Can proteomics of snake venoms help drug discovery? A study on the venom of spectacled cobra (*Naja naja*) from the Western Ghats

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Running Title: Venom proteomics of *Naja naja* from the Western Ghats of India

Abstract

Venom proteome profiling is important to understand the toxicology and treatment of persons poisoned by animal venoms. An in depth understanding of the pharmacological mechanisms induced by venom toxins could help in the discovery of novel drug molecules. In the current study, we aimed to delineate the venom toxins of Indian cobra (*Naja naja*) from the Western Ghats of India through SDS-PAGE and reversed-phase HPLC followed by Q-TOF LC-MS/MS analysis, incorporating PEAKS and Novor assisted *de novo* sequencing methodologies. A total of 143 proteins distributed across 17 different enzymatic and non-enzymatic venom protein families were identified. The *de novo* analysis exclusively yielded 59 peptides representing 28 venom protein families. Among these, glutathione peroxidase and endonuclease were reported for the first time in Indian cobra venom. Immunological cross-reactivity of cobra venom assessed using Indian polyvalent antivenoms suggested that VINS showed better EC_{50} (2.48 µg/mL) values than that of PSAV (6.04 µg/mL) and Virchow (6.03 µg/mL) antivenoms. Also, immunoaffinity chromatography performed using VINS antivenom indicated that it failed to detect few low molecular mass proteins (<10 kDa) that include three-finger toxins, phospholipase A_2s and kunitz- type serine protease inhibitors. Taken together, the present study enabled a large-scale characterization of the venom proteome of *Naja naja* that offers valuable insights on the possible pharmacological mechanisms and future therapeutic potential of hitherto unexplored snake venom constituents.

Significance

The present work describes the venom proteome characterization of *Naja naja* collected from the Western Ghats region in India, incorporating conventional proteomics approaches as well as *de novo* sequencing methods. Interestingly, we were able to determine proteins belong to glutathione peroxidase and endonuclease family, which was not reported in any of the previous

studies on *Naja naja* venom. Notably, our study has reported the highest number of proteins from cobra venom so far. Also, the current study highlights the importance of developing region-specific antivenoms for improving the specificity and cross-neutralization potential of antivenoms.

Key Words: Snake venom proteomics; *Naja naja*; Mass Spectrometry; *De novo* sequencing; Immunological cross-reactivity

Introduction

Small molecules continue to represent a significant part of the current drug discovery and development. However, there is a need to accelerate therapeutic innovation building on newer molecular leads and omics-based high throughput technologies. In this context, proteins and peptides, the major components of animal venoms present new opportunities for drug discovery as an alternative to traditional small molecule drugs [1, 2]. Snake venom is a cocktail of pharmacologically active components including proteins and peptides that primarily help snakes in capturing their prey [3]. Once injected, the venom interferes with the victims' body homeostasis and muddles up with the central and peripheral nervous system, blood coagulation machinery, cardiac functioning and muscle coordination [4]. Though there are numerous pharmacologically active components present in snake venom, reports suggest that only 0.01% of them are explored and investigated for possible therapeutic purposes [5]. To date, six of the FDA approved snake venom-derived drugs such as Integrilin, Aggrastat, Captopril, Defibrase, Hemocoagulase and Exanta have been used in the treatment of pulmonary embolism, stroke, heart attack, deep vein thrombosis, acute coronary syndrome and hypertension [2, 5]. Moreover, a few of them including Alfimeprase and Viprinex are under clinical trials aiming for the treatment of acute peripheral arterial occlusion and ischemic stroke [5]. All these are suggestive of the fact that characterisation of snake venom proteins and peptides and an in

depth understanding of their pharmacological mechanisms may lead to the discovery of novel drug candidates. The advancement of mass spectrometry assisted proteomics platforms have enabled wider opportunities in the drug discovery process. First and foremost, it helps in the identification and characterization of venom proteins that are important in the development of anti-venom treatment strategies. Secondly, it may help in the discovery of novel drug candidates.

Naja naja belonging to elapidae family is native to the Indian subcontinent and is also found in Sri Lanka, Bangladesh and Southern Nepal [6]. Envenomation by cobra often results in neurotoxicity leading to neuromuscular paralysis and in the most cases, it leads to cardiac arrest or respiratory failure that often results in death [7, 8]. It is evident from the previous reports that snake species belonging to elapidae family are rich in three-finger toxins (3FTxs) and phospholipases (PLA_2s) [9]. Proteins fall in 3FTxs family includes post-synaptic neurotoxins, cardiotoxins and cytotoxins. Though these proteins are smaller in size, they are highly toxic and are responsible for most of the morbidity and mortality cases associated with its envenomation. However, apart from these adverse biological effects, many of them are being characterised for evaluating their potential pharmacological mechanisms [10]. Similarly, PLA_2s are evaluated for their anticancer properties [11, 12]. Apart from 3FTxs and PLA_2s, studies reveal that cobra venom also contains various proteins belonging to enzymatic and non-enzymatic families including snake venom metalloproteases, nerve growth factors, phosphodiesterases, 5'-nucleotidases, snake venom serine proteases, cobra venom factors and L-amino acid oxidases [9].

Since *Naja naja* is distributed throughout India, studies were conducted to understand constituent proteins of *Naja naja* venom collected from different geographical locations and the data clearly indicate intra-species variation in the venom composition [6, 13-17]. The observed difference can be correlated to multiple factors including the geographical and prey-

specific variations [18, 19]. Irrespective of all these, it was interesting to note that cobra venom from all the geographical areas were predominant with proteins belonging to three-finger toxin class. However, due to lack of inadequate protein sequence information in public repositories, in-depth characterisation of snake venom proteome remains a challenging one. Previous studies from our laboratory on venom proteome of various snake species had clearly demonstrated the importance of homology-assisted sequencing strategies in elucidating the venom profiles of snake species, whose sequence information is under-represented in the databases [20, 21].

While snake venomics approaches enables characterising the toxic venom arsenal of various snake species, antivenomics on the other hand utilises this information for extending the possibilities in improving antivenom preparations and in assessing the immunological cross reactivity between homologous toxins present within various snake species [22]. Since antivenoms are the only treatment of choice available for treating envenomed victims, there is a need in improving the efficacy of the available antivenoms [8]. It is important to note that though Indian polyvalent antivenoms contains enough antibodies against antigenic epitopes present in the cobra venom, they often fail to neutralise few of the low-molecular weight but highly toxic components including several 3FTxs and PLA_2s [13, 15]. Even the immunological cross reactivity profiles of various antivenoms towards cobra venom seem to be varied [13, 16].

We report here, new observations on the venom proteome of *Naja naja* venom from the Western Ghats of India through orthogonal separation and identification strategies. Also, we have evaluated the specificity and binding potential of commercially available antivenoms towards *Naja naja* venom.

Materials and methods

Reagents and chemicals

Formic acid (FA), methanol, acetonitrile (ACN), trifluoroacetic acid (TFA), acrylamide, bisacrylamide, ammonium persulphate, coomassie brilliant blue R-250 (CBB R-250), beta-mercaptoethanol, bromophenol blue, glycerol, glycine, iodoacetamide (IAm), ammonium bicarbonate, Tetramethyl benzidine/Hydrogen Peroxide (TMB/H_2O_2), HRP-conjugated secondary antibody, tetramethylethylenediamine, dithiothreitol (DTT), trypsin, chymotrypsin and V8 proteases were purchased from Sigma-Aldrich. ECL substrate, Tris, SDS and PVDF membrane were procured from Bio-Rad. NHS-activated Sepharose 4 Fast Flow was obtained from GE Healthcare and protein ladder from Thermo Fisher Scientific.

Venom collection

Venom from two snake species of *Naja naja* from the Western Ghats of India (NNwg) was collected in captive. Venom milking was done in-house (as per the order, GO (Rt) No. 94/2009/F & WLD dated 25/02/2009) in the Government serpentarium (Thiruvananthapuram, Kerala, India) by an experienced herpetologist. Followed by lyophilization, venom was stored at -80 ^{o}C until analysis.

Antivenoms

Lyophilized equine antivenoms raised against the 'Big Four' snakes of India (*Echis carinatus*, *Naja naja*, *Bungarus caeruleus*, and *Daboia russelli*) obtained from VINS (batch number: 01AS16040, expiry date: 07/2020), Virchow (batch number: PAS00116, expiry date: 01/2020) and Premium Serums and Vaccines (PSAV; batch number: ASVS (I) Ly-010, expiry date: 08/2022) were used for the entire immunological cross-reactivity assays.

SDS-PAGE and In-gel digestions of crude NNwg venom

12% SDS gel was used to resolve the crude NNwg venom (170 µg) proteins. The bands obtained were stained and visualized using CBB R-250 stain followed by scanning using a gel

doc system. The gels bands separated under reducing conditions were then processed for in-gel digestion procedures. For this, the gel bands were cut into small pieces and excess stain was removed through 25 mM ammonium bicarbonate and 50% ACN washing. This step was repeated until the gel bands were clear. Subsequently, the gel pieces were subjected to reduction using 100 mM DTT. The reaction was performed at 56 °C for 45 min. After incubation, the tube was allowed to cool at room temperature and excess of DTT was aspirated. Further, alkylation was performed using IAm (250 mM in ammonium bicarbonate). This was kept for incubation in the dark at room temperature for 30 min. After this, 25 mM ammonium bicarbonate buffer solution was used to wash the gel pieces for removing any leftover IAm. Subsequently, the gel pieces were dehydrated using 100% ACN for removing water content. Followed by this, in-gel digestion was done using trypsin, chymotrypsin and V8 protease on three different gel preparations. For trypsin digestion, 25 µL of enzyme (from 12.5 ng/µL stock) was added to the gel pieces after dehydration (using 100% ACN). Roughly, 200 ng of chymotrypsin or V8 protease were added on to the individual gel pieces for the respective digestion processes. All the three enzymatic digestions were allowed to proceed at 37 °C for 16 h. The extraction of the digested peptides was done using ACN and water containing 0.1 % TFA. The extraction step was repeated twice, and the resulting supernatants were pooled and were completely dried using a vacuum concentrator. The samples resuspended in 5% ACN solution were subjected to mass spectrometric analysis or stored at -80 ºC until analysis.

Reversed-phase HPLC of NNwg venom

3.2 mg of crude venom was dissolved in 80 µL of Milli-Q water and was centrifuged at 12000 rpm for 15 min at 4 ºC. From this, 5 µL was loaded on to a reversed-phase column (Agilent Zorbax SB C8, 150 x 2.1 mm, 3.5 µM) through an Agilent 1260 HPLC system for the separation of various venom components. Solvent (A: water + 0.1% FA; B: ACN + 0.1% FA) flowrate was maintained at 200 µL/min. The following time program was used for the fraction

collection: 5% B for 5 min, 5-15 % for 10 min, 15-45 % B for 60 min, 45-70 % B for 12 min, 70-95% B for 5 min and equilibration for 13 min. The absorbance was monitored at 210 nm and the peaks were manually collected and stored.

In-gel and In-solution enzymatic digestions of HPLC separated protein fractions

In-gel digestion of RP-HPLC separated fractions were performed as mentioned above. The fractions collected after reversed-phase HPLC was subjected to in-solution enzymatic digestions also. The pH of the samples was adjusted to 8.0 using 50 mM ammonium bicarbonate solution prior to in-solution digestion. Initially the samples were subjected to reduction using 100 mM DTT. This was then kept for incubation at 56 °C for 45 min. Alkylation was performed on the resulting solution using IAm (250 mM in ammonium bicarbonate solution). The tubes were incubated at room temperature in the dark for 30 min. Followed by this, trypsin, chymotrypsin or V8 protease were added and kept for incubation at 37 °C (overnight). The resultant solutions were acidified and dried completely using a vacuum concentrator.

Tandem mass spectrometry

The digested samples were reconstituted in 5% ACN containing 0.1% FA. Sample analysis was performed using an Agilent 6540 Q-TOF LC-MS/MS equipped with a chip cube source. The samples were infused to the MS through an HPLC system containing capillary and nano pumps that deliver solvents to the enrichment (40 nL) and analytical columns (Zorbax 300 SB-C18, 150 mm x 75 µm, 5 µ) present in the chip, respectively. The solvent (A: water with 0.1% formic aid; B: 90% acetonitrile with 0.1% formic acid) flowrate was maintained at 3 µL/min for the capillary and 0.3µL/min for the nano pump. The gradient conditions used for the separation of the peptide samples are as mentioned: initial equilibration using 5% B for 2 min, 5-45 % B in 30 min, 45-90% B in 45 min, 90% B for 2 min. All the MS data were collected in a data-dependent and positive ionisation mode using nitrogen as the collision gas. The MS

parameters used for data acquisition are as follows: MS range- 250-3000 m/z; MS/MS-50-3000 m/z; Capillary voltage: 2000 V; Dry gas temperature: 325 ^{o}C; Dry gas flow: 4 L/min; Fragmentation voltage: 150 V; MS and MS/MS scan speeds were set at 4 spectra/sec and 3 spectra/sec, respectively. A maximum of five precursors were selected for the fragmentation. The charge states for tryptic digested samples were selected as 2, 3, >3 and for chymotrypsin and V8 digests: 1, 2, 3, >3. Active exclusion was enabled after 3 spectra with a releasing time of 0.5 min. MassHunter software from Agilent was used for the data acquisition and analysis.

Identification and validation of proteins using Mascot and Scaffold

The mgf files generated from the raw mass spectrometric data were analysed using Mascot Daemon (version 2.5.0) search with the following parameters: Database: Serpentes (Taxonomy ID:8570); peptide tolerance: 10 ppm, MS/MS tolerance: 0.5 Da; amino acid modifications: cysteine (carbamidomethylation as fixed modification), glutamine, asparagine (deamidation) and methionine (oxidation) as variable modifications.; number of missed cleavages: 2; Enzymes (selected charge states), trypsin (2^+, 3^+, 4^+), chymotrypsin and V8 protease (1^+, 2^+ and 3^+); the database searches were decoy enabled. The obtained data was further validated using Scaffold software by setting the protein and peptide identification probabilities to 99% and 95%, respectively [23, 24]. The data was filtered using the inbuilt peptide and protein prophet algorithms and those proteins which are represented by a minimum of 2 peptides were included for the protein identification and classification process.

Protein identification through PEAKS

The obtained MS data was also subjected to protein identification using PEAKS Studio (version 8.5) software. The desired experimental conditions were set accordingly prior to data analysis [20].

De novo sequencing through Novor and MS-BLAST

In order to identify peptides with possible amino acid mutations, Novor followed by MS-BLAST sequence similarity search was performed. For this, the raw data converted to mgf format was subjected to Novor analysis using the DeNovoGUI (Version 1.15.11) user interface [25]. Parameters used for the identification of proteins and peptides are: 10 ppm and 0.5 Da error tolerance for precursor and fragment masses, respectively; amino acid modifications: cysteine (carbamidomethylation as fixed modification), glutamine, asparagine (deamidation) and methionine (oxidation) as variable modifications. The obtained data after Novor analysis was pre-processed manually and subjected to MS-BLAST [26] analysis using Serpentes database as described previously [21].

BLASTp and ClustalW analysis

The authenticity of the mutated peptides obtained through *de novo* analysis were validated through BLASTp analysis. For this, the obtained sequences were searched against non-redundant database from NCBI. Also, the same procedure was applied for confirming the sequences obtained for newly reported proteins from NNwg venom. Multiple sequence alignment of the peptides representing these proteins were performed through ClustalW analysis.

Data availability

The raw mass spectrometry data and processed files have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) [27] via the PRIDE partner repository [28] with the dataset identifier PXD018936.

End point titration ELISA

ELISA was performed as described previously [20]. 1000 ng of NNwg venom added on to high bind ELISA plate (Costar) was kept for overnight incubation at 4℃. The unbound venom was removed from the wells by inverting the plates and flick drying. Followed by this, any non-

reactive sites in the wells were blocked by adding 2.5% BSA in PBS and incubated for 2 h at room temperature. Primary antibodies were serially diluted using 2.5% BSA by a factor of three (1:100, 1:300, 1:900, 1:2700, 1:8100, 1:24300, 1:72900, 1:218700, 1:656100, 1:1968300, 1: 5004900) and was incubated for 2 h at room temperature. The unbound antibodies were removed through 0.1% phosphate buffered saline (PBST) wash and this was repeated thrice. Followed by this, secondary antibody (HRP conjugated) at a dilution of 1:32,000 in 2.5 % BSA was added on to each well and kept for incubation at room temperature for 2 h. Excess of secondary antibodies were removed through 0.1% PBST wash as mentioned above. To the wells, 100 µL of HRP substrate solution, TMB/H_2O_2, was added and incubated in the dark for 30 min at room temperature. The end point readings were taken by terminating the reaction by adding 100 µL of 0.5 M sulphuric acid to each well. The optical density values were recorded at 450 nm using a plate reader. The EC_{50} values were computed from the obtained OD values through non-linear regression analysis using Prism (version 6.01) software [20].

Western blotting

For immunoblotting experiments, 50 µg of crude NNwg venom resolved on a 15% SDS gel was transferred (25 V for 1 hr) to a PVDF membrane. To minimize non-specific binding, the membrane was blocked using 5% BSA at room temperature for 1 hr. Antivenoms obtained from Virchow, PSAV and VINS were used as primary antibodies (dilution used-1:500) and was kept for incubation at 4 ℃ (overnight). The membrane was washed thrice for 10 min using 0.1% PBST solution. To this, secondary antibody (HRP conjugated) at 1:5000 dilution in 5% BSA was added and kept at room temperature for 1 hr. The excess of secondary antibody was then removed through 0.1% PBST wash as mentioned above. Followed by this, ECL substrate was added on to the membrane and signals indicating the venom-antivenom interaction were captured using a plate reader (Bio-Rad).

Second generation antivenomics

To determine the venom-antivenom interaction, second generation antivenomics protocol was adapted from a previous study [22]. For this, 600 µL of NHS-activated Sepharose 4 fast flow was packed on to a column. The column was washed using 15 column volumes of ice cold 1 mM HCl for removing isopropanol (used for storing column material) and to make sure that the pH of the column was maintained between 7.0- 8.0. After this, the column was pre-equilibrated using coupling solution (0.2 M $NaHCO_3$ and 0.5 M NaCl, pH 8.3). To this 30 mg of anti-venom dissolved in the coupling buffer was added and kept for overnight incubation at 4 °C. Subsequently, unbound anti-venom was eluted out and the non-reacted groups were blocked using 0.1M Tris-HCl at room temperature for 4 hr. The column was then alternatively washed using buffers with high (0.1 M Tris-HCl; pH 8.5) and low pH (0.1 M acetate buffer containing 0.5 M NaCl; pH 4.5). After this, the column was equilibrated using 5 column volumes of working buffer solution (1X PBS, pH 7.4). Followed by this, 200 µg of venom dissolved in PBS was added. After keeping it for incubation (4 h at room temperature), the unbound venom was washed out using PBS. The bound venom proteins were eluted using 0.1 M glycine-HCl, pH 2.0 and the resultant solution was immediately neutralised using 1M Tris buffer, pH 9.0. The bound and unbound fractions after desalting was subjected to RP-HPLC analysis as mentioned above.

Statistical analysis

Prism (GraphPad software Inc., San Diego, CA; version 5) was used for performing statistical comparisons. The data obtained from triplicate experiments were plotted on to the software and is represented as mean ± standard deviation or as stated, otherwise.

Results

SDS gel-based separation of crude NNwg venom

The crude NNwg venom components were resolved on a 12% SDS gel. Bands observed after CBB staining indicated that the venom components were distributed across a wider mass range (Figure 1). All the visible bands excised from the samples run under reducing conditions were subjected to various enzymatic digestion protocols followed by tandem mass spectrometric data collection.

HPLC fractionation followed by SDS-PAGE analysis of NNwg venom

The fractionation of NNwg venom components were performed on a reversed-phase column as mentioned in the methodology section. As seen in Figure 2a, a total of 12 peaks were collected. All these peaks were further separated under reducing conditions on a 12% SDS gel. From the results (Figure 2b), it is evident that most of the fractions (labelled 1—8) fall around 10 kDa molecular weight region. All these peaks were eluted within 42 min (26% B) of the chromatographic run and the rest of the peaks (labelled 9-12) found to contain proteins with molecular weights range between 10-238 kDa. However, the LC chromatogram indicates that almost all the observable proteins got eluted within 65 minutes of the chromatographic run.

Proteomics of NNwg venom

The proteins and peptides identified through different enzymatic digestion procedure from NNwg venom are shown in Tables 1 and 2. From the Tables and Figure 3a, it is evident that NNwg venom contains proteins belonging to enzymatic and non-enzymatic families. A total of 143 proteins representing 17 different snake venom protein families were identified. Of the 143 proteins, 74 belong to enzymatic class and 69 were of non-enzymatic in nature (Tables 1 and 2). The enzymatic proteins identified includes snake venom metalloproteases (SVMP), glutathione peroxidase (GPrx), phospholipase A_2s (PLA_2), acetylcholinesterase (ACE), phospholipase B (PLB), endonuclease (END), phosphodiesterase (PDE), snake venom serine

proteases (SVSP),5-nucleotdase (5'-NU) and L-amino acid oxidases (LAAO). Some of the non-enzymatic protein families identified include three-finger toxins (3FTxs), nerve growth factor (NGF), cysteine-rich secretory proteins (CRISP), cobra venom factor (CVF), C-type lectins (CTL), Ohanin/Vespryn (OLP) and kunitz-type serine protease Inhibitors (KSPI). As seen in Figure 3a, 28% of the identified proteins belongs to 3FTx family followed by SVMP (11%) and PLA_2 (9%). The identified proteins classified according to their molecular masses indicated that almost 28% of them falls under 10 kDa region (Figure 3b). 3FTxs, the highly abundant proteins commonly found in elapidae snake species are seen in this mass range.

Importance of *de novo* sequencing strategies in elucidating NNwg venom proteome

Peptides that failed to appear in conventional database searches were subjected to homology-driven proteomic approaches using PEAKS and Novor based *de novo* sequencing followed by MS-BLAST analysis and manual validation. As seen in Table 2, a total of 59 peptides with various amino acid mutations were exclusively determined through *de novo* approaches. From the analysis, it is conclusive that 23% of the total proteome identified from NNwg venom was obtained through *de novo* approaches (Figure 3c). This finding highlights the importance of following multiple data analysis approaches in elucidating the venom proteome of snake species whose protein sequence information is limited in the public repositories. *De novo* analysis of a peptide representing alpha-cobratoxin identified from NNwg is shown in Figure 4. Manual inspection of the doubly charged peptide at m/z 651.2766 indicated that NNwg derived peptide has a glycine instead of alanine (A28G). Similarly, we could identify a total of 59 peptides representing 28 proteins through *de novo* analysis. The identified peptides harbouring one or more amino acid mutations along with other relevant information are shown in Table 2.

Newly identified proteins from NNwg venom

Though a total of 17 different snake venom protein families were identified, it is interesting to note that glutathione peroxidase and endonucleases were reported for the first time in Indian cobra venom (Table 3). The peptide information representing these families of proteins are included in Tables 1 and 2. For confirming the authenticity of the derived peptides, sequences were aligned from N-C terminus and subjected to pBLAST using the non-redundant database from NCBI. The sequences obtained were further subjected to multiple sequence alignment using ClustalW. Figures 5a and b show the sequence similarity of the obtained sequences with respect to various snake species. As seen in Table 1, the peptides representing GPrx 3 identified from NNwg venom showed 77% sequence similarity to a glutathione peroxidase reported in *Ophiophagus hannah*. Similarly, the endonuclease identified in this study shown to have 53% sequence similarity with an *O.hannah* protein (accession no- ETE56723).

Commercial antivenoms showed varied immunological cross-reactivity profiles towards NNwg venom

All the tested Indian antivenoms showed a dose-dependent increase in reactivity towards NNwg venom components. However, as seen in Figure 6a, the binding potential seems to be varied across the tested antivenoms. Though the end point titration values of all the antivenoms were same (1:72,900), the strength of binding seen at the same dilution was higher in Virchow antivenom. At the same time, EC_{50} values computed from the graph suggest that VINS (2.48 µg/mL) showed better reactivity towards NNwg venom compared to that of PSAV (6.04 µg/mL) and Virchow (6.03 µg/mL) antivenoms. Similarly, the specificity of antivenom binding towards NNwg venom was assessed through immunoblot analysis. As seen in Figure 6b (i), the resolved proteins were transferred on to a PVDF membrane and immunoblotting was performed using various polyvalent antivenoms as primary antibodies. From the results (Figure

6b (ii-iv)), it can be inferred that the binding specificities are varied across different antivenom preparations.

VINS antivenom failed to detect NNwg venom proteins falling under 10 kDa

Since the immuno cross-reactivity assays showed a significant difference in binding potential of various antivenoms towards NNwg venom, second generation antivenomics approach was used in determining the identity of antivenom bound and unbound protein fractions (Figure 7a-c). It was observed from the obtained EC_{50} values that VINS antivenom showed better reactivity towards NNwg venom (Figure 6a). Hence, we performed the immunoaffinity experiments using VINS antivenom. Our results reveal that HPLC peaks numbered 1-4 were not present in the bound fractions (Figure 7c). Further analysis confirmed that these peaks contain proteins belonging to 3FTx, KSPI and PLA_2 venom families (Figure 7a).

