Chapter 3

Proteomics and partial characterization of crude *Daboia russelii* venom

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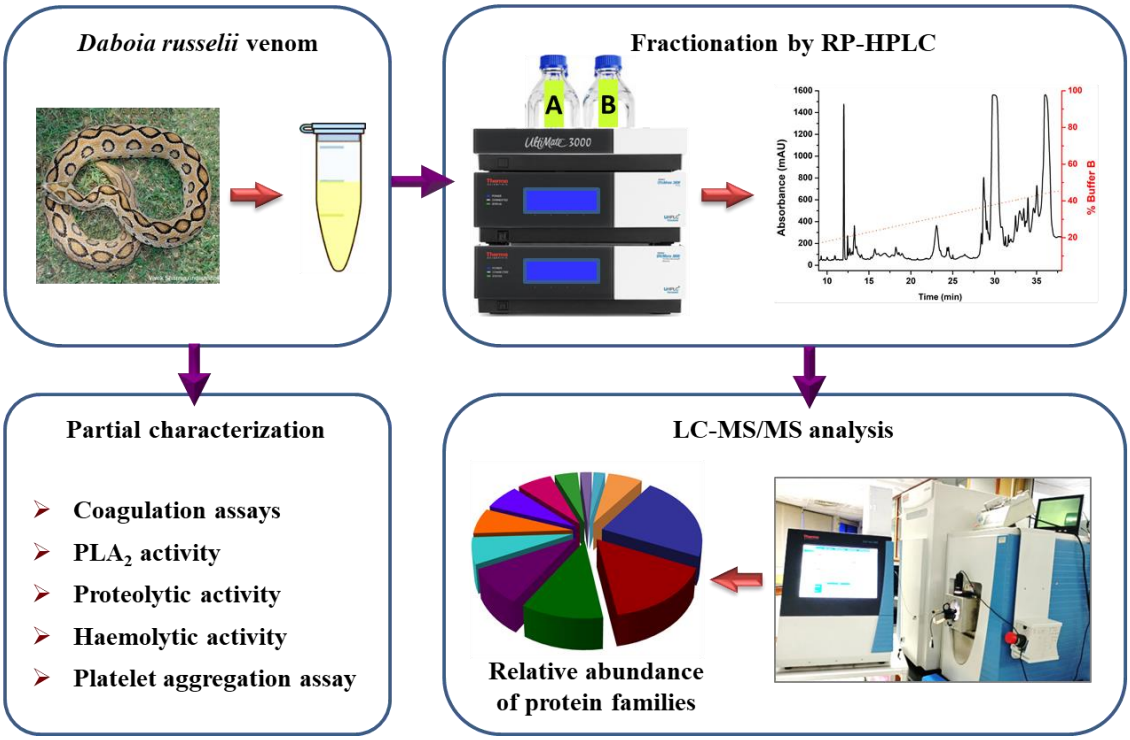
GRAPHICAL ABSTRACT



3.1 INTRODUCTION:

Snake venom is a multifarious assortment of proteins and peptides with therapeutic as well as toxicological potential. These are responsible for manifestation of diverse pathological effects in the envenomated victims leading to incidences of morbidity and mortality every year around the world. The only available antidote for snakebite is the polyvalent antivenom which is often accompanied by various limitations such as inefficacy and adverse reactions. These are mainly due to differential expression of immunogenic components and the variable generation of neutralizing antibodies owing

to variation in venom composition [89]. As such, an inclusive understanding of venom profiles from venomous snakes belonging to different geographical locations becomes crucial for designing effective antidotes [90].

Elucidation of venom composition using proteomics tools have helped unveil the complexities of the venom proteomes aiding in identification of major as well as trace toxin components present and assisted in understanding the signs and symptoms of envenomation triggered by these components. Besides providing strategies for developing specific and effective antivenom, this understanding has provided in-depth knowledge about venom variation and similarities [2,127]. *Daboia russelii* being a category 1 medically important snake across most of the Indian sub-continent, numerous studies have been undertaken to understand its venom composition, variation and correlate the pathophysiological symptoms observed post envenomation [84,85]. For instance, recent proteomic studies have revealed significant inter-specific venom variation among *Daboia russelii*, *Daboia siamensis* and *Daboia palestinae* alongwith intra-specific geographic variation in the venom profile of *Daboia russelii* from India (south, east and west), Sri Lanka and Pakistan [2,87,128–132].

In this chapter, we profiled the proteome of *Daboia russelii* venom from Tanore, Rajshahi, Bangladesh in order to gain in-depth understanding of the venom composition from this region. Additionally, we partially characterized this venom to understand its effect of on the haemostatic system.

