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In silico analysis of κ-theraphotoxin-Cg2a from *Chilobrachys guangxiensis*

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κ-theraphotoxin-Cg2a is a 29- residue polypeptide extracted from the venomous glands of the Chinese earth tiger tarantula *Chilobrachys guangxiensis*. Plethoras of cancers are being associated with irregular functions of potassium ion channels. An extensive understanding of the toxin's interaction with the voltage-gated potassium channels is of utmost necessity for it to be screened as a potential pharmacological molecule which may perhaps serve as toxin-based therapy to manage various cancer channelopathies. Physicochemical properties were studied, the evolutionary analysis was done to visualize the conserved domain among different toxins of tarantula family, docking studies between κ-theraphotoxin-Cg2a and a voltage-gated potassium ion channel was done by ClusPro 2.0. The presence of signal peptide was observed using PSIPRED. Cysteine – disulfide bonds present in the amino acid sequence was predicted by DiANNA server. Multiple sequence alignment illustrated conserved residues with other families of tarantula's toxin. The docking of κ-theraphotoxin-Cg2a with the voltage-gated potassium channel was found to be interactive. The presence of cysteine –disulfide bonds were observed potentially playing a crucial role in the docking process. The interaction between the receptor and the ligand was found to be interactive which could turn out to help develop strategies to assist in creating potential pharmacological drug-based therapies.

Keywords: Chinese earth tiger tarantula, Ion channels, Signal peptide, Voltage-gated potassium channel

A group of large spiders with hairy legs and a body called tarantulas belongs to the family Theraphosidae that possesses 140 genera with around 983 species[1]. These spiders have the capability of consuming larger insects and birds making them the largest spiders in the world[2]. Just like any other spider they own venom glands[3] which aids them primarily in capturing and digesting prey and is also being used as a defense mechanism against predator as an alternative function[4-6]. Almost 100 of tarantula species have been identified so far. Tarantulas are extensively distributed across Mexico, the United States and regions of the world. Other species are also found in Australia, Africa, Asia, and some parts of Europe. Most of them hunt near or on the ground while some hunt their prey in trees. Like all other spiders, all tarantulas do produce silk. They also feed on millipedes and centipedes and some large tarantulas are capable of feeding on lizards, mice, and bats.

Tarantulas are venomous as they are capable of producing different types of toxins. Venom collected from tarantula is a multifaceted cocktail of toxins consisting of chiefly three categories of components mainly organic and inorganic molecules with low molecular weight (1 kDa), variety of peptides with molecular weights ranging from 2-8 kDa and a tiny group of proteins with high molecular weights (10kDa)[7] with impending uses which are being used as tools in pharmacological industries for the production of bioinsecticides and beneficial drugs to manage and treat cancer. κ-theraphotoxin-Cg2a is a toxin that is derived from the Chinese earth tiger tarantula *Chilobrachys guangxiensis*. Venom from [illegible] exceptional source of toxins for shal related subfamily channels. The shal related subfamily of the voltage-gated potassium ion channel is comprised of three separate genes that encode for $K_V4.1$, $K_V4.2$, and $K_V4.3$ that play a role in expressing transitory potassium currents which aid in normal cellular physiology. The genetic expressions for these proteins are extremely homologous that lies inside the transmembrane area with diverse carboxy and amino termini. Jingzhao toxins are short peptides that are highly arranged and constructed by disulfide bridges which are considered as potent $K_V4.2$ and $K_V4.3$ channel blockers[8]. These toxins alter the

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channel kinetics with regards to activation or inactivation gating mechanism by having interaction with the voltage sensor domains of the potassium ion channels[9-10].

Potassium ion channels are the largest group of ion channels among the ion channel family that is vital for the normal function of cellular processing. These channels are fundamental regulators of the distribution of K^+ ions for cellular ionic homeostasis and contribute to essentially all the elementary cellular processes. They play a characteristic role in selectively facilitating the transport of only K^+ ions via the plasma membrane of cells across an electrochemical gradient which results in the formation of membrane potential across the cell membrane[11]. During an action potential, the potassium ion channel takes part dynamically in the repolarisation of the cellular membrane which is followed by depolarization that is aided by the sodium ion channels resulting in the movement of K^+ ions via voltage-gated potassium ion channel controlling cell proliferation, cell cycle progression, and cell migration[12]. Toxins from other species of tarantulas such as *Grammostola rosea*, *Pterinochilus murinus*, and *Paraphysa scrofa* have also shown to act as blockers for specific members of the voltage-gated potassium ion channel families. As these toxins have shown to have interaction with K^+ channels, it leads a path of exploration and understanding which could pave a path towards toxin-based therapies which will aid in the treatment of several diseases and disorders. This paper aims to explore the toxin's protein sequence, secondary structures, phylogenetic relationships, and molecular docking studies on a voltage-gated potassium ion channel.