Discussion

Proteins and peptides, the major components of animal venom open up new opportunities for therapeutic innovation as an alternative to the traditional small molecule drugs. Several snake venom-derived components have been used towards drug discovery against cancer, diabetes, cardiovascular diseases and inflammatory disorders [29-31]. In this context, snake venom profiling using mass spectrometry-based proteomics enables in the identification of toxins oblating various pharmacological activities.

Reports suggest that 3FTxs and PLA_2s are the major proteins present in snake species belonging to elapidae family [9]. Agreeing with this observation, our study also shows that proteins representing these two families are the major toxin components present in NNwg venom (Table 1 and Figure 3a). Three finger toxins present in cobra venom belong to low molecular mass proteins having 57 to 82 amino acids and include several neurotoxins, cytotoxins/cardiotoxins and muscarinic toxins [10, 32]. Neurotoxins and muscarinic toxins bind to muscle nicotinic acetylcholine receptors (nAChRs) and muscarinic acetylcholine

receptors, respectively [10, 33, 34]. Though the exact target of cardiotoxins are not identified, they are known to induce cytolysis of cardiomyocytes, erythrocytes and also prevent platelet aggregation [10, 35]. Proteins targeting nAChRs will be useful in developing drugs for neurodegenerative diseases and as analgesics [36]. As an example, cobratoxin from *Naja kaouthia* was tested for neuropathic pain in rats and has shown to have antinociceptive effect [37]. In our studies too, we were able to identify multiple peptides representing proteins belonging to alpha-cobratoxins (Table 1). Apart from this, the *de novo* approach resulted in the identification of two additional peptides TWCDGFCSIR and VRTGVDLQCCSTDNCNPF representing an alpha-cobratoxin (*Naja siamensis*; accession no-P01391). Compare to the *N.siamensis* counterpart, doubly charged NNwg peptides with m/z, 651.276 and 1071.961 were having A28G and K49R mutations, respectively. Since muscarinic toxin acts as a α2B-adrenoceptor antagonist, it has potential applications in treating blood pressure. Similarly, cardiotoxin-I has shown to have insulinotropic activity through induction of insulin secretion from β-cells and can be used in developing drugs for type 2 diabetes [32]. As seen in Tables 1 and 2, almost all subclasses of 3FTxs including long and short neurotoxins, weak toxins, muscarinic toxin-like protein, cytotoxins and cardiotoxins were identified from NNwg venom. Cytotoxins present in snake venoms trigger non-selective disruption of cell membranes that eventually alter their structure and function [38]. Because of this property, cytotoxins isolated from various snake venoms are being evaluated for their anticancer properties [39-41]. For example, cytotoxin NN-32 purified from Indian cobra venom showed anti-proliferative effects on MDA-MB-231 breast cancer cells [42]. It is proposed that cytotoxins from cobra venom mediates anticancer effects through lysosomal damage [40, 41]. As seen in Table 1, our analysis resulted in the identification of 18 proteins representing various cytotoxins such as cytotoxin-1, 2, 7, 8, 9, 13 and 14. Additionally, four peptides harbouring multiple amino acid

mutations were identified through our *de novo* analysis (Table 2). These identified peptides might open up the possibilities of expanding the library of anticancer molecules.

PLA_2s, the collection of proteins belonging to enzymatic venom protein family, are mainly involved in releasing lysophosphoglycerides and fatty acids by the catalytic cleavage of phosphoglycerides. They mediate multiple pharmacological effects such as platelet aggregation, anticoagulation, neurotoxic and cardiotoxic activities [43]. Most importantly, the myotoxic, neurotoxic and cytotoxic potential of several purified PLA_2s from the Indian cobra venom has been studied in detail [44-46]. Apart from the toxic effects, PLA_2s are being evaluated as potential anticancer molecules. BnSP-6, isolated from *Bothrops pauloensis* showed in vitro cytotoxic activities on MDA-MB-231 and MCF10A breast cancer cells [47]. Similarly, BthTX-I, a PLA_2 isolated from *Bothrops jararacus* has shown to induce cytotoxic effects on various cancer cells [48]. As seen in Table 1, twelve proteins representing several PLA_2s reported from various Naja species are identified through our analysis. Additionally, a doubly charged tryptic peptide with m/z 594.779, NSGTPVDDLDR, representing a PLA_2 protein was determined through *de novo* analysis. On manual inspection, it was identified that NNwg derived peptide has asparagine instead of glycine in the 38th position (Table 2). Altogether, the identified PLA_2s constitutes to 9% of the total proteome of NNwg venom (Figure 3a).

Like cytotoxins and PLA_2s, LAAOs isolated from various snake species are also evaluated for their anti-cancer properties [49]. LAAOs are flavoenzymes with flavin adenine dinucleotide as cofactors. Flavins are accountable for the yellow colour of snake venom and they mediate cell toxicity by inducing oxidative stress through hydrogen peroxide release [49]. They are also involved in arbitrating platelet aggregation activation or inhibition, anticoagulation, myonecrotic and haemorrhagic activities [49, 50]. OH-LAAO isolated from *O. hannah* has shown to have anti-proliferative potential on MCF-7 human breast cancer and A549 human

lung adenocarcinoma cells [11]. Similarly, ACTX-8, an LAAO isolated from *Agkistrodon acutus* induced DNA fragmentation and phosphotidylserine externalization, the prominent markers for apoptosis, in HeLa cervical cancer cells [12]. From our LC-MS characterization, it is evident that 6% of the proteins identified from NNwg venom represents LAAO family (Figure 3a and Tables 1 and 2). All the NNwg venom derived LAAO peptides showed sequence similarity to the peptides reported from *Bungarus multicinctus*, *Oxyuranus scutellatus* and several other Naja species. On the other hand, *de novo* sequencing yielded two more additional peptides harbouring different amino acid mutations. As seen in Table 2, two peptides, FWEADGLHNK and IAETVHLNAR, representing an LAAO, showed sequence similarity with a *Naja atra* protein. Manual inspection of the MS/MS data of FWEADGLHNK confirmed that the NNwg venom derived peptide has asparagine and lysine at the 360th and 361st positions, respectively (G360N and G361K). Similarly, the triply charged peptide (IAETVHLNAR) at m/z, 375.213, was found to contain threonine instead on methionine (M273T). The lysine mutation (G361K) identified possibly explains why this tryptic digested peptide was not determined through conventional database analysis. All the identified proteins/peptides can be further isolated and purified for checking their potential pharmacological mechanisms.

Snake venom proteases, another major venom protein belonging to the enzymatic family, are known to cleave fibrinogen and affect haemostasis through diverse anti- and pro-coagulant mechanisms [51]. Proteases with fibrinogenolytic and anti-coagulation properties have potential application in drug development for treating thrombotic disorders including stroke and heart attack [52]. Unlike viper venoms (72%), both serine and metalloproteases are least abundant in elapidae species (12-19%) [9]. In accordance with this, our analysis indicated that SVMPs (11%) and SVSPs (7%) contribute only 18% of the NNwg venom proteome identified (Figure 3a). SVMPs are zinc dependent high molecular mass proteins with multidomain

organization, divided in to four main classes, PI, PII, PIII and PIV. They possess fibrinolytic, haemorrhagic and platelet aggregation inhibitory activities [53]. For example, kaouthiagin, a metalloprotease that cleaves and binds to von Willebrand factor (vWF) was isolated and characterised from *Naja kaouthia* venom. Cleavage of vWF results in collagen mediated platelet aggregation inhibition [54]. As seen in Table 1, several peptides representing two kaouthiagin proteins were identified from our study on NNwg venom. Additionally, two more peptides, KTPAYQFSSCSVR (T178K, A179T, F182Y, I189V) and LYCTEKNTMSCR (L367R), harbouring various amino acid mutations representing a kaouthiagin protein was determined through *de novo* analysis (Table 2). Also, it is reported that NN-PF3, a non-toxic anticoagulant SVMP protein inhibits collagen induced plated aggregation through binding to α2β1 integrin receptors [55]. Like cobra venom SVMPs (NN-PF3 and kaouthiagin), several other PIII-SVMPS from different snake species were characterised for their platelet-aggregation inhibitory activities [56-58]. As seen in Table 1, most of the SVMPs identified through our study belong to PIII class. Apart from this, a total of 19 peptides representing multiple SVMPs were identified through *de novo* approach. The details of all the *de novo* derived peptides are provided in Table 2. SVSPs, on the other hand, are mainly composed of thrombin-like and fibrinolytic proteins. Thrombin-like proteins are mainly used as defibrinogenating agents because fibrin formed during clotting can be easily removed from circulation [59]. As seen in Table 1, all the NNwg venom derived thrombin-like proteins showed sequence similarity to peptides reported from *Naja naja* and *Gloydius ussuriens* species. In addition to this, tissue-type plasminogen activator proteins were also present in NNwg venom (Table 1). Since plasmin is involved in degrading clots formed by fibrin, tissue type plasminogen activators can be used as thrombolytic agents for treating deep vein thrombosis [60]. Apart from the proteins reported in Table 1, our *de novo* analysis resulted in

the identification of four additional peptides harbouring various amino acid mutations representing different tissue-type plasminogen activator proteins (Table 2).

Apart from these, some of the minor venom protein families identified from NNwg venom includes KSPI (2%), PDE (6%), 5'-NUC (5%), ACE (3%), OLP (5%) and CTL (1%). Kunitz-type serine protease inhibitors are known to act on several serine proteases and MMP-2s [61]. They inhibit activity of coagulation factors including thrombin, protease C, factors Xa and XIIa and also found to regulate blood pressure [62]. Considerable number of KSPIs are identified through proteomics analysis of *Naja naja* venom from different parts of India [6, 13-17]. Similarly, our study also resulted in the identification of eight different peptides representing KSPI (Table 1). The *de novo* sequencing on the other hand has resulted in the identification of an additional peptide representing a kunitz-type inhibitor protein reported from *Walterinnesia aegyptia* venom. On manual inspection, the doubly charged peptide with m/z, 927.9134, was contained to have four amino acid mutations (P43T, S44A, H50D, and H52R). Besides the inhibitory activities, KSPIs possess anti-myonecrotic, anti-haemorrhagic, trypsin and chymotrypsin inhibitory activities too [63-65]. Contrary to KSPI, PDEs and 5'-NUC are enzymes involved in the release of endogenous nucleosides resulting in venom-induced hypotension [66, 67]. They were reported to have platelet aggregation inhibitory activities also. Hence these molecules can be evaluated as potential anticoagulant molecules in atherosclerotic conditions including myocardial infarction and stroke [68, 69]. Apart from the peptides reported in Table 1, through *de novo*, we were able to identify two additional peptides harbouring single amino acid mutations representing 5'-NUC. One of the peptides contains V338L amino acid substitution when comparing it with a peptide representing a homologous protein from *M.fulvius*. Similarly, the other tryptic digested peptide, EVVQFMNSLR, has a glutamine replaced by histidine. In addition, two peptides representing PDEs, having multiple amino acid mutations were also identified through our *de novo* analysis (Table 2).

Snaclecs, the C-type lectins found in snake venom are known to activate and inhibit platelet aggregation mechanisms [70]. One of the earlier studies demonstrated that Jerdonibitin, a glycoprotein Ib (GPIb) binding snaclec, has a potential to inhibit platelet aggregation in a dose-dependent manner [71]. In another notable study, vipegitide, a α2β1 integrin antagonist with potential anti-platelet activity was synthesised using a CTL, vixapatin, isolated from viper venom as a template [72, 73]. It's efficiency to inhibit platelet binding on to collagen α2β1 integrin was proved and can be further evaluated in the development of anti-platelet drugs [73]. Peptides representing homologous C-type lectin BfL-1 from *Bungarus fasciatus* was also identified through our analysis (Tables 1 and 2). Through *de novo* approach, two peptides representing the same protein (accession no- Q90WI8) were identified. The peptides, YNCPLDWLPK and NNEFCVELVK, were contained to have one amino acid mutation (T25N, N134K) each. However, apart from our findings, CTL was reported only in one of the cobra venom proteomics study from India [17]. Apart from the above-mentioned protein families, peptides representing acetylcholinesterases and ohanin were also identified from NNwg venom. ACE, a subclass of cholinesterase, are involved in the hydrolysis of neurotransmitter acetylcholine to acetic acid and choline [74]. Our LC-MS/MS analysis resulted in the identification of four proteins representing ACEs (Table 1). However, since ohanin like proteins are present only in smaller amounts, they are not isolated or characterised in detail from snake venoms. Nevertheless, ohanin identified from king cobra has shown to induce hyperalgesia and hypolocomotion in mice [75]. In addition to six proteins identified through conventional approaches, our *de novo* analysis yielded a doubly charged tryptic peptide, FNGSPCVLGSPGFR, having a single amino acid mutation (S100G) representing ohanin.

Earlier studies have clearly indicated the use of complementary protein/peptide detection techniques in providing information on protein function-structure relationships and its possible

role in accelerating the drug discovery process. Though venom gland transcriptomics has opened up the possibilities in exploring snake venom proteome profiles, due to its limitations, details on protein isoforms might be missed [2, 76]. For example, venom proteomics of *Sistrurus catenatus edwardsii* studied using different ionisation mechanisms using MALDI and ESI mass spectrometry enabled the identification of various protein isoforms that were not detected through the transcriptomics study [76]. Similarly, the peptide/protein information obtained from NNwg venom (Tables 1 and 2) was compared with the proteome information of cobra venom elucidated from its transcriptomics data [17]. Notably, many peptide data obtained from our study were not identified through the transcriptomic analysis. Though the protein families identified from NNwg venom have been already reported in the previous studies, our analysis exclusively resulted in the identification of two protein families, endonuclease and glutathione peroxidase (Tables 1, 2 and 3; Figures 5 a and b). Endonucleases are known to hydrolyse nucleic acids to their respective nucleotides. 5'-NUC utilises these nucleotides as substrates to generate nucleosides, resulting in venom-induced hypotension [67]. However, the exact pharmacological role of GPrx in snake venom is yet to be understood. In addition to these protein families, considerable number of protein isoforms with potential pharmacological properties were also identified through our study (Tables 1 and 2). While comparing with the other studies on Indian cobra venom proteome, more number of proteins were identified from our analysis (Table 4). In summary, while 77% of the total proteins were identified through conventional proteomics approaches incorporating Mascot and PEAKS tools, the remaining 23% protein identities were established by *de novo* sequencing approaches (Figure 3c). These results are on par with our previous findings on venom characterisation of two pit viper species that showed the importance of following *de novo* sequencing workflows in developing snake venom proteome profiles [20, 21] and thereby identify novel protein candidates towards drug discovery.

Further, from a clinical perspective, detailed information on the toxin components present in venom helps in streamlining antivenom production strategies. As recommended by World Health Organisation, various in vitro preclinical studies including ELISA, western blotting and immunoaffinity chromatography are widely used in determining the specificity and reactivity of commercially available antivenoms towards various snake venoms. In India, polyvalent antivenoms are manufactured by using pooled venom from 'Big 4' snake species that include *Naja naja, Bungarus caeruleus, Daboia russelii and Echis carinatus*. But still the commercial antivenoms from major Indian manufacturers' present varied immunological reactivity towards cobra venom collected from different geographical locations in India [13, 15, 16]. In agreeing with the above observation, our results obtained through end-point titration ELISA indicate that the immuno-cross-reactivity of VINS antivenom (EC_{50}: 2.48 µg/mL) is better compared to Virchow (6.03 µg/mL) and PSAV antivenoms (6.04 µg/mL) (Figure 6a). Also, as seen in Figure 6b, there are notable differences in detecting the antigenic epitopes of NNwg venom by various antivenoms. It was evident that low molecular mass proteins were abundant in NNwg venom (Figure 6b (i)). However, all the antivenoms tested were showing varying specificity profiles towards NNwg venom epitopes (Figure 6b (ii-iv)). Most importantly, compared to low molecular mass proteins, all the antivenoms recognized high molecular mass proteins with better specificity. Previous reports indicate that high molecular mass proteins are less toxic but highly immunogenic compared to highly toxic low molecular mass proteins that are poorly immunogenic [77], and the same was observed from our findings. The differences in the antivenom neutralisation efficiencies might be contributed by the differences in the venom antigens that were used for immunizing horses while generating polyvalent antivenoms [78]. Though ELISA and western blotting can provide an overall immunoreactive profile of venom and antivenom interaction, they are not successful enough to distinguish the antivenom binding and non-binding protein components. To verify this, we have followed a second generation

antivenomics approach combining immunoaffinity chromatography and LC-MS based proteomic characterization [22]. Though most of the major proteins are found in the bound fraction (Figure 7c), some proteins (represented by the peaks, 1-4) are not getting recognized by the antivenom. From the MS analysis it was identified that 3FTxs, PLA_2s and KSPIs were present in those peaks, suggesting that VINS antivenom failed to detect few proteins falling under 10 kDa region (Figure 7a). Altogether, our study reveals that the specificity of antivenoms varies across different manufactures. Hence, as recommended by WHO, the production and purification strategies need to be streamlined for improving the efficacy of antivenoms [79].

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Conflict of Interest

There are no potential conflict of interests.

Figure Legend

Figure 1. 12% SDS-PAGE analysis of crude NNwg venom. 1,molecular mass ladder; 2, Crude NNwg venom resolved without β-ME (non-reducing) condition; 3, Crude NNwg venom subjected to β-ME treatment (reducing) condition. The bands resolved under reducing

conditions were further subjected to various enzymatic digestion (trypsin, chymotrypsin and V8) strategies followed by Q-TOF LC-MS/MS analysis.

Figure 2 (a). Reversed-phase HPLC profile of crude NNwg venom. Crude *Naja naja* venom (500 µg) was subjected to reversed-phase HPLC separation using an Agilent 1260 HPLC system. X-axis and Y-axis corresponds to the retention time and peak intensity observed at 210 nm, respectively.

Figure 2 (b). 12% SDS-PAGE of RP-HPLC peaks of NNwg venom. The 12 peaks collected after HPLC separation were resolved on a 12% SDS gel under reducing conditions. Each of these bands were further diced and subjected to trypsin, chymotrypsin and V8 protease digestion. The resulting peptides were further subjected to Q-TOF LC-MS/MS analysis. (MW; molecular weight marker)

Figure 3. Relative abundance of protein families identified from NNwg venom (a) after different separation and digestion strategies and (b) according to molecular weight. (c) Percentage distribution of total proteome identified from NNwg venom through conventional proteomics and *de novo* sequencing methods. (Relative abundance = (Number of proteins identified representing a snake venom protein family/ Total number of proteins identified) X 100). (PLA_2-Phospholipase A_2, PLB-Phospholipase B, SVMP- Snake Venom Metalloprotease, NGF- Nerve Growth Factor, CRISP- Cysteine Rich Secretory Protein, PDE-Phosphodiesterase, 5'-NUC- 5 Nucleotidase, SVSP- Snake Venom Serine Protease, 3FTx- 3 Finger Toxin, CVF- Cobra Venom Factor, LAAO- L-Aminoacid Oxidase, GPrx- Glutathione peroxidase, ACE- Acetylcholinesterase, OLP- Ohanin/ Vespryn family proteins, END-Endonuclease, CTL-C Type Lectin, GPrx-Glutathione Peroxidase)

Figure 4. *De novo* analysis of peptide representing NNwg venom protein. Cobra venom derived peptide (inset) showing sequence similarity to alpha-cobratoxin (P01391; *Naja kaouthia*) harbouring one amino acid mutation. The m/z values and corresponding ion

intensities are labelled in the x- and y- axis, respectively. MS/MS spectrum representing and y ions are labelled along with the immonium ions (depicted by single letter amino acid code along with 'i' as subscript). The b5 and y6 ions representing the mutated amino acid glycine (G) are highlighted in red colour in the spectra.

Figure 5. pBLAST and ClustalW analysis: (a) Endonuclease and (b) Glutathione peroxidase peptide sequences identified from NNwg venom after Mascot search were subjected to pBLAST followed by multiple sequence alignment using known sequences from various snake species. Those highlighted in-boxes are the shared amino acid sequences between NNwg and various other snake species.

Figure 6. Assessing the immunological cross-reactivity of various polyvalent antivenoms towards NNwg venom. (a) Titration curves obtained through ELISA using various dilutions of VINS, PSAV, Virchow antivenoms. Results are represented as mean ± SD of triplicates. The EC_{50} values for various antivenoms obtained from this graph are shown in insets. (b) (i) 15% SDS-PAGE gel electrophoretic profile of crude NNwg venom under reducing conditions. Immunoblot of the SDS-PAGE with (ii) VINS (iii) PSAV and (iv) Virchow polyvalent antivenoms.

Figure 7. Immunoaffinity chromatography of NNwg using VINS antivenom (a) The protein peaks observed from crude NNwg venom on reversed-phase HPLC column are labelled from 1-12 (b) the unbound and (c) bound fractions were eluted using PBS and glycine buffers, respectively. Proteins present in the bound and unbound fractions were compared using the chromatogram obtained for crude venom (a). The proteins present in each peaks were determined through proteomics analysis.

Figures

Figure 1. 12% SDS-PAGE analysis of crude NNwg venom

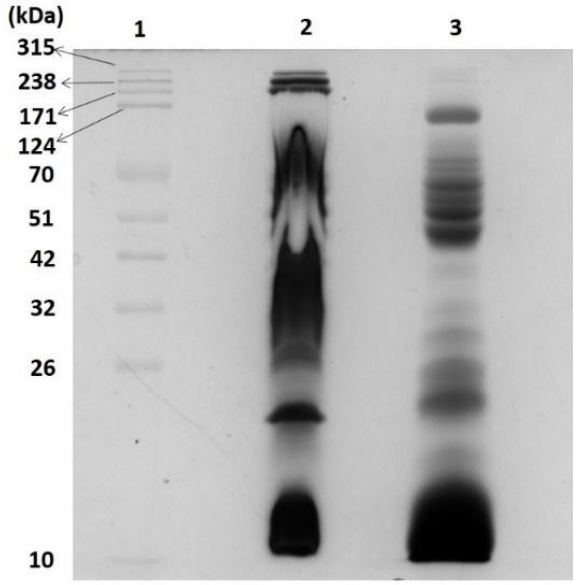

Figure 2 (a). Reversed-phase HPLC profile of crude NNwg venom

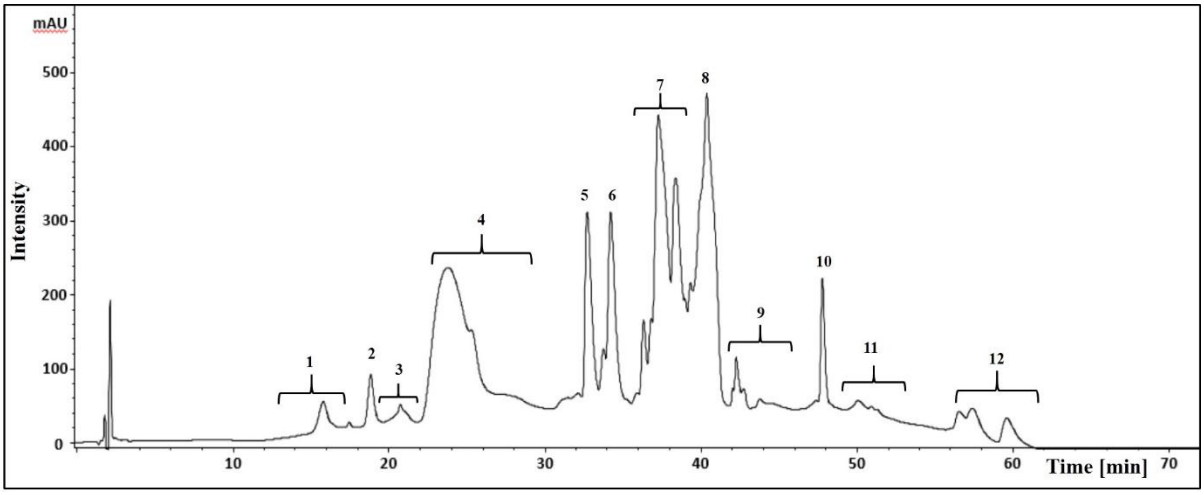

Figure 2 (b). 12% SDS-PAGE of RP-HPLC peaks of NNwg venom

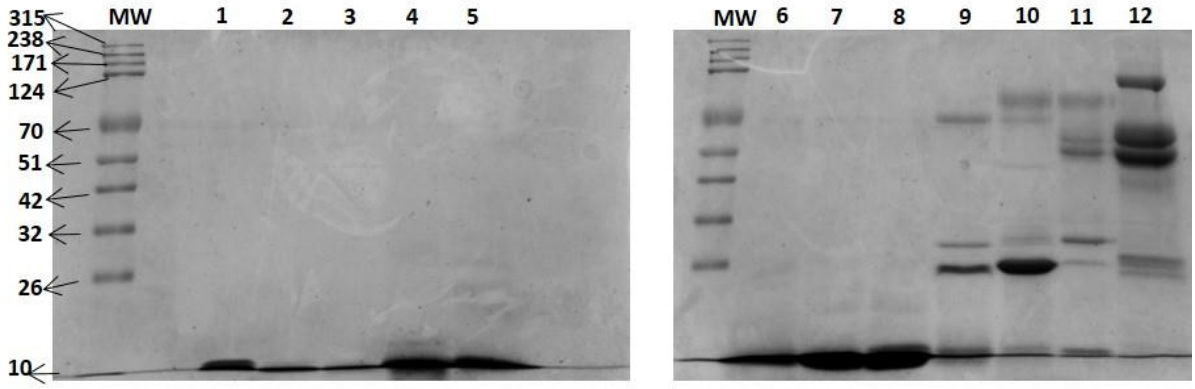

Figure 3. Relative abundance of protein families identified from NNwg venom

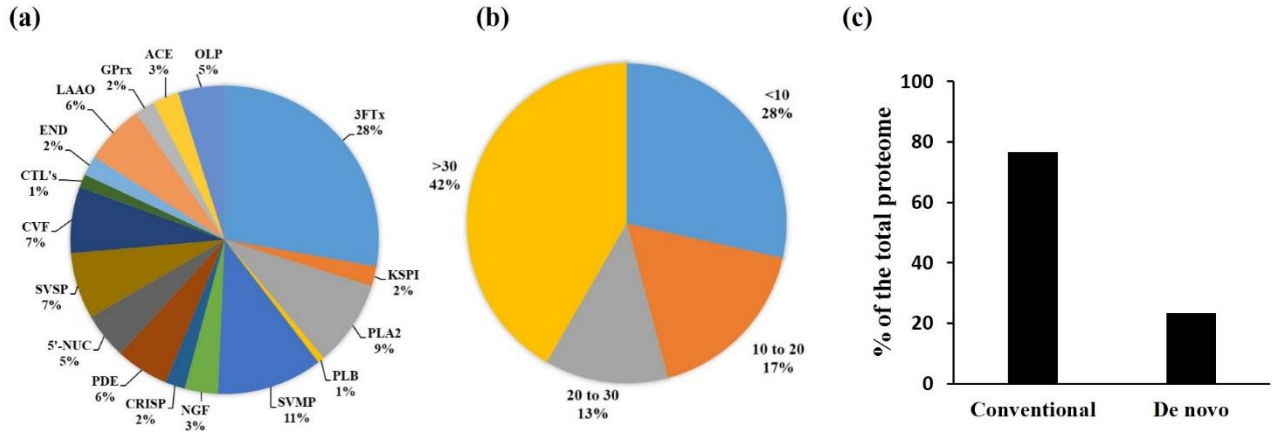

Figure 4. *De novo* analysis of peptide representing NNwg venom protein

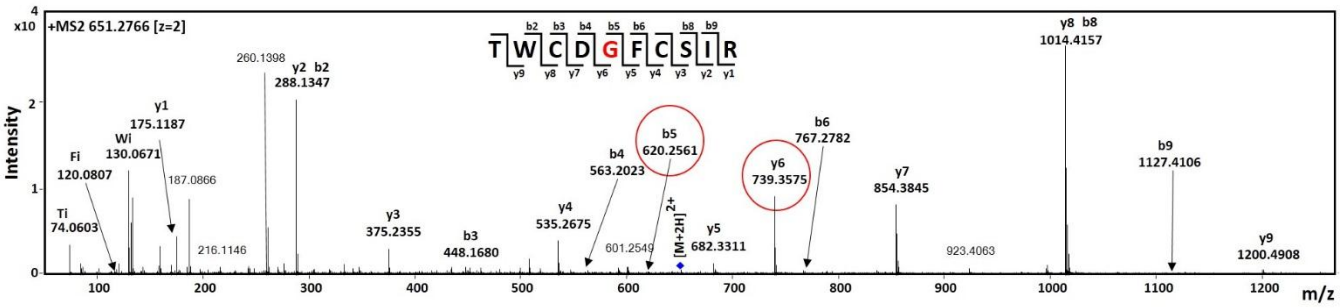

Figure 5. pBLAST and ClustalW analysis

(a)