3.2 RESULTS:

3.2.1 Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE):

Electrophoretic separation of crude *Daboia russelii* venom exhibited the presence of different protein bands of varying intensity in the range of ~150 – 15 kDa (Figure 2.1). Under reducing conditions, a total of 14 bands were observed with 7 prominent bands at ~100, 72, 60, 28, 25, 20 and 15 kDa; and 7 relatively faint bands at ~120, 95, 50, 40, 30, 26 and 18kDa. Moreover under non-reducing conditions, in addition to the bands at 120, 100, 95, 75, 60, 50, 40, 30, 28, 26, 25, 20, 18 and 15 kDa, a prominent band at 27 kDa and a faint band at 150 kDa were observed. under non-reducing conditions, apart from the bands observed in the reducing gel, a prominent band at 27 kDa and a faint band at 150 kDa were observed.

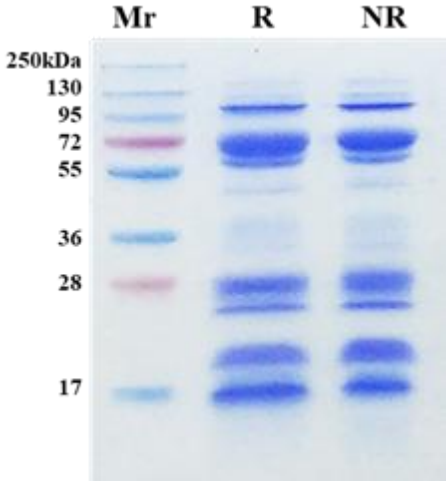


Figure 3.1: Electrophoretic profiling of crude *Daboia russelii* venom: Coomassie brilliant blue stained 12.5% SDS-PAGE (Mr: Page ruler plus protein marker, R: reduced, NR: non-reduced).

3.2.2 Proteomic analysis of crude *Daboia russelii* venom:

The RP-HPLC profile revealed that crude *Daboia russelii* venom separated into 14 fractions (Figure 3.2). The fractions were named as P1 to P14 corresponding to their fraction number. Each of the fractions were individually digested with trypsin and subjected to LC-MS/MS analysis. The peptide fragments obtained were analysed using homology search based on sequence similarity and m/z ratio with the existing *Daboia* NCBI database. Peak-wise identification of proteins is shown in Figure 3.2. The RP-HPLC fractions P1, P2 and P3 revealed the presence of peptide fragments having sequence similarity to proteins each belonging to snake venom protein families VEGF, disintegrin and KSPI respectively. P4 showed homology to 2 proteins, 1 isoform each from KSPI and PLA_2. P5 consisted of 7 proteins with 3 isoforms of PLA_2 enzymes and 1 isoform each belonging to LAAO, VEGF, VNGF and disintegrin. P6 displayed the presence of 11 proteins having similarity with 3 isoforms of PLA_2 enzymes, 2 isoforms of SVSP and 1 soform each of VEGF, VNGF, CRISP, LAAO, SVMP and snaclec. P7 revealed 9 proteins with 3 isoforms each of PLA_2 and SVSP; and 1 each of CRISP, LAAO and snaclec. P8 consisted of 4 proteins having homology with 2 isoforms of SVSP and 1 each of PLA_2, LAAO and snaclec. P9 comprised of 10 proteins consisting of 4 isoforms of snaclec, 3 isoforms of SVSP, 2 isoform of PLA_2 and 1 isoform of LAAO. P10 consisted of 10 proteins, 4 isoforms of snaclecs, 2 isoforms each of PLA_2 and SVSP; and 1 isoform each of CRISP and LAAO. P11 consisted of 12 proteins, 6

isoforms of snaclec, 2 each of PLA_2 and SVSP; and 1 each of SVMP and LAAO. P12 displayed the presence of 11 proteins with 4 snaclecs, 3 SVSPs, 2 PLA_2 enzymes and 1 each of CRISP and LAAO. P13 revealed 7 proteins with 3 isoforms each of snaclec, 2 of PLA_2 and 1 eah of SVSP and LAAO. P14 consisted a total of 13 proteins comprising of 5 scanclec isoforms, 3 SVSPs, 2 PLA_2 enzymes and 1 each of CRISP, SVMP and glutaminyl-peptide cyclotransferase. Overall, the *Daboia russelii* venom from Rajshahi, Bangladesh comprised of a blend of at least of 37 different protein isoforms belonging to 11 different snake venom protein families. A brief summary of the different proteins identified is given in Table 3.1. A detailed overview of the peptide fragments identified in each RP-HPLC fractions assigned to proteins from different snake venom protein families is shown in Table 3.2.