Materials and Methods

Sequence retrieval

The sequence of the potassium channel κ-theraphotoxin-Cg2a toxin was retrieved from Uniprot (www.uniprot.org) (Table 1).

Composition of the amino acid and prediction of κ-theraphotoxin-Cg2a

The composition of the amino acid and the physicochemical properties[13] of the potassium channel toxin κ-theraphotoxin-Cg2a was analyzed with the aid of the EMBOSS Pepstat tool (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/). With the aid of this tool different physicochemical properties were analyzed for the given protein sequence which included parameters such as molecular weight, theoretical pI, extension coefficient[14], aliphatic, aromatic, Grand Average of Hydropathy (GRAVY)[15] polar and nonpolar.

Secondary structure prediction of κ-theraphotoxin-Cg2a

The Secondary structure prediction for κ-theraphotoxin-Cg2a toxin was performed by using the PSIPRED[16] tool online for protein analysis (www. https:// bioinf.cs.ucl.ac.uk/psipred/)

Transmembrane prediction of κ-theraphotoxin-Cg2a

To identify the presence or absence of any transmembrane helices for potassium ion channel κ-theraphotoxin-Cg2a toxin, TMHMM[17] online server tool V. 2.0 was used to predict it (https:// www.cbs.dtu.dk/services/TMHMM/).

Hydrophobicity prediction of κ-theraphotoxin-Cg2a

The hydrophobicity prediction for κ-theraphotoxin-Cg2a was carried out through the Protscale tool (https://web.expasy.org/protscale/)

Cysteine-disulfide prediction of κ-theraphotoxin-Cg2a

The location of cysteine-disulfide for κ-theraphotoxin-Cg2a was located through DiANNA 1.1 Web Server (https://clavius.bc.edu/~clotelab/DiANNA/). It aims to find the connectivity of disulfide predictions.

Signal peptide sequence prediction of κ-theraphotoxin-Cg2a

Identification of signal peptide sequence for κ-theraphotoxin-Cg2a was carried out through the SignalP 4.1[18] server (https://www.cbs.dtu.dk/services/ SignalP-4.1/).

Phylogenetic tree construction of κ-theraphotoxin-Cg2a

Phylogenetic tree construction was performed by using CLUSTALW Omega, a multiple sequence alignment tool. The evolutionary relationship of the phrixotoxin family amongst the related tarantulas was considered (https://www.ebi.ac.uk/Tools/msa/

Table 1 — Retrieval of sequence

Toxin Name	Species origin	Uniprot Identifier	The peptide sequence of toxin	Sequence Length
κ-theraphotoxin-Cg2a	Chilobrachys guangxiensis	P0C5X7 (JZT45_CHIGU)	MKGSAFAIILGLVVLCACSFAEDEQDQFA SPNELLRSMFLESRHELIPEVEGRYCQKW MWTCDSERKCCEGYVCELWCKYNLG	83aa

clustalo/). The conserved domains between different tarantula toxins were visualized through PRALINE[19] (http://zeus.few.vu.nl).

Protein disordered region prediction of κ-theraphotoxin-Cg2a

The protein disordered region prediction for κ-theraphotoxin-Cg2a was carried out through the PrDOS server[20] (https://prdos.hgc.jp/cgi-bin/top.cgi). They help in recognizing any natively disordered protein region from its amino acid sequence.

Selection of a voltage-gated potassium ion channel (receptor) for docking study

A voltage-gated potassium ion channel (PDB ID: 1S1G) was selected for the docking study.

Protein-Protein docking of κ-theraphotoxin-Cg2a

Docking between κ-theraphotoxin-Cg2a (ligand) with the voltage-gated potassium ion channel (receptor) was carried out through ClusPro 2.0 Server (https://cluspro.bu.edu). Sequence retrieval for κ-theraphotoxin-Cg2a was taken from Uniprot and the potassium voltage-gated ion channel sequence retrieval was acquired from the protein databank PDB.

Results and Discussion

In this study, the physicochemical properties of the potassium channel toxin κ-theraphotoxin-Cg2a were predicted by using the PEPSTAT tool and the results as shown in (Table 2). It illustrates that glutamic acid is in abundance (12.0%), leucine (10.8%), and cysteine (9.63%). Histidine and threonine being the least abundant amino acid with (1.20%) each of the total amino acid composition. Cysteine is an amino acid that is usually abundant as L-form. They are predisposed to get oxidized very easily which results in a dimer containing disulfide bridges between two cysteines. They play a pivotal role in the analysis of primary protein structures, changes to secondary protein structures, and stabilization of tertiary and quaternary structures.

