudi Arabia. The collected samples were placed on tissue paper and then pressed between hard boards, with the upper board applied evenly to weigh it down and flatten the samples. The samples were dried for 21 days. The dried plant samples to be processed for protein extraction were ground to powder. There were three replicates. The plant powder was dissolved in 50 mM ammonium bicarbonate solution. Proteins were precipitated with ice-cold acetone, and the protein pellets were then resuspended in 50 mM ammonium bicarbonate. After reduction by 10 mM DL-dithiothreitol (DTT) at 56°C for 1 hour and alkylation by 20 mM iodoacetamide (IAA) at room temperature in the dark for 1 hour, the suspension was centrifuged at 12,000 g at 4°C for 10 min. The proteins were washed once with 50 mM ammonium bicarbonate. Then, 100 µL of 50 mM ammonium bicarbonate and free trypsin were added to the protein solution at a ratio of 1:50 and incubated at 37°C overnight. The samples were centrifuged at 12,000 g at 4°C for 10 min, followed by the addition of 100 µL of 50 mM ammonium bicarbonate to the samples with two cycles of centrifugation. Finally, the samples were lyophilized, and the peptides were extracted to near dryness. The peptides were resuspended in 2-20 µL of 0.1% formic acid before LC-MS/MS analysis.

Data analysis

The raw MS file protein sequences were analyzed and searched against the Solanaceae protein database according to the sample species. MaxQuant (1.5.6) software was used for quantitative proteomics MS data analyses. The parameters were set as follows: the protein modifications were carbamidomethylation (C) (fixed), oxidation (M) (variable); the enzyme

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specificity was set to trypsin; the maximum missed cleavages were set to 2; the precursor ion mass tolerance was set to 10 ppm and the MS/MS tolerance was 0.6 Da. Only confidently identified peptides were chosen for the downstream protein identification analysis.

Phylogenetic tree analysis

The sequences of 358 *Solanum nigrum* proteins were used to search for homologous snake proteins using PSI-Blast, which resulted in 101 snake protein sequences. These sequences were filtered using PROMALS3D (PROfile Multiple Alignment with predicted Local Structures and 3D constraints) by aligning multiple protein sequences. The balance alignment using the two-stage alignment strategy described by Pei et al. [17] reduced the number of protein sequences from 459 to 80 [17,18]. The alignment parameters were as follows: identity threshold=0.6; weight for constraints derived from sequences=1; weight for constraints derived from homologs with structures=1.5; weight for constraints derived from input structures=1.5; profile – profile comparison; weight for amino acid scores=0.8; and weight for predicted secondary structure scores=0.2. The parameters for deriving sequence profiles from PSI-BLAST searches were set as follows: PSI-BLAST iteration number=3; PSI-BLAST e-value inclusion threshold=0.001; identity cut off below which distant homologs are removed=0.25; and the maximum number of homologs kept for PSI-BLAST alignment=300. The parameters for detecting and using homologs with 3D structures (homologed) were as follows: PSI-BLAST e-value cut off against structural database=0.001, and the identity cut off below which 3D structures are not used=0.2. The multiple sequence alignment was performed using MAFFT [19]. The resulting phylogenetic tree was visualized and edited using iTOL [20].

Protein homology modeling and similarity evaluation

The sequence structures of *Solanum nigrum* and snake proteins found in the same clade were extracted from the Protein Data Bank (PDB) using Easy Modeller and the Python script Homology-modeling-preprocessing.py. All the PDB files were visualized in UCSF Chimera [21,22]. *Solanum nigrum* and snake proteins clustered in the same clades were aligned to assess their similarity.

RESULTS

Three snake proteins were identified as similar to *Solanum nigrum* proteins: 5Z2G, 5THP, and 6IMF [23-25]. The phylogenetic tree analysis showed that *Solanum nigrum* proteins clustered with snake proteins in three clades (Cluster 1: O65819 and Q9M4G5 with 5THP; Cluster 2: O04899 with 6IMF; Cluster 3: SN Q84P52, SN Q96557, and SN O48658 with 5Z2G) (Figure 1). Homology modeling showed that 5Z2G shared 2.08% similarity with Q84P52 (Figure 2), and it shared higher similarity with two proteins from *Solanum nigrum*, Q96557 (Figure 3) and O48658 (Figure 4), whereas 5THP shared only 3.85% similarity with Q9M4G5 (Figure 5), and 6IMF B shared 1.96% similarity with O04899 (Figure 6).