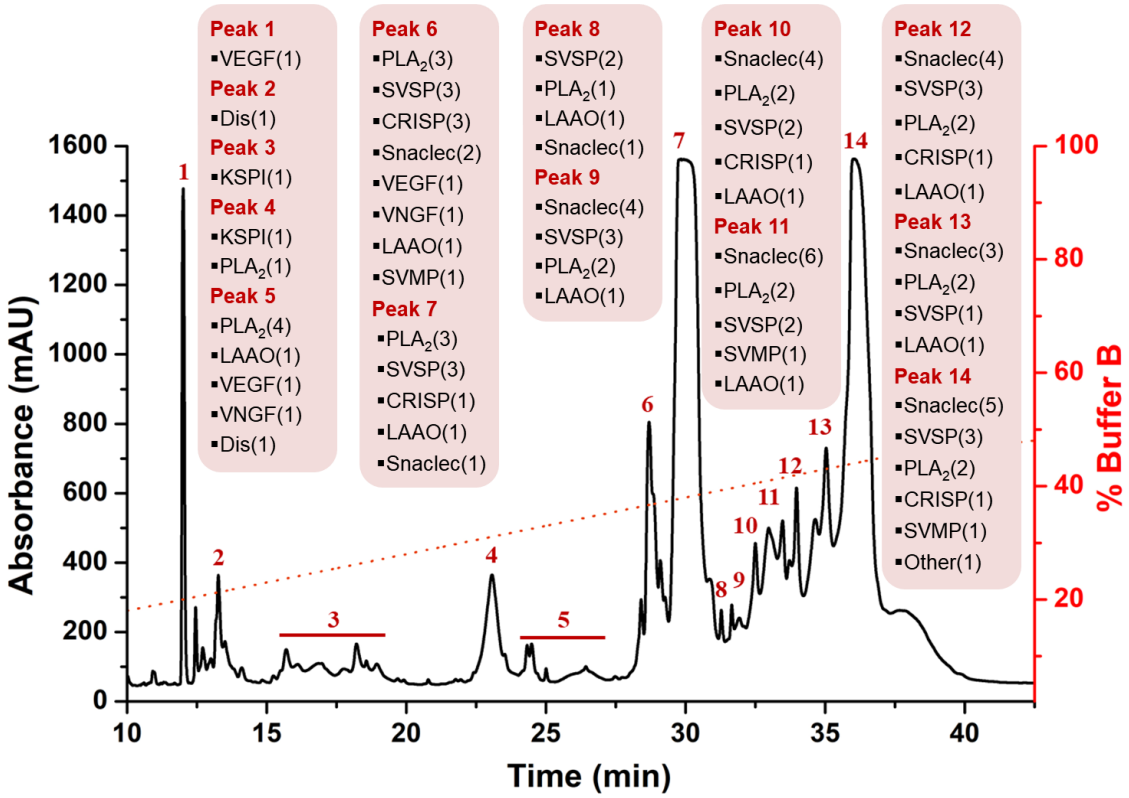


Figure 3.2: RP HPLC profile of *Daboia russelii* venom and distribution of snake venom protein families present in each RP-HPLC fraction: (The digits in parenthesis represent the number of proteins belonging to the indicated protein family, VEGF: vascular endothelial growth factor, Dis: disintegrin, KSPI: kunitz-type serine protease inhibitor, PLA_2: phospholipase A_2, VNGF: vascular nerve growth factor, SVSP: snake venom serine protease, CRISP: cysteine rich secretory peptide, LAAO: L-amino acid oxidase, SVMP: snake venom metalloprotease, Snaclec: snake venom C-type lectin)

Table 3.1: An overview of different proteins identified from *Daboia russelii* venom by LC-MS/MS analysis (VEGF: vascular endothelial growth factor, Dis: disintegrin, KSPI: kunitz-type serine protease inhibitor, PLA_2: phospholipase A_2, VNGF: vascular nerve growth factor, SVSP: snake venom serine protease, CRISP: cysteine rich secretory peptide, LAAO: L-amino acid oxidase, SVMP: snake venom metalloprotease, Snaclec: snake venom C-type lectin)

S.N.	Protein	Accession No.	Family	Homology with
1	A Chain A, Phospholipase A2 VRV-PL-VIIIa	1Q6V	PLA_2	*D. russelii*
2	Acidic phospholipase A2 daboiatoxin A	2H4C	PLA_2	*D. russelii*
3	Acidic phospholipase A2 daboiatoxin B	Q7T3T5.1	PLA_2	*D. siamensis*
4	Basic phospholipase A2 3	P86368.1	PLA_2	*D. russelii*
5	Basic phospholipase A2 Drk-b1	A8CG89.1	PLA_2	*D. russelii*
6	Basic phospholipase A2 Drk-b2	AAZ53183.1	PLA_2	*D. russelii*
7	Basic phospholipase A2 DsM-S1	A8CG84.1	PLA_2	*D. siamensis*
8	Basic phospholipase A2 RVV-VD	P81458.1	PLA_2	*D. russelii*
9	Daboxin P	C0HK16	PLA_2	*D. russelii*
10	Phospholipase A2-II	ABD24037.1	PLA_2	*D. russelii*
11	Dabocetin alpha subunit	ABA86561.1	Snaclec	*D. siamensis*
12	Dabocetin beta subunit	AAY63876.1	Snaclec	*D. siamensis*
13	P31 alpha subunit	ADK22829.1	Snaclec	*D. limitis*
14	P31 beta subunit	ADK22832.1	Snaclec	*D. siamensis*
15	P68 alpha subunit	ADK22825.1	Snaclec	*D. siamensis*
16	Snaclec 6	Q4PRC7.1	Snaclec	*D. siamensis*
17	Snaclec coagulation factor X-activating enzyme light chain 1	Q4PRD1	Snaclec	*D. siamensis*
18	Snaclec coagulation factor X-activating enzyme light chain 2	ADJ67473.1	Snaclec	*D. siamensis*
19	Factor V activator RVV-V alpha	P18964.1	SVSP	*D. russelii*
20	A Chain A, Vipera russelli proteinase RVV-V gamma	3S9C	SVSP	*D. russelii*
21	RVV-V gamma-like protein precursor	ADP88558.1	SVSP	*D. siamensis*
22	Serine alpha-fibrinogenase-like protein precursor	ADP88559.1	SVSP	*D. siamensis*
23	Serine beta-fibrinogenase-like protein precursor	ADP88560.1	SVSP	*D. siamensis*
24	Cysteine-rich seceretory protein Dr-CRPK	ACE73567.1	CRISP	*D. russelii*
25	Cysteine-rich seceretory protein Dr-CRPB	ACE73568.1	CRISP	*D. russelii*