The isoelectric (pI) was found to be 4.42 Mol% which indicates that the protein is acidic in nature. Aliphatic index of a protein is usually defined as the volume which is relatively occupied by the aliphatic side chain amino acids such as valine, alanine, leucine, and isoleucine. It shows the thermostability of the globular protein with regards to its protein charge stability. Generally, the aliphatic index of cytotoxins lies between 66.5-84.33 which shows that they are thermally stable. The aliphatic index depends on the mole fraction of Ile, Leu, Val, and Ala which is present in the protein[21]. The aliphatic index value for κ-theraphotoxin-Cg2a is at 25.30 Mol% which indicates less thermostability. The aromatic acid content of this toxin is at 13.25 Mol %. Aromatic acids have a natural tendency to support the peptide self-assembly and sequence of the protein into cross-β amyloid. With the toxin being hydrophilic, the opportunity for a cross-β amyloid formation is very low. The toxin shows low basic properties due to its low basic amino acid content which is at 10.84 Mol% when compared with the acidic amino acid content for the toxin at 15.66 Mol% which indicates that it's a proton donator which is responsible for the acidic properties of the toxin. The extension co-efficient value for this toxin (20970 M^{-1} cm^{-1}) indicates the amount of light absorption at a particular wavelength. The extinction coefficient value for conserved protein at 280 nm wavelength ranges between 18006 to 71170 with regards to the concentration of cysteine (Cys), tryptophan (Trp), and tyrosine (Tyr)[22]. In this case, the toxin absorbs a higher amount of light which is visible in the UV spectrum. The secondary structure prediction was performed with the aid of PSIPRED software as seen in (Fig. 1). It does illustrate the presence of helix and coils within the toxin sequence. The helix position was found to be from 2-21 and 31-43 amino acid positions. Random coils in four stretches (1, 22-30, 44-76, 81-83 amino acid position) were found that represent the structure and energetic stabilization.

The toxin is envisaged to be a soluble protein and globular with a vast amount of alpha helix. In a globular protein, the amino acid chain can twists and folds in such a style that it augments the protein's solubility in water by inserting polar groups of atoms at the protein's surface where they can have desirable interactions with water molecules. This twisting and folding which determines the overall shape of a protein molecule are achieved by a tertiary structure that is chiefly due to the extremely multifaceted interplay of intramolecular forces that subsist between diverse groups of atoms within the molecule, and to intermolecular forces working among groups of atoms on the protein and molecules in the protein's direct surroundings.

The hydrophobicity of κ-theraphotoxin-Cg2a toxin which is represented by the GRAVY score. Kyte and Doolittle is the most extensively applied scale used to

Table 2 — Analysis of κ-theraphotoxin-Cg2a

Potassium voltage-gated ion channel KCND3

Protein Name	Source	PDB ID	Uniprot Identifier	Sequence length
Potassium channel subunit KCND3/ K_v 4.3	Homo sapiens	1S1G	Q9UK17	655aa

Protein Sequence

MAAGVAAWLPFARAAAIGWMPVANCPMPLAPADKNKRQDELIVLNVSGRRFQTWRTTLERYPD
TLLGSTEKEFFFNEDTKEYFFDRDPEVFRCVLNFYRTGKLHYPRYECISAYDDELAFYGILPEIIGDC
CYEEYKDRKRENAERLMDDNDSENNQESMPSLSFRQTMWRAFENPHTSTLALVFYYVTGFFIAVS
VITNVVETVPCGTVPGSKELPCGERYSVAFFCLDTACVMIFTVE
YLLRLFAAPSRYRFIRSVMSIIDVVAIMPYYIGLVMTNNEDVSGAFVTLRVFRVFRIFKFSRHSQGL
RILGYTLKSCASELGFLLFSLTMAIIIFATVMFYAEKGSSASKFTSIPASFWYTIVTMTTLGYGDMVP
KTIAGKIFGSICSLSGVLVIALPVPVIVSNFSRIYHQNQRADKRRAQKKARLARIRVAKTGSSNAYL
HSKRNGLLNEALELTGTPEEEHMGKTTSLIESQHHHLLHCLEKTTGLSYLVDDPLLSVRTSTIKNHE
FIDEQMFEQNCMESSMQNYPSTRSPSLSSHPGLTTTCCSRRSKKTTHLPNSNLPATRLRSMQELSTI
HIQGSEQPSLTTSRSSLNLKADDGLRPNCKTSQITTAIISIPTPPALTPEGESRPPPASPGPNTNIPSIAS
NVVKVSAL

Amino acid composition of κ-theraphotoxin-Cg2a

Type of Amino Acid	Amino Acid Composition (%)	Type of Amino Acid	Amino Acid Composition (%)
Ala	6.024	Leu	10.843
Arg	4.819	Lys	4.819
Asp	3.614	Met	3.614
Asn	2.410	Phe	4.819
Cys	9.639	Pro	2.410
Glu	12.048	Ser	7.229
Gln	3.614	Thr	1.205
Gly	6.024	Trp	3.614
His	1.205	Tyr	3.614
Ile	3.614	Val	4.819