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Notechis scutatus      1 MLLYFILLSLSWSQMPTSGEVVDSFQDHCPQFFLRETPPVIGVPLSPVRICQRYQNLYRF
Pseudonaja textilis    1 MLLCFILLSLSWSQTPTSGEVVDSFQDHCPQFFLRETPPVIGVPLSPVRICQRYQNLYRF
Thamnophis elegans     1 MRLLFILLSVSWSQMPTSGEVVDSFQDTCPQFFLRETPPLIRIPPSPVRICQRYQNLYRF
Ophiophagus hannah     1 ------------------------------------------------------------
NNwg                   1 ------------------------------------------------------------

Notechis scutatus     61 ATLYDKHNRIPVYSAYVYNPGKAKRPGKWMVEPQLISSTFQSEMETERDFLNKKGPITAM
Pseudonaja textilis   61 ATLYDKHNRIPVYSAYVYNPGKAKRPGKWMVEPQLISSTFQSEMETERDFLNKKGPKAAM
Thamnophis elegans    61 ATLYDKDNRIPVYSAYVYNPGSAKRPKKWMVEPQLINSSFQPEMETEWDFLNKKGPKEAL
Ophiophagus hannah     1 ---------------------------------------------METEWDFLNKKGSPKAL
NNwg                   1 ------------------------------------------------------------

Notechis scutatus    121 QNSQAILEDYKNLTDCNRGHLNPNGHQPDYSAKSSTFTLTNIVPQFIKLNGGAWNNYEQT
Pseudonaja textilis  121 QDSQAVLEDYKNLTDCNRGHLNPNGHQPDYSAKSSTFTLTNIVPQFIKLNGGAWNNYEQT
Thamnophis elegans   121 HVSQAVLEDYKNLSDYNRGHLNPNGHQPDYSAKSSTFTLTNIVPQFIKLNGGAWNNYEQT
Ophiophagus hannah    18 QDSQAVLEDYKNLADCNRGHLNPNGHQPDYSAKSSTFTLTNIVPQFIKLNGGAWNNYEQT
NNwg                   1 ------------------GHLNPNGHQPDYSAKSSTFTLTNIVPQFIKG-----------

Notechis scutatus    181 TMQQMTRGCHQTFAVVGAVPGDTYIARGRVNRPSHLWSAACCVIDNNHLRSWAILARNDQ
Pseudonaja textilis  181 TMQQMTRGCHQTFAVVGAVPGDTYISGGRVNRPSHLWSAACCVIDNNHLRSWAILARNDQ
Thamnophis elegans   181 TMVQMTKGCQETFAVVGAVPGDTYVAGGRVNKPSHMWSAACCMIDNNHLRSWAILARNDQ
Ophiophagus hannah    78 TMQQMTRGCQQTFAVVGAVPGDTYIARGRVNRPSHLWSAACCLIDNNHLRSWAILARNX-
NNwg                  32 --------CQQTFAVVGAVPGDTYIAR----------SAACCLIDNNHLRSWLAQLYN--

Notechis scutatus    241 NVVQRFTLGQLEEKLAQLYNVNHVSLFHSDCPRQ
Pseudonaja textilis  241 NVVQRFTLGQLEEKLAQLYNVNHVSLFHSDCPRQ
Thamnophis elegans   241 NVVEKLTLGQLEAHLAQLYNINQVSLFHSDCPRQ
Ophiophagus hannah   137 ----------LEEKLAQLYNVNHVSLFHSDCPRQ
NNwg                  72 --------------------VNHVSLFHSDCPRQ
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(b)

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Pseudonaja textilis     1 ----------------------------------------------MKGRLKGAWILSLL
Notechis scutatus       1 ----------------------------------------------MKGRLKGAWILSLL
P.mucrosquamatus        1 ----------------------------------------------MKGRLKGAWILSLL
Ophiophagus hannah      1 VELEGDNVLTQRQRERNTKEHLEQTQISVEARPGRGAEALSLLLSNHEGQTERSLDPFLA
NNwg                    1 ------------------------------------------------------------

Pseudonaja textilis    15 LAGLFQPSLGQEQQ----------------------------EVDCYDSVKGSAHDYGAF
Notechis scutatus      15 LAGLFQPSLGQEQQ----------------------------EVDCYDSVKGSAHDYGAL
P.mucrosquamatus       15 LAGLFQPNLGQEQQEILTGLQLSHQHDEARSCLHAQPIPDRAKVDCYDSVQGSAHDYGAL
Ophiophagus hannah     61 LGWFISAQPGTGTTGGLQLSHQHEEASSCSYAPNSQPIPDRAKVDCYDSVKGSAHDYGAL
NNwg                    1 ------------------------------------------AKVDCYDSVK---------

Pseudonaja textilis    47 SLSGEYIAFQKYVGKYILFVNVATYUGLTAQYLELNALQNSLRTDRLVILGFPCNQFGKQ
Notechis scutatus      47 SLTGEYIAFQNYVGKYILFVNVATYUGLTQQYLELNALQNSLRTDRLVILGFPCNQFGKQ
P.mucrosquamatus       75 SLSGEYIPFRNYAGKYILFVNVATYUGLTGQYLELNALQTSLGIDRLVILGFPCNQFGKQ
Ophiophagus hannah    121 SLT------------------------------ELNALQNSLKTDRLVILGFPCNQFGKQ
NNwg                   11 ------------------------------------------TDRLVILGFPCNQFGKQ

Pseudonaja textilis   107 EPGQNSEILQGIKYVRPGGGYVPNFQLFQKGDVNGENEQQIYTFLKNSCPPVVETFGDTT
Notechis scutatus     107 EPGQNSEILQGIKYVRPGGGYVPNFQLFQKGDVNGENEQQIYTFLKNSCPPVVETFGDTT
P.mucrosquamatus      135 EPGQNSEILQGIKHVRPGGGFVPNFQLFQKGDVNGENEQRIYTFLKNSCPPVVENFGDPA
Ophiophagus hannah    151 EPGQNSEILQGIKHVRPGGGFVPNFQLFQKGDVNGENEQKIYTFLKNSCPPVVETFGDTA
NNwg                   28 EPGQNSEILQGIK---------------QKGDVNGENEQKIYTFLKNSCPPVVETFGDTA

Pseudonaja textilis   167 RLFWSPLKIHDIKWNFEKFLVNPQGKPVMRWFHRTNVSTVKNDIIRYMRKNRNG
Notechis scutatus     167 RLFWSPLKIHDIKWNFEKFLVNPQGKPVMRWFHRTNVSTVKNDIIRYMRKNRNG
P.mucrosquamatus      195 KLFWSPLKTHDIKWNFEKFLVNPQGKPVMRWFQRTNVSSVKNDIIRYMRKNG--
Ophiophagus hannah    211 KLFWSPLKIHDIKWNFEKFLVNPQGKPVMRWFHRTNVSTVKNDIIRFMRKKRSG
NNwg                   73 KLFWSPLKIHDIKWNFEFLVN-PQGKPVMRWTN---VSTVKNDIIR--------
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Figure 6. Assessing the immunological cross-reactivity of various polyvalent antivenoms towards NNwg venom

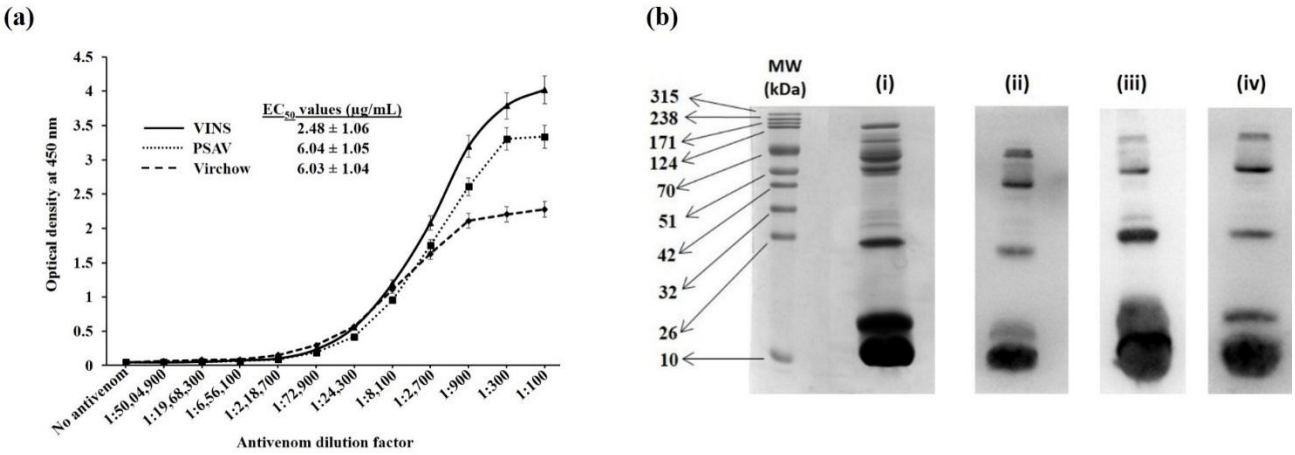
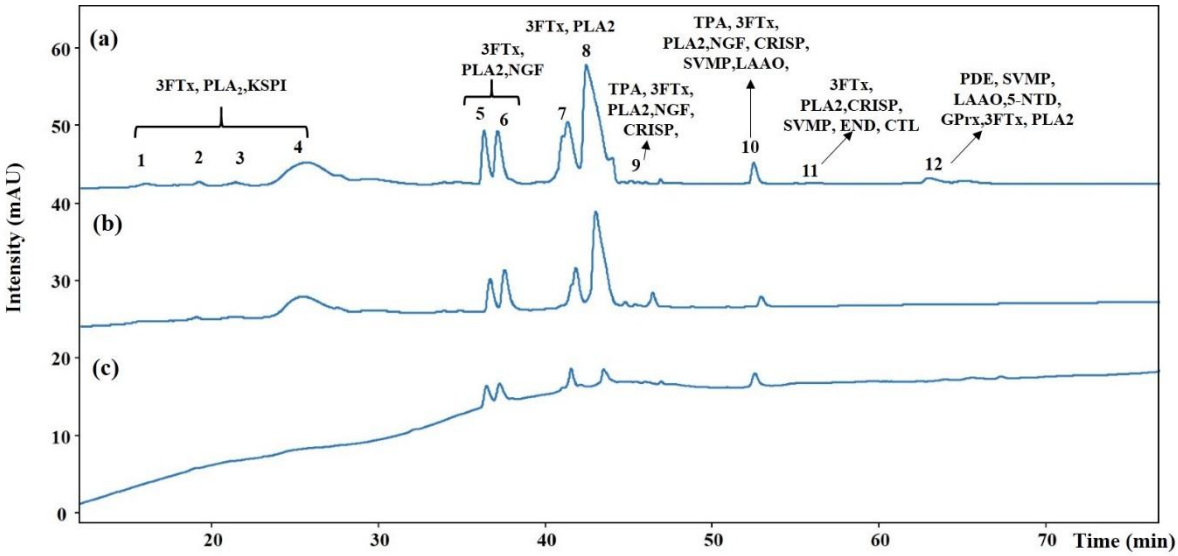


Figure 7. Immunoaffinity chromatography of NNwg using VINS antivenom



Tables

Table 1: Proteins identified from crude NNwg venom through SDS-PAGE and HPLC analysis. The proteins separated through SDS-PAGE and HPLC fractionation were subjected to in-gel and in-solution digestion using trypsin, chymotrypsin and V8 protease. The digests were then subjected to Q-TOF LC-MS/MS data collection and the obtained proteins were

assigned to different venom protein families through Mascot and PEAKS analysis. Proteins marked in bold were identified through both conventional proteomics and *de novo* methods. N/Q; Deamidation at Asn/Gln, M; Oxidation at Met, c;Carbamidomethylated Cysteine, #;peptides identified through chymotrypsin digestion, ^;peptides identified through V8 protease digestion, P#; peptides derived through PEAKS analysis after chymotrypsin digestion, P^; peptides derived through PEAKS analysis after V8 digestion. All the other peptides were obtained through trypsin digestion.

Table 2: Peptides representing various snake venom proteins identified through *de novo* analysis. *De novo* sequencing was performed using PEAKS and Novor softwares followed by MS-BLAST. The mutated amino acids obtained are highlighted in bold and underlined. Proteins marked in bold were identified through both conventional proteomics and *de novo* methods.

Table 3: Comparison of protein families identified in cobra venom collected from different geographical location of India.

Table 4: Summary of cobra venom proteomic studies from India.

Highlights

- Proteomics of cobra venom resulted in the identification of 143 proteins.
- *De novo* approaches exclusively yielded 59 peptides representing 28 proteins.
- Glutathione peroxidase and endonuclease were identified for the first time in Indian cobra venom.
- Indian polyvalent antivenoms showed varying cross-reactivity towards cobra venom.
- VINS antivenom failed to detect few low molecular mass proteins (< 10 kDa).

References

[1] C.Y. Koh, R.M. Kini, From snake venom toxins to therapeutics--cardiovascular examples, Toxicon 59(4) (2012) 497-506.

[2] S.D. Robinson, E.A.B. Undheim, B. Ueberheide, G.F. King, Venom peptides as therapeutics: advances, challenges and the future of venom-peptide discovery, Expert Rev Proteomics 14(10) (2017) 931-939.

[3] J.M. Jimenez-Porras, Pharmacology of peptides and proteins in snake venoms, Annu Rev Pharmacol 8 (1968) 299-318.

[4] S.E. Gasanov, R.K. Dagda, E.D. Rael, Snake Venom Cytotoxins, Phospholipase A2s, and Zn(2+)-dependent Metalloproteinases: Mechanisms of Action and Pharmacological Relevance, J Clin Toxicol 4(1) (2014) 1000181.

[5] T. Mohamed Abd El-Aziz, A. Garcia Soares, J.D. Stockand, Snake Venoms in Drug Discovery: Valuable Therapeutic Tools for Life Saving, Toxins (Basel) 11(10) (2019).

[6] M. Choudhury, R.J.R. McCleary, M. Kesherwani, R.M. Kini, D. Velmurugan, Comparison of proteomic profiles of the venoms of two of the 'Big Four' snakes of India, the Indian cobra (Naja naja) and the common krait (Bungarus caeruleus), and analyses of their toxins, Toxicon 135 (2017) 33-42.

[7] C.D. Cher, A. Armugam, Y.Z. Zhu, K. Jeyaseelan, Molecular basis of cardiotoxicity upon cobra envenomation, Cell Mol Life Sci 62(1) (2005) 105-18.

[8] A. Silva, W.C. Hodgson, G.K. Isbister, Antivenom for Neuromuscular Paralysis Resulting From Snake Envenoming, Toxins (Basel) 9(4) (2017).

[9] T. Tasoulis, G.K. Isbister, A Review and Database of Snake Venom Proteomes, Toxins (Basel) 9(9) (2017).

[10] R.M. Kini, Molecular moulds with multiple missions: functional sites in three-finger toxins, Clin Exp Pharmacol Physiol 29(9) (2002) 815-22.

[11] M. Li Lee, I. Chung, S. Yee Fung, M.S. Kanthimathi, N. Hong Tan, Antiproliferative activity of king cobra (Ophiophagus hannah) venom L-amino acid oxidase, Basic Clin Pharmacol Toxicol 114(4) (2014) 336-43.

[12] L. Zhang, L.J. Wei, ACTX-8, a cytotoxic L-amino acid oxidase isolated from Agkistrodon acutus snake venom, induces apoptosis in Hela cervical cancer cells, Life Sci 80(13) (2007) 1189-97.

[13] A. Chanda, B. Kalita, A. Patra, W. Senevirathne, A.K. Mukherjee, Proteomic analysis and antivenomics study of Western India Naja naja venom: correlation between venom composition and clinical manifestations of cobra bite in this region, Expert Rev Proteomics 16(2) (2019) 171-184.

[14] S. Dutta, A. Chanda, B. Kalita, T. Islam, A. Patra, A.K. Mukherjee, Proteomic analysis to unravel the complex venom proteome of eastern India Naja naja: Correlation of venom composition with its biochemical and pharmacological properties, J Proteomics 156 (2017) 29-39.

[15] A. Chanda, A. Patra, B. Kalita, A.K. Mukherjee, Proteomics analysis to compare the venom composition between Naja naja and Naja kaouthia from the same geographical location of eastern India: Correlation with pathophysiology of envenomation and immunological cross-reactivity towards commercial polyantivenom, Expert Rev Proteomics 15(11) (2018) 949-961.

[16] K. Sintiprungrat, K. Watcharatanyatip, W.D. Senevirathne, P. Chaisuriya, D. Chokchaichamnankit, C. Srisomsap, K. Ratanabanangkoon, A comparative study of venomics of Naja naja from India and Sri Lanka, clinical manifestations and antivenomics of an Indian polyspecific antivenom, J Proteomics 132 (2016) 131-43.

[17] K. Suryamohan, S.P. Krishnankutty, J. Guillory, M. Jevit, M.S. Schroder, M. Wu, B. Kuriakose, O.K. Mathew, R.C. Perumal, I. Koludarov, L.D. Goldstein, K. Senger, M.D. Dixon, D. Velayutham, D. Vargas, S. Chaudhuri, M. Muraleedharan, R. Goel, Y.J. Chen, A. Ratan, P. Liu, B. Faherty, G. de la Rosa, H. Shibata, M. Baca, M. Sagolla, J. Ziai, G.A. Wright, D. Vucic, S. Mohan, A. Antony, J. Stinson, D.S. Kirkpatrick, R.N. Hannoush, S. Durinck, Z. Modrusan, E.W. Stawiski, K. Wiley, T. Raudsepp, R.M. Kini, A. Zachariah, S. Seshagiri, The Indian cobra reference genome and transcriptome enables comprehensive identification of venom toxins, Nat Genet 52(1) (2020) 106-117.

[18] H.L. Gibbs, S.P. Mackessy, Functional basis of a molecular adaptation: prey-specific toxic effects of venom from Sistrurus rattlesnakes, Toxicon 53(6) (2009) 672-9.

[19] S.P. Mackessy, J. Leroy, E. Mocino-Deloya, K. Setser, R.W. Bryson, A.J. Saviola, Venom Ontogeny in the Mexican Lance-Headed Rattlesnake (Crotalus polystictus), Toxins (Basel) 10(7) (2018).

[20] M. Vanuopadath, S.K. Shaji, D. Raveendran, B.G. Nair, S.S. Nair, Delineating the venom toxin arsenal of Malabar pit viper (Trimeresurus malabaricus) from the Western Ghats of India and evaluating its immunological cross-reactivity and in vitro cytotoxicity, Int J Biol Macromol 148 (2020) 1029-1045.

[21] M. Vanuopadath, N. Sajeev, A.R. Murali, N. Sudish, N. Kangosseri, I.R. Sebastian, N.D. Jain, A. Pal, D. Raveendran, B.G. Nair, S.S. Nair, Mass spectrometry-assisted venom profiling of Hypnale hypnale found in the Western Ghats of India incorporating de novo sequencing approaches, Int J Biol Macromol 118(Pt B) (2018) 1736-1746.

[22] D. Pla, J.M. Gutierrez, J.J. Calvete, Second generation snake antivenomics: comparing immunoaffinity and immunodepletion protocols, Toxicon 60(4) (2012) 688-99.

[23] A. Keller, A.I. Nesvizhskii, E. Kolker, R. Aebersold, Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search, Anal Chem 74(20) (2002) 5383-92.

[24] A.I. Nesvizhskii, A. Keller, E. Kolker, R. Aebersold, A statistical model for identifying proteins by tandem mass spectrometry, Anal Chem 75(17) (2003) 4646-58.

[25] T. Muth, L. Weilnbock, E. Rapp, C.G. Huber, L. Martens, M. Vaudel, H. Barsnes, DeNovoGUI: an open source graphical user interface for de novo sequencing of tandem mass spectra, J Proteome Res 13(2) (2014) 1143-6.

[26] A. Shevchenko, S. Sunyaev, A. Loboda, P. Bork, W. Ens, K.G. Standing, Charting the proteomes of organisms with unsequenced genomes by MALDI-quadrupole time-of-flight mass spectrometry and BLAST homology searching, Anal Chem 73(9) (2001) 1917-26.

[27] J.A. Vizcaino, E.W. Deutsch, R. Wang, A. Csordas, F. Reisinger, D. Rios, J.A. Dianes, Z. Sun, T. Farrah, N. Bandeira, P.A. Binz, I. Xenarios, M. Eisenacher, G. Mayer, L. Gatto, A. Campos, R.J. Chalkley, H.J. Kraus, J.P. Albar, S. Martinez-Bartolome, R. Apweiler, G.S. Omenn, L. Martens, A.R. Jones, H. Hermjakob, ProteomeXchange provides globally coordinated proteomics data submission and dissemination, Nat Biotechnol 32(3) (2014) 223-6.

[28] J.A. Vizcaino, A. Csordas, N. del-Toro, J.A. Dianes, J. Griss, I. Lavidas, G. Mayer, Y. Perez-Riverol, F. Reisinger, T. Ternent, Q.W. Xu, R. Wang, H. Hermjakob, 2016 update of the PRIDE database and its related tools, Nucleic Acids Res 44(D1) (2016) D447-56.

[29] L. Li, J. Huang, Y. Lin, Snake Venoms in Cancer Therapy: Past, Present and Future, Toxins (Basel) 10(9) (2018).

[30] S.D. Robinson, H. Safavi-Hemami, Venom peptides as pharmacological tools and therapeutics for diabetes, Neuropharmacology 127 (2017) 79-86.