S.N.	Protein	Accession No.	Family	Homology with
26	Cysteine-rich seceretory protein	ASU45033.1	CRISP	*D. russelii*
27	RVV-X heavy chain	AUF41652.1	SVMP	*D. siamensis*
28	Zinc metalloproteinase-disintegrin-like daborhagin-K	B8K1W0.1	SVMP	*D. russelii*
29	DSAIP	AUF41660.1	SVMP	*D. siamensis*
30	Kunitz-type protease inhibitor	AFD04724.1	KSPI	*D. russelii*
31	Kunitz-type serine protease inhibitor B5	A8Y7P5.1	KSPI	*D. siamensis*
32	Vascular endothelial growth factor A isoform 1	ASU45062.1	VEGF	*D. russelii*
33	VR-1 precursor	ACN22046.1	VEGF	*D. russelii*
34	Disintegrin	AUF41656.1	Dis	*D. siamensis*
35	Secreted L-amino acid oxidase precursor	ACF70483.1	LAAO	*D. russelii*
36	Beta-nerve growth factor	ASU45040.1	VNGF	*D. russelii*
37	Glutaminyl-peptide cyclotransferases	AFE84762.1	GPC	*D. russelii*

Relative abundance: Among the identified protein families, Phospholipase A_2 was found to be the most abundant family with 26%. This was closely followed by snake C-type lectin (snaclec) with 17% and snake venom serine protease (SVSP) with 15%. Snake venom metalloprotease (SVMP) and cysteine-rich secretory peptide (CRISP) each contributed 9% to the proteome. Disintegrins, kuntiz-type serine protease (KSPI) and vascular endothelial growth factor (VEGF) contributed 6% each while L-amino acid oxidase (LAAO), vascular nerve growth factor (VNGF) and glutaminyl-peptide cyclotransferase (GPC) were present in low abundance with only 3% each. Relative distribution of various protein families identified is shown in Figure 3.3.

Multiple sequence alignment and phylogenetic analysis: Multiple sequence alignment of the PLA_2s identified in the proteome of *Daboia russelii* from Bangladesh revealed the presence of both the s-type and p-type PLA_2s (Figure 3.4). Furthermore, the phylogenetic analysis exhibited that these two types separated into different branches of the phylogram suggesting them to be evolutionarily distinct clades (Figure 3.5).

Table 3.2 A summary of the peptide fragments obtained in each RP-HPLC fraction (MH+ stands for mass/charge (m/z) of the protonated molecular ions (peptide), z stands for the number of charges a peptide carries after ionization, PLA_2: phospholipase A_2, Snaclec: snake venom C-type lectin, SVSP: snake venom serine protease, CRISP: cysteine rich secretory peptide, SVMP: snake venom metalloprotease, KSPI: kunitz-type serine protease inhibitor, VEGF: vascular endothelial growth factor, Dis: disintegrin, LAAO: L-amino acid oxidase, VNGF: vascular nerve growth factor, GPC: glutaminyl-peptide cyclotransferase)

