Journal of Medicinal Plants and By-products (2025) 06: 624– 631

In Silico Investigation on the Effect of Extraction of *Rhazya stricta* on the Venom of *Androctonus rostami* in the Sistan Region by Molecular Docking

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Article Info

Article Type
Original Article

Article History
Received: 14 February 2025
Accepted: 05 April 2025

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ABSTRACT

Scorpion stings are a major health issue in Iran, with around 50,000 cases yearly. Current antivenom treatments face challenges like availability and side effects, prompting the search for alternatives. Medicinal plants, such as *Rhazya stricta* Decne., offer potential due to their cost-effectiveness, fewer side effects, and environmental benefits. This study investigates the interactions between *R. stricta* phytochemicals and venom components of *Androctonus rostami*, a scorpion species in eastern Iran. Using in silico methods, the research aims to identify bioactive compounds that neutralize venom, potentially leading to plant-based antivenom therapies. Gene and protein sequences from *R. stricta* were analyzed using NCBI data. In silico analysis was performed using NCBI, Expasy, and ClustalW. Protein tertiary structures were predicted using Swiss-Model, ESYPred3D, and Galaxy Web, visualized with Weblab ViewerLite and RasMol, and assessed via PROCHECK and Ramachandran plots. ACRA4 peptide effects on Quebrachamine protein expression were investigated using PubMed, HGNC, MBC, and ProtScale databases, focusing on sodium channel and neuronal changes. The *in silico* analysis revealed significant interactions between several phytochemicals of *R. stricta* and key venom components of *A. rostami*. Specifically, compounds like quebrachamine, rhazyaminine, and certain alkaloids exhibited strong binding affinities to neurotoxins such as Acra4, a sodium channel neurotoxin, and phospholipases A2. These interactions suggest a potential mechanism for neutralizing the venom's neurotoxic effects and inflammatory responses. Furthermore, flavonoids and saponins present in *R. stricta* showed interactions with hyaluronidases, potentially inhibiting venom spread. These findings indicate that *R. stricta* harbors bioactive compounds capable of counteracting multiple facets of *A. rostami* venom. *In silico* analysis suggests *R. stricta* may contain antivenom compounds against *A. rostami*. Further research is needed to validate these interactions and explore *R. stricta*'s potential as a cost-effective, plant-based antivenom alternative.

Keywords: *Androctonus*, Antivenom, *In silico* analysis, *Rhazya stricta*, Scorpion sting

How to cite this paper
Barahoei, H., Fazeli-Nasab, B., Saberi, M. *In Silico* Investigation on the Effect of Extraction of *Rhazya stricta* on the Venom of *Androctonus rostami* in the Sistan Region by Molecular Docking. Journal of Medicinal Plants and By-products, 2025; 14(6): 624-631. doi: 10.22034/jmpb.2025.368636.1899

INTRODUCTION

Scorpion venom is a rich mixture of proteins and peptides with diverse biological activities. Key components include Phospholipases A2, which disrupt membranes and trigger inflammation; Serine Proteases, involved in blood coagulation and immune responses; and Metalloproteases, which degrade proteins and contribute to tissue remodeling [1]. Lipolysis Activating Peptides (LVPs) regulate lipid metabolism and have therapeutic potential [2, 3]. Neurotoxins, such as Sodium and Potassium Channel Toxins, target ion channels, causing paralysis or disrupting nerve signals [1, 4]. Hyaluronidases facilitate venom spread by breaking down hyaluronic acid, while Acetylcholinesterase affects neuromuscular transmission. Oligopeptides exhibit antimicrobial properties, and Cytotoxins induce cell death or inhibit cellular functions [3]. Low-Molecular-Weight Peptides (3-10 kDa) often display neurotoxic effects by disrupting ion channels [1-3]. The composition of scorpion venom varies by species, and its proteins not only aid in envenomation but also hold promise for medical applications, particularly in drug development targeting ion channels and metabolic pathways. Further research is needed to fully explore their pharmacological potential and therapeutic uses.

The venom of *Androctonus rostami* contains a variety of proteins and peptides, many of which have been characterized in related species. While specific studies on *A. rostami* may be limited, the following proteins have been identified in the venom of *Androctonus* species, particularly *A. crassicauda*, which shares similarities with *A. rostami*. Some notable proteins and peptides identified in various studies include: 1. Neurotoxins (Acra4: A sodium channel neurotoxin peptide characterized from *A. crassicauda* venom, known for its role in affecting voltage-gated sodium channels [5], Consensus Scorpion Toxin (SccTx): Another neurotoxin used to generate antibodies for antivenom production, derived from various scorpion venoms [5]); 2. Phospholipases A2 (PLA2): Enzymes that hydrolyze phospholipids, contributing to the inflammatory response and membrane disruption [6]. PLA2 is a diverse group of enzymes that play a crucial role in lipid metabolism and cell signaling. These enzymes catalyze the hydrolysis of the sn-2 ester bond of glycerophospholipids, releasing a fatty acid and a lysophospholipid. PLA2 enzymes specifically cleave the second fatty acid "tail" from phospholipids.