[31] H. Waheed, S.F. Moin, M.I. Choudhary, Snake Venom: From Deadly Toxins to Life-saving Therapeutics, Curr Med Chem 24(17) (2017) 1874-1891.

[32] Y.N. Utkin, Last decade update for three-finger toxins: Newly emerging structures and biological activities, World J Biol Chem 10(1) (2019) 17-27.

[33] V. Tsetlin, Snake venom alpha-neurotoxins and other 'three-finger' proteins, Eur J Biochem 264(2) (1999) 281-6.

[34] L.T. Potter, Snake toxins that bind specifically to individual subtypes of muscarinic receptors, Life Sci 68(22-23) (2001) 2541-7.

[35] T.K. Kumar, G. Jayaraman, C.S. Lee, A.I. Arunkumar, T. Sivaraman, D. Samuel, C. Yu, Snake venom cardiotoxins-structure, dynamics, function and folding, J Biomol Struct Dyn 15(3) (1997) 431-63.

[36] E.V. Kryukova, N.S. Egorova, D.S. Kudryavtsev, D.S. Lebedev, E.N. Spirova, M.N. Zhmak, A.I. Garifulina, I.E. Kasheverov, Y.N. Utkin, V.I. Tsetlin, From Synthetic Fragments of Endogenous Three-Finger Proteins to Potential Drugs, Front Pharmacol 10 (2019) 748.

[37] S. Gong, Q. Liang, Q. Zhu, D. Ding, Q. Yin, J. Tao, X. Jiang, Nicotinic acetylcholine receptor alpha7 subunit is involved in the cobratoxin-induced antinociception in an animal model of neuropathic pain, Toxicon 93 (2015) 31-6.

[38] A.G. Konshina, P.V. Dubovskii, R.G. Efremov, Structure and dynamics of cardiotoxins, Curr Protein Pept Sci 13(6) (2012) 570-84.

[39] S.E. Gasanov, M.A. Alsarraj, N.E. Gasanov, E.D. Rael, Cobra venom cytotoxin free of phospholipase A2 and its effect on model membranes and T leukemia cells, J Membr Biol 155(2) (1997) 133-42.

[40] A.V. Feofanov, G.V. Sharonov, M.V. Astapova, D.I. Rodionov, Y.N. Utkin, A.S. Arseniev, Cancer cell injury by cytotoxins from cobra venom is mediated through lysosomal damage, Biochem J 390(Pt 1) (2005) 11-8.

[41] M. Wu, W. Ming, Y. Tang, S. Zhou, T. Kong, W. Dong, The anticancer effect of cytotoxin 1 from Naja atra Cantor venom is mediated by a lysosomal cell death pathway involving lysosomal membrane permeabilization and cathepsin B release, Am J Chin Med 41(3) (2013) 643-63.

[42] S.S. Attarde, S.V. Pandit, Cytotoxic activity of NN-32 toxin from Indian spectacled cobra venom on human breast cancer cell lines, BMC Complement Altern Med 17(1) (2017) 503.

[43] J.M. Gutierrez, B. Lomonte, Phospholipases A2: unveiling the secrets of a functionally versatile group of snake venom toxins, Toxicon 62 (2013) 27-39.

[44] M.K. Bhat, T.V. Gowda, Purification and characterization of a myotoxic phospholipase A2 from Indian cobra (Naja naja naja) venom, Toxicon 27(8) (1989) 861-73.

[45] M.K. Bhat, B.N. Prasad, T.V. Gowda, Purification and characterization of a neurotoxic phospholipase A2 from Indian cobra (Naja naja naja) venom, Toxicon 29(11) (1991) 1345-9.

[46] L.M. Rudrammaji, T.V. Gowda, Purification and characterization of three acidic, cytotoxic phospholipases A2 from Indian cobra (Naja naja naja) venom, Toxicon 36(6) (1998) 921-32.

[47] F.V. Azevedo, D.S. Lopes, S.N. Cirilo Gimenes, D.C. Ache, L. Vecchi, P.T. Alves, O. Guimaraes Dde, R.S. Rodrigues, L.R. Goulart, M. Rodrigues Vde, K.A. Yoneyama, Human

breast cancer cell death induced by BnSP-6, a Lys-49 PLA(2) homologue from Bothrops pauloensis venom, Int J Biol Macromol 82 (2016) 671-7.

[48] C. Prinholato da Silva, T.R. Costa, R.M. Paiva, A.C. Cintra, D.L. Menaldo, L.M. Antunes, S.V. Sampaio, Antitumor potential of the myotoxin BthTX-I from Bothrops jararacussu snake venom: evaluation of cell cycle alterations and death mechanisms induced in tumor cell lines, J Venom Anim Toxins Incl Trop Dis 21 (2015) 44.

[49] K.K. Tan, B.H. Bay, P. Gopalakrishnakone, L-amino acid oxidase from snake venom and its anticancer potential, Toxicon 144 (2018) 7-13.

[50] M. Pessatti, J.D. Fontana, M.F. Furtado, M.F. Guimaraes, L.R. Zanette, W.T. Costa, M. Baron, Screening of Bothrops snake venoms for L-amino acid oxidase activity, Appl Biochem Biotechnol 51-52 (1995) 197-210.

[51] F.S. Markland, Jr., Inventory of alpha- and beta-fibrinogenases from snake venoms. For the Subcommittee on Nomenclature of Exogenous Hemostatic Factors of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, Thromb Haemost 65(4) (1991) 438-43.

[52] D.K. Jagadeesha, R. Shashidhara murthy, K.S. Girish, K. Kemparaju, A non-toxic anticoagulant metalloprotease: purification and characterization from Indian cobra (Naja naja naja) venom, Toxicon 40(6) (2002) 667-75.

[53] J.W. Fox, S.M. Serrano, Structural considerations of the snake venom metalloproteinases, key members of the M12 reprolysin family of metalloproteinases, Toxicon 45(8) (2005) 969-85.

[54] J. Hamako, T. Matsui, S. Nishida, S. Nomura, Y. Fujimura, M. Ito, Y. Ozeki, K. Titani, Purification and characterization of kaouthiagin, a von Willebrand factor-binding and -cleaving metalloproteinase from Naha kaouthia cobra venom, Thromb Haemost 80(3) (1998) 499-505.

[55] M.S. Kumar, K.S. Girish, B.S. Vishwanath, K. Kemparaju, The metalloprotease, NN-PF3 from Naja naja venom inhibits platelet aggregation primarily by affecting alpha2beta1 integrin, Ann Hematol 90(5) (2011) 569-77.

[56] A.S. Kamiguti, Platelets as targets of snake venom metalloproteinases, Toxicon 45(8) (2005) 1041-9.

[57] W.J. Wang, C.H. Shih, T.F. Huang, Primary structure and antiplatelet mechanism of a snake venom metalloproteinase, acurhagin, from Agkistrodon acutus venom, Biochimie 87(12) (2005) 1065-77.

[58] W.J. Wang, Purification and functional characterization of AAV1, a novel P-III metalloproteinase, from Formosan Agkistrodon acutus venom, Biochimie 89(1) (2007) 105-15.

[59] F.S. Markland, Snake venoms and the hemostatic system, Toxicon 36(12) (1998) 1749-800.

[60] E.F. Sanchez, R.J. Flores-Ortiz, V.G. Alvarenga, J.A. Eble, Direct Fibrinolytic Snake Venom Metalloproteinases Affecting Hemostasis: Structural, Biochemical Features and Therapeutic Potential, Toxins (Basel) 9(12) (2017).

[61] W.M. Chou, W.H. Liu, K.C. Chen, L.S. Chang, Structure-function studies on inhibitory activity of Bungarus multicinctus protease inhibitor-like protein on matrix metalloprotease-2, and invasion and migration of human neuroblastoma SK-N-SH cells, Toxicon 55(2-3) (2010) 353-60.

[62] Y. Yamazaki, T. Morita, Snake venom components affecting blood coagulation and the vascular system: structural similarities and marked diversity, Curr Pharm Des 13(28) (2007) 2872-86.

[63] J. Shafqat, O.U. Beg, S.J. Yin, Z.H. Zaidi, H. Jornvall, Primary structure and functional properties of cobra (Naja naja naja) venom Kunitz-type trypsin inhibitor, Eur J Biochem 194(2) (1990) 337-41.

[64] J. Shafqat, Z.H. Zaidi, H. Jornvall, Purification and characterization of a chymotrypsin Kunitz inhibitor type of polypeptide from the venom of cobra (Naja naja naja), FEBS Lett 275(1-2) (1990) 6-8.

[65] K.N. Suvilesh, M. Yariswamy, M.N. Savitha, V. Joshi, A.N. Nanjaraj Urs, A.P. Urs, M. Choudhury, D. Velmurugan, B.S. Vishwanath, Purification and characterization of an anti-hemorrhagic protein from Naja naja (Indian cobra) venom, Toxicon 140 (2017) 83-93.

[66] S.D. Aird, Taxonomic distribution and quantitative analysis of free purine and pyrimidine nucleosides in snake venoms, Comp Biochem Physiol B Biochem Mol Biol 140(1) (2005) 109-26.

[67] S.D. Aird, Ophidian envenomation strategies and the role of purines, Toxicon 40(4) (2002) 335-93.

[68] M.C. Boffa, G.A. Boffa, Correlations between the enzymatic activities and the factors active on blood coagulation and platelet aggregation from the venom of Vipera aspis, Biochim Biophys Acta 354(2) (1974) 275-90.

[69] L. Peng, X. Xu, D. Shen, Y. Zhang, J. Song, X. Yan, M. Guo, Purification and partial characterization of a novel phosphodiesterase from the venom of Trimeresurus stejnegeri: inhibition of platelet aggregation, Biochimie 93(9) (2011) 1601-9.

[70] P. Lazarovici, C. Marcinkiewicz, P.I. Lelkes, From Snake Venom's Disintegrins and C-Type Lectins to Anti-Platelet Drugs, Toxins (Basel) 11(5) (2019).

[71] Z. Chen, J. Wu, Y. Zhang, X. Yang, G. Yu, S. Zhu, W. Lee, Q. Lu, A novel platelet glycoprotein Ib-binding protein with human platelet aggregation-inhibiting activity from Trimeresurus jerdonii venom, Toxicon 57(5) (2011) 672-9.

[72] T. Momic, F.T. Arlinghaus, H. Arien-Zakay, J. Katzhendler, J.A. Eble, C. Marcinkiewicz, P. Lazarovici, Pharmacological aspects of Vipera xantina palestinae venom, Toxins (Basel) 3(11) (2011) 1420-32.

[73] T. Momic, J. Katzhendler, E. Shai, E. Noy, H. Senderowitz, J.A. Eble, C. Marcinkiewicz, D. Varon, P. Lazarovici, Vipegitide: a folded peptidomimetic partial antagonist of alpha2beta1 integrin with antiplatelet aggregation activity, Drug Des Devel Ther 9 (2015) 291-304.

[74] Y. Frobert, C. Creminon, X. Cousin, M.H. Remy, J.M. Chatel, S. Bon, C. Bon, J. Grassi, Acetylcholinesterases from Elapidae snake venoms: biochemical, immunological and enzymatic characterization, Biochim Biophys Acta 1339(2) (1997) 253-67.

[75] Y.F. Pung, P.T. Wong, P.P. Kumar, W.C. Hodgson, R.M. Kini, Ohanin, a novel protein from king cobra venom, induces hypolocomotion and hyperalgesia in mice, J Biol Chem 280(13) (2005) 13137-47.

[76] A. Chapeaurouge, M.A. Reza, S.P. Mackessy, P.C. Carvalho, R.H. Valente, A. Teixeira-Ferreira, J. Perales, Q. Lin, R.M. Kini, Interrogating the Venom of the Viperid Snake Sistrurus catenatus edwardsii by a Combined Approach of Electrospray and MALDI Mass Spectrometry, PLoS One 10(5) (2015) e0092091.

[77] G. de la Rosa, F. Olvera, I.G. Archundia, B. Lomonte, A. Alagon, G. Corzo, Horse immunization with short-chain consensus alpha-neurotoxin generates antibodies against broad spectrum of elapid venomous species, Nat Commun 10(1) (2019) 3642.

[78] R. Teixeira-Araujo, P. Castanheira, L. Brazil-Mas, F. Pontes, M. Leitao de Araujo, M.L. Machado Alves, R.B. Zingali, C. Correa-Netto, Antivenomics as a tool to improve the neutralizing capacity of the crotalic antivenom: a study with crotamine, J Venom Anim Toxins Incl Trop Dis 23 (2017) 28.

[79] WHO Guidelines for the Production, Control and Regulation of Snake Antivenom Immunoglobulins. <https://www.who.int/bloodproducts/AntivenomGLrevWHO_TRS_1004_web_Annex_5.pdf?ua=1>, 2018).

Table 1: Proteins identified from crude NNwg venom through SDS-PAGE and HPLC analysis. The proteins separated through SDS-PAGE and HPLC fractionation were subjected to in-gel and in-solution digestion using trypsin, chymotrypsin and V8 protease. The digests were then subjected to Q-TOF LC-MS/MS data collection and the obtained proteins were assigned to different venom protein families through Mascot and PEAKS analysis. Proteins marked in bold were identified through both conventional proteomics and de novo methods. N/Q; Deamidation at Asn/Gln, M; Oxidation at Met, c;Carbamidomethylated Cysteine, #;peptides identified through chymotrypsin digestion, ^;peptides identified through V8 protease digestion, P#; peptides derived through PEAKS analysis after chymotrypsin digestion, P^; peptides derived through PEAKS analysis after V8 digestion. All the other peptides were obtained through trypsin digestion.

Accession number	**Protein name**		**Snake species**	**Molecular mass (kDa)**	**Total no.of peptides**	**No.of unique peptides**	**Summed MS/MS score**	**Sequence Coverage**	**Identified Peptides**	**Observed m/z (Z)**	**ppm**
Three finger toxins											
1	Short neurotoxin 1	P01427	*Naja oxiana*	7	2	2	97	37%	VKPGVNLNccR	439.5582 (3)	3
									WWSDHRGTIIER	519.2664 (3)	5
2	Weak neurotoxin 6	P29180	*Naja naja*	8	4	3	112	52%	LTcLIcPEK	567.2889 (2)	1
									YIRGcADTcPVR	489.9011 (3)	4
									EIVqccSTDKcNH	551.2202 (3)	3
									LTcLIcPE^	1005.4723 (1)	2
3	Chain A, Short neurotoxin 1	2MJ4_A	*Naja Oxiana*	7	1	1	59	39%	LEcHNQQSSQPPTTKTcSGETNcY#	943.063 (3)	1

4	Chain A, Alpha-cobratoxin	1CTX_A	*Naja siamensis*	8	10	10	793	93%	cFITPDITSK	591.2994 (2)	3
									VDLGcAATcPTVK	696.3388 (2)	3
									TGVDIQccSTDNcNPFPTR	747.9849 (3)	3
									RVDLGcAATcPTVK	516.5957 (3)	4
									IRcFITPDITSK	725.8918 (2)	3
									IRcFITPDITSKDcP**N**GHVcYTK	696.582 (4)	4
									cFITPDITSKDcP**N**GHVcYTK	838.7116 (3)	4
									TWcDAFcSIR	658.2843 (2)	4
									VDLGcAATcPTVKTGVDIQccSTDNcNPFPTR	904.4042(4)	4
									IRcFITPD P^	511.2638 (2)	7
5	**Long neurotoxin 1**	P25668	*Naja naja*	8	8	8	758	69%	TWcDGFcSIR	651.2766 (2)	4
									RVDLGcAATcPTVR	525.931 (3)	3
									DcPNGHVcYTKTWcDGFcSIR	659.0287 (4)	5
									VDLGcAATcPTVR	710.3422(2)	4
									ITPDITSKDcP**N**GHVcY#	659.9621 (3)	2

									IRcFITPDITSKDcPNGHVcY#	851.728 (3)	1
									ITPDITSKDcPNGHVcYTKTW#	624.0448 (4)	1
									GKRVDLGcAATcPTVR	587.6371 (3)	4
6	Muscarinic toxin-like protein 2	P82463	*Naja kaouthia*	7	7	7	374	60%	SIFGVTTEDcPDGQNLcFKR	782.0311 (3)	4
									EKSIFGVTTEDcPDGQNLcFKR	651.0602(4)	4
									SIFGVTTEDcPDGQNLcFK	729.9981(3)	0
									LTcVKEKSIFGVTTEDcPDGQNL#	871.0853 (3)	1
									GVTTEDcPDGQNLcF#	856.8486 (2)	1
									GcAATcPIAENR	660.2914 (2)	6
									KSIFGVTTE^	491.2668 (2)	1
7	Three-finger toxin precursor T.A	AKO63248	*Micrurus tener*	9	1	1	47	9%	AVVTIVcL#	874.5022 (1)	5
8	Toxin B	764177A	*Naja naja*	8	6	3	460	83%	TGVDI**Q**ccSTDDcPFPTRKRP	837.7108 (3)	5
									IRcFITPDITSK	725.8918(2)	3

									IRcFITPDITSKDcP**N**GHVcYTK	696.582 (4)	4
									cFITPDITSK	591.2987(2)	2
									cFITPDITSKDcP**N**GHVcYTK	838.7116(3)	4
									RVDLGcAATcPTVR	525.931(3)	3
									VDLGcAATcPTVR	473.8915(3)	9
9	Weak toxin CM-9a	P25679	*Naja kaouthia*	7	2	1	106	42%	LTcLNcPE**M**F[#]	1300.5356 (1)	1
									ITPDITSKDcP**N**GHVcY[#]	659.9624 (3)	2
10	Cytotoxin 8	P86540	*Naja naja*	7	6	6	204	83%	LIPLAYKTcPAGK	477.9417 (3)	5
									MYMVSDKTVPVKR	518.6127 (3)	4
									GcIDVcPK	474.7189 (2)	3
									NSLLVKYEccNTDRcN	682.6346 (3)	3
									GCIDVCPKNSLL [P#]	688.3450 (2)	9
									GCIDVCPKNSLLVKY [P#]	883.4595 (2)	8
11	Neurotoxin-like protein NTL2	Q9W717	*Naja atra*	10	1	1	60	12%	GITRLPWVIR	605.8765 (2)	3

12	Cytotoxin 9	A0A0U5AUY6	*Naja naja*	8	12	12	522	90%	cNKLVPLFYK	641.3564 (2)	2
									LVPLFYKTcPAGK	498.613 (3)	4
									LVPLFYKTcPAGKNLcYK	543.7929 (4)	7
									NLcYKMYMVATPK	540.2676 (3)	6
									MYMVATPK	470.7368 (2)	3
									MYMVATPKVPVKR	380.7189 (4)	3
									GcIDVcPK	474.7181 (2)	1
									GcIDVcPKSSLLVK	525.9475 (3)	3
									SSLLVKYVccNTDR	572.2832 (3)	6
									SSLLVKYVccNTDRcN	663.639 (3)	2
									YVccNTDRcN	681.2563 (2)	2
									TcPAGKNLcYK	437.8775 (3)	1
13	Cytotoxin 1	P01447	*Naja naja*	7	10	3	430	100 %	MY**M**VS**N**KTVPVKR	523.9463 (3)	7
									NSLVLKYEccNTDRcN	682.6346 (3)	3
									LIPLAYKTcPAGK	477.9417 (3)	5
									GcIDVcPK		3

										474.7189 (2)	
									NSLVLKYEccNTDR	591.2777 (3)	5
									GcIDVcPK**N**SLVLK	535.281 (3)	6
									LIPLAYKTcPAGKNLcYK	528.2877 (4)	5
									LKcNKLIPLAYK	487.6289 (3)	4
									cNKLIPLAYK	610.3487 (2)	3
									KTcPAGKNL[#]	987.5221(2)	5
14	Cytotoxin 2	P01445	*Naja kaouthia*	7	10	7	460	100 %	**M**F**M**VS**N**KTVPVKR	523.95 (3)	7
									GcIDVcPK	474.72 (2)	3
									LIPLAYKTcPAGK	477.94 (3)	5
									LKcNKLIPLAYK	487.6289 (3)	4
									cNKLIPLAYK	610.3487 (2)	3
									LIPLAYKTcPAGK	477.941 (3)	4
									LIPLAYKTcPAGKNLcYK	528.2877 (4)	5
									GcIDVcPKNSLLVK	801.924 (2)	4
									NSLLVKYVccNTDRcN	672.6441 (3)	4

									YVccNTDRcN	681.2563 (2)	2
15	Muscarinic toxin-like protein 3	P82464	*Naja kaouthia*	8	8	8	324	94%	TSETTEIcPDSWYFcYK	1093.959 (2)	3
									ISLADGNDVR	530.2762 (2)	2
									TcPELRPTGIY#	653.827 (2)	1
									TcPELRPTGIYVY#	784.8949 (2)	3
									GcTFTcPELRPTGIYVYccR	837.3771 (3)	3
									TRTSETTEIcPDSWYF#	996.9373 (2)	1
									cYKISLADGNDVRIKRGcTF#	594.2957 (4)	2
									TIcYNHLTR	393.1971 (3)	1
16	Neurotoxin-like protein	CAB45156	*Naja naja*	10	6	6	294	65%	SIFSETIEIcPDGHNF#	742.8895 (4)	2
									SETIEIcPDGHNF#	831.8872 (2)	1
									SETIEIcPDGHNFcF#	649.8186 (2)	1
									VIRGcAATcPKAEARVY#	724.8637 (2)	1
									ARVYVDccARDKcNR^	643.3226 (2)	3

									GITRLPWVIR	404.2532 (3)	3
17	Chain A, Cardiotoxin V4II	1CDT_A	*Naja mossambica*	7	3	3	87	18%	LKcNKLIPIAY#	666.8889 (2)	0
									KcNKLIPIAY#	610.3474 (2)	1
									LKCNKLIPIAY P#	666.8954 (2)	10
									IPIAY P#	576.3439 (1)	8
18	Muscarinic toxin-like protein 1	P82462	*Naja kaouthia*	7	1	1	59	17%	NQAHLIYPGKY#	652.3433 (2)	1
19	Chain A, Ringhalexin	4ZQY_A	*Hemachatus haemachatus*	7	2	2	92	34%	RLcLSDYSIFSE^	745.3546 (2)	2
									GITRLPWVIR	404.254 (3)	5
20	Chain A, Cardiotoxin V	1CVO_A	*Naja atra*	7	8	8	319	77%	LKcHNTQLPFIYK	554.6351 (3)	4
									cHNTQLPFIYK	474.2421 (3)	5
									NLcFKATLK	365.5405 (3)	4
									KFPLKFPVK	368.5727 (3)	5
									FPLKFPVK	488.3065 (2)	3
									NSALLKYVccSTDKcN	644.9608 (3)	3
									FPLKFPVKR	377.9074 (3)	3

									LKcHNTQLPFIYKTcPE^	717.0243(3)	1
21	Cytotoxin 13, partial	BAU24670	*Naja naja*	8	2	2	134	33%	GcIDVcPK	474.721(2)	7
									NSLLVKYVccNTDR	581.286 (3)	4
									NSLLVKYVccNTDRcN	672.6443 (3)	4
22	Cytotoxin 1	P0CH80	*Naja kaouthia*	7	9	3	417	100 %	GcIDVcPK**N**SLVLK	535.281 (3)	6
									NSLVLKYVccNTDRcN	672.6443 (3)	4
									GcIDVcPK	474.721 (2)	7
									MFMVSNKTVPVKR	384.9681 (4)	8
									LKcNKLVPLFYK	761.9469 (2)	3
									cNKLVPLFYK	641.3564 (2)	2
									LVPLFYKTcPAGK	498.613 (3)	4
									LVPLFYKTcPAGKNLcYK	543.7929 (4)	7
									YVccNTDRcN	681.2563 (2)	2
23	Cytotoxin 14, partial	BAU24671	*Naja naja*	8	8	3	414	81%	NSLLVKYEccNTDR	591.2777 (3)	5
									NSLLVKYEccNTDRcN	682.636 (3)	5

									cNKLIPLAYK	610.3487 (2)	3
									LIPLAYKTcPAGK	477.941 (3)	4
									LIPLAYKTcPAGKNLcYK	528.2877(4)	5
									GcIDVcPK	474.7181 (2)	1
									GcIDVcPKNSLLVK	801.924 (2)	4
									MYMVSNKTVPVKR	388.9647 (4)	2
24	Cytotoxin D1	1007132A	*Naja atra*	7	2	1	105	40%	SSLLVKYVccNTDRcN	663.6377 (3)	1
									GcIDVcPK	474.721 (2)	7
25	Cytotoxin 2	P01440	*Naja naja*	7	10	3	457	100 %	SSLVLKYVccNTDRcN	663.639 (3)	2
									GcIDVcPK	474.7181 (2)	1
									LKcNKLVPLFYK	761.9469 (2)	1
									SSLVLKYVccNTDR	572.2832 (3)	6
									MYMVATPKVPVKR	507.2897 (3)	4
									cNKLVPLFYK	641.3564 (2)	2
									LVPLFYKTcPAGK	498.613 (3)	4
									LVPLFYKTcPAGKNLcYK	543.7929 (4)	7