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
1	SKQLELNER	1116.596	2	558.8014	Vascular endothelial growth factor A isoform 1 (ASU45062.1)	VEGF	*Daboia russelii*	8.22	5	2
	QLELNER	901.469	2	451.2382						
2	TSVSSHYCTGR	1254.553	3	418.8537	Disintegrin (AUF41656.1)	Dis	*Daboia siamensis*	14.87	22	3
	QCKLKPAGTTCWR	1605.799	3	535.9344						
	LKPAGTTCWR	1189.615	2	595.3076						
	TTSGYAGGLSSSYGGLTSPGFSYGMSSFQPGFGSVGGSSTYSR	4209.862	5	842.7706						
	LALDIEIATYR	1277.71	2	639.3603						
	SFYYDSESKK	1253.568	2	627.2879						
	SFYYDSESKKCK	1541.694	3	514.5689						
	CRQTCGAPR	1105.499	2	553.2546						
4	SFYYDSESKK	1253.568	3	418.5312	Kunitz-type protease inhibitor (AFD04724.1)	KSPI	*Daboia russelii*	29.87	19	3
	SFYYDSESK	1125.474	2	563.2401						
	QTCRAPR	888.4469	2	444.7279						
	HTCGASGK	817.3621	2	409.1879						
4	AAAICFR	808.4134	2	404.7129	Basic phospholipase A2 DsM-S1 (A8CG84.1)	PLA_2	*Daboia siamensis*	21.97	18	2
	VNGAIVCEK	989.5084	2	495.2582						
	GTPKDATDR	960.4745	2	480.7451						
	TQCMPR	808.344	2	404.6753						
	SKAEAESLYQSK	1340.669	2	670.839						
	SLDLDSIIAEVK	1302.715	2	651.8642						
	SISISVAR	832.4887	2	416.7471						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	SLVNLGGSK	874.4993	2	437.7553						
	TLLEGEESR	1033.516	2	517.2634						
	AEAESLYQSK	1125.542	2	563.2741						
	AQYEDIAQK	1065.521	2	533.265						
	FLEQQNQVLQTK	1475.785	2	738.399						
	GSGGGSSGGSIGGR	1092.503	2	546.7555						
	NKYEDEINKR	1308.654	3	436.8888						
	YEELQITAGR	1179.6	2	590.3048						
5	AAAICFR	808.4134	2	404.7098	Basic phospholipase A2 3 (P86368.1)	PLA_2	*Daboia russelii*	27.45	48	3
	VNGAIVCEQGTSCENR	1793.791	2	897.396						
	ICECDKAAAICFR	1613.723	3	538.5824						
	IYMLYPDFLCK	1478.706	2	739.858						
	LAVPFYSSYGCYCGWGGK	2071.904	2	1036.459						
5	SAGQLYQESLGK	1280.648	2	640.8281	Secreted L-amino acid oxidase precursor (ACF70483.1)	LAAO	*Daboia russelii*	7.74	9	4
	VTVLEASERPGGR	1370.739	3	457.583						
	FWEDDGIQGGK	1251.564	2	626.2838						
	IFLTCTK	882.4754	2	441.7424						
5	VAAICFR	836.4447	2	418.7257	Acidic phospholipase A2 daboiatoxin B chain (Q7T3T5.1)	PLA_2	*Daboia siamensis*	4.37	12	1
	LVEYSYSYR	1179.568	2	590.2876						
5	AAAICFR	808.4134	2	404.7098	A Chain A, Phospholipase A2 VRV-PL-VIIIa (2O1N)/ Daboxin P (C0HK16.1)	PLA_2	*Daboia russelii*	23.07	40	5
	YMLYPDFLCK	1349.627	2	675.3173						
	VNGAIVCEK	989.5084	2	495.2543						
	KYMLYPDFLCK	1477.722	3	493.2464						
	ICECDKAAAICFR	1613.723	3	538.5824						
	MILEETGK	936.4707	2	468.7357						
5	QGEPEGPKEPR	1223.601	3	408.5368	VR-1 precursor (ACN22046.1)	VEGF	*Daboia russelii*	74.89	24	5
	WKQGEPEGPKEPR	1537.776	3	513.2613						
	WKQGEPEGPK	1155.579	2	578.2911						
	HTADIQIMR	1084.557	2	542.7814						
	FMEHTACECRPR	1593.672	3	531.8934						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
5	TATYSYSFENGGIVCGDR	1996.871	2	998.9373	Basic phospholipase A2 / Basic phospholipase A2 Drk-b2 (AAZ53183.1)	PLA_2	*Daboia russelii*	38.59	50	7
	TATYSYSFENGGIVCGDRDPCKR	2653.177	4	664.0504						
	VAATCFR	824.4083	2	412.7064						
	NPLSSYSNYGCYCGWGGK	2069.848	2	1035.427						
	DNLNTYDKK	1110.543	2	555.7726						
	CCFVHDCCYEK	1577.564	3	526.5244						
	DNLNTYDK	982.4476	2	491.7247						