The reaction produces a free fatty acid (often arachidonic acid) and a lysophospholipid [7]. These products are precursors for important signaling molecules like eicosanoids, which are involved in inflammation and immune responses [7].

There are several main types of PLA2 enzymes [7]; Secreted PLA2 (sPLA2), Cytosolic PLA2 (cPLA2), Calcium-independent PLA2 (iPLA2), PAF acetylhydrolases, Lysosomal PLA2. PLA2 enzymes hydrolyze the bond between the second fatty acid and the glycerol molecule in phospholipids. Some PLA2 enzymes show specificity for arachidonyl-containing phospholipids. Many PLA2 enzymes require calcium for their activity, though some (like iPLA2) are calcium-independent. PLA2 enzymes are involved in various physiological processes like: Eicosanoid production (prostaglandins, leukotrienes), Inflammation and immune response, Cell signaling, Membrane remodeling, and Potential roles in insulin secretion and calcium entry. PLA2 enzymes are associated with several medical conditions: Increased activity in cerebrospinal fluid of patients with Alzheimer's disease and multiple sclerosis, Potential pharmacological targets for treating atherosclerosis, immune disorders, cardiovascular diseases, and cancer, and involvement in inflammatory disorders. Understanding the biochemistry and functions of PLA2 enzymes continues to be an important area of research, with potential implications for developing new therapeutic strategies for various diseases; 3. Serine Proteases: These enzymes play roles in blood coagulation and immune responses [8]; 4. Metalloproteases: Proteins involved in degrading other proteins, significant for tissue remodeling and inflammation [6]; 5. Lipolysis Activating Peptides (LVPs): These peptides can stimulate lipid metabolism; variants like LVP1 have been identified across several scorpion species [2]; 6. Hyaluronidases: Enzymes that degrade hyaluronic acid, facilitating venom spread through tissues [8]; 7. Cytotoxins: Peptides that can induce cell death or inhibit cellular functions [6]; 8. Low-molecular-weight peptides: Often exhibit neurotoxic effects and disrupt ion channel functions [8].

Medicinal plant studies have gained particular importance worldwide in discovering new therapeutic methods with fewer side effects and higher economic value [9]. More than 30% of herbal medicines are used in hospitals and clinics. Herbal medicines are more popular among the public, and the factors mentioned above have led to a new wave of extensive global studies and the introduction of the antibacterial effects of various plants in recent years [10]. *R. stricta*, a medicinal plant, has been studied for its genomic and proteomic traits. Its plastid genome contains 80 protein-coding genes, while the mitochondrial genome has 38. Key proteins include Heat Shock Proteins (HSPs) for thermotolerance, enzymes like Sucrose-phosphate synthase for sugar metabolism during heat stress, and Rubisco Subunit Binding-Protein Alpha for photosynthesis under stress. Late-Emergence-Associated Proteins are linked to turion formation. Alkaloids like rhazinilam and strictamine contribute to pharmacological effects. Other proteins involved in secondary metabolite biosynthesis and stress responses were also noted, highlighting the plant's complex adaptation and medicinal properties [11-13].

Rhazya stricta is valued for its therapeutic properties, rich in proteins and alkaloids with significant health benefits. Key compounds like Rhazyaminine exhibit anticancer effects by inducing apoptosis and inhibiting metastasis in breast cancer cells. The plant's alkaloids also demonstrate antimicrobial, antihypertensive, and antitumor activities, supporting its traditional use for ailments like syphilis and hyperglycemia. Flavonoids in R. stricta provide antioxidant and cardiovascular benefits, while saponins boost immunity and may lower cholesterol. Additionally, neuroprotective extracts show promise in treating neurodegenerative diseases like Alzheimer's, highlighting the plant's diverse medicinal potential [14-16].

Rhazya stricta is recognized for its neuroprotective properties due to bioactive compounds like quebrachamine, rhazyaminine, alkaloids, and flavonoids. Quebrachamine, an indole alkaloid, enhances neural stem cell viability and proliferation, showing potential in neuroprotection. Rhazyaminine exhibits anticancer effects by inducing apoptosis and inhibiting metastasis, with possible neuroprotective benefits. Alkaloids and flavonoids in R. stricta provide antioxidant properties, protecting neural cells from oxidative stress and supporting its use in treating neurodegenerative diseases. These compounds highlight R. stricta's therapeutic potential, though further research is needed to fully understand their mechanisms and clinical efficacy [14-17]. Quebrachamine A has been associated with anti-inflammatory and analgesic properties, making it significant for therapeutic applications. Quebrachamine B, known for its role in promoting wound healing and reducing oxidative stress, this protein is another candidate for therapeutic use. Quebrachamine C exhibits antimicrobial activity, which can be beneficial in treating infections and enhancing immune responses [18]. These proteins are part of a broader group of bioactive compounds in *R. stricta* that contribute to its traditional uses in medicine. The therapeutic properties of these proteins highlight the potential for further research and development into pharmaceutical applications based on natural products derived from this plant.

Considering the hot climate of the region, the diversity and large number of scorpion species in Sistan, the lack of sufficient information about the venom's side effects, the inefficiency and availability of existing antivenoms against scorpion venom, and the lack of identification of effective plants in the treatment of scorpion stings, this research seems necessary. The project's findings can be used for treatment management and medical and health education to prevent and treat scorpion stings and for use in the pharmaceutical sector to produce specific antivenoms.