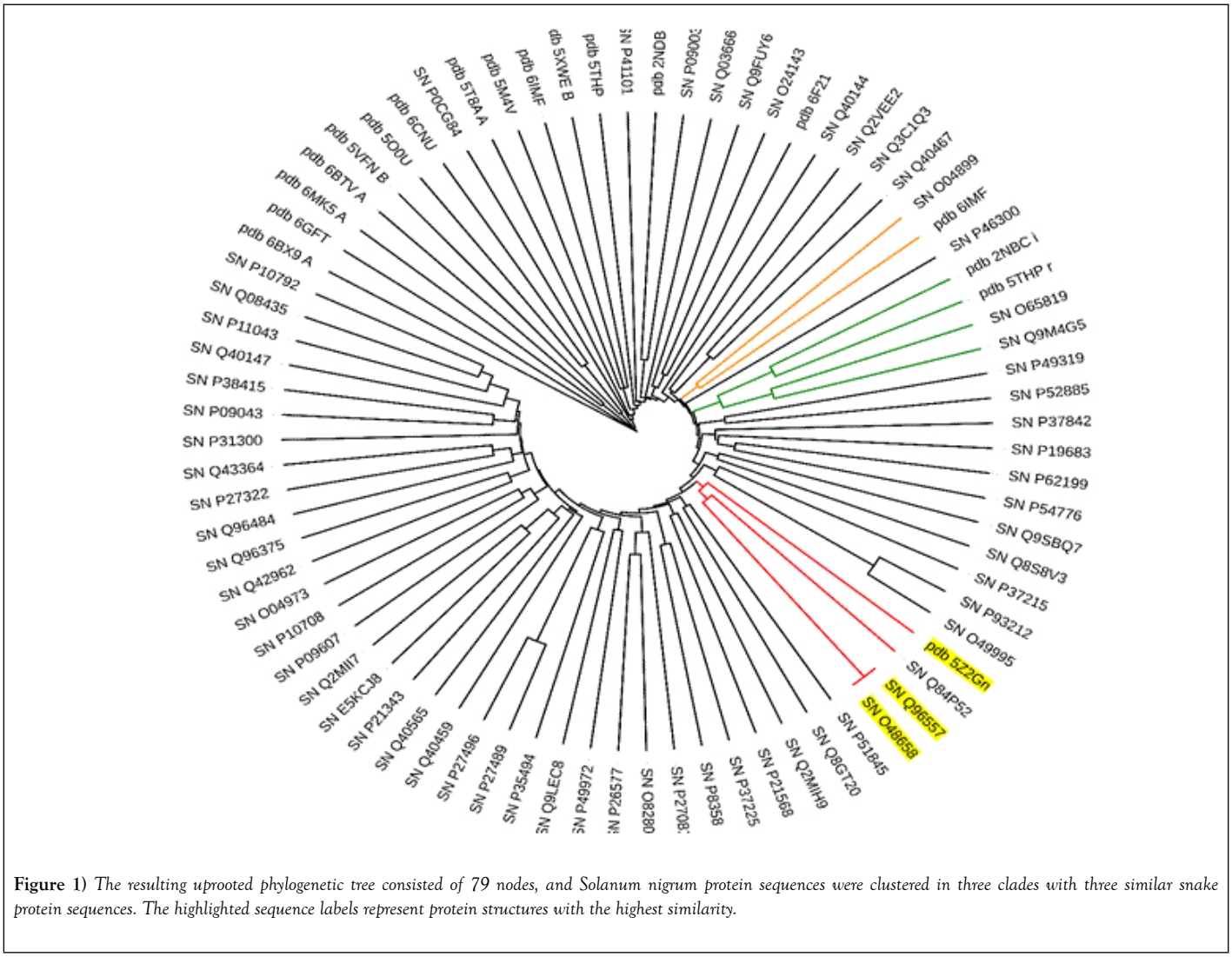

Figure 1) *The resulting uprooted phylogenetic tree consisted of 79 nodes, and Solanum nigrum protein sequences were clustered in three clades with three similar snake protein sequences. The highlighted sequence labels represent protein structures with the highest similarity.*

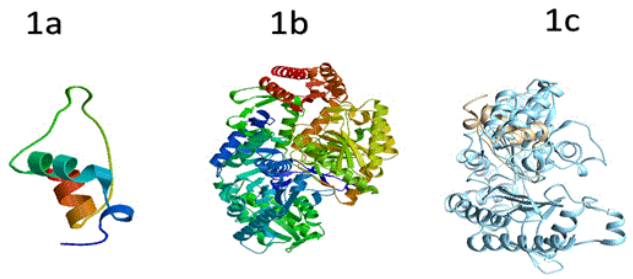

Figure 2) *(1a) 5Z2G B Chain B L-amin, (1b) Q84P52, (1c) Protein structural comparison similarity identified as 2.08%.*

Figure 3) *(2a) 5Z2G B Chain B L-amin, (2b) Q96557, (2c) Protein structural comparison similarity identified as 18.75%.*

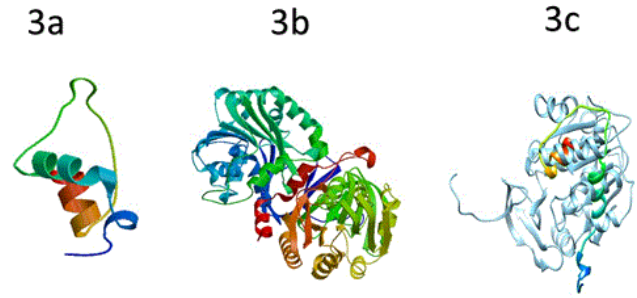

Figure 4) *(3a) 4: 5Z2G B Chain B L-amin, (3b) O48658, (3c) Protein structural comparison similarity identified as 18.75%.*