	VTMQNLNDR	1090.531	2	545.7679						
	VLDELTLAR	1029.594	2	515.2999						
	LASYLDK	809.4404	2	405.2247						
	LAADDFR	807.3995	2	404.2047						
	LASYLDKVR	1064.61	2	532.8096						
	ALEEANADLEVK	1301.658	2	651.3348						
	SGGGGGGGLGSGGSIR	1232.598	2	616.799						
	QVLDNLTMEK	1190.609	2	595.8075						
	QGVDADINGLR	1157.591	2	579.2994						
	QFSSSYLSR	1074.521	2	537.7659						
	QEYEQLIAK	1121.584	2	561.2961						
	VQALEEANNDLENK	1586.766	2	793.8907						
	STMQELNSR	1065.499	2	533.251						
	TLLDLDNTR	1060.563	2	530.785						
	LASYLDK	809.4404	2	405.2247						
	HGVQELEIELQSQLSK	1837.965	3	613.3254						
	GGSGGSYGGGGSGGGYGGGSGSR	1791.728	2	896.3617						
	FSSSSGYGGGSSR	1235.529	2	618.2643						
	DIENQYETQITQIEHEVSSSGQEVQSSAK	3264.514	3	1088.846						
	EIETYHNLLEGGQEDFESSGAGK	2510.132	3	837.3815						
	GGSGGSHGGGSGFGGESGGSYGGGEEASGSGG GYGGGSGK	3223.282	3	1075.094						
	MTLDDFR	913.4084	2	457.2086						
	IKFEMEQNLR	1307.678	3	436.5647						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	IQDWYDK	967.452	2	484.2326						
	IQDWYDKK	1095.547	2	548.2772						
	YPGSPGSYAVR	1153.564	2	577.2861						
5	QYFFETK	962.4618	2	481.734	Beta-nerve growth factor (ASU45040.1)	VNGF	*Daboia russelii*	29.29	24	6
	NPNPVPSGCR	1097.516	2	549.2573						
	HWNSYCTTTDTFVR	1787.781	3	596.5999						
	CKNPNPVPSGCR	1385.641	3	462.5494						
	DEQSVEFLDNADSLNR	1851.836	2	926.4205						
	INTACVCVISR	1292.645	2	646.827						
	QLDSIVGER	1016.537	2	508.7722						
	WTLLQEQGTK	1203.637	2	602.3238						
	FASFIDKVR	1082.599	2	541.8018						
	NLDLDSIIAEVK	1329.726	2	665.3673						
	AIGGGLSSVGGGSSTIK	1447.775	2	724.3923						
	AQYEEIAQR	1107.543	2	554.2732						
	SLNNQFASFIDKVR	1638.86	3	546.9601						
	SLNNQFASFIDK	1383.69	2	692.3494						
	SLDLDSIIAEVK	1302.715	2	651.8621						
	SGGGFSSGSAGIINYQR	1657.793	2	829.4022						
	SISISVAR	832.4887	2	416.7483						
	QISNLQQSISDAEQR	1716.851	3	572.9549						
	SLVNLGGSK	874.4993	2	437.7518						
	YEELQITAGR	1179.6	2	590.3057						
	WELLQQVDTSTR	1475.749	2	738.3781						
	TNAENEFVTIK	1265.637	2	633.3202						
	TNAENEFVTIKK	1393.732	3	465.2512						
	TLLEGEESR	1033.516	2	517.2609						
	GGGGGGYGSGGSSYGSGGGSYGSGGGGGGGR	2383.952	3	795.3183						
	FLEQQNQVLQTK	1475.785	2	738.3955						
	DYQELMNTK	1141.519	2	571.2642						
	DVDGAYMTK	999.4452	2	500.2261						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	LLRDYQELMNTK	1523.789	3	508.6029						
	AQYEDIAQK	1065.521	2	533.2623						
	IEISEINR	973.5313	2	487.2692						
	NMQDMVEDYR	1316.525	2	658.7703						
	THNLEPYFESFINNLR	1993.977	3	665.332						
5	QCISLFGSR	1067.53	2	534.27	DSAIP (AUF41660.1)	SVMP	*Daboia siamensis*	20.74	9	3
	KIPCAPQDVK	1155.619	2	578.3098						
	GMVDLGTK	820.4233	2	410.7166						
	LYCFDNLPEHK	1435.667	3	479.226						
	IPCAPQDVK	1027.524	2	514.2642						
	ILFSEDYSETHYSPDGR	2015.898	3	672.6398						
	SFSTASAITPSVSR	1410.722	2	705.8673						
	QLDSIVGER	1016.537	2	508.7722						
	SLYNLGGSK	938.4942	2	469.7516						
	YEELQQTAGR	1194.575	2	597.7886						
	WTLLQEQGTK	1203.637	2	602.3238						
	LAELEEALQK	1143.626	2	572.3153						
	FASFIDKVR	1082.599	2	541.8018						
	NLDLDSIIAEVK	1329.726	2	665.3673						
	ISISTSGGSFR	1111.574	2	556.2929						
	AQYEEIANR	1093.527	2	547.2659						