									NLcYKMYMVATPK	540.2676 (3)	6
									MYMVATPK	470.7368 (2)	3
26	Cardiotoxin isoform 1, Cytotoxin isoform 1	AAB25732	*Naja naja*	7	4	2	188	40%	GcIDVcPKSNLLVK	801.924 (2)	4
									SNLLVKYVccNTDRcN	672.6441 (3)	4
									GcIDVcPK	474.7181 (2)	1
									YVccNTDRcN	681.2563 (2)	2
27	Cytotoxin SP15d	P60309	*Naja atra*	7	2	1	89	23%	Gc**I**NVcPKSSLLVK	788.4181 (2)	3
									Gc**I**NVcPK	474.7181 (2)	1
28	Toxin,cardio	0406231A	*Naja atra*	7	12	6	554	100 %	**M**FMVATPKVPVKR	507.2897 (3)	4
									LKcNKLVPLFYK	508.3007 (3)	4
									cNKLVPLFYK	641.3564 (2)	2
									LVPLFYKTcPAGK	498.613 (3)	4
									LVPLFYKTcPAGKNLcYK	543.7929(4)	7
									GcIDVcPK	474.7181 (2)	1
									SSLLVKYVccNTDR	572.2832 (3)	6
									MFMVATPK	470.7366 (2)	3

									SSLLVKYVccNTDRcN	663.6368 (3)	1
									KTcPAGKNL#	494.7678 (2)	4
									KTcPAGK**N**LcY#	656.8085 (2)	6
									VccNTDRcN#	599.724 (2)	1
29	Cytotoxin sagitoxin	P83345	*Naja sagittifera*	7	2	1	93	38%	SLLVKYEccNTDRcN	644.6205 (3)	3
									GcIDVcPK	474.7181 (2)	1
30	Chain A, Neurotoxin II	1NOR_A	*Naja Oxiana*	7	2	2	119	43%	LEcHNQQSSQPPTTK	585.6074 (3)	3
									VKPGVNLNccR	439.5553 (3)	4
31	Cytotoxin 7	P86382	*Naja naja*	7	3	3	144	48%	LIPLAYKTcPAGK	477.9402 (3)	2
									NSLLVKYEccNTDR	591.2762 (3)	2
									NSLLVKYEccNTDRcN	682.6347 (3)	3
32	Cardiotoxin N	CAA90966	*Naja naja*	9	1	1	13	10%	VVATIVcL#	874.5021 (1)	5
33	Long chain neurotoxin precursor	AAT97250	*Ophiophagus hannah*	10	1	1	35	14%	ccSTDNcNPFPTR	543.5418 (3)	9
Kunitz-type serine protease Inhibitor (KSPI)											
34	Kunitz-type serine	P20229	*Naja naja*	6	4	4	195	60%	RPGFcELPAAK	415.8872 (3)	5

	protease inhibitor								AHKPAFYYNK	413.5516 (3)	7
									FIYGGcGGNANR	643.2932 (2)	4
									IYGGcGGNANRF#	643.2911 (2)	1
									RPGFcELPAAKGL P#	643.2955 (2)	8
35	Kunitz-type serine protease inhibitor	Q5ZPJ7.1	*Naja atra*	9	3	3	94	42%	RPRFcELAPSAGScF P#	585.6139 (3)	10
									FcELAPSAGScF P#	1345.5602 (1)	4
									AFVPSYY P#	846.4078 (1)	5
Phospholipase A_2 (PLA_2)											
36	Chain A, Phospholipase A2	1A3D_A	*Naja naja*	13	26	25	1448	100 %	ISGcWPYFK	579.2771(2)	2
									NLYQFKNMIK	657.8491(2)	3
									NMIKcTVPSR	402.5434(3)	2
									cTVPSRSWWDFADYGcYcGR	848.3523(3)	4
									SWWDFADYGcYcGR	921.8565(2)	3
									GGSGTPVDDLDR	594.7802(2)	3
									ccQVHDNcYNEAEKISGcWPYFK	742.0635(4)	5

									TYSYEcSQGTLTcK	566.578 (3)	3
									LAAIcFAGAPYNDNNYNIDLK	1179.0696(2)	4
									DFADYGcY#	1010.3547 (1)	1
									cGRGGSGTPVDDLDRccQVHDNcY#	700.2852 (4)	2
									NEAEKISGcWPY#	727.324 (2)	1
									NEAEKISGcWPYFKTY#	664.9779 (3)	2
									SYEcSQGTLTcKGDNNAcAASVcDcDRL#	1071.4335 (3)	2
									AEKISGcWPYFKTYSYE^	710.3292 (3)	2
									NLYQFKNMIKcTVPSR	667.017 (3)	6
									ISGcWPYFKTYSYEcSQGTLTcK	946.0887 (3)	3
									TYSYEcSQGTLTcKGDNNAcAASVcDcDR	841.5856 (4)	4
									GDNNAcAASVcDcDRLAAIcFAGAPYNDNNYNIDLK	1341.5809 (3)	3
									LAAIcFAGAPYNDNNYNIDLKAR	646.8242 (4)	5
									TcKGDNNAcAASVcDcDRL#	729.6284 (3)	1

									TcKGDNNAcAASVcDcDRLAAIcF#	1375.0691 (2)	2
									AGAPYNDNNYNIDL#	777.3489 (2)	2
									NDNNYNIDLKARcQ#	579.9395 (3)	4
									GDNNAcAASVcDcDR	842.8049 (2)	5
									NDNNYNIDL#	1094.4761 (1)	1
									KNMIKCTVPSRSWW P#	598.3008 (3)	6
									DNCYNEAEKISGCWPY P#	1003.4136 (2)	7
37	Phospholipase A2 E3	0508173A	*Naja naja*	13	20	13	1083	100 %	SWLDFA**N**YGcYcGR	885.3587 (2)	3
									LAAIcFAGAPYNNDNYNINLK	1178.5765 (2)	3
									LAAIcFAGAPYNNDNYNINLKAR	861.77 (3)	4
									NLYQFKN**M**IK	657.85 (2)	3
									NLYQFKNMIKcTVPSR	500.52 (4)	9
									NMIKcTVPSR	402.54 (3)	2
									GGSGTPVDDLDR	594.78 (2)	3
									DFA**N**YGcY#	1010.3547 (1)	1

									AGAPYNNDNYNINL[#]	776.8552 (2)	1
									NNDNYNINL[#]	547.2497 (2)	1
									NNDNYNI**N**LKARcQ[#]	579.9383 (3)	2
									cTVPSRSWLDFA**N**YGcYcGR	824.02 (3)	5
									GGSGTPVDDLDRccQIHDNcYNEAGKISGcWPYFK	816.15 (5)	2
									ccQIHDNcY**N**EAGKISGcWPYFK	727.81 (4)	5
									TYSYEcSQGTLTcKGDNNScAASVcDcDR	845.59 (4)	10
									ISGcWPYFK	579.278 (2)	3
									ISGcWPYFKTYSYEcSQGTLTcK	946.0887 (3)	3
									TYSYEcSQGTLTcK	566.5788 (3)	4
									NEAGKISGcWPY[#]	691.8074 (2)	2
									AGKISGcWPYFKTYSYE[^]	686.3242 (3)	5
38	Chain A, Phospholipase A2	1LN8_A	*Naja sagittifera*	13	3	2	185	39%	TYSYEcTQGTLTcK	856.3705 (2)	2
									LAAIcFAGAPYNDDNYNIDLK	786.7134 (3)	787

									GGSGTPVDDLDR	594.7802 (2)	3
39	Phospholipase A2	BAA36404	*Naja kaouthia*	16	18	7	1002	94%	LAAIcFAGAPYNNNNYNIDLK	1179.0696 (2)	4
									LAAIcFAGAPYNNNNYNIDLKAR	861.7676 (3)	4
									SWWDFADYGcYcGR	921.8565 (2)	3
									GGSGTPVDDLDR	594.7802 (2)	3
									ccQVHDNcYNEAEKISGcWPYFK	742.0635 (4)	5
									ISGcWPYFK	579.2774(2)	2
									TYSYEcSQGTLTcK	566.578 (3)	3
									DFADYGcY#	1010.3547 (1)	1
									cGRGGSGTPVDDLDRccQVHDNcY#	700.2852 (4)	2
									NEAEKISGcWPY#	727.324 (2)	1
									NEAEKISGcWPYFKTY#	664.9779 (3)	2
									GGSGTPVDDLDRccQVHDNcYNEAEKISGcWPYFK	827.7593 (5)	5
									ccQVHDNcYNEAEKISGcWPYFK	742.0653 (4)	8

									ISGcWPYFKTYSYEcSQGTLTcK	849.363 (2)	2
									AGAPYN**N**NNYNIDL#	777.3489 (2)	2
									NNN**N**YNIDLKARcQ#	579.9395 (3)	4
									N**N**NNYNIDL#	1094.4763 (1)	1
									NMIQcTVPSR	603.2881 (2)	6
40	Chain A, Phospholipase A2	1POA_A	*Naja atra*	13	8	1	482	91%	LAAIcFAGAPYNDNDYNINLK	786.3834 (3)	5
									SWWDFADYGcYcGR	921.8565 (2)	3
									GGSGTPVDDLDR	594.7802 (2)	3
									ccQVHDNcYNEAEKISGcWPYFK	742.0635 (4)	5
									ISGcWPYFK	579.2774 (2)	2
									TYSYEcSQGTLTcK	566.578 (3)	3
									GGSGTPVDDLDRccQVHDNcYNEAEKISGcWPYFK	1034.4458 (4)	3
									ISGcWPYFKTYSYEcSQGTLTcK	946.0887(3)	3
41	Chain A, Phospholipase A2 Isoform 3	1T37_A	*Naja sagittifera*	13	9	4	544	67%	TYSYEcTQGTLTcK	856.3708(2)	2
									TYSYEcTQGTLTcKGDNNAcAASVcDcDR	1126.4497 (3)	3

									LAAIcFAGAPYNDDNYNIDLK	1179.5669(2)	8
									LAAIcFAGAPYNDDNYNIDLKAR	862.424(3)	5
									GGSGTPVDDLDR	594.779(2)	1
									TcKGDNNAcAASVcDcDRL#	729.6284 (3)	1
									TcKGDNNAcAASVcDcDRLAAIcF#	917.049 (3)	2
									NLYQFKNMIQcTVPSR	667.0049 (3)	6
									NMIQcTVPSR	603.2928 (2)	1
42	Chain A, Phospholipase A2, Acidic.	2WQ5_A	*Naja naja*	13	12	3	620	79%	TYSYKcSQGTLTcKGG**NN**AcAASVcDcDR	1102.7773 (3)	2
									NLYQFKNMIK	649.8522 (2)	3
									NLYQFKNMIKcTVPSR	667.017 (3)	6
									cTVPSRSWWDFADYGcYcGR	848.3534 (3)	5
									SWWDFADYGcYcGR	921.8565 (2)	3
									GGSGTPVDDLDR	594.779 (2)	1
									LAAIcFAGAPYNDNNYNIDLK	786.3829 (3)	5
									LAAIcFAGAPYNDNNYNIDLKAR	646.8242 (4)	5

									TcKGGN**N**AcAASVcDcDRLAAIcF[#]	898.0412 (3)	2
									DFADYGcY[#]	1010.3569 (1)	1
									NMIKcTVPSR	402.8665 (3)	10
									TYSYKcS**Q**GTLTcK	849.3706 (2)	10
43	Phospholipase A2	BAB72252	*Laticauda colubrina*	17	1	1	46	13%	AALcFAKSPY**NN**NNYNIDIK	778.0418 (3)	8
44	Phospholipase A2	PSNJ3K	*Naja kaouthia*	13	9	2	516	82%	NLYQFKNMIQcTVPSR	667.0049 (3)	6
									NMIQcTVPSR	603.2881 (2)	6
									SWW**N**FADYGcYcGR	614.9076 (3)	4
									GGSGTPVDDLDR	594.7795 (2)	2
									ISGcWPYFK	579.2781(2)	3
									TYSYEcSQGTLTcK	849.361 (2)	1
									LAAIcFAGAPYN**NN**NYNIDLK	786.7116 (3)	6
									NFADYGcY[#]	1010.3559 (1)	1
									N**N**NNYNIDLKARcQ[#]	579.9384 (3)	2
45		CAM34525	*Naja atra*	12	4	2	189	36%	IQcTVPSRSWW[#]	710.3474 (2)	3

	Phospholipase A2, partial								DFADYGcY#	1010.3569(1)	1
									NEAEKISGcWPY#	727.3268 (2)	3
									SYEcSQGTL	1044.4353(1)	5
46	Chain B, Phospholipase A2 isoform 2	1S6B_B	*Naja sagittifera*	13	4	2	195	41%	TcTGRNNAcAASVcDcDRLAAIcF#	921.7215 (3)	1
									AGAPYNDNNYNIDL#	1010.356 (1)	1
									DFADYGcY#	777.3489 (2)	2
									NDNNYNIDL#	1094.4763 (1)	1
47	Phospholipase A2	1MH7_A	*Naja sagittifera*	13	1	1	63	29%	TYTYqcTQGTLTcK	863.3789 (2)	3
Phospholipase B (PLB)											
48	Phospholipase B	JAC94989	*Opheodrys aestivus*	64	3	3	109	7%	KQVVPESLLAW#	635.3642 (2)	1
									AINGPPVEKGLPIFSW#	862.9733 (2)	1
									NFDFVTMKPVL#	655.8445 (2)	1
Vespryn /Ohanin family (Vesp/OLP)											

49	Vespryn	F8S122	*Crotalus adamanteus*	22	1	1	50	5%	EWAVGIAGK	465.7572 (2)	3
50	Vespryn	JAC96417	*Hypsiglena sp. JMG-2014*	20	2	2	129	16%	ADVTFDS**N**TAFKSLVVSPDKK	757.3839 (3)	8
									EWAVGLAGK	465.7572 (2)	3
51	Ohanin precursor	AAR07992	*Ophiophagus hannah*	21	1	1	37	11%	ADVTFDSNTAFESLVVSPDKK	757.3804 (3)	3
52	Thaicobrin	P82885	*Naja kaouthia*	12	13	13	652	85%	ADVTFDSNTAFESLVVSPDKK	757.3839 (3)	8
									TVENVGVSQVAPDNPER	905.953 (2)	3
									TVENVGVSQVAPDNPERFDGSPcVLGSPGFR	1096.5327 (3)	3
									FDGSPcVLGSPGFR	748.3528(2)	1
									EWAVGLAGK	465.7572 (2)	3
									SPPGNWQKADVTFDSNTAFESLVVSPDKK	791.8965 (4)	3
									YGTQREWAVGLAGK	512.6065 (3)	5
									SPPGNWQKADVTF[#]	723.8549 (2)	1
									VVSPDKKTVENVGVSQVAPDNPERF[#]	678.6057 (4)	1
									SPPGNWQKADVTFDSNTAFE[^]	737.6713 (3)	1

									DSNTAFESLVVSPDKKTVENVGVSQVAPDNPERF[#]	919.7082 (4)	1
									NVGVSQVAPDNPE[^]	663.3209 (2)	1
									LRLVPEERIW [P#]	442.9255 (3)	7
									RLVPEERIW [P#]	399.900 (3)	10
53	Ophanin	AAO62996	*Ophiophagus hannah*	27	2	2	65	6%	KM**Q**WYPEAASNAERW[#]	623.2855 (3)	1
									MqWYPEAASNAER	777.3405 (2)	3
54	Opharin precursor	ACN93671.1	*Ophiophagus hannah*	26	165	3	2	19%	NMLQMEWNSNAAQNAK	925.4233 (2)	4
									cSFAHSPPHLR	327.9121 (4)	1
									FScGENLFmSSQPYAWSR	728.3113 (3)	6
Snake venom metalloprotease (SVMP)											
55	**Metalloproteinase atrase A**	ADF43026	*Naja atra*	68	9	9	456	12%	TRVYEMVNYLNTK	544.2818 (3)	3
									VYE**M**VNYLNTK	695.3404 (4)	1
									ERPQcILNKPSR	499.937 (3)	1
									cGTLYcTEIK	622.7862 (2)	1

									HIALIGLEIW#	582.8415 (2)	2
									GSVAVVQDY#	937.4582 (1)	5
									VYE**M**VNYLNTKYR	570.2841 (3)	1
									cGTLYcTEIKK	458.2257 (3)	2
									LGPNYTVGPAGcF#	676.8201 (2)	2
56	**Hemorrhagic metalloproteinase-disintegrin-like kaouthiagin**	P82942	*Naja kaouthia*	44	21	20	1090	51%	YYNYDKPAIK	425.5516 (3)	1
									IRVYEMINAVNTK	517.6175 (3)	1
									VYEMINAVNTK	641.3299 (2)	1
									TAPAFQFSScSIR	736.3567 (2)	4
									DYQEYLLR	550.2765 (2)	3
									AAKHDcDLPELcTGQSAEcPTDSLQR	740.5821 (4)	3
									HDcDLPELcTGQSAEcPTDSLQR	897.0514 (3)	4
									cPTLTNQcIALLGPHFTVSPK	785.4074 (3)	3
									GcFDLNMR	506.7214 (2)	2
									GcFDLN**M**RGDDGSFcR	641.591 (3)	1
									MEDGTKIPcAAK	660.8176 (2)	1

									GQcVDVQTAY	570.7528 (2)	1
									EMINAVNTKF#	583.796 (2)	2
									EMINAVNTKFRPL#	511.6128 (3)	1
									NYDKPAIKIRVY#	493.9484 (3)	1
									SScSIRDYQEY#	704.2942 (2)	2
									TNTPEQDRYLQAEK	564.946 (3)	3
									YIEFYVIVDNR	715.8756 (2)	9
									TAPAFQFSScSIRDYQEYLLR	851.4133 (3)	1
									STDIVSPAIcGNY#	698.8252 (2)	1
									NGKcPTLTNQcIAL#	795.8866 (2)	3
57	K-like metalloprotease precursor, partial	ACN50005	*Naja atra*	66	9	2	531	17%	VAKDDcDLPELcTGQSAEcPTDSLQR	989.1071 (3)	3
									SFGEWRETVLLPR	530.6213 (3)	4
									IRVYEMINAVNTK	517.6175 (3)	1
									VYEMINAVNTK	641.3299 (2)	1
									TAPAFQFSScSIR	736.3567 (2)	4
									GcFDLNMR	506.7214 (2)	2

									GcFDLN**M**RGDDGSFcR	641.591 (3)	1
									MEDGTKIPcAAK	660.8176 (2)	1
									GQcVDVQTAY	570.7528 (2)	1
58	Snake Venom Metalloprotease	3K7L_A	*Naja atra*	48	23	20	1427	62%	TNTPEQDRYLQAK	521.93 (3)	1
									ATL**N**LFGEWR	604.3111 (2)	4
									ATL**N**LFGEWREK	488.9225 (3)	3
									TKPAYQFSScSVR	510.919 (3)	4
									AAKDDcDLPELcTGQSAEcPTDVFQR	995.1032 (3)	3
									cPIMTNQcIALR	738.8614 (2)	2
									VSRDScFTLNQR	494.9097 (3)	2
									DScFTLNQR	570.7601 (2)	2
									DPNYGMVEPGTK	654.3011 (2)	1
									KRNDNAQLLTGIDFNGTPVGLAYIGSIcNPK	837.4404 (4)	5
									RNDNAQLLTGIDFNGTPVGLAYIGSIcNPK	1073.55 (3)	3
									NDNAQLLTGIDFNGTPVGLAYIGSIcNPK	1531.7691 (2)	2

									RTKPAYQFSScSVR	562.9542 (3)	6
									TKPAYQFSScSVR	765.8754 (2)	4
									DDcDLPELcTGQSAEcPTDVFQR	905.0475 (3)	4
									NGLPcQNNQGYcYNGK	943.9026 (2)	4
									cPIMTNQcIALRGPGVK	638.9951 (3)	0
									NS**M**IcNcSISPR	727.8159 (2)	4
									QcVDVKTAY	542.2624 (2)	3
									QR**N**GLPcQNNQGY#	775.3461 (2)	2
									TGIDFNGTPVGLAY#	712.8567 (2)	1
									TGIDFNGTPVGL#	595.806 (2)	1
									LQHEAQcDSEEccEK	641.5876 (3)	9
59	Metalloproteinase	ADI47610	*Echis coloratus*	69	1	1	64	2%	VTL**N**LFGEWR	618.3277 (2)	5
60	Mocarhagin 1	AAM51550	*Naja mossambica*	68	6	5	227	10%	DRPQcILNKPSR	495.2666 (3)	4
									AAK**N**DcDFPELcTGR	585.5891 (3)	3
									RVYEMVNALNTMYR	587.2954 (3)	6
									SVAVVQDHSK	535.2900 (2)	8

									FKGAGAEcR	498.2449 (2)	10
61	**Cobrin precursor**	AAF00693	*Naja naja*	68	32	18	1901	59%	DPSYG**M**VEPGTK	648.7924 (2)	1
									ATLDLFGEWR	604.3115 (2)	4
									TSAAVVQDYSK	584.7976 (2)	3
									DScFTLNQR	570.7616 (2)	5
									TSAAVVQDYSKSTR	504.9276 (3)	2
									ATLDLFGEWREK	604.3111 (2)	3
									TKPAYQFSScSVR	510.919 (3)	4
									VSRDScFTLNQR	494.9097 (3)	2
									YIEFY**M**VVDNIMYR	936.4425 (2)	4
									KRNDNAQLLTGIDFNGTPVGLAYIGSIcNPK	837.4404 (4)	5
									RNDNAQLLTGIDFNGTPVGLAYIGSIcNPK	1073.55 (3)	3
									NDNAQLLTGIDFNGTPVGLAYIGSIcNPK	1021.517 (3)	4
									RTKPAYQFSScSVR	562.9542 (3)	6
									TKPAYQFSScSVR	765.8754 (2)	4
									AAKDDcDLPELcTGQSAEcPTDVFQR	746.5811 (4)	5

									DDcDLPELcTGQSAEcPTDVFQR	905.0475 (3)	4
									cPIMTNQcIALR	738.8618 (2)	3
									cPIMTNQcIALRGPGVK	638.9951 (3)	1
									NS**M**IcNcSISPR	727.8159 (2)	4
									QcVDVKTAY	542.2624 (2)	3
									QAEKYIEFY#	595.7939 (2)	6
									TGIDFNGTPVGL#	595.806 (2)	1
									TGIDFNGTPVGLAY#	712.8567 (2)	1
									LQHEAQcDSEEccEK	641.5876 (3)	9
									FKGAGAEcR	994.4753 (2)	10
									YMVVDNIMY#	574.2642 (2)	4
									SNINEINVQSDVKATL#	582.3073 (3)	1
									HIALIGLEIW#	582.8419 (2)	1
									IGSIcNPKTSAAVVQDY#	608.3064 (3)	2
									AYIGSIcNPKTSAAVVQDY#	686.3396 (3)	2

									NGKcPIMTNQcIAL#	810.3895 (2)	1
									KGAGAEcRAAKDDcDLPELcTGQSAEcPTDVF#	882.8868 (4)	3
62	Metalloprotease	XP_015671647	*Protobothrops mucrosquamatus*	133	1	1	2	1%	QKPIAS**Q**Ac**N**INF#	746.8542 (2)	6
63	P-III snake venom metalloprotease, partial	AHZ08819.1	*Micropechis ikaheca*	68	1	1	57	1%	cPTDSFQR	505.7263 (2)	9
64	Scutellatease-1	ABQ01136	*Oxyuranus scutellatus*	69	2	1	88	4%	LTNTPEQER	544.2776 (2)	9
									LQHEAQcDSEEccEK	641.5876 (3)	9
Nerve growth factor (NGF)											
65	Nerve growth factor beta chain precursor	A59218	*Naja kaouthia*	28	8	8	477	48%	EDHPVHNLGEHSVcDSVSAWVTK	651.558 (4)	6
									TTATDIKGNTVTVMENVNLDNKVYK	692.8625 (4)	5
									GIDSSHWNSYcTETDTFIK	754.3351 (3)	4
									ALTMEGNQASWR	682.3266 (2)	3

									TTATDIKGNTVTVMENVNLDNK	793.4036 (3)	4
									HSVcDSVSAWVTKTTATDIKGNTVTVME^	760.1237 (4)	4
									NVNLDNKVYKQYFFE^	960.9794 (2)	1
									TDTFIKALTME^	635.3242 (2)	1
66	Chain A, Venom growth factor	4EC7_A	*Naja atra*	13	10	10	422	83%	VTKTTATDIKGNTVTVMENVNLDNKVY#	742.8895 (4)	2
									NSYcTETDTFIKAL#	831.8872 (2)	1
									cTETDTFIKAL#	649.8186 (2)	1
									IKALTMEGNQASW#	724.8637 (2)	1
									TDTFIKALT**M**E^	643.3226 (2)	3
									TTATDIKGNTVTVMENVNLDNKVYK	692.8623 (4)	5
									GIDSSHWNSYcTETDTFIK	754.3369 (3)	6
									ALTMEGNQASWR	682.3261 (2)	2
									TTATDIKGNTVTVMENVNLDNK	793.4070 (3)	8
									EDHPVHNLGEHSVcDSVSAWP#	759.0060 (3)	