MATERIALS AND METHODS

Gene and Protein Sequence Retrieval and Bioinformatics Analysis

Gene and protein sequences related to scorpion sting treatment in *R. stricta* Decne. were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov). Bioinformatics and comparative analyses of DNA and proteins were conducted using NCBI and Expasy tools (http://expasy.org/tools). Sequence alignment was performed using ClustalW (http://www.ebi.edu.au/tools/clustalw) to assess DNA and protein similarities.

Protein Structure Prediction and Visualization

The tertiary structures of proteins were predicted through homology modeling using Swiss-Model, ESYPred3D, and Galaxy Web. Molecular docking studies were also performed. The resulting 3D models were visualized using WebLab ViewerLite 4.0 and RasMol. Protein structure validation was carried out using PROCHECK, and Ramachandran plots were generated to assess structural integrity.

Study on ACRA4 Peptide and Quebrachamine Protein

This study investigated the ACRA4 peptide and its influence on Quebrachamine protein expression in humans, referencing the PubMed database. The role of ACRA4 in neuronal activity was

examined using the HGNC database. The 3D structure of Quebrachamine was determined using the MBC database, while its molecular weight and structural characteristics were analyzed via ProtScale. Since ACRA4 modulates sodium channels by altering membrane permeability and stimulating neurons, its effect on Quebrachamine expression across different tissues was analyzed. Additionally, changes in Quebrachamine protein levels in neurons were evaluated.

RESULTS

Quebrachamine, also known as (-)-Quebrachamine Kamassine, is a fascinating alkaloid with the molecular formula C19H26N2. This compound has a molecular weight of 282.4 g/mol and consists of 21 heavy atoms. Its structural properties reveal a topological polar surface area of 19Å², indicating a relatively small area of polar atoms in the molecule. Quebrachamine is composed of a single covalently bonded unit, suggesting a compact and unified structure. As an alkaloid, it likely possesses nitrogen-containing heterocyclic rings, which are characteristic of this class of compounds. The specific structural features of Quebrachamine contribute to its unique chemical and potentially biological properties, making it an interesting subject for further study in fields such as pharmacology and natural product chemistry (Table 1). Table 1 shows the properties of the Quebrachamine protein. Figs. 1 and 2 also show the two-dimensional and three-dimensional structures of the Quebrachamine protein and also the total synthesis of Quebrachamine and Kopsiyunnanine D.

Table 1 Structural properties of the Quebrachamine protein.

Quebrachamine	
Name	(-)-Quebrachamine Kamassine
Molecular Formula	$C_{19}H_{26}N_2$
Description	Quebrachamine is an alkaloid
Topological Polar Surface Area	19Å²
Heavy Atom Count	21
Molecular weight (Da)	282.4 g/mol
Covalently-Bonded Unit Count	1

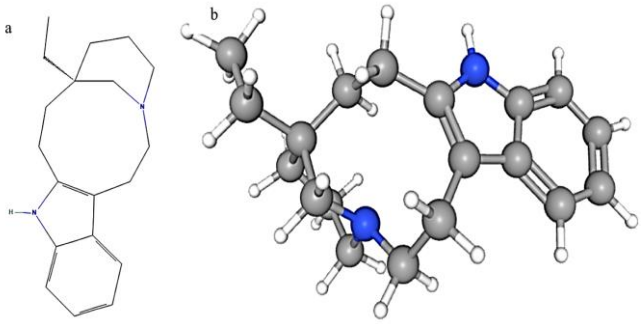

Fig. 1 2D (a) and 3D (b) structures of Quebrachamine protein.

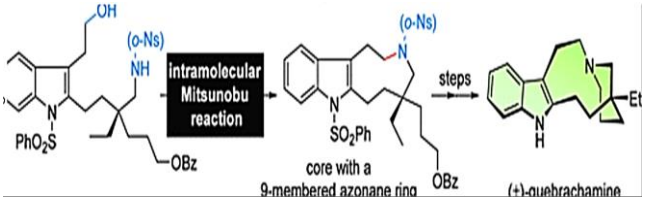

Fig. 2 Total synthesis of Quebrachamine and Kopsiyunnanine D [19].