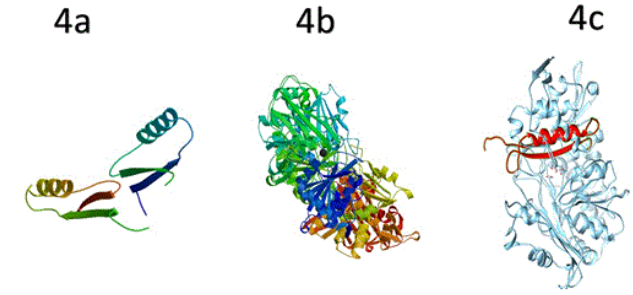

Figure 5) *(4a) 5THP R Chain R, (4b) Q9M4G5, (4c) Protein structural comparison similarity identified as 3.85%.*

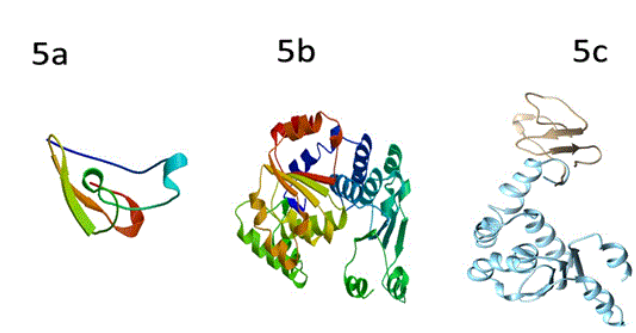

Figure 6) *(5a) 6IMF B Chain B, (5b) O04899, (5c) Protein structural comparison similarity identified as 1.96%.*

DISCUSSION

The approach to neutralizing snake venom toxicity by applying serum antivenom was developed within the last century. Snakebites are treated by medicinal plants alone or in combination with processed edible items [26]. Medicinal plants are vital sources of bioactive compounds that are useful for treating snakebites by boosting the effects of conventional serum therapy and reducing side effects [14]. In the current study, *Solanum nigrum* proteins were clustered with the snake proteins 5Z2G, 5THP and 6IMF [23-25]. The protein similarity was identified as 3.85% between 5THP from the Malayan ground pit viper snake (*Calloselasma rhodostoma*) and the *Solanum nigrum* protein Q9M4G5, which is chloroplastic phosphoglucomutase, an enzyme that participates in both the breakdown and synthesis of glucose. The similarity only reached 3.85% between 6IMF from *Protobothrops flavoviridis*-a venomous pit viper endemic to the Ryukyu Islands of Japan and the *Solanum nigrum* protein O04899, caffeoyl-CoA O-methyltransferase 5 (CCoAMT-5). These proteins interact selectively and noncovalently with any metal ion. CCoAMT-5 plays a role in the synthesis of feruloylated polysaccharides that increase the formation of plant cell walls after a wound or pathogen attack. Some snake proteins were similar to a lesser extent: 2.08% similarity was found between 5Z2G-a protein from the Chinese cobra (*Naja atra*) and the *Solanum nigrum* protein Q84P52, which is chloroplastic gamma aminobutyrate transaminase 3, an enzyme that degrades gamma-aminobutyric acid (GABA) and uses pyruvate or glyoxylate as an amino-group acceptor. The highest protein similarity was identified as 18.75% between 5Z2G and the *Solanum nigrum* protein Q96557, which is spermidine synthase 2, and O48658, which is spermidine synthase-1. The spermidine synthase is among the ubiquitous cellular polyamines associated with the growth and developmental process of plants, and it plays a key role in plant stress signalling pathways and stress tolerance mechanisms [27,28]. Moreover, spermidine is involved in plant abiotic stress tolerance processes *via* reactive oxygen species (ROS) signaling and modulation of ion channel activities and Ca2+ homeostasis [29].

Solanum nigrum proteins might function as co-neutralizing agents and might reduce the toxicity of venomous snakes such as *Naja atra*, the venom of which shares high sequence homology with the venom of *Naja haje*, whose natural habitat is in the Arabian Peninsula [30].

CONCLUSION

The main conclusion of this study is that *Solanum nigrum* proteins have potential use as co-neutralizing agents to reduce the toxicity of certain venomous snakes, such as *Naja atra*. The potency of antivenom antibodies can be increased with the use of highly concentrated plant protein extracts. Therefore, this study serves as a validation report for *Solanum nigrum* as plant-based antivenom.

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AUTHOR CONTRIBUTIONS

The study was conducted solely by the author Faten Dhawi.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY

Data that are supplementary to the manuscript will be available upon request.

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