67	Nerve growth factor II	AAS94269	*Naja sputatrix*	27	15	15	800	48%	EDHPVHNLGEHSVcDSVSAWVTK	651.5576 (4)	5
									TTATDIKGNTVTVMENVNLDNK	793.404 (3)	4
									TTATDIKGNTVTVMENVNLDNKVYK	692.8624 (4)	5
									GNTVTVMENVNLDNKVYK	680.0197 (3)	8
									GIDSSHWNSYcTETDTFIK	754.3358 (3)	5
									ALTMEGNQASWR	682.3267 (2)	3
									FIRIDTAcVcVITK	565.9709 (3)	3
									FIRIDTAcVcVITKK	456.7553 (4)	7
									IDTAcVcVITK	640.3291 (2)	10
									GNTVTVMENVNLDNK	824.405 (2)	1
									VYKQYFFETK	676.8492 (2)	2
									GIDSSHWNSYcTETDTFIKALT**M**EGNQASWR	906.1592 (4)	4
									IDTAcVcVITKK	469.9174 (3)	3
									TDTFIKALT**M**E^	643.3222 (2)	2

68	Venom nerve growth factor precursor	ABA60123	*Pseudechis porphyriacus*	27	2	1	147	13%	GIDSSHWNSYcTTTqTFVRALTMEGNQASWR	902.1619 (2)	2
									ALTMEGNQASWR	682.3267 (2)	3
69	Venom nerve growth factor	P61899	*Naja kaouthia*	13	3	3	145	35%	VTKTTATDIKGNTVTVMENVNLDNKVY#	742.8912 (4)	4
									NSYcTETDTFIKAL#	831.8878 (2)	1
									cTETDTFIKAL#	649.8201 (2)	2
Cysteine-rich secretory proteins (CRISP)											
70	Kaouthin-2 precursor	ACH73168.1	*Naja kaouthia*	26	17	17	1089	77%	ENQKQIVDKHNALR	423.9856 (4)	4
									N**M**LQ**M**EWNSNAAQNAK	627.9481 (3)	4
									NMLQMEWNSNAAQNAKR	669.3174 (3)	2
									cSFAHSPPHLR	436.8813 (3)	2
									TVGKIGcGENLFMSSQPYAWSR	830.0661 (3)	4
									IGcGENLF**M**SSQPYAWSR	706.9863 (3)	3
									VIQSWYDENK	641.3098 (2)	1
									VIQSWYDENKK	470.5747 (3)	2

									YLYVcQYcPAGNIIGSIATPYK	851.0855 (3)	2
									SGPPcGDcPSAcVNGLcTNPcK	803.3262 (3)	3
									HHNVFSNcQSLAK	514.5816 (3)	2
									QNAcQTEWMK	648.2808 (2)	3
									cAAScFcRTEII	744.3279 (2)	3
									VYGVGANPPGSVIGHY#	793.9009 (2)	1
									GVGANPPGSVIGHY#	662.8339 (2)	3
									cPAGNIIGSIATPY#	717.3552 (2)	4
									SRVIQSWYDENKKF P#	600.6443 (3)	6
71	**Natrin**	1XTA_A	*Naja atra*	25	27	27	1500	86%	NVDFNSESTR	584.7662 (2)	2
									VSPTASNMLK	524.2794 (2)	1
									MEWYPEAASNAER	785.3376 (2)	3
									VLEGIQcGESIY**M**SSNAR	1015.4722 (2)	3
									VSPTASN**M**LK**M**EWYPEAASNAER	872.071 (3)	3
									SNcPAScFcR	629.7443 (2)	4

									NVDFNSESTRR	442.2138 (3)	2
									KMEWYPEAASNAERW#	623.2863 (3)	1
									TEIIHLWHDEY#	485.9007 (3)	2
									KQKEIVDLHNSLR	527.3047 (3)	5
									QKEIVDLHNSLR	484.6051 (3)	3
									QKEIVDLHNSLRR	402.7301 (4)	1
									RVSPTASNMLK	602.3298 (2)	1
									RVSPTASN**M**LKMEWYPEAASNAER	689.333 (4)	5
									MEWYPEAASNAERWANTcSLNHSPDNLR	830.6245 (4)	4
									TATPYKLGPPcGDcPSAcD**N**GLcTNPcTIYNK	901.6417 (4)	4
									WANTcSL**N**HSPDNLR	595.9396 (3)	4
									TWTEIIHLWHDEYK	468.4854 (4)	4
									LGPPcGDcPSAcDNGLcTNPcTIYNK	1471.1084 (2)	2
									ANTcSLNHSPDNLRVL#	604.2994 (3)	2
									RVLEGIQcGESIY#	762.376 (2)	5

									EIVDLHNSLR	598.3245 (2)	1
									VSPTASN**M**LK	532.2771 (2)	2
									ANTcSLNHSPDNLRVL#	604.3004 (3)	1
									NHSPDNLRVLEGIQcGESIY#	767.7023 (3)	1
									AASNAERWANTcSLNHSPDNLRVLE^	707.0907 (4)	1
									RWANTcSLNHSPDNLRVLE^	571.2838 (4)	2
Phosphodiesterase (PDE)											
72	Phosphodiesterase 1	JAB52814	*Micrurus fulvius*	97	34	34	1720	40%	**M**ANVLcScSEDcLTK	902.3754 (2)	3
									MANVLcScSEDcLTKK	644.618 (3)	3
									DccTDYKSIcKR	402.1783 (4)	6
									AEYLETWDTLMPNINK	969.472 (2)	3
									AEYLETWDTL**M**PNINKLK	732.3765 (3)	4
									AATYFWPGSEVK	678.3368 (2)	2
									AKRPDFSTLYIEEPDTTGHK	577.045 (4)	5

									RPDFSTLYIEEPDTTGHK	527.2597 (4)	1
									TLGMLMEGLK	546.7952 (2)	3
									TLG**M**LMEGLKQR	464.9182 (3)	4
									NLHNcVNLILLADHGMEAIScNR	666.8294 (4)	6
									SKNVPKDFYTFDSEAIVK	522.7749 (4)	6
									NVPKDFYTFDSEAIVK	624.9855 (3)	2
									DFYTFDSEAIVK	717.8457 (2)	4
									IDKVNLMVDR	401.5591 (3)	5
									IDKVNLMVDRQWLAVR	489.7796 (4)	6
									VNLMVDRQWLAVR	533.966 (3)	3
									YcSGGTHGYDNEFK	545.5581 (3)	3
									NPFYNPSPAK	567.7837 (2)	2
									cSSITDLEAVNQR	746.8596 (2)	4
									YISAYSQDIL**M**PLWNSYTISK	837.0864 (3)	5
									LWNYFHSTLLPK	506.945 (3)	6

									VLSFILPHRPDNSEScADKSP**N**NLWVEER	682.9384 (5)	6
									SP**N**NLWVEER	622.7999 (2)	2
									QPLSETLR	472.2655 (2)	3
									cScSEDcLTKKDccTDY#	734.6032 (3)	2
									VNHYTIVTGLY#	640.3384 (2)	1
									GPVSGQVIKSL#	542.8232 (2)	3
									cLLHQTKY#	531.7742 (2)	1
									DALITSNIVP**M**YKEF#	878.9474 (2)	3
									NcPPGSLKVL#	542.7957 (2)	2
									NcPPGSLKVLSF#	659.8455 (2)	1
									KQPLSETL#	458.2616 (2)	1
									EARV**M**EVL#	481.7536 (2)	3
73	Phosphodiesterase, partial	ALA20853	*Macropisthodon rudis*	91	7	2	390	11%	TEVTSFENIEVYNLMcDLLK	1209.5852 (2)	3
									EKTEVTSFENIEVYNLMcDLLK	892.4386 (3)	2
									LWNYFHSTLLPK	506.945 (3)	6

									NLHNcVNLILLADHGMEAIScNR	666.8294 (4)	6
									NPFYNPSPAK	567.7837 (2)	2
									QPLSETLR	472.2655 (2)	3
									SPDNLWVEER	622.7999 (2)	2
74	Phosphodiesterase	AHJ80885	*Macrovipera lebetina*	96	5	1	273	6%	S**MQ**AIFLAHGPGFK	507.593 (3)	5
									AATYFWPGSEVK	678.3368 (2)	2
									TLG**M**LMEGLK	554.7938 (2)	5
									TLGMLMEGLKQR	459.5866 (3)	4
									NPFYNPSPAK	567.7837 (2)	2
75	Phosphodiesterase family member 3, partial	XP_007430581	*Python bivittatus*	94	9	4	399	9%	AATYFWPGSDIK	678.3368 (2)	2
									TLG**M**LMEGLK	554.7938 (2)	5
									TLGMLMEGLKQR	459.5866 (3)	4
									IDKVNLMVDR	401.5591 (3)	5
									YIEEPDTTGHKF[#]	479.563 (3)	4
									IEEPDTTGHKF[#]	425.2086 (3)	4
									cLLHQTKY[#]	531.7742 (2)	1

									KQPLSETL#	458.2616 (2)	1
									DYQDTcVLPTQSW#	806.8536 (2)	3
76	Phosphodiesterase family member 3	JAG67101	*Boiga irregularis*	101	12	3	542	11%	AEYLETWDTLMPNINK	969.472 (2)	3
									AEYLETWDTL**M**PNINKLK	732.3765 (3)	4
									AATYFWPGSEVK	678.3368 (2)	2
									NPFYNPSPAK	567.7837 (2)	2
									LWNYFHSTLLPK	506.945 (3)	6
									QPLSETLR	472.2655 (2)	3
									TFDSEAIVKKL#	625.854 (2)	1
									DSEAIVKKL#	501.7958 (2)	1
									ANNIRIDKVNL#	423.9153 (3)	3
									EARV**M**EVL#	481.7536 (2)	3
									KQPLSETL#	458.2616 (2)	1
									DTLMPNINKL#	579.8135 (2)	1
77	Phosphodiesterase	JAS05142	*Micrurus tener*	97	9	9	486	13%	RPDFSTLYIEEPDTTGHK	527.2624 (4)	6
									NPFYNPSPAK	567.7848 (2)	4

									cSSITDLEAVNQR	746.858 (2)	2
									SPNNLWVEER	622.8051 (2)	10
									DTLMPNINKL#	579.8135 (2)	1
									VNHYTIVTGLY#	640.3389 (2)	2
									GPVSGQVIKSL#	542.8226 (2)	2
									GPVPSPDLSGcKcSSITDLEAVNQRL#	934.1275 (3)	3
									DALITSNIVPMYKEF#	870.949 (2)	2
78	Chain A, Snake Venom Phosphodiesterase P#^	pdb|5GZ4|A	*Naja atra atra*	94	9	8	620	15%	TIVTGLYAETHGIIDNNMY	1071.0222 (2)	7
									LYPPDFSSSGPEQY	793.8597 (2)	7
									YNPSPAKEQSPPLY	795.8998 (2)	8
									GPVSGQVIKSL	542.8268 (2)	10
									NLIDQAKMQSEADNLPY	975.4763 (2)	9
									WGGQPIWHTASY	701.8361 (2)	8
									SLSGSNMRNAAW	647.3091 (2)	8
									RAVYPTKTF	541.8075 (2)	8

									LLTGLDFYSALKQPLSE	948.0171 (2)	6
5' nucleotidase (5-NTD)											
79	Ecto-5'-nucleotidase	JAS05143	*Micrurus tener*	63	30	30	1585	57%	LTILHTNDVHAR	463.9257 (3)	2
									YDA**M**ALGNHEFDNGLNGLLDPLLK	882.7688 (3)	2
									NVKFPILSANIRPK	399.9963 (4)	3
									FPILSANIRPK	419.257 (3)	3
									IINVGSEKVGIIGYTTK	598.0133 (3)	2
									VGIIGYTTK	476.28 (2)	2
									ETPVLSNPGPYLEFRDEVEELQK	897.1196 (3)	3
									QVPVVQAYAFGK	653.8614 (2)	1
									ASGNPILLNK	513.8015 (2)	1
									FHEcNLGNLIcDAVVYNNLR	807.7188 (3)	1
									AN**N**GIITLEELTAVLPFGGTFDLLQIK	963.5335 (3)	7
									HGQGTGELLQVSGIK	508.612 (3)	2
									VVSLNVLcTEcR	725.3642 (2)	2

									VPTYVPL**Q**MEK	653.3439 (2)	3
									VLLPSFLAAGGDGYY**M**LK	966.0096 (2)	7
									MGKVFPAMEGR	408.2085 (3)	3
									VFPAMEGR	453.7298 (2)	2
									IINVGSEK	430.2492 (2)	3
									SIQEDPAVK	493.7645 (2)	6
									QAFEHSVHR	555.7784 (2)	5
									KIINVGSEKVGIIGY[#]	795.469 (2)	3
									TTKETPVLSNPGPY[#]	752.3899 (2)	2
									LEFRDEVEEL[#]	639.8155 (2)	2
									GVNKIIAL[#]	827.5274 (1)	9
									GVNKIIALGHSGF[#]	656.8721 (2)	1
									MQLSDDGRQVPVVQAY[#]	903.4476 (2)	1
									SDDGRQVPVVQAY[#]	717.3557 (2)	1
									HEcNLGNLIcDAVVY[#]	888.9075 (2)	2
									cTEcRVPTYVPL**Q**MEKTY[#]	759.3554 (3)	5

									KVLLPSFL#	916.5877 (1)	1
80	Snake venom 5'-nucleotidase	JAG67188	*Boiga irregularis*	65	15	3	796	30%	GREVVQFMNSLR	479.2564 (3)	5
									EVVQFMNSLR	611.8174 (2)	2
									QVPVVQAYAFGK	653.8614 (2)	1
									VGIIGYTTK	476.28 (2)	2
									ETPVLSNPGPYLEFRDEVEELQK	897.1196 (3)	3
									HGQGTGELLQVSGIK	508.612 (3)	2
									VPTYVPLE**M**EK	661.3424 (2)	5
									FHEcNLGNLIcDAVVYNNLR	807.7188 (3)	1
									TTKETPVLSNPGPY#	752.3899 (2)	2
									LEFRDEVEEL#	639.8155 (2)	2
									GVNKIIALGHSGF#	656.8721 (2)	1
									SDDGRQVPVVQAY#	717.3557 (2)	1
									HEcNLGNLIcDAVVY#	888.9075 (2)	2
									KVLLPSFL#	916.5877 (1)	1
									QVSGIKVVY#	992.579 (1)	2

81	5' nucleotidase, partial	BAN82018	*Protobothrops flavoviridis*	57	9	3	422	23%	GREVVKFMNSLR	479.2695 (3)	7
									FHEcNLGNLIcDAVIYNNVR	807.7188 (2)	1
									GDSSNHSSGNLDISIVGDYIKR	584.292 (4)	6
									IINVGSEKVGIIGYTTK	598.0133 (3)	2
									VGIIGYTTK	476.28 (2)	2
									ETPVLS**N**PGPYLEFR	573.9617 (3)	7
									QVPVVQAYAFGK	653.8614 (2)	1
									ASGNPILLNK	513.8015 (2)	1
									VVSLNVLcTEcR	725.3642 (2)	2
82	5'-nucleotidase, partial	ETE67245	*Ophiophagus hannah*	28	6	1	348	30%	HADKLTTLGVNK	432.9155 (3)	4
									HGQGTGELLQVSGIK	508.612 (3)	2
									VVSLNVLcTEcR	725.3642 (2)	2
									VPTYVPLEMEK	653.3439 (2)	3
									VLLPSFLAAGGDGYY**M**LK	966.0096 (2)	7
									VFPAMEGR	453.7298 (2)	2
83	Snake venom 5'-	JAS04365	*Agkistrodon*	65	7	5	302	13%	LLDAGDQY[#]	447.7135 (2)	1

	nucleotidase		*contortrix*						KIINVGSEKVGIIGY#	530.6476 (3)	1
									TTKETPVLSNPGPY#	752.3889 (2)	1
									DLLQIKGSTL#	544.3216 (2)	1
									QVSGIKVVY#	496.7928 (2)	1
									LEFRDEVEEL#	639.8155 (2)	2
									GVNKIIALGHSGF#	656.8721 (2)	1
84	Ecto-5' nucleotidase, partial	AEJ31979.1	*Crotalus adamanteus*	58	108	3	2	6%	VGIIGYTTK	476.2776 (2)	3
									QVPVVQAYAFGK	653.8606(2)	1
									VLSLNVLcTEcR	732.3704 (2)	1
Snake venom serine proteinase (SVSP)											
85	Thrombin-like serine protease	AAL68708	*Gloydius ussuriensis*	29	1	1	51	3%	TKVFDYTEW#	594.7799 (2)	4
86	Thrombin-like enzyme TLP	P86545.1	*Naja naja*	4	1	1	37	28%	cAGTLLNHEW#	600.7764 (2)	1
87	Tissue-type plasminogen activator	JAI11009	*Crotalus adamanteus*	60	4	4	234	10%	AVcYQDIGDTYR	730.829 (2)	2
									GTWSTTESGAEcVNWK	906.897(2)	2

									TLDNDIALLK	558.3208 (2)	3
									TVTENMLcAGDTR	734.3349 (2)	4
88	Tissue-type plasminogen activator, partial	ETE66683	*Ophiophagus hannah*	32	4	3	152	15%	GGAYSYIESHPWQAGIFVK	704.0202 (3)	3
									LYPDSFcTSAR	658.8021 (2)	2
									TVTEN**M**LcAGDTR	742.3355 (2)	8
									SYIESHPWQAGIF P#	767.8721 (2)	3
89	Tissue-type plasminogen activator, partial	XP_007443169	*Python bivittatus*	38	2	2	70	4%	LIGIISWGVGcGK	680.3778 (2)	2
									IGIISW P#	704.3993 (1)	2
90	Tissue-type plasminogen activator	XP_013924331	*Thamnophis sirtalis*	51	3	2	190	6%	LPDWTEcEVSGYGK	820.8695 (2)	3
									TVTEN**M**LcAGDTR	742.3355 (2)	8
									SYIESHPW#	509.7357 (2)	1
									KLPDWTEcEVSGY#	792.3562 (2)	1
91	Snake venom serine protease NaSP	A8QL53.1	*Naja atra*	31	67.88	1	1	5%	FPcAQLLEPGVYTK	811.9148 (2)	2
Cobra Venom Factor (CVF)											

92	Complement decay-accelerating factor transmembrane isoform, partial	ETE63384	*Ophiophagus hannah*	79	1	1	55	1%	IVSS**N**DLR	452.7419 (2)	3
93	Pseutarin C	AAO38805	*Pseudonaja textilis*	166	1	1	10	1%	KQEEPRDALSGL[#]	671.8464 (2)	8
94	**Chain B, Cobra Venom Factor**	3PRX_B	*Naja kaouthia*	185	53	53	2739	37%	QLDIFVHDFPR	462.9135 (3)	7
									QNQYVVVQVTGPQVR	857.9704 (2)	6
									GIYTPGSPVLYR	661.8634 (2)	7
									YVLPSFEVR	555.3068 (2)	7
									IPIIDGDGK	464.2642 (2)	7
									IPIIDGDGKATLKR	374.9726 (4)	8
									LILNIPLNAQSLPITVR	938.0822 (2)	8
									VGLVAVDK	400.7512 (2)	9
									VGLVAVDKAVYVLNDKYK	499.293 (4)	9
									DDNEDGFIADSDIISR	891.399 (2)	4

									YRINYENALLAR	499.2721 (3)	1
									INYENALLAR	588.8223 (2)	1
									LNQDITVTASGDGK	709.8604 (2)	1
									ATMTILTFYNAQLQEK	936.4821 (2)	1
									VAVIIYLNK	516.8269 (2)	1
									HFEVGFIQPGSVK	482.2579 (3)	1
									VYSYYNLDEK	647.3094 (2)	9
									IDVPLQIEK	527.8109 (2)	1
									AcETNVDYVYK	681.3118 (2)	9
									IEEQDGNDIYVMDVLEVIK	741.3712 (3)	4
									KcQEALNLK	552.2982 (2)	1
									KcQEALNLKVNDDYLIWGSR	808.0767 (3)	1
									VNDDYLIWGSR	669.3328 (2)	7
									SDLLPTKDKISYIITK	612.3611 (3)	9
									QTRVDMNPAGGML[#]	695.3375 (2)	4
									VVVQVTGPQVRL[#]	647.8958 (2)	1

									VVVQVTGPQVRLEKVVLL#	988.6198 (2)	3
									LFIQTDKGIY#	599.3298 (2)	1
									IQTDKGIYTPGSPVLY#	876.4661 (2)	2
									NLPDLVSLGTW#	1214.6428 (1)	1
									LYGEEVEGVAF#	606.7939 (2)	2
									TRIPIIDGDGKATL#	735.4209 (2)	1
									YQVGNNEIVADSVW#	797.3836 (2)	3
									VDVKDTcMGTL#	619.7919 (2)	1
									KISQAKIWDTIEKSDF#	637.0084 (3)	2
									TPTKGIcVAEPY#	668.3351 (2)	1
									LILNRQQPDGAF#	686.3739 (2)	2
									ALAAADQLNDDRVL#	742.8890 (2)	1
									DQTGPIVRW#	536.2831 (2)	1
									EIQMPTHKDLNLDITIELPDREVPIRY#	812.9323 (4)	3
									YIDGNENFHVSITARY#	633.6415 (3)	4

									NLDITIELPDREVPIRY#	686.0439 (3)	5
									RINYENALL#	553.3039 (2)	1
									NAQLQEKANVcNKF#	555.2797 (3)	2
									TGFLPDAEDLTRL#	724.3776 (2)	3
									EVDNNMAQKVAVIIY#	853.9431 (2)	1
									EVGFIQPGSVKVY#	711.8854 (4)	1
									HQERIDVPLQIEKAcETNVDY#	668.5794 (4)	7
									QIEKAcETNVDYVY#	866.3968 (2)	2
									NHQERIDVPLQIEKAcETNVDYVY#	734.1098 (4)	2
									TKIIIQGDPVAQIIE^	819.4784 (2)	1
									NALLARTVE^	493.7859 (2)	2
									FQKLcDDFAQFSYTLTE^	1056.9837 (2)	1
95	Chain A, Cobra Venom Factor Alpha Chain	3FRP_A	*Naja kaouthia*	70	9	2	471	17%	ALYTLITPAVLR	665.91 (2)	2
									YVLPSFEVR	555.3068 (2)	7
									IPIIDGDGK	464.2642 (2)	7

									QLDIFVHDFPR	462.9135 (3)	7
									GIYTPGSPVLYR	661.8634 (2)	7
									LILNIPLNAQSLPITVR	938.0822 (2)	8
									VGLVAVDK	400.7512 (2)	9
									VGLVAVDKAVYVLNDKYK	499.293 (4)	9
									ALYTLITPAVLRTDTEE[^]	953.51 (2)	2
96	OVF precursor protein	AFJ59923	*Ophiophagus hannah*	184	20	4	982	14%	TDTEEQILVEAHGDNTPK	666.3263 (3)	9
									QLDIFVHDFPR	462.9135 (3)	7
									GIYTPGSPVLYR	661.8634 (2)	7
									HFEVGFIQPGSVK	482.2579 (3)	1
									RTDTEEQILVEAHGDNTPKQL[#]	599.3055 (4)	4
									RTDTEEQILVEAHGDNTPKQLDIF[#]	693.1002 (4)	3
									ALAAADRLNDDRVL[#]	504.9446 (3)	4
									VVVQVTGPQVRL[#]	647.8958 (2)	1
									IQTDKGIYTPGSPVLY[#]	876.4661 (2)	2

									LYGEEVEGVAF#	606.7939 (2)	2
									YQVGNNEIVADSVW#	797.3836 (2)	3
									VDVKDTcMGTL#	619.7919 (2)	1
									KISQAKIWDTIEKSDF#	637.0084 (3)	2
									TPTKGIcVAEPY#	668.3351 (2)	1
									LILNRQQPDGAF#	686.3739 (2)	2
									DQTGPIVRW#	536.2831 (2)	1
									NAQLQEKANVcNKF#	555.2797 (3)	2
									TGFLPDAEDLTRL#	724.3776 (2)	3
									EVDNNMAQKVAVIIY#	853.9431 (2)	1
									EVGFIQPGSVKVY#	711.8854 (2)	1
97	Complement-depleting factor, partial	ABN72542	*Bungarus fasciatus*	143	6	3	319	5%	IEEKDGNDIYVMDVLEVIK	741.3753 (3)	7
									GNANSLNQIK	529.7876 (2)	8
									VGLVAVDK	400.751 (2)	9
									VGLVAVDKAVYVLNDKYK	499.293 (4)	9

									HFEVGFIQPGSVK	482.2579 (3)	1
									KcQEALNLK	552.2982 (2)	1
									AVPFVIVPLqQGLHDIEVK	701.7338 (3)	1
									SDFGcTAGSGQNNLGVFEDAGLALTTSTNLNTK	1120.8595 (3)	1
98	Venom factor	AAX86641.1	*Australaps superbus*	185.00	291	6	2	4%	YFTYLILTK	581.3316 (2)	1
									AVYVLNDKYK	606.8333 (2)	3
									VGLVAVDK	400.7464 (2)	3
									HFEVGFIQPGSVK	482.2569 (3)	2
									DTcMGTLVVK	562.2756 (2)	4
									VELLYNPAFcSASTEGQR	681.328 (3)	2
C-type lectin (CTL)											
99	**C-type lectin BfL-1**	Q90WI8	*Bungarus fasciatus*	19	5	5	96	23%	YIWEWTDR	584.7768 (2)	3
									ISDYLTGQGHVW[#]	688.3337 (2)	3
									SEYISDYLTGQGHVW [P#]	877.8997 (2)	6