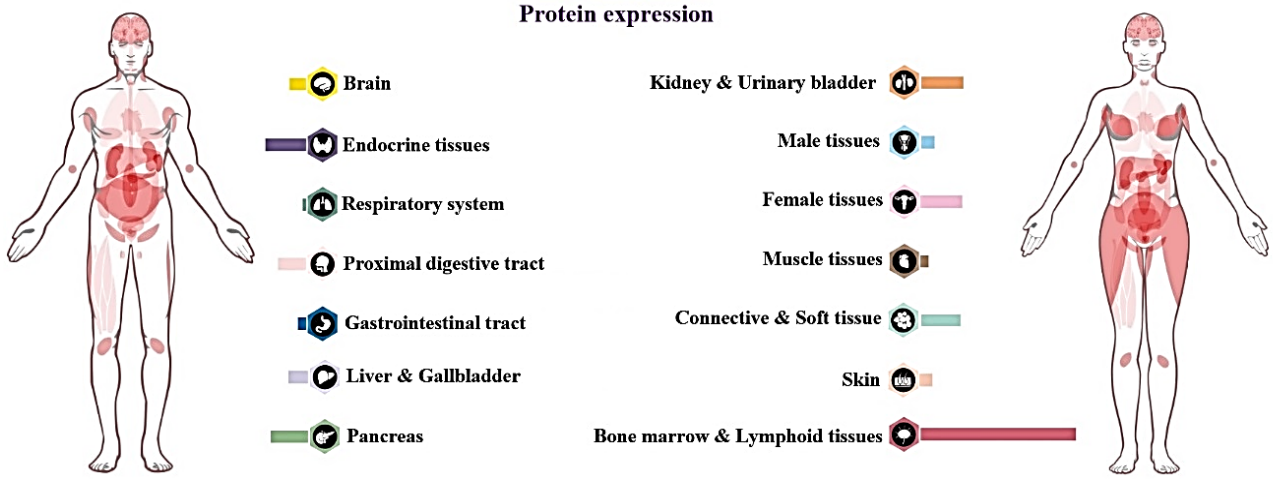

Fig. 3 Quebrachamine protein expression in different organs of the human body. Quebrachamine protein expression in different organs of the human body. Each bar represents the highest expression score found in a particular group of tissues. Protein expression scores are based on the best estimate of true protein expression.

Figure 3 shows the analysis of Quebrachamine protein expression in different organs of the body. The expression profile of this protein showed that this protein is expressed in most organs, but its expression level in bone marrow and lymphoid tissue is relatively higher than in other organs of the body. The results related to expression also show that this protein is less expressed in muscle tissues. In general, it can be said that this protein has a high expression in the nervous system and its related cells and has more effects. The results of the study of Quebrachamine protein expression in neural tissues also showed that this protein is expressed more in neurons than in other neural cells (Fig. 4).

Changes in Quebrachamine protein expression under the influence of the ACRA4 gene are shown in diagram 1. Quebrachamine protein expression analysis showed that Quebrachamine protein expression is influenced by the ACRA4 gene. These results showed that Quebrachamine protein expression increases in the Kidney, Adrenal gland, Adipose tissue, and Breast tissues. However, as can be seen in Fig. 5, the expression level in Spleen is higher than in other organs (Fig. 5).

The results of GC-MS analysis showed that this protein has 131 Total Peaks. Also, m/z top peak is 282, m/z 2nd Highest is 125 and m/z 3rd highest is 110. Fig. 6 shows the peaks related to Quebrachamine protein.

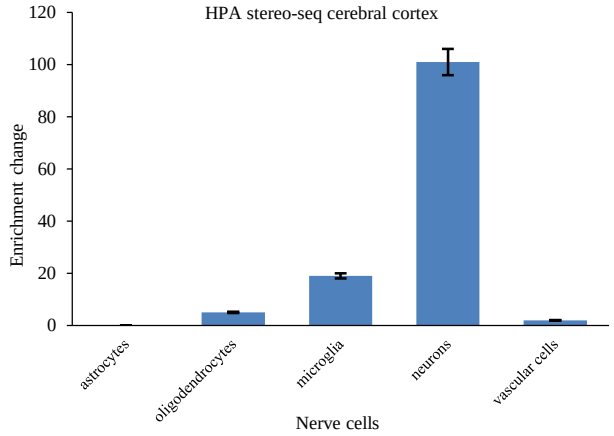

Fig. 4 Quebrachamine protein expression in nerve cells. HPA stereo-seq cerebral cortex associated with Quebrachamine in nerve cells. In this graph, each bar represents the number per million copies in each cell-type mask.

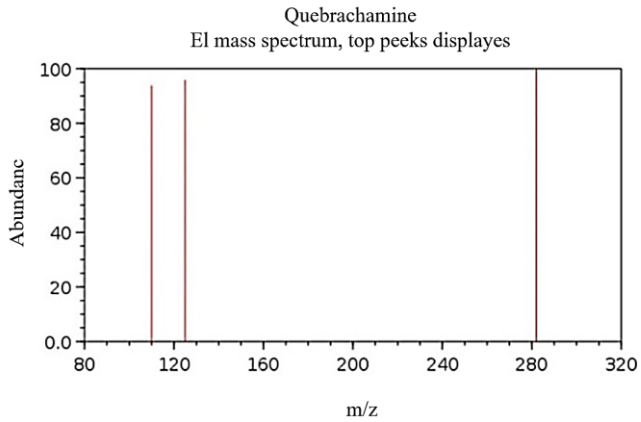

Fig. 6 GC-MS results on Quebrachamine protein. GC-MS results on Quebrachamine protein. m/z (mass-to-charge ratio): In mass spectrometry the ratio of an ion's mass (m) in atomic mass units (amu) to its formal charge (z).