Physicochemical properties of κ-theraphotoxin-Cg2a

Toxin Name	Mol. wt.	pI	Aromatic amino acid (Mol%)	Aliphatic amino acid (Mol%)	Non-Polar amino acid (Mol%)	Polar amino acid (Mol%)	Basic amino acid (Mol%)	Acidic amino acid (Mol%)	Extension co-efficient ($M^{-1}cm^{-1}$)
κ-theraphotoxin-Cg2a	9610.0	4.42	13.253	25.301	59.036	40.964	10.843	15.663	20970

Secondary structure prediction of κ-theraphotoxin-Cg2a

Toxin Name	Alpha helix position	Strand position	Coil position
κ-theraphotoxin-Cg2a	2-21 and 31-43	77-80	1,22-30,44-76 and 81-83

Transmembrane prediction of κ-theraphotoxin-Cg2a

Toxin Name	Sequence length	No. of Transmembrane helix
κ-theraphotoxin-Cg2a	83	0

Hydrophobicity prediction of κ-theraphotoxin-Cg2a

Toxin Name	Maximum value	Minimum value	GRAVY
κ-theraphotoxin-Cg2a	3.4	−1.9	−0.081

establish the hydrophobicity of a protein. When the region is greater than zero, it indicates its hydrophobic nature. As shown in (Fig. 2), the GRAVY value of κ-theraphotoxin-Cg2a initially increased and made a peak ~3.4 on the 13th amino acid position after which the GRAVY value declines downward toward the 23rd amino position for a value of −1.9. Another peak rise was seen from the 30th amino acid position for a GRAVY value of −0.1 with a decline till the 68th amino acid position with a −1.7 GRAVY value

and final rise in the peak at 0.8 followed by a decline at –0.08 at the 79th amino acid position. The final GRAVY score was at –0.081. Low range GRAVY score indicates that the protein could perhaps be globular (hydrophilic in nature) rather than being membranous protein (hydrophobic in nature).

Hydrophobicity and hydrophilicity increases and decreases respectively based on the formation of hydrogen bonds, therefore aiding in stabilizing the structure. These hydrophobic forces have also revealed to play a role in binding behavior[23]. This information could be constructive in localizing such proteins.

As predicted through DIANNA 1.1 server, potassium channel toxin κ-theraphotoxin-Cg2a has at least 4 (3 no.) disulfide bonds between cysteines in 16-62; 18-69; 55-74 and 68-78 positions as seen in (Table 3).

Stabilizing the tertiary and quaternary protein structures is achievable through disulfide bonds. Disulfide bonds present within the protein is a significant post-translational modification which is crucial for stabilizing the protein organization concerning its structure and also helps in a better understanding of the relationships between its function and structure[24].

Bond formation takes place between different amino acids of a protein chain; as a result, it leads to stabilization leading to maintaining a three-dimensional structure. Such bonding does occur in proteins and not in amino acids, but they do form a covalent bond between two amino acids *i.e.* cysteine and cysteine. The diversity of peptides mainly relies on point mutations, alteration of the termination codon, insertion, and deletion and shuffling section of oligonucleotide sequences. Spider toxins are known to contain an assorted group of disulfide patterns that fold into a range of three-dimensional structures. In general, 1136 toxin precursors are recognized in the transcriptome of venomous glands and from those nearly 65.8% of the matured peptide was reported to contain two adjacent residues of cysteine. Scaffolds of the toxin are present in an extensive range of bioactive peptide that is seen in snakes, spiders, scorpions, and other venomous animals. Research has shown that the venomous spider *H. Hainanum*, contains mature peptides with twelve cysteine residues making it the paramount number in a toxin peptide. The Earth Tiger Tarantula (*Selenocosmia*