									HSNADAVEFSEY [P#]	692.7882 (2)	3
									ASLHSNADAVEFSEY [P#]	828.3643 (2)	2
Endonucleases (END)											
100	Endonuclease domain-containing 1 protein	ETE56723	*Ophiophagus hannah*	18	9	6	525	53%	GHLNPNGHQPDYSAK	545.5948 (3)	2
									SSTFTLTNIVPQFIK	565.9838 (3)	3
									GcQQTFAVVGAVPGDTYIAR	704.0215 (3)	5
									LAQLYNVNHVSLFHSDcPR	568.2862 (4)	4
									LAQLYNVNHVSLFHSDcPRQ	600.3018 (4)	6
									AVVGAVPGDTY[#]	524.7672 (2)	4
									TLTNIVPQFIKL[#]	693.9181 (2)	5
									SAAccLIDNNHL[#]	694.3016 (2)	10
									SAAccLIDNNHLRSW[#]	606.2776 (3)	2
101	Endonuclease domain-containing 1 protein	JAB54304	*Micrurus fulvius*	32	4	1	176	14%	TLTNIVPQFIKL[#]	693.9181 (2)	5
									SAAccLIDNNHL[#]	694.3016 (2)	10
									SAAccLIDNNHLRSW[#]	606.2776 (3)	2

									ARNDQNVVQKF#	440.2333 (3)	1
L- aminoacid oxidases (LAAO)											
102	L-amino-acid oxidase	A8QL58	*Naja atra*	51	47	47	2445	88%	VTLLEASER	509.2818 (2)	1
									VIKIQYDAEK	402.8965 (3)	2
									VIKIQYDAEKVR	487.954 (3)	3
									TFVTADYVIVcSTSR	573.6209 (3)	4
									IYFEPPLPPK	600.8379 (2)	2
									TcADIVINDLSLIHDLPK	679.696 (3)	2
									TcADIVINDLSLIHDLPKR	549.0504 (4)	4
									EIQALcYPSIK	661.3424 (2)	4
									EIQALcYPSIKK	483.9317 (3)	2
									VLADDSDFF#	1028.4577 (1)	1
									IQYDAEKVR	561.3017 (2)	1
									TSNPKHVVVVGAGMAGLSAAYVLAGAGHK	553.3035 (5)	4
									HVVVVGAG**M**AGLSAAYVLAGAGHK	563.5583 (4)	4

									HVVVVGAGMAGLSAAYVLAGAGHKVTLLEASER	809.1965 (4)	6
									EGWYVNMGPMR	670.3011 (2)	2
									LNEFFQENENAWYYINNIR	1239.0828 (2)	3
									LNEFFQENENAWYYINNIRK	869.0918 (3)	5
									VWEVKKDPSLLK	361.2174 (4)	4
									KDPSLLKYPVKPSEEGK	479.5207 (4)	4
									KVIEELKR	338.8827 (3)	3
									YDSYSTKEYLIK	503.9226 (3)	4
									SDALFSYEK	530.2547 (2)	3
									SDALFSYEKR	405.8724 (3)	2
									VRVTYQTPAK	388.2247 (3)	1
									VTYQTPAKTFVTADYVIVcSTSR	869.7785 (3)	3
									RIYFEPPLPPK	452.9286 (3)	3
									RIYFEPPLPPKK	371.9726 (4)	4
									IYFEPPLPPKK	443.5934 (3)	3

									IFLTcSKK	332.8576 (3)	3
									KFWEADGIHGGK	448.8964 (3)	4
									FWEADGIHGGK	406.1977 (3)	3
									STTDLPSR	438.7257 (2)	2
									REIQALcYPSIK	493.2672 (3)	2
									REIQALcYPSIKK	402.227 (4)	5
									DAEKVRVTY[#]	540.7887 (2)	2
									WEADGIHGGKSTTDLPSRF[#]	519.2571 (4)	4
									YPVKPSEEGK	567.297 (2)	3
									VTYQTPAK	454.2491 (2)	3
									VLAGAGHKVTLL[#]	589.8667 (2)	1
									AGAGHKVTLL[#]	483.7905 (2)	1
									SRGAVD**M**IGDLLNEDSSY[#]	979.4456 (2)	2
									EKRFDEIVGGFDQLPISMY[#]	748.7078 (3)	4
									EADGIHGGKSTTDLPSRF[#]	629.9805 (3)	4

									DTKTcADIVINDLSLIHDLPKREIQAL[#]	773.6705 (4)	5
									NLDKYTMGSITSF[#]	738.8577 (2)	3
									GWYVNMGPMRLPE[^]	775.3707 (2)	3
									VKKDPSLLKYPVKPSEE[^]	490.0318 (4)	2
103	L-amino-acid oxidase	A8QL51	*Bungarus multicinctus*	59	3	1	142	2%	T**M**GALTSFTPY[#]	1204.5557 (1)	1
									VLADDSDFF[#]	1028.4578 (1)	1
									EADGIHGGKSTTDLPSRF[#]	629.9805 (3)	4
104	L-amino-acid oxidase	P0DI91	*Naja oxiana*	11	12	3	688	82%	RSPLEEcFQQNDYEEFLEIAR	891.75 (3)	4
									SPLEEcFQQNDYEEFLEIAR	839.7163 (3)	4
									SDALFSYEK	530.2547 (2)	3
									KFWEADGIHGGK	448.8964 (3)	4
									FWEADGIHGGK	406.1977 (3)	3
									REIQALcYPSIK	493.2672 (3)	2
									REIQALcYPSIKK	402.227 (4)	5
									EIQALcYPSIK	661.3424 (2)	4

									EIQALcYPSIKK	483.9317 (3)	2
									HLSFIESL[#]	473.26 (2)	9
									DDRRSPLEEcFQQNDYEEFLEIAR	765.5999 (4)	1
									cFQQNDYEEFLE[^]	811.3284 (2)	1
105	L-amino acid oxidase precursor	AAY89682	*Pseudechis australis*	59	3	1	110	6%	SASQLYRESLQK	1408.7372 (2)	4
									FWEADGIHGGK	1215.5713 (3)	3
									STTDLPSR	438.7257 (2)	2
106	L-amino acid oxidase, partial	JAC94991	*Opheodrys aestivus*	40	3	1	123	8%	QAIAETVHL[#]	491.2726 (2)	2
									WEADGIHGGKSTTDLPSRF[#]	519.2571 (4)	4
									EADGIHGGKSTTDLPSRF[#]	472.737 (4)	3
107	L-Amino Acid Oxidase	3KVE_A	*Vipera ammodytes ammodytes*	55	2	2	105	7%	HVVVVGAGMSGLSAAYVLAGAGHK	563.5583 (4)	4
									STTDLPSR	438.7257 (2)	2
108	Secreted L-amino acid oxidase precursor	ACF70483	*Daboia russelii*	57	1	1	61	3%	NLLLETADYVIVcTTSR	656.6753 (3)	1
109	L-amino acid	AAY89680.1	*Oxyuranus*	59	1	5	239	15%	nPLEEcFREADYEEFLEIAR	844.3866 (3)	2

	oxidase precursor		*scutellatus*						HVVVVGAGMAGLSAAYVLAGAGHK	745.7428 (3)	3
									VTLLEASER	509.2811 (2)	3
									IYFEPPLPPK	600.8363 (2)	1
									KFWEADGIHGGK	448.8939 (2)	2
Gluthathione peroxidase (GPrx)											
110	Glutathione peroxidase 3, partial	ETE68810	*Ophiophagus hannah*	30	20	20	943	77%	LVILGFPcNQFGK	746.9027 (2)	1
									QEPGQNSEILQGIK	770.9036 (2)	2
									GDVNGENEQKIYTFLK	618.9817 (3)	4
									NScPPVVETFGDTAK	811.3824 (2)	3
									AKVDcYDSVK	592.7864 (2)	3
									TDRLVILGFPcNQFGK	622.3319 (3)	4
									LVILGFPcNQFGKQEPGQNSEILQGIK	1005.532 (3)	1
									FLVNPQGKPVMR	462.5971 (3)	3
									TNVSTVKNDIIR	453.9265 (3)	4
									GKQEPGQNSEIL[#]	650.3313 (2)	1

									QKGDV**N**GENEQKIY[#]	541.5946 (3)	2
									TFLKNScPPVVETF[#]	819.9152 (2)	2
									LKNScPPVVETF[#]	695.8555 (2)	1
									KNScPPVVETF[#]	639.3131 (2)	1
									KNScPPVVETFGDTAKL[#]	621.6494 (3)	1
									KNScPPVVETFGDTAKLF[#]	670.6727 (3)	2
									VDcYDSVK	493.2219 (2)	7
									LVNPQGKPVMRW[#]	475.6001 (3)	2
									QKIYTFLKNScPPVVE[^]	641.6744 (3)	2
									TFGDTAKLFWSPLKIHDIKWNFE[^]	559.4953 (5)	3
111	Glutathione peroxidase 3	XP_015679695	*Protobothrops mucrosquamatus*	28	3	2	143	9%	ILFVNVATY[#]	1039.5821 (1)	1
									GKQEPGQNSEIL[#]	650.3313 (2)	1
									LVNPQGKPVMRW[#]	475.6001 (3)	2
Acetylcholinesterase (ACE)											

112	ACN-Sut-1, partial	JAA74986	*Suta fasciata*	63	3	3	145	6%	SLPVLDGHVSAF[#]	621.3313 (2)	2
									GESAGAASVGMHLL[#]	650.3216 (2)	1
									TASRPQYVQL[#]	581.8148 (2)	1
113	Acetylcholinesterase	XP_007444166	*Python bivittatus*	67	2	1	106	5%	GVPHANDIATEAVVL[#]	753.407 (2)	7
									GESAGAASVGMHLL[#]	650.3216 (2)	1
114	Acetylcholinesterase	XP_015668657.1	*Protobothrops mucrosquamatus*	65	94	2	2	3%	VATQTGWVR	509.2783 (2)	2
									VYAYLFDHR	395.2006 (3)	2
115	Acetylcholinesterase	4QWW_A	*Bungarus fasciatus*	60	171	2	2	8%	VGAFGFLGLPGSPEAPGNMGLLDQR	834.0948 (3)	2
									LLNATVDPPR	548.3103 (2)	2

Table 2: Peptides representing various snake venom proteins identified through *de novo* analysis. *De novo* sequencing was performed using PEAKS and Novor softwares followed by MS-BLAST. The mutated amino acids obtained are highlighted in bold and underlined. Proteins marked in bold were identified through both conventional proteomics and *de novo* methods.

Sl. No.	**Protein name**	**Accession number**	**Family**	**Snake species**	**Molecular mass (kDa)**	**Summed MS/MS score**	**Identified Peptides**	**Mutated amino acid position**	**Observed m/z (Z)**	**ppm**
1	Alpha-cobratoxin	P01391	3FTxs	*Naja siamensis*	7	168	TWCD**G**FCSIR	A28G	651.2766 (2)	4
							V**R**TGVDLQCCSTDNCNPF	K49R	1071.9618 (2)	2
2	Long neurotoxin B	P13495	3FTxs	*Pseudonaja textilis*	7	57	CFLTPD**LT**SK	V9L K10T	591.2994 (2)	4
3	Alpha-elapitoxin-Ast2b	P01381	3FTxs	*Astrotia stokesii*	7	60	**D**V**L**GCAATCPT**V**K	I37D M39L A48V	696.3388 (2)	3
4	Long neurotoxin 1	P25674	3FTxs	*Naja haje haje*	7	67	LRCFLTPN**L**TS**K**	V9L Q12K	484.2621(3)	1
5	Cytotoxin 3	P24780	3FTxs	*Naja naja*	6	63	NSLVLKY**E**CCNTD**N**C**R**	V52E R58N N60R	682.6360 (2)	5
6	Cardiotoxin 7a	AAB03220	3FTxs	*Naja atra*	9	61	FPL**R**FPVKR	K54R	387.2393(3)	6
7	Cytotoxin 3a	P86539	3FTxs	*Naja naja*	4	148	NLCY**R**MYM**WNK**	K23R V27W A28N T29K	789.8619 (2)	10
							LIPLAY**R**TCPAGK	K12R	730.4047 (2)	6

8	**Long neurotoxin 1**	P25668	3FTxs	*Naja naja*	7	76	**G**TWCDGFCSLR	K23G	679.7821(2)	6
9	Tissue-type plasminogen activator	XP_026570965	SVSP	*Pseudonaja textilis*	62	78	SS**V**TEQLFQVER	A394V	711.8667 (2)	3
10	Tissue-type plasminogen activator-like, partial	XP_025033187	SVSP	*Python bivittatus*	10	73	**G**LCYQDLG**D**TYR	A41GH49D	730.8290 (2)	2
11	Tissue-type plasminogen activator, partial	XP_026544671	SVSP	*Notechis scutatus*	58	139	**GL**CYQD**L**GDTYR	A103G M104L G109L	730.8283 (2)	5
							L**F**PDSFCTSAR	Y449F	650.8045 (2)	2
12	C4b-binding protein alpha chain-like isoform X1	XP_026571379	CVF	*Pseudonaja textilis*	81	214	**V**A**DL**FQATYTYQNK	I328V S330D V331L	554.6120 (2)	3
							CP**L**PNLENGK	V96L	571.2884 (2)	2
							LG**DK**V**TF**GCDYGFR	E115D E116K I118T L119F	545.5937 (3)	2
13	C4b-binding protein alpha chain-like	XP_026532545	CVF	*Notechis scutatus*	81	62	VT**F**GCDYGFR	L117F	611.2727 (2)	2
14	**Chain B, Cobra**	3PRX_B	CVF	*Naja kaouthia*	180	294	LLLN**T**PLNAQSLPLTVR	I402T	932.0644(2)	8

	Venom Factor						VPVVSEAFHS**AD**TTL SDGTAK	M387A G388D	711.360 4(3)	8
							DDNED**NM**LAD**MA**LL SR	G737N F738M S743M D744A	920.399 4(2)	10
15	Venom factor	JAG68918	CVF	*Boiga irregularis*	185	127	ACE**TN**VDYVYK	P1530T S1531N	681.311 8(2)	9
							VNDDYLLWG**SR**	L1598S S1599R	669.332 8(2)	7
16	**Metalloprotei nase atrase A**	ADF43026	SVMP	*Naja atra*	68	244	SFAEWR**E**K	A281E	351.510 9 (3)	2
							TGCLV**T**VSP**H**DPDSR	P576T R580H	547.596 0 (3)	3
							DKAVVQDYSR	S318D V319K	590.801 9 (2)	1
							DHQEYLLR	E381D	537.237 0 (2)	1
17	Metalloprotei nase, partial	ADI47661	SVMP	*Echis coloratus*	42	77	VTLDLFGEW**K**ER	R43K	498.263 2 (3)	3
18	Venom metalloprotein ase TSV-DM	ABC73079	SVMP	*Trimeresur us stejnegeri*	68	63	LAYLG**S**LCNP**K**	A309S M314K	618.327 5 (2)	2
19	Acutusin 2	ADB4312 4	SVMP	*Deinagkistr odon acutus*	46	77	VYEMLN**M**LR**D**SYR	T226M E229D	845.413 5 (2)	7
20	**Cobrin precursor**	AAF00693	SVMP	*Naja kaouthia*	67	170	T**A**AAVVQDYS**R**	S309A K318R	394.204 3 (3)	2

							CPIMTNQCIALRGP**R** **K**		644.3293 (3)	2
21	Zinc metalloproteinase-disintegrin-like cobrin	Q9PVK7	SVMP	*Naja kaouthia*	67	237	NDNAQLLTGLDF**D**G **K**	N291D T293K	810.9036(2)	9
							DP**N**YGMVEPGTK	S572N	662.3037(2)	10
							NS**F**LCNCA**E**SPR	M560F I566E	727.8141(2)	8
22	Snake venom metalloproteinase 1	JAB52758	SVMP	*Micrurus fulvius*	69	240	TS**A**AVVQDY**S**K	V322A G329S	584.8009(2)	8
							NSM**L**C**N**CS**L**SPR	T574L R576N V579L	727.8191(2)	6
							D**S**CFTLNQR	M531S	570.7622(2)	6
							YLEFYV**E**V**V**NR	V212ED214V	715.8756(2)	9
23	**Hemorrhagic metalloproteinase-disintegrin-like kaouthiagin**	P82942	SVMP	*Naja kaouthia*	44	160	**KT**PA**Y**QFSSCS**V**R	T178K A179T F182Y I189V	510.9186(3)	6
							LYCTEKNTMSC**R**	L367R	521.5637 (3)	1
24	Metalloproteinase type III 6b	JAS05275	SVMP	*Sistrurus tergeminus*	67	64	AWVYE**F**VN**Y**LN**TK**	I228F T231Y E234T I235K	549.6121(3)	1

25	Endonuclease domain-containing 1 protein-like	XP_026579205	END	*Pseudonaja textilis*	30	94	NDE**L**VVQ**Q**FTLGQLEEK	N241L R245Q	664.0157 (3)	10
26	**C-type lectin BfL-1**	Q90WI8	CTL	*Bungarus fasciatus*	18	142	Y**N**CPLDWLPK	T25N	653.3204 (2)	3
							NNEFCVELV**K**	N134K	626.3071 (2)	3
27	L-amino acid oxidase	AVX27607	LAAO	*Naja atra*	57	122	FWEADGLH**NK**	G360N G361K	406.1977 (3)	3
							IAE**T**VHLNAR	M273T	375.2134 (3)	3
28	GPX3	AFS63888	Gprx	*Thamnophis elegans*	28	60	SITG**N**YIAFR	E78N	571.7955 (2)	1
29	Phosphodiesterase	JAS05035	PDE	*Micrurus fulvius*	96	143	AATYFWPG**TD**VK	S251T E252D	678.3412(2)	9
							FGPVSGQVLK	Y308F	516.3001(2)	7
30	Kunitz inhibitor II	ABX82869	KSPI	*Walterinnesia aegyptia*	8	64	NCAYL**TA**FYYNP**D**S**R**	P43T S44A H50D H52R	927.9134 (2)	4
31	Acidic phospholipase A2 5	Q5G291	PLA2	*Naja sagittifera*	13	71	**N**SGTPVDDLDR	G38N	594.7790 (2)	1
32	Vespryn-21	XP_026576508	OLP	*Pseudonaja textilis*	24	71	FN**G**SPCVLGSPGFR	S100G	748.3528 (2)	8
33	**Natrin**	1XTA_A	CRISP	*Naja Atra*	24	56	LTNCDSL**V**K	L196V	525.272(2)	7

34	Natrin	AAP85301	CRISP	*Naja atra*	26	185	N**G**DFNSQSTR	V20G	563.7368 (2)	9
							Q**N**SCQDDWLK	S217N	647.2816 (2)	2
							LTNCDSL**V**K	L214V	525.2693(2)	2
35	Ecto-5'-nucleotidase 1a	JAI09049	5-NU	*Micrurus fulvius*	63	150	LSQEDPAVKAE**L**SR	V338L	514.9465(3)	8
							EVV**Q**FMNSLR	H117Q	611.8199(2)	6

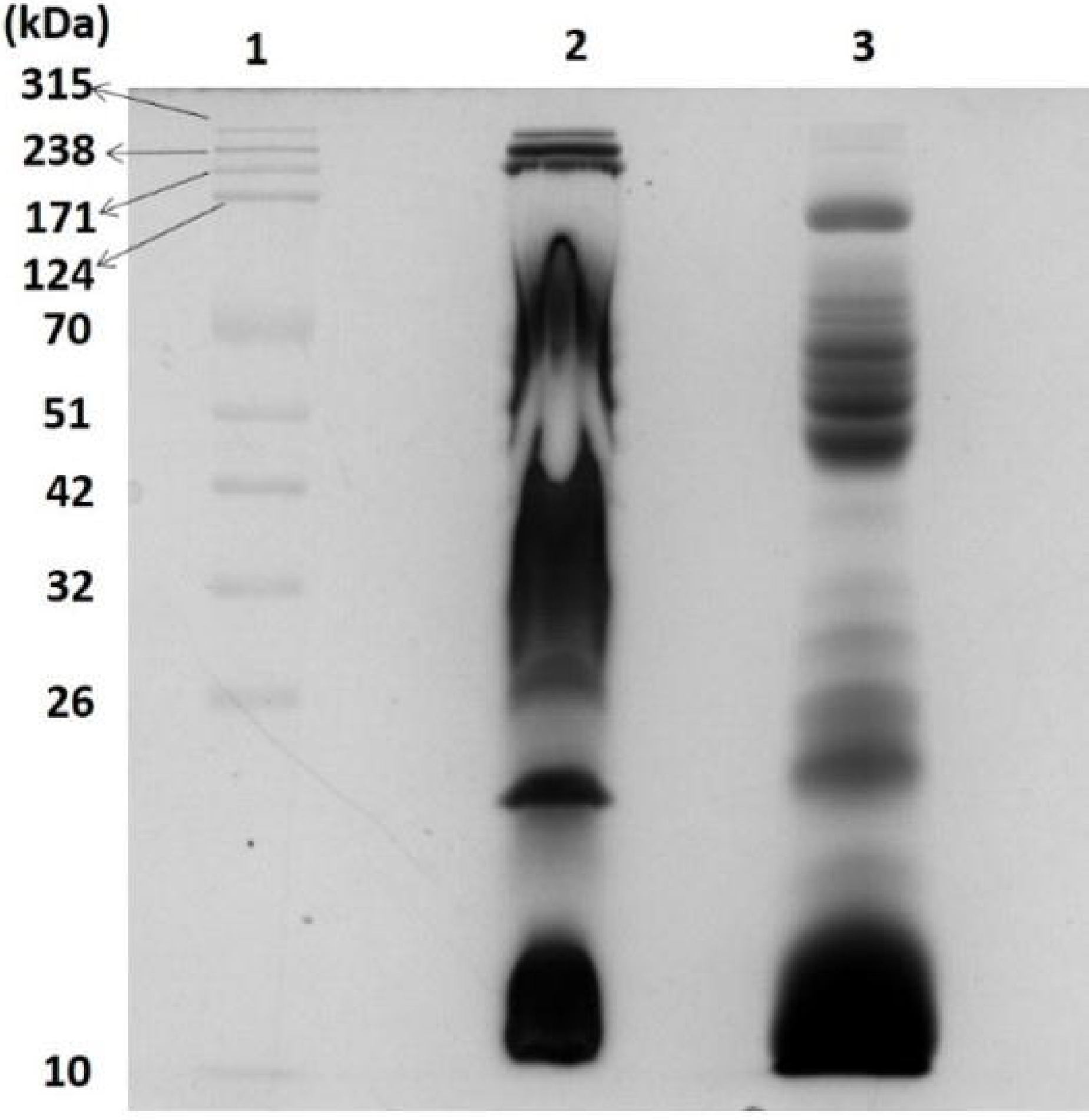
Table 3: Comparison of protein families identified in cobra venom collected from different geographical location of India.

SI. No.	Venom family	*Naja naja*					
		Western India [13]	**Eastern India [14, 15]**	**Northern India [16]**	**Southern India [6]**	**Unknown [17]***	**Western Ghats #**
1	Phospholipase A_2	+	+	+	+	+	+
2	Phospholipase B	+	+	-	-	+	+
3	Snake venom metalloprotease	+	+	+	+	+	+
4	Snake venom serine protease	+	-	-	+	+	+
5	Cysteine-rich secretory proteins	+	+	+	+	+	+
6	Cystatin	+	+	-	-	+	-
7	Kunitz-type serine protease inhibitor	+	+	+	+	+	+
8	3-Finger toxins	+	+	+	+	+	+
9	Nerve growth factor	+	+	+	+	+	+
10	Cholinesterase	+	+	-	-	+	+
11	Phosphodiesterase	+	+	-	+	-	+
12	L-amino acid oxidase	+	+	-	+	+	+
13	Cobra venom factor	+	+	-	+	+	+
14	5'-Nucleotidase	+	+	-	+	+	+
15	Glutathione Peroxidase	-	-	-	-	-	+
16	Natriuretic peptide	-	+	-		-	-
17	Aminopeptidase	+	-	-	+	-	-
18	Vespryn/Ohanin-like proteins	+	+	-	+	+	+
19	C-type lectin	-	-	-	-	+	+
20	Endonucleases	-	-	-	-	-	+
21	Hyaluronidase	-	-	-	-	+	-
22	Vascular endothelial growth factor	-	-	-	-	+	-

	*- cobra venom obtained from Kentucky Reptile Zoo , #-current study

Table 4: Summary of cobra venom proteomic studies from India.