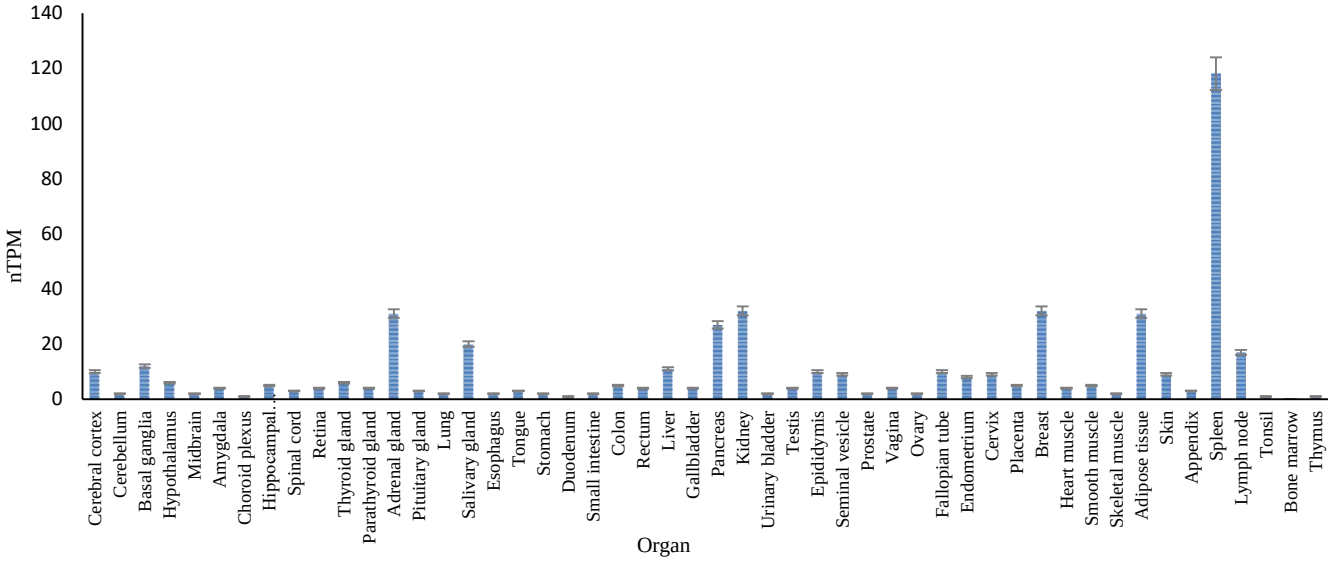

Fig. 5 Changes in Quebrachamine protein expression under the influence of the ACRA4 gene. Changes in Quebrachamine protein expression under the influence of the ACRA4 gene. For genes with low expression levels, expression data in cell clusters are evaluated to produce normalized transcripts per million by cell type, which is indicated by the (nTPM) index.

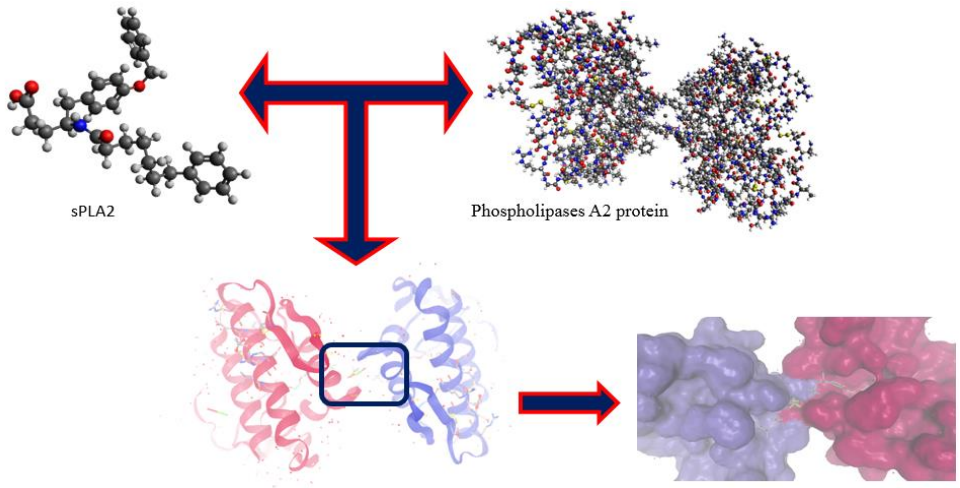

Fig. 7 Docking of sPLA2 on Phospholipases A2 protein.

Molecular docking is a precise computational method that predicts molecular binding sites by identifying the three-dimensional structure of two molecules and establishing a strong connection between the molecules. The docking protein design is similar to a lock and key, in that it organizes the binding of molecules to each other by accurately predicting the target site. In docking designs, considering the interaction of molecules and the orientation of the ligand and protein is very important. In this study, the active site of