Fig. 2 — Hydrophobicity plot of κ-theraphotoxin-Cg2a

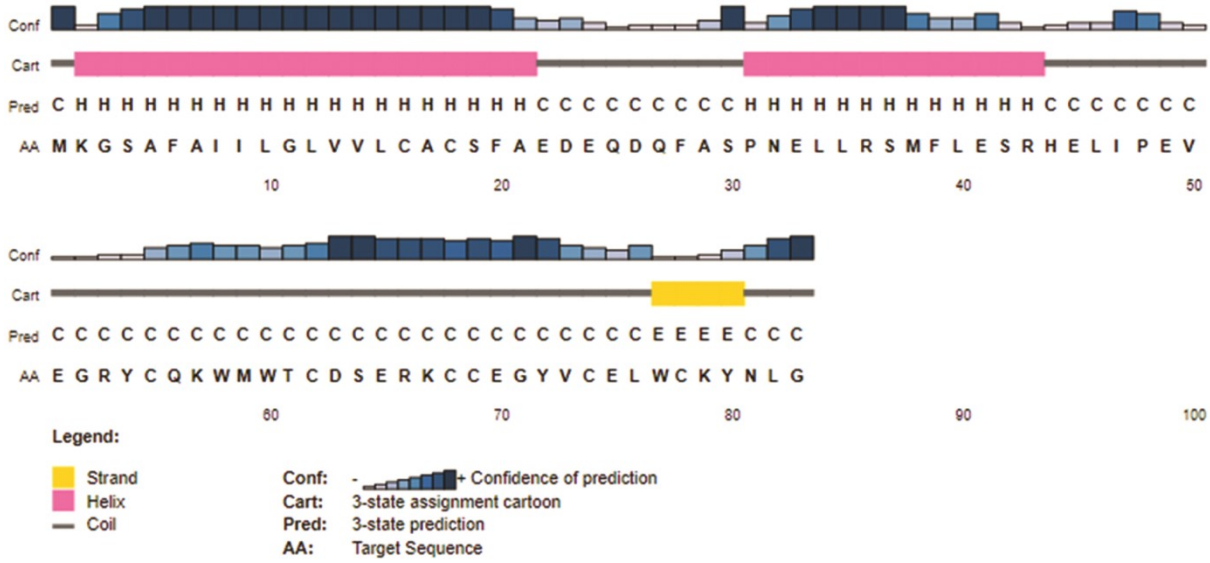

Fig. 1 — Secondary structure prediction of κ-theraphotoxin-Cg2a visualized through PSIPRED

Table 3 — Prediction of Cysteine-disulphide bonds for potassium channel κ-theraphotoxin-Cg2a toxin

Toxin Name	Sequence length	Cysteine sequence position		Presence of disulfide bond (Cysteine in Red)
κ-theraphotoxin-Cg2a	83	1	16-62	MKGSAFAIILGLVVLCACSF
		2	18-69	AEDEQDQFASPNELLRSMFL
		3	55-74	ESRHELIPEVEGRYCQKWMW
		4	68-78	TCDSERKCCEGYVCELWCKYNLG
		3 nos. Cysteine disulfide bonds		

Table 4 — Prediction of signal peptide sequence for potassium channel toxin κ-theraphotoxin-Cg2a

Toxin Name	Presence of peptide sequence	Cleavage site	Cut off value	Y-Score
κ-theraphotoxin-Cg2a	Yes	21-22	0.45	0.70

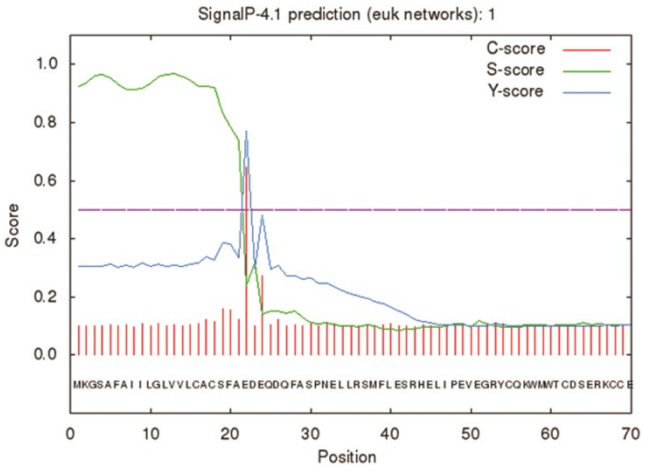

Fig. 3 — Signal sequence analysis of κ-theraphotoxin-Cg2a (Uniprot ID: P0C5X7). The C-score indicates a raw cleavage site, S-score indicates signal peptide score and Y-score is the combination of C-score and S-score. The cut-off value of more than 0.45 shows the presence of the signal peptide in a protein sequence.

huwena) possesses a double knot toxin (DkTx) that contains a C-C-CC-C-C-C-C-CC-C-C pattern which illustrates sequence similitude of 87% when compared with HN-Aa. DkTx functions by selectively and irreversibly activating the capsaicin and the heat-sensitive channel TRPV1.

The signal peptide is those 12-30 small amino acid sequence that is generally present in newly synthesized proteins that are en route to the secretory pathway[25]. In this study, κ-theraphotoxin-Cg2a is predicted to have a signal peptide within its protein sequence. The cleavage site is depicted by the C- Score and aids in distinguishing the signal of cleavage site from the rest of the known associated particles. Another important data obtained from SignalP 4.1 is the S-score, which indicates the score of the signal peptide. It facilitates in identifying the position of various signal peptides from the signaling proteins and non-signaling peptides of protein. Similarly, Y-score is a culmination of the C-score and S-score which also helps in identifying the signal peptides from the non- signal peptides. Any value above 0.45 shows the occurrence of the signal peptide within a protein sequence (Fig. 3). Table 4 shows the presence of signal peptide present in the toxin from 21-22 with a cut value of 0.45 and a Y-score of 0.70. It is seen that during the evolutionary progress of toxin families, the spider venoms experienced hypermutation within the regions of the mature peptide while preserving the fundamental disulfide framework. This framework seems to be connected with a definite signal sequence.