	TEAESWYQTK	1242.564	2	621.7854						
	LVPLFYK	879.5339	2	440.2704						
6	QGEPEGPKEPR	1223.601	3	408.5367	VR-1 precursor (ACN22046.1)	VEGF	*Daboia russelii*	22.09	34	4
	WKQGEPEGPKEPR	1537.776	3	513.2612						
	ETLVSILQEHPDEISDIFRPSCVAVLR	3123.614	4	781.663						
	HTADIQIMR	1084.557	2	542.7791						
6	NIRNEDEQIR	1286.645	3	429.5509	Factor V activator RVV-V alpha (P18964.1)	SVSP	*Daboia russelii*	609.42	92	4
	NIRNEDEQIRVPR	1638.867	3	546.957						
	RPVTYSTHIAPVSLPSR	1881.034	3	627.6797						
	VVGGDECNINEHPFLVALYTSTSSTIHCGGALINR	3801.832	5	761.1729						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	WCEPLYPWVPADSR	1775.821	2	888.4164						
	YFCLNTK	945.4499	2	473.2259						
	YFCLNTKFPNGLDK	1716.841	2	858.9219						
	YFCLNTKFPNGLDKDIMLIR	2458.262	4	615.3239						
	TLCAGILKGGR	1145.646	2	573.3237						
	VFDYNNWIQNIIAGNR	1936.966	2	968.9887						
	EWVLTAAHCDR	1357.632	3	453.2133						
	EWVLTAAHCDRR	1513.733	3	505.2469						
	FPNGLDKDIMLIR	1531.83	2	766.4183						
	GGRDTCHGDSGGPLICNGQIQGIVAGGSEPCGQHLKPAVYTK	4349.061	5	870.6105						
	GKYFCLNTK	1130.566	2	565.7838						
	DTCHGDSGGPLICNGQIQGIVAGGSEPCGQHLKPAVYTK	4078.917	3	1360.31						
	HKWCEPLYPWVPADSR	2040.975	2	1020.988						
	LRRPVTYSTHIAPVSLPSR	2150.219	3	717.4077						
	ISTTEDTYPDVPHCTNIFIVK	2450.191	2	1225.6						
	ISTTEDTYPDVPHCTNIFIVKHK	2715.345	4	679.5878						
	NEDEQIR	903.4167	2	452.2093						
	NEDEQIRVPR	1255.639	3	419.2163						
6	SGCAAAYCPSSEYNYFYVCQYCPAGNIIGK	3470.458	3	1157.49	Cysteine-rich seceretory protein Dr-CRPK (ACE73567.1)	CRISP	*Daboia russelii*	475.69	67	13
	RSVTPTASNMLK	1304.699	3	435.5679						
	RPEIQNEIVDLHNSLR	1933.025	3	645.0082						
	RPEIQNEIVDLHNSLRR	2089.126	3	697.0436						
	SVTPTASNMLK	1148.598	2	574.7993						
	WTAIIHEWHK	1320.685	2	660.8414						
	SVTPTASNMLK	1164.593	2	582.7962						
	SVTPTASNMLKMEWYPEAAANAER	2667.254	3	889.7565						
	TKCPASCFCHNEII	1736.755	2	868.8764						
	VIGGIKCGENIYMSPYPMK	2157.054	3	719.688						
	EKKDFVYGQGASPANAVVGHYTQIVWYK	3155.595	5	631.9261						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	CGENIYMSPYPMK	1589.68	2	795.3403						
	CILNHSPYNSR	1360.643	3	454.2168						
	CILNHSPYNSRVIGGIK	1928.017	3	643.3406						
	DFVYGQGASPANAVVGHYTQIVWYK	2770.362	3	924.1222						
	DFVYGQGASPANAVVGHYTQIVWYKSYR	3176.559	4	794.8956						
	MEWYPEAAANAER	1537.674	3	513.2285						
	MEWYPEAAANAERWAFR	2097.96	3	699.9943						
	KDFVYGQGASPANAVVGHYTQIVWYK	2898.457	3	966.8253						
	KDFVYGQGASPANAVVGHYTQIVWYKSYR	3304.654	5	661.7394						
6	CILDHSPYNSR	1361.627	3	454.5454	Cysteine-rich seceretory protein Dr-CRPB (ACE73568.1)	CRISP	*Daboia russelii*	168.95	5	2
	MEWYPEAAANAER	1537.674	3	513.2285						
	MEWYPEAAANAER	1553.669	2	777.331						
	MEWYPEAAANAERWAFR	2097.96	3	699.9943						
6	RSVTPTASNMLK	1304.699	3	435.5679	Cysteine rich secretory protein (ASU45033.1)	CRISP	*Daboia russelii*	240.87	22	2
	SVTPTASNMLK	1148.598	2	574.7993						
	SVTPTASNMLKMEWYPEAAANAER	2667.254	3	889.7565						
	CILNHSPYNSR	1360.643	3	454.2168						
	MEWYPEAAANAER	1537.674	3	513.2285						
	MEWYPEAAANAERWAFR	2097.96	3	699.9943						
	KPEIQNEIVDLHNSLR	1905.019	3	635.676						
	KPEIQNEIVDLHNSLRR	2061.12	4	516.0331						
6	LGVYYAYCR	1164.551	2	582.7757	Zinc metalloproteinase-disintegrin-like daborhagin-K (B8K1W0.1)	SVMP	*Daboia russelii*	5.54	2	2