Sl. No.	Geographical location/place from venom was collected or procured	Number of snakes used for the study	Total number of proteins identified	Number of venom families identified	Reference
1	North-Western (Rajasthan, Gujarat)	17	26	6	K. Sintiprungrat et al., 2016 [16]
2	Eastern India (Nadia District, West Bengal)- Calcutta Snake park	4	43	15	S. Dutta et al.,2017 [14]
3	Eastern India-(Burdwan District, West Bengal)- Calcutta snake park, Kolkata	4	52	14	Chanda A et al., 2018 [15]
4	Southern India-Tamil Nadu-Irula snake catcher's society	Pooled venom	81	13	Choudhury M et al., 2017 [6]
5	Western India-Haffkine Institute, Mumbai	4	54	16	Chanda A et al., 2018 [13]
6	Indian cobra venom obtained from Kentucky Reptile Zoo (exact geographical location not mentioned)	Pooled venom	48	17	Suryamohan K et al, 2020 [17]
7	Western Ghats of India- Kerala	2	143	17	Current study*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3
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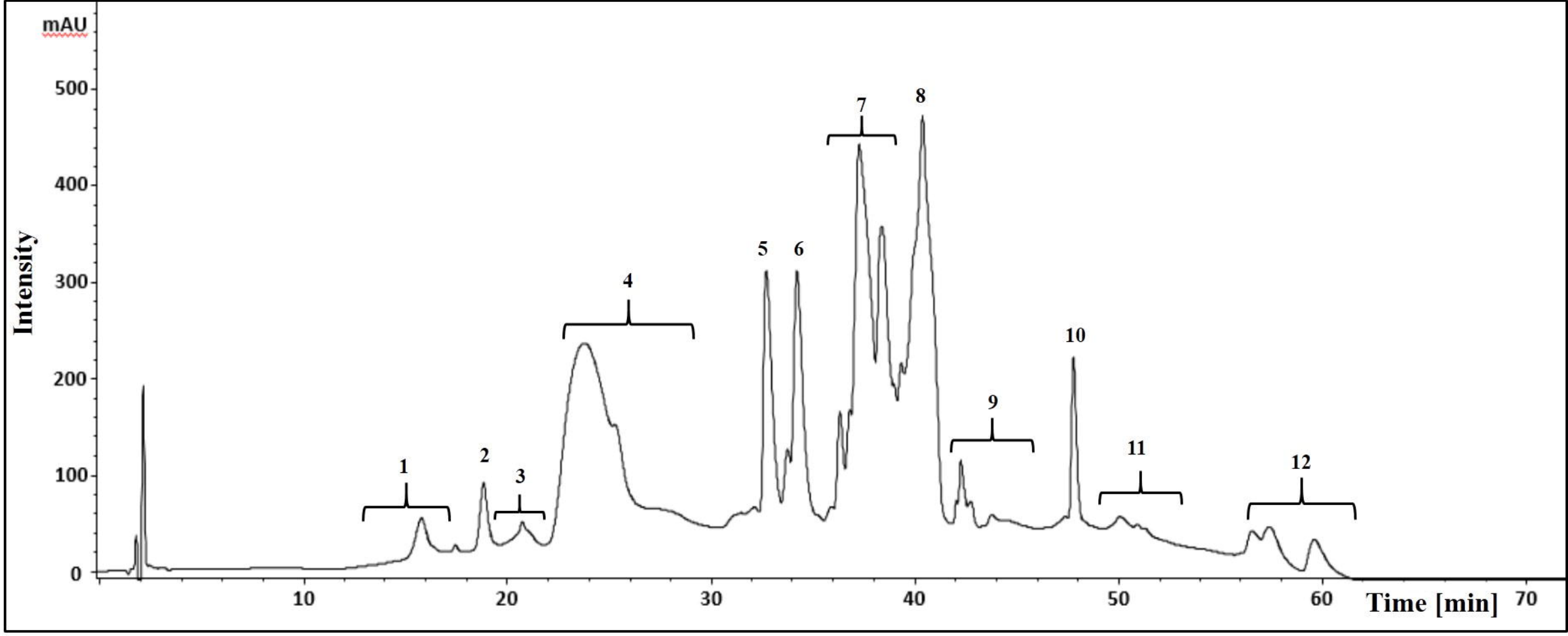
124
70
51
42
32
26
10


mAU
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100
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Intensity
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2
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7
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11
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20
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50
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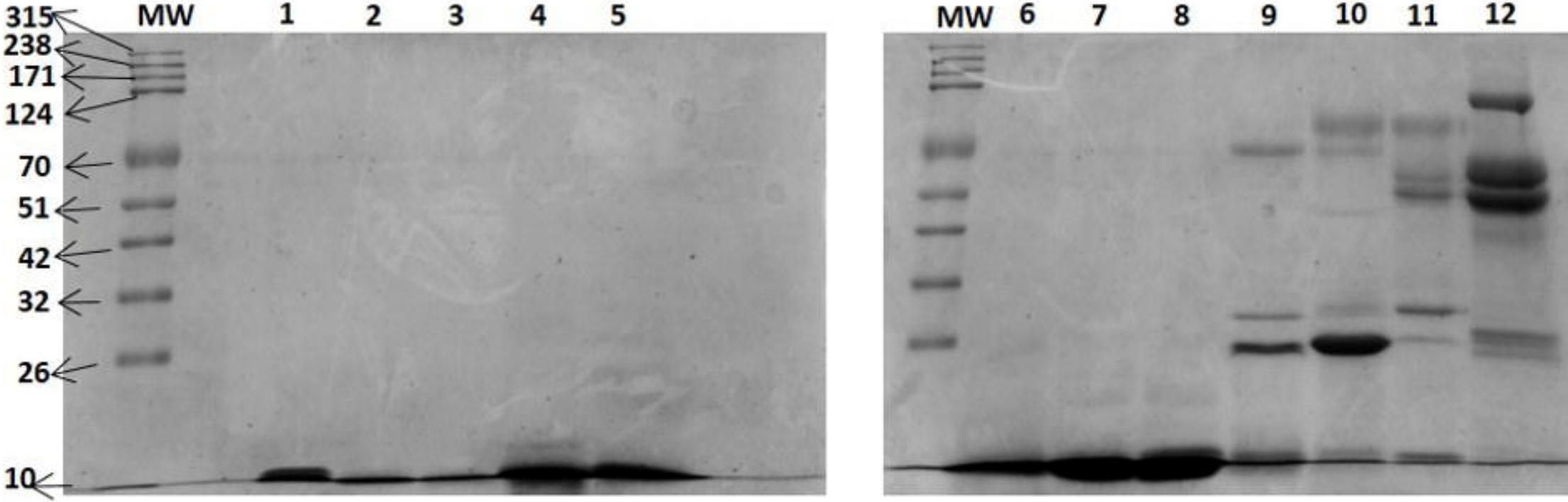
Time [min]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MW
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MW
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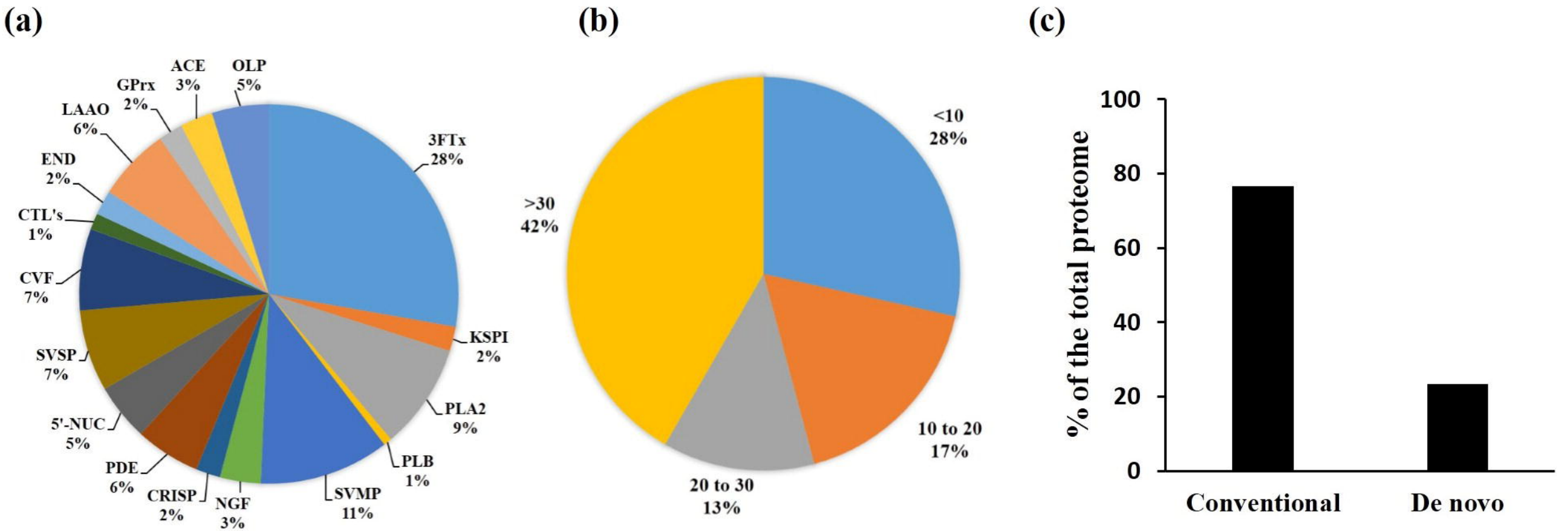
10
11
12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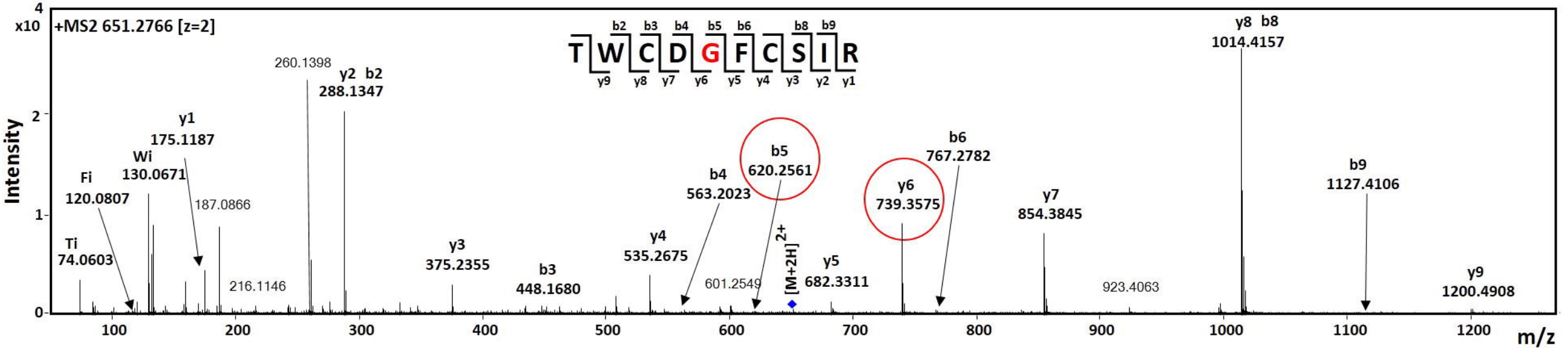
PLA2 9%
PLB 1%
SVMP 11%
NGF 3%
CRISP 2%
PDE 6%
5'-NUC 5%
SVSP 7%
CVF 7%
CTL's 1%
END 2%
LAAO 6%
GPrx 2%
ACE 3%
OLP 5%
(b)
<10 28%
10 to 20 17%
20 to 30 13%
>30 42%
(c)
% of the total proteome
100
80
60
40
20
0
Conventional
De novo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260.1398
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b3 448.1680
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b4 563.2023
601.2549
b5 620.2561
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y7 854.3845
923.4063
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b9 1127.4106
y9 1200.4908

(a)

```
Notechis scutatus      1 MLLYFILLSLSWSQMPTSGEVVDSFQDHCPQFFLRETPPVIGVPLSPVRICQRYQNLYRF
Pseudonaja textilis    1 MLLCFILLSLSWSQTPTSGEVVDSFQDHCPQFFLRETPPVIGVPLSPVRICQRYQNLYRF
Thamnophis elegans     1 MRLLFILLSVSWSQMPTSGEVVDSFQDTCPQFFLRETPPLIRIPPSPVRICQRYQNLYRF
Ophiophagus hannah     1 ------------------------------------------------------------
NNwg                   1 ------------------------------------------------------------

Notechis scutatus     61 ATLYDKHNRIPVYSAYVYNPGKAKRPGKWMVEPQLISSTFQSEMETERDFLNKKGPITAM
Pseudonaja textilis   61 ATLYDKHNRIPVYSAYVYNPGKAKRPGKWMVEPQLISSTFQSEMETERDFLNKKGPKAAM
Thamnophis elegans    61 ATLYDKDNRIPVYSAYVYNPGSAKRPKKWMVEPQLINSSFQPEMETEWDFLNKKGPKEAL
Ophiophagus hannah     1 -------------------------------------------METEWDFLNKKGSPKAL
NNwg                   1 ------------------------------------------------------------

Notechis scutatus    121 QNSQAILEDYKNLTDCNRGHLNPNGHQPDYSAKSSTFTLTNIVPQFIKLNGGAWNNYEQT
Pseudonaja textilis  121 QDSQAVLEDYKNLTDCNRGHLNPNGHQPDYSAKSSTFTLTNIVPQFIKLNGGAWNNYEQT
Thamnophis elegans   121 HVSQAVLEDYKNLSDYNRGHLNPNGHQPDYSAKSSTFTLTNIVPQFIKLNGGAWNNYEQT
Ophiophagus hannah    18 QDSQAVLEDYKNLADCNRGHLNPNGHQPDYSAKSSTFTLTNIVPQFIKLNGGAWNNYEQT
NNwg                   1 ------------------GHLNPNGHQPDYSAKSSTFTLTNIVPQFIKG-----------

Notechis scutatus    181 TMQQMTRGCHQTFAVVGAVPGDTYIARGRVNRPSHLWSAACCVIDNNHLRSWAILARNDQ
Pseudonaja textilis  181 TMQQMTRGCHQTFAVVGAVPGDTYISGGRVNRPSHLWSAACCVIDNNHLRSWAILARNDQ
Thamnophis elegans   181 TMVQMTKGCQETFAVVGAVPGDTYVAGGRVNKPSHMWSAACCMIDNNHLRSWAILARNDQ
Ophiophagus hannah    78 TMQQMTRGCQQTFAVVGAVPGDTYIARGRVNRPSHLWSAACCLIDNNHLRSWAILARNX-
NNwg                  32 --------CQQTFAVVGAVPGDTYIAR----------SAACCLIDNNHLRSWLAQLYN--

Notechis scutatus    241 NVVQRFTLGQLEEKLAQLYNVNHVSLFHSDCPRQ
Pseudonaja textilis  241 NVVQRFTLGQLEEKLAQLYNVNHVSLFHSDCPRQ
Thamnophis elegans   241 NVVEKLTLGQLEAHLAQLYNINQVSLFHSDCPRQ
Ophiophagus hannah   137 ----------LEEKLAQLYNVNHVSLFHSDCPRQ
NNwg                  72 --------------------VNHVSLFHSDCPRQ
```

(b)

```
Pseudonaja textilis    1 ----------------------------------------------MKGRLKGAWILSLL
Notechis scutatus      1 ----------------------------------------------MKGRLKGAWILSLL
P.mucrosquamatus       1 ----------------------------------------------MKGRLKGAWILSLL
Ophiophagus hannah     1 VELEGDNVLTQRQRERNTKEHLEQTQISVEARPGRGAEALSLLLSNHEGQTERSLDPFLA
NNwg                   1 ------------------------------------------------------------

Pseudonaja textilis   15 LAGLFQPSLGQEQQ----------------------------EVDCYDSVKGSAHDYGAF
Notechis scutatus     15 LAGLFQPSLGQEQQ----------------------------EVDCYDSVKGSAHDYGAL
P.mucrosquamatus      15 LAGLFQPNLGQEQQEILTGLQLSHQHDEARSCLHAQPIPDRAKVDCYDSVQGSAHDYGAL
Ophiophagus hannah    61 LGWFISAQPGTGTTGGLQLSHQHEEASSCSYAPNSQPIPDRAKVDCYDSVKGSAHDYGAL
NNwg                   1 ------------------------------------------AKVDCYDSVK---------

Pseudonaja textilis   47 SLSGEYIAFQKYVGKYILFVNVATYUGLTAQYLELNALQNSLRTDRLVILGFPCNQFGKQ
Notechis scutatus     47 SLTGEYIAFQNYVGKYILFVNVATYUGLTQQYLELNALQNSLRTDRLVILGFPCNQFGKQ
P.mucrosquamatus      75 SLSGEYIPFRNYAGKYILFVNVATYUGLTGQYLELNALQTSLGIDRLVILGFPCNQFGKQ
Ophiophagus hannah   121 SLT------------------------------ELNALQNSLKTDRLVILGFPCNQFGKQ
NNwg                  11 ---------------------------------------------TDRLVILGFPCNQFGKQ

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Notechis scutatus    107 EPGQNSEILQGIKYVRPGGGYVPNFQLFQKGDVNGENEQQIYTFLKNSCPPVVETFGDTT
P.mucrosquamatus     135 EPGQNSEILQGIKHVRPGGGFVPNFQLFQKGDVNGENEQRIYTFLKNSCPPVVENFGDPA
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Notechis scutatus    167 RLFWSPLKIHDIKWNFEKFLVNPQGKPVMRWFHRTNVSTVKNDIIRYMRKNRNG
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Ophiophagus hannah   211 KLFWSPLKIHDIKWNFEKFLVNPQGKPVMRWFHRTNVSTVKNDIIRFMRKKRSG
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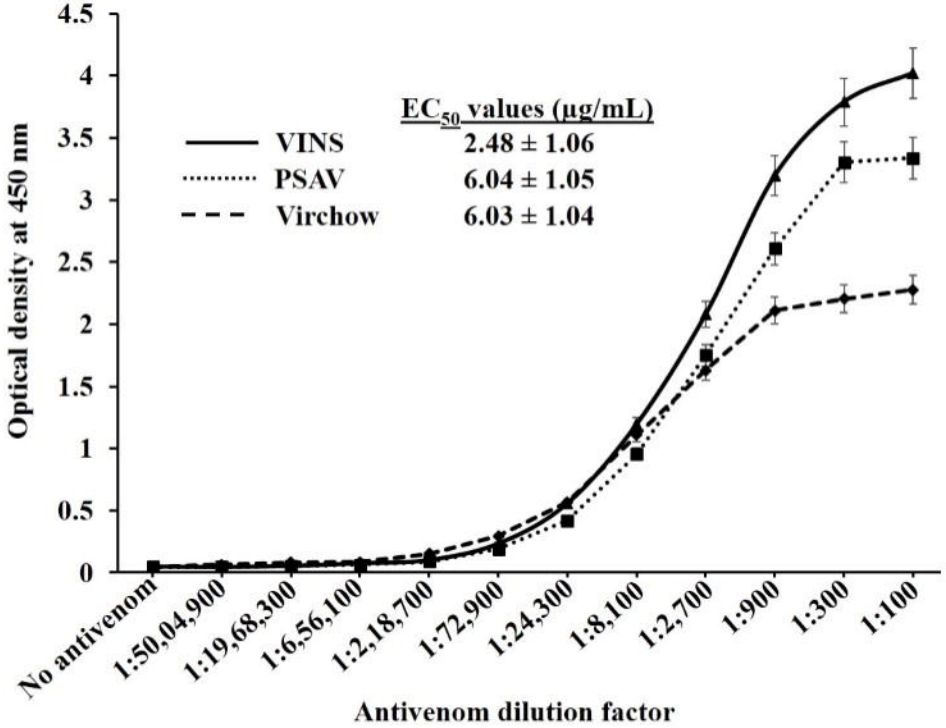
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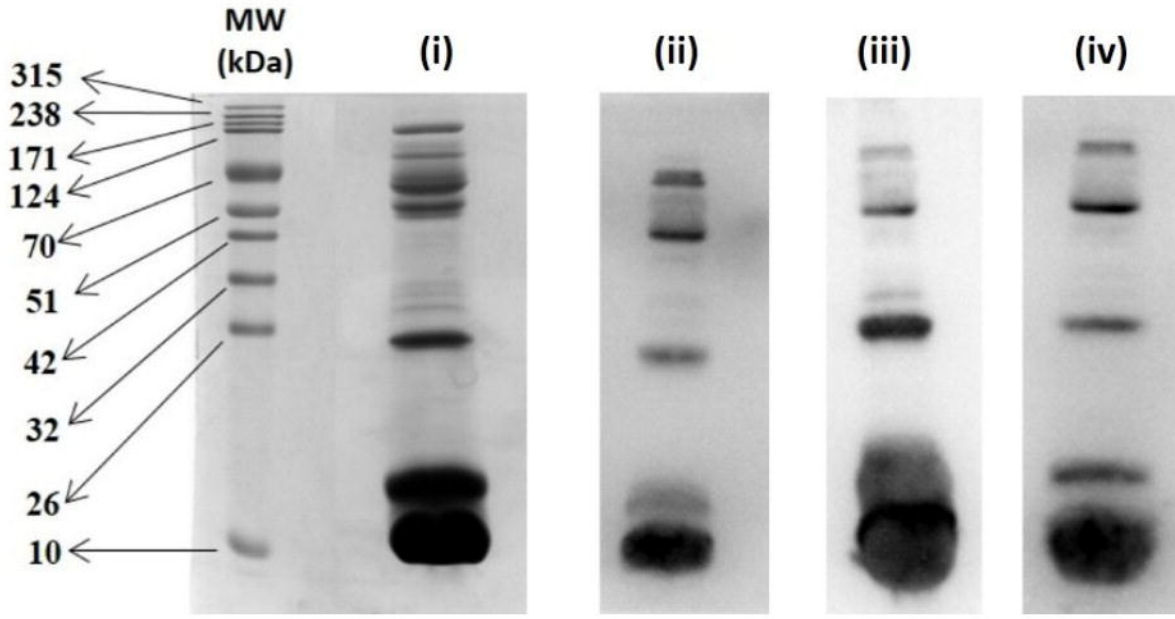
Antivenom dilution factor

(b)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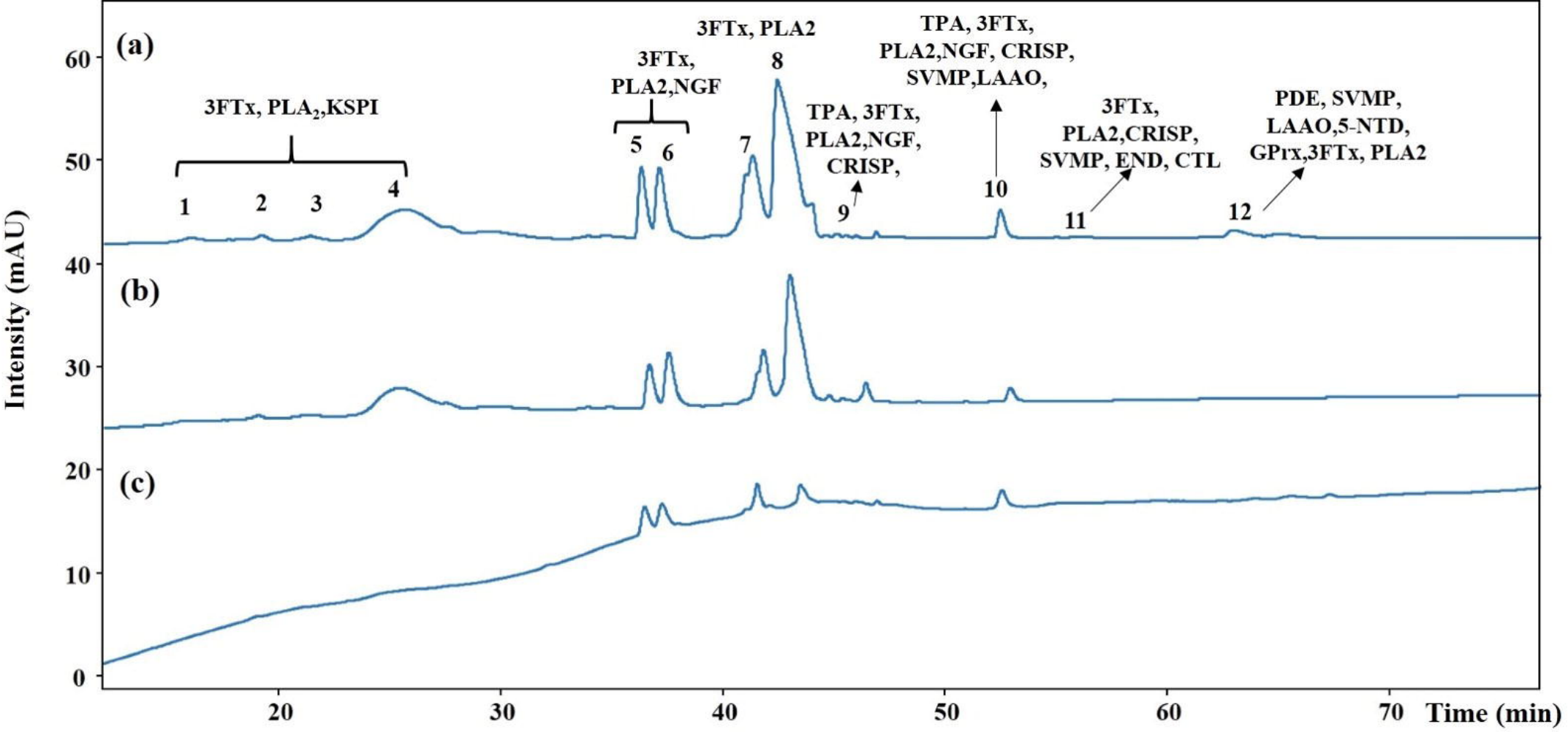
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