Nevertheless, the exact role of pro peptide regions is not clear but they may perhaps most likely take part in post translational modification such as N-terminal pyroglutamine and trimming of C-terminal and proteolytic processing[26].

A better quality of information on molecular evolution can be achieved from an enormous amount of sequence data. Although many strategies and techniques in evolutionary studies do rely on sequence-based data, the one widely used technique in the current scenario is the construction of a phylogenetic evolutionary tree which is made with the aid of multiple sequence alignment. (Fig. 4), shows the sequence alignment of toxins belonging to different families of tarantulas. A phylogenetic tree was constructed using Clustal W Omega as shown in (Fig. 5), which includes toxins taken from other families of tarantulas. P85117 (*Grammostola rosea)*, B1P1B6 (*Chilobrachys guangxiensis*), Q2PAY4 (*Chilobrachys jingzhao*), P61231 (*Paraphysa scrofa*), P61409 (*Grammostola spatulata*) and P61230 (*Phrixotrichus auratus*). The phylogenetic tree shows that P61231 and P61409 are sister toxins so is B1P1B6 and Q2PAY4. B1P1B6 and Q2PAY4, both the sister toxins are clad to P85117. Similarly, P61231 and P61409 sister toxins are clad to P61230. The idea of

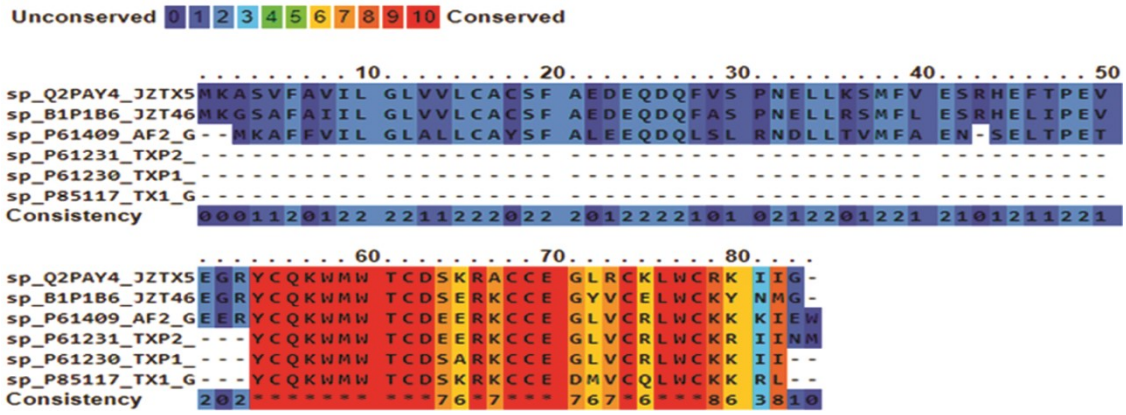

Fig. 4 — Multiple sequence alignment from diverse species of tarantula toxins with conserved residues highlighted as red

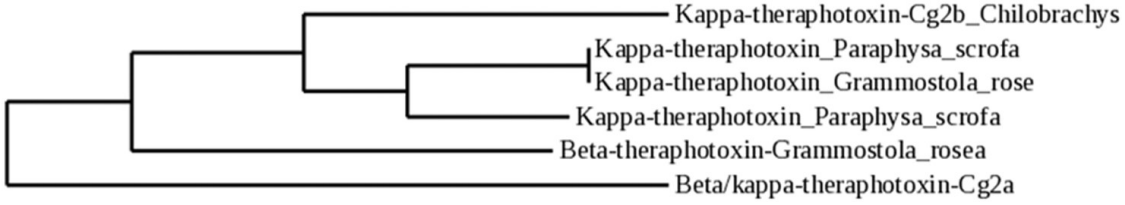

Fig. 5 — Phylogenetic tree prediction of κ-theraphotoxin-Cg2a from different tarantula toxins

protein evolutionary analysis revolves around comparing the homolog's sequences to identify common ancestors that could provide information on proteins that possess a similar structure or function.

So far it is being acknowledged that a bulky proportion of proteins in most proteomes do include disordered regions. Disordered regions are those fragments of the protein chain which do not take on a stable structure. Studies have shown that nearly 30% of the human proteins are fundamentally disordered that do not possess any 3D structure[27]. As they don't possess stable structures, they end up regulating other protein based functions which include binding with other proteins, protein interactions with nucleic acids cell cycle control, and signaling pathways. (Table 5) shows the disordered region of κ-theraphotoxin-Cg2a toxin with the aid of the PrDos server.