the target protein was identified by reviewing related articles and accurately identifying the three-dimensional structure of the Phospholipases A2 protein. Then, the best orientation of the sPLA2 ligand for binding was examined. After going through these steps, the appropriate binding site was finally identified. Then, using the AutoDock software, the sPLA2 ligand was placed in its appropriate position, which in this study was the binding site of the two molecules between the two Phospholipases A2 protein complexes (Fig. 7). Rhazyaminine, an alkaloid derived from the plant *R. stricta*, shows promise as a potential ligand for molecular docking studies. The docking process involves simulating the interaction of Rhazyaminine with sPLA2 from scorpion venom, specifically from *Androctonus rostami*, to explore binding affinity and interaction patterns. These investigations include predicting the binding pose of Rhazyaminine within the active site of sPLA2, assessing the stability of the resulting complex through molecular dynamics simulations, and evaluating the impact of binding on the enzyme's activity. The success of molecular docking is assessed using various scoring functions and structural analyses. For example, a Ramachandran Plot analysis evaluates the quality of the protein model by examining the distribution of phi-psi angles of amino acid residues. In a favorable protein model, a high percentage of non-glycine and non-proline residues should be located within the most favored regions of the Ramachandran Plot. Additionally, G-factors, which assess the normalcy of structural parameters, provide further validation. Any deviations in parameters such as the Omega angle score may require further investigation to ensure the reliability of the protein model used in docking studies. The molecular docking of Rhazyaminine (Fig. 8) to iPLA2 (Fig. 9) from *A. rostami* was successful, leading to a stable ligand-protein complex that potentially inhibits the protein's function (Figs. 10 and 11). Such inhibition could be valuable in developing therapeutic strategies against scorpion venom-induced toxicity. The results of these simulations can guide experimental validation, paving the way for novel drug development targeting phospholipase enzymes in scorpion venom.

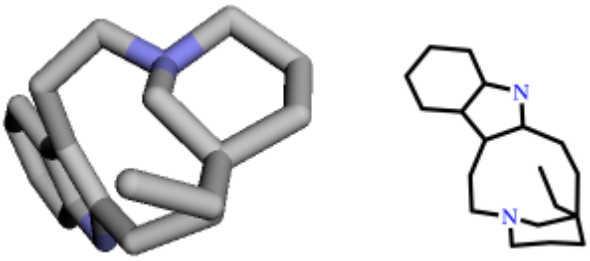

Fig. 8 Rhazyaminine ligand.

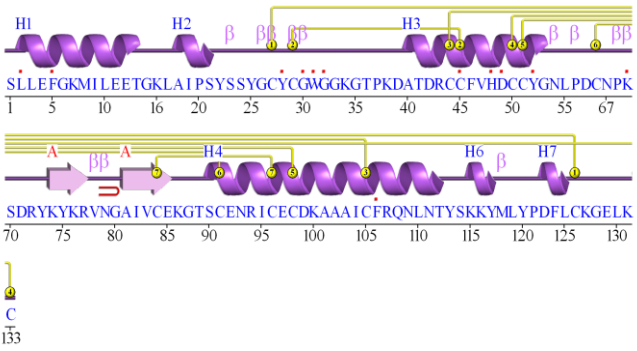

Fig. 9 Calcium-independent PLA2 (iPLA2) protein.

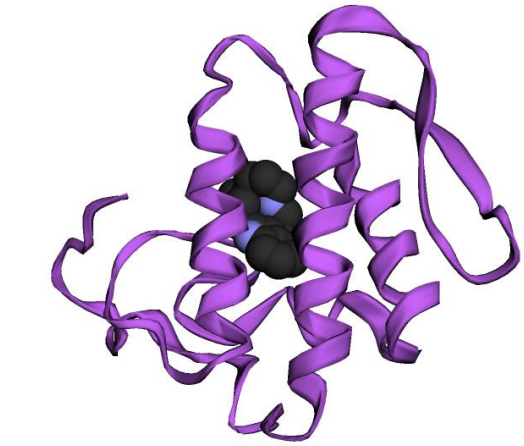

Fig. 10 Docking of Rhazyaminine on Calcium-independent PLA2 (iPLA2) protein.

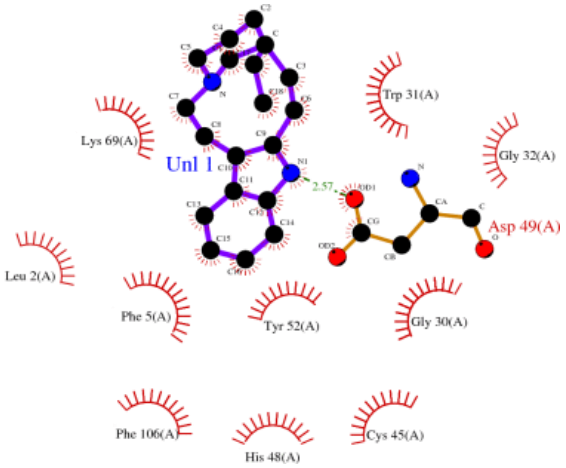

Fig. 11 Interactions involving ligand with protein.