The computer-aided design which is known as molecular docking is a modeling technique that is widely used in the fields of bioinformatics. It facilitates the prediction of interactions between two molecules *i.e.* ligand and receptor. A tool that is extensively being used to predict protein-protein interactions and three-dimensional structures[28]. The binding affinity relies on the most favoured orientation which is the key component for scoring function. These scoring functions are generally physico based in nature with the force fields of

Table 5 — Disordered region prediction for κ-theraphotoxin-Cg2a

Toxin name	Protein disordered region (Disordered region in red)
κ-theraphotoxin-Cg2a	MKGSAFAIILGLVVLCACSFAEDEQDQFA SPNELLRSMFLESRHELIPEVEGRYCQKW MWTCDSERKCCEGYVCELWCKYNLG

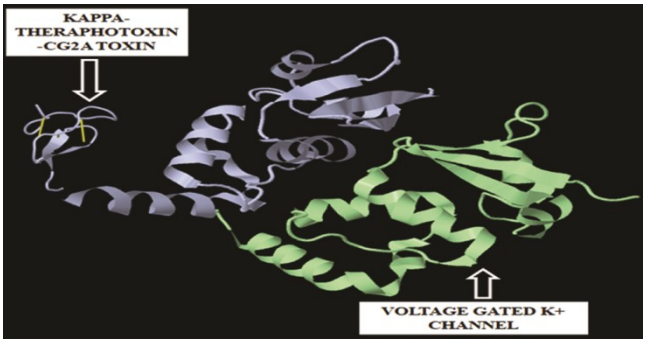

Fig. 6 — Predicted Chinese earth tiger tarantula κ-theraphotoxin-Cg2a Voltage-gated potassium channel bound complex visualized with JSmol First Glance software

molecular mechanics which roughly approximates the energy value. A low (negative) energy value generally demonstrates a stable orientation with good binding interaction. (Fig. 6), illustrates the molecular docking between the voltage-gated potassium channel and κ-theraphotoxin-Cg2a toxin which is being visualized by JS mol First Glance software[29]. The highest negative weighted score models were selected from

Table 6 — Molecular docking score prediction between voltage-gated potassium channel and κ-theraphotoxin-Cg2a

Cluster	Members	Representative	Weighted score
1	172	Center	–750.0
		Lowest energy	–962.4

the list of predicted models which signify an efficient docking (Table 6).

The weighted score is based on the following coefficient equation:

$$E=0.40E_{rep}+-0.40E_{att}+600E_{elec}+1.00E_{DARS}^{30}$$

where E_{rep} and E_{att} represent the repulsive and attractive contributions to van der Waals interaction energy. E_{elec} represents the electrostatic energy term. DARS signifies Decoy as Reference State as it represents desolvation contribution which is the change in the free energy which is caused by the removal of a water molecule from the interface. Molecular interactions rely on electrostatic forces as one of their foremost influence. Electrostatic energy interactions play a significant role in the biological process by controlling the ligand-protein interactions. Numerous proteins are being analyzed with their characteristics electrostatic potentials alongside with their functions[31].

Conclusion

Studies have shown the amino acids that are involved in molecular docking are usually occurring around the amino acid position 6, 13, 23, 25, and 26. In this study, the disulfide bonds were found to be between the amino acid residues range for docking parameters 16-62, 18-69, 55-74, and 68-78. This result illustrates how disulfide bonds play a chief role in protein docking prediction and shed light on their protein-protein interaction. On the other hand, disordered amino acids were found at 1-4, 81-83. Such a region of disordered region of protein generally tends to coalesce with domains that are structured in nature that mediate interaction within and between proteins. In the present study, the structure and sequence analysis including docking of the potassium channel κ-theraphotoxin-Cg2a toxin was done through a selection of bioinformatics software and tools. In molecular docking analysis, the interaction between the toxin and potassium channel was found to be viable. Molecular dynamics studies will be the next approach to further understand the movement and interaction between atoms and molecules over a period of time. With the data obtained further finding and characterization of κ-theraphotoxin-Cg2a toxin will be important for screening novel molecules with pharmacological properties that can play a role in the identification of new drug-based therapies for treating channelopathies.

Conflict of interest

All authors declare no conflict of interest.

References

1 World Spider Catalog. Natural History Museum Bern. Current valid spider genera and species. https://wsc.nmbe.ch/statistics/.

2 Coddington JA & Levi HW, Systematics and evolution of spiders (Araneae). *Annu Rev Ecol Syst*, 2 (1991) 565.

3 King GF, The wonderful world of spiders: Preface to the special Toxicon issue on spider venoms. *Toxicon*, 43 (2004) 471.

4 Escoubas P, Diochot S & Corzo G, Structure and pharmacology of spider venoms neurotoxins. *Biochimie*, 82 (2000) 893.

5 Sannaningaiah D, Subbaiah GK & Kempaiah K, Pharmacology of spider venom toxins. *Toxin Rev,* 33 (2014) 206.

6 Vassilevski AA, Kozlov SA & Grishin EV, Molecular diversity of spider venom. *Biochimie*, 74 (2009)1505.

7 Liao Z, Cao J, Li S, Yan X, Hu W, He Q, Chen J, Tang J, Xie J & Liang S, Proteomic and peptidomic analysis of the venom from Chinese tarantula *Chilobrachys jingzhao*. *Proteomics*, 11 (2007) 1892.

8 Zeng X, Deng M, Lin Y, Yuan C, Pi J & Liang S, Isolation and characterization of Jingzhaotoxin-V, a novel neurotoxin from the venom of the spider *Chilobrachys jingzhao*. *Toxicon*, 49 (2007) 388.

9 Yuan C, Liao, Zeng X, Dai L, Kuang F & Liang S, Jingzhaotoxin-XII, a gating modifier specific for Kv4.1 channels. *Toxicon*, 5 (2007) 646.

10 Zarayskiy, VV, Balasubramanian G, Bondarenko & VE, Morales MJ, Heteropoda toxin 2 is a gating modifier toxin specific for voltage-gated K^+ channels of the Kv4 family. *Toxicon* 45 (2005) 431.

11 González C, Baez-Nieto D, Valencia I, Oyarzún I, Rojas P, Naranjo D & Latorre R, K ($^+$) channels: function-structural overview. *Compr Physiol*, 2 (2012) 2087.

12 Kim DM & Nimigean CM, Voltage-Gated Potassium Channels: A Structural Examination of Selectivity and Gating. *Cold Spring Harb Perspect Biol*, 8 (2016) 5.

13 Olson SA, EMBOSS opens up sequence analysis. European Molecular Biology Open Software Suite. *Brief Bioinform*, 3 (2002) 87.

14 Priyadarsini KI, Gandhi VV & Kunwar A, Important chemical structural features of curcumin and its derivatives: How do they influence their anticancer activity?. *Indian J Biochem Biophys*, 57 (2020) 228.

15 Bashyal BM, Zaidi NW, Singh US & Aggarwal R, Effect of fungal biocontrol agents on enhancement of drought stress tolerance in rice (*Oryza sativa* L.). *Indian J Biochem Biophys*, 57 (2020) 101.

16 Buchan DWA & Jones DT, The PSIPRED Protein Analysis Workbench: 20 years on. *Nucleic Acids Res*, 47 (2019) 402.
17 Moller S, Croning MDR & Apweiler R, Evaluation of methods for the prediction of membrane spanning regions. *Bioinformatics*, 17 (2001) 646.
18 Emanuelsson O, Brunak S, Heijne GV & Nielsen H, Locating proteins in the cell using Target P, SignalP, and related tools. *Nature protocols*, 2 (2007) 953.
19 Pirovano W, Feenstra AK & Heringa J, PRALINE^{TM}: A strategy for improved multiple alignment of transmembrane proteins. *Bioinformatics*, 24 (2008) 492.
20 Ishida T & Kinoshita K, PrDOS: prediction of disordered protein regions from amino acid sequence. *Nucleic Acids Res*, 35 (2007) 460.
21 Bansal Ruchi, Sharma S, Tripathi K, Gayacharan & Kumar A, Waterlogging tolerance in black gram [*Vigna mungo* (L.) Hepper] is associated with chlorophyll content and membrane integrity. *Indian J Biochem Biophys*, 56 (2019) 81.
22 Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD & Bairoch A. In: Walker JM, editor. The Proteomics Protocols Handbook. (*Humana Press*) 2005, 571.
23 Zuo YQ, Yi ZS, Jie Xu, Rui YF, Wei YC & Liu HY, Molecular simulation and spectroscopic studies on the interaction between perfluoro hexadecanoic acid and human serum albumin. *Indian J Biochem Biophys*, 56 (2019) 185.
24 Tang HY & Speicher DW**,** Determination of disulfide-bond linkages in proteins. *Curr Protoc Protein Sci*, 11 (2004).
25 Barudin MA, Isa MLM & Yusof AM, Signal Peptide Sequence Analysis of Selected Protein Sequences from Cryptosporidium parvum. *Trends in Bioinformatics*, 11 (2018) 33.
26 Graham Nicholson M, Spider Venom Peptides. *Handbook of Biologically Active Peptides*, (2006).
27 Faust O, Grunhaus D, Shimshon O, Yavin E & Friedler A, Protein Regulation by Intrinsically Disordered Regions: A Role for Subdomains in the IDR of the HIV-1 Rev Protein. *Chembiochem*, 19 (2018) 1618.
28 Dar AM & Mir S, Molecular Docking: Approaches, Types, Applications and Basic Challenges. *J Anal Bioanal Tech*, 8 (2017) 356.
29 Jmol: An open –source browser based HTML5 viewer and a standalone viewer for chemical structures in 3D. http://www.jmol.org/.
30 Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, Yueh C, Beglov D & Vajda S, The ClusPro web server for protein-protein docking. *Nature Protocols*, 12 (2017) 255.
31 Basu A, Sarkar A, Maulik U & Basak P, Three dimensional structure prediction and ligand-protein interaction study of expansin protein ATEXPA23 from *Arabidopsis thaliana* L. *Indian J Biochem Biophys*, 56 (2019) 20.