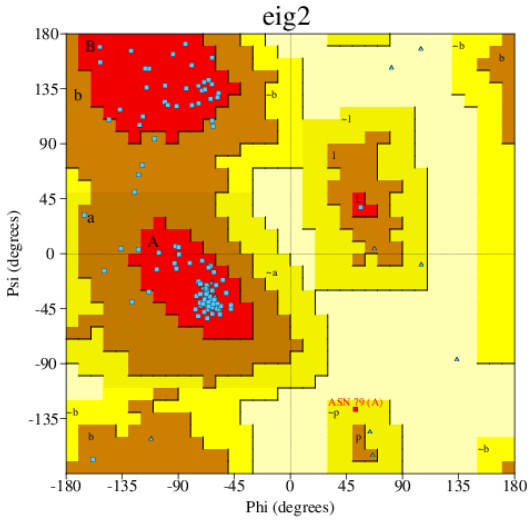

Fig. 12 Ramachandran analyses of Rhazyaminine on Calcium-independent PLA2 (iPLA2) protein.

The Ramachandran Plot analysis shows that 85.4% of non-glycine and non-proline residues are located in the most favored regions [A, B, L], with an additional 13.6% found in the additionally allowed regions. Only 1.0% of residues are in the generously allowed regions, and none are in the disallowed regions. Out of a total of 122 residues, which includes end-residues, glycine, and proline, these findings suggest a reasonably good protein model, albeit slightly below the expectation of over 90% in the most favored regions for high-resolution structures. The G-factors,

which evaluate the normalcy of various structural parameters, indicate some deviations. The average score for the Phi-psi distribution is -0.14, while the Omega angle score is -1.15, suggesting a highly unusual value. Other parameters, such as Chi1-chi2 distribution, Chi1 only, Chi3 & Chi4, main-chain bond lengths, and main-chain bond angles, have positive scores or scores close to zero, indicating they are within the normal range. The overall average G-factor is -0.11. It is important to note that main-chain bond lengths and bond angles are compared to ideal values from Engh & Huber [20], which may result in significant deviations if the structure is refined using different restraints. The unusual Omega angle score requires further investigation, while the rest of the structure appears to fall within acceptable norms based on these statistics (Fig. 12).

DISCUSSION

Quebrachamine's biological activities have been the focus of ongoing investigation. It has been found to exhibit adrenergic blocking properties, particularly in urogenital tissues, similar to the structurally related compound yohimbine [21]. This discovery suggests potential applications for treating urological disorders. Additionally, Quebrachamine shows promise in cancer research, with preliminary studies indicating that it may possess antitumor activity by inhibiting tumor growth. While these initial findings are encouraging, further research is necessary to fully understand its mechanisms of action and potential therapeutic uses [21, 22]. The synthesis of Quebrachamine has also garnered attention, with various methods developed to produce both racemic and enantiomerically pure forms of the compound [23]. These synthetic strategies not only facilitate access to natural products but also provide platforms for developing new catalysts and methodologies in organic synthesis. In conclusion, Quebrachamine’s unique structural properties, alongside its diverse biological activities, make it an intriguing subject for continued research. As our understanding of this alkaloid deepens, it may lead to novel medical applications and contribute to advancements in synthetic organic chemistry.

Molecular docking is a crucial technique in structural molecular biology and computer-aided drug design. It aims to predict how a molecule (ligand) orients itself when binding to a receptor molecule (protein) to form a stable complex. This process resembles a lock-and-key mechanism at the microscopic level, where the ligand fits into the protein's binding site based on shape and physicochemical properties. The goal is to identify the optimal conformation and relative orientation between the protein and ligand that minimizes the overall system's free energy. In the context of scorpion venom, secreted phospholipase A2 (sPLA2) serves as a key enzyme with specific biological activities. sPLA2, especially that derived from the Iranian *Scorpio maurus* (Maurolipin), has been molecularly characterized for its ability to inhibit ryanodine receptor channels and its neurotoxic effects. These enzymes catalyze the hydrolysis of phospholipids, producing lysophospholipids and free fatty acids, and are associated with various inflammatory and neurodegenerative diseases [24, 25]. Targeting sPLA2 with specific inhibitors can potentially mitigate the venom's toxicity and therapeutic intervention.

The expression profile of Quebrachamine protein displays a complex distribution pattern across various human tissues, consistent with the general understanding of protein expression in the human body. Similar to the findings reported by Uhlen *et al*. which indicated that a significant number of proteins are expressed in multiple cell types, Quebrachamine is also widely present in most organs [26]. However, its significantly higher expression in bone marrow and lymphoid tissue indicates a potential role in hematopoietic or immune functions. This variation in expression levels across different tissues aligns with the idea of cell-specific regulation of protein expression, as shown in studies of other proteins, such as vimentin [27]. The reduced expression of Quebrachamine in muscle tissues aligns with findings from other studies that have noted a decrease in certain proteins within muscular structures. In contrast, the high expression of this protein in the nervous system, especially in neurons, suggests it may play a crucial role in neuronal function or signaling. This neuron-specific expression pattern resembles that of other proteins enriched in neurons, as identified in extensive studies of the nervous system, including those conducted in *C. elegans* [28]. The diverse expression profile of Quebrachamine in various tissues highlights the significance of protein mapping projects for understanding the functional diversity of human proteins in different physiological contexts [29].

The expression profile of the Quebrachamine protein influenced by the ACRA4 gene shows a complex pattern of tissue-specific regulation, similar to the control mechanisms found in other protein expression studies. The upregulation of Quebrachamine in the kidneys, adrenal glands, adipose tissue, and breast tissues suggests a potential role in endocrine or metabolic functions, akin to the tissue-specific expression patterns seen with steroidogenic enzymes in adipose tissue [30]. The increased expression observed in the spleen is particularly interesting, as it may suggest a role in immune function or hematopoiesis. This organ-specific difference in expression levels supports the idea of cell-specific regulation of protein expression, a concept backed by studies of other proteins. The effect of ACRA4 on Quebrachamine expression resembles the regulation of other genes by transcriptional activators, such as MarA's role in regulating the acrAB operon [31]. Furthermore, the increased expression in adipose and breast tissues may have implications for breast cancer research, particularly as adipose tissue is increasingly recognized as a significant endocrine organ in the progression of breast cancer [30, 32]. This study enhances our understanding of gene-protein interactions and tissue-specific expression patterns, highlighting the complexity of protein regulation across various physiological contexts.

The GC-MS analysis of the Quebrachamine protein shows a complex mass spectral profile that reflects its structural intricacy. The detection of 131 total peaks indicates a highly fragmented molecule, a characteristic commonly observed in proteins analyzed by this technique [33]. The three highest peaks at m/z 282, 125, and 110 offer essential insights into the protein's fragmentation pattern and possible structural components [34]. The highest peak at m/z 282 likely indicates a significant molecular fragment or a stable ion formed during the ionization process. The secondary peaks at m/z 125 and 110 may correspond to smaller structural units or characteristic fragmentation products of the Quebrachamine protein [33, 34]. This spectral pattern is consistent with the typical fragmentation behavior seen in protein mass spectrometry, where peptide bonds are cleaved to generate a variety of fragment ions [35]. The numerous peaks in the spectrum indicate that Quebrachamine experienced significant fragmentation during analysis, a factor that aids in detailed structural elucidation. Comparing these peaks with spectral databases may yield valuable insights into the amino acid sequence and potential post-translational modifications of Quebrachamine [35].

Scorpion venom is a complex cocktail of bioactive compounds, including the enzyme phospholipase A2 (PLA2) [24]. PLA2s are

secretory enzymes that catalyze the hydrolysis of glycerophospholipids in cell membranes, releasing fatty acids and lysophospholipids [36]. These enzymes play diverse roles in various biological processes, including inflammation, neurotoxicity, and even antiviral activity [24, 37]. Due to their pharmacological potential, PLA2s from scorpion venom are of significant medical interest, particularly regarding the development of novel therapeutics [25].

PLA2 enzymes found in scorpion venom are classified as group III sPLA2s. They are distinct from groups I and II, except for the conserved active site and the Ca2+-binding loop. Typically, group III sPLA2s from scorpion venom are heterodimeric, consisting of a long enzymatic chain linked to a short chain by a disulfide bridge. The long chain contains the active site residues and the Ca2+-binding loop, which are essential for phospholipase activity. Given the role of PLA2 in various diseases, research into these enzymes and their inhibition is actively ongoing [38].

In silico analyses, including molecular docking, are essential for understanding the structure-function relationships of scorpion venom PLA2s and for identifying potential inhibitors [38]. These computational approaches enable researchers to model how PLA2 enzymes interact with various ligands, offering insights into binding affinities and mechanisms of action [24]. By identifying molecules that effectively bind to and inhibit PLA2, researchers can pave the way for the development of novel therapeutic interventions [37].

One potential research avenue is the exploration of natural compounds as PLA2 inhibitors. For example, while Rhazyaminine from *Rhazya stricta* was not directly mentioned in the search results, it could be investigated for its ability to bind to and inhibit sPLA2 from *A. rostami* [24, 38]. Molecular docking studies can be used to evaluate the binding affinity and identify key interactions between Rhazyaminine and the enzyme's active site. If the docking results are favorable, additional in vitro and in vivo studies may be conducted to confirm the inhibitory effect and assess its therapeutic potential.

Exploring PLA2 inhibitors derived from natural sources offers a promising strategy for developing new therapeutics for various diseases [25, 37]. Further research is necessary to fully understand the structure-function relationships of scorpion venom PLA2s and to identify effective inhibitors that can modulate their activity [38]. By utilizing a combination of in silico analyses, biochemical assays, and pharmacological evaluations, scientists can uncover the therapeutic potential of these enzymes and create innovative treatments for various conditions [24, 37].

CONCLUSION

Quebrachamine, an alkaloid found in various organs, particularly the nervous system, exhibits notable expression in bone marrow and lymphoid tissue. Its expression is influenced by the ACRA4 gene, leading to increased levels in the kidney, adrenal gland, adipose tissue, and breast tissues, with the highest expression in the spleen. Molecular docking studies reveal the potential of Rhazyaminine, another alkaloid, as a ligand for inhibiting secreted phospholipase A2 (sPLA2) from scorpion venom. Successful docking of Rhazyaminine to iPLA2 suggests the formation of a stable complex, potentially inhibiting the protein's function and offering therapeutic strategies against venom-induced toxicity.

Acknowledgments

This research was supported by project number PR-RIOZ-1402-8576-1 of the Research Institute of Zabol, Zabol, Iran.

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