	YSVGVVQDHSK	1218.611	2	609.8051						
6	EWVLTAAHCDR	1357.632	3	453.2133	A Chain A, Vipera russelli proteinase RVV-V gamma (3S9C)	SVSP	*Daboia russelii*	532.86	67	2
	EWVLTAAHCDRR	1513.733	3	505.2469						
	FPNGLDKDIMLIR	1531.83	2	766.4183						
	GKYFCLNTK	1130.566	2	565.7838						
	HKWCEPLYPWVPADSR	2040.975	2	1020.988						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	LRRPVTYSTHIAPVSLPSR	2150.219	3	717.4077						
	NEDEQIRVPR	1255.639	3	419.2163						
	NIRNEDEQIR	1286.645	3	429.5509						
	NIRNEDEQIRVPR	1638.867	3	546.957						
	RPVTYSTHIAPVSLPSR	1881.034	3	627.6797						
	TLCAGILKGGR	1145.646	2	573.3237						
	VFDYNNWIQSIIAGNR	1909.956	2	955.4851						
	VVGGDECNINEHPFLVALYTSASSTIHCAGALINR	3785.837	4	947.2159						
	WCEPLYPWVPADSR	1775.821	2	888.4164						
	YFCLNTK	945.4499	2	473.2259						
	YFCLNTKFPNGLDK	1716.841	2	858.9219						
	YFCLNTKFPNGLDKDIMLIR	2458.262	4	615.3239						
6	SKYHAWIGLR	1230.674	3	410.8937	C-type lectin-like protein subunit 8 / Dabocetin alpha subunit (ABA86561.1)	Snaclec	*Daboia siamensis*	22.78	44	5
	YHEWITLPCGDKNPFICK	2278.078	3	760.0319						
	FCTQQANGWHLASIESVEEANFVAQLASETLTK	3679.77	4	920.701						
6	QEAFSFFK	1003.488	1	1003.487	Phospholipase A2-II (ABD24037.1)	PLA_2	*Daboia russelii*	86.4	59	2
	YISYGCYCGWGGQGTPK	1953.826	2	977.4143						
	TGKIVCETYNR	1340.663	2	670.8311						
	VAAICLGQDVNTYNK	1665.826	2	833.4183						
	VAAICLGQDVNTYNKGYMFLSSYYCR	3093.427	3	1031.822						
	GYMFLSSYYCR	1446.618	2	723.8135						
	CCFVHDCCYAR	1547.565	2	774.28						
	IVCETYNR	1054.499	2	527.7508						
	LVEYSYSYR	1179.568	2	590.2839						
	LVEYSYSYRTGK	1465.732	2	733.3652						
6	SPATPDLSDTSCAK	1449.653	2	725.3259	Beta-nerve growth factor (ASU45040.1)	VNGF	*Daboia russelii*	28.67	23	5
	NPNPVPSGCR	1097.516	2	549.2579						
	INTACVCVISR	1292.645	2	646.8221						
	HWNSYCTTTDTFVR	1787.781	2	894.3882						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
6	SAGQLYQESLGK	1280.648	2	640.824	Secreted L-amino acid oxidase precursor (ACF70483.1)	LAAO	*Daboia russelii*	11.1	11	4
	VTVLEASERPGGR	1370.739	3	457.5815						
	EDDYEEFLEIAK	1500.674	2	750.8444						
	LNEFVQETENGWYFIK	2016.97	2	1008.992						
6	AAAICFR	808.4134	2	404.7081	A Chain A, Phospholipase A2 VRV-PL-VIIIa (1Q6V)	PLA_2	*Daboia russelii*	114.4	65	4
	SLEEFGKMILEETGK	1694.903	2	847.9564						
	QNLNTYSGK	1024.506	2	512.7533						
	YMLYPDFLCK	1365.622	2	683.3158						
	YMLYPDFLCKGELK	1776.87	3	592.9636						
	VNGAIVCEK	989.5084	2	495.2558						
	ICECDKAAAICFR	1613.723	2	807.3605						
	RVNGAIVCEK	1145.61	2	573.3054						
6	NLFQFAR	895.4785	2	448.243	F Chain F, Phospholipase A2-II / Acidic phospholipase A2 daboiatoxin A (2H4C)	PLA_2	*Daboia russelii*	71.16	14	2
	QEAFSFFK	1003.488	1	1003.487						
	YISYGCYCGWGGQGTPK	1953.826	2	977.4143						
	VAAICFR	836.4447	2	418.7229						
	CCFVHDCCYAR	1547.565	2	774.28						
	LVEYSYSYR	1179.568	2	590.2839						
	LVEYSYSYRTGK	1465.732	2	733.3652						
6	VAATCFR	824.4083	2	412.7056	Basic phospholipase A2 RVV-VD (P81458.1)	PLA_2	*Daboia russelii*	6.09	15	2
	NLFQFAEMIVK	1355.703	2	678.3565						
6	NNAEIRLPEER	1340.692	2	670.8452	Serine beta-fibrinogenase-like protein precursor (ADP88560.1)	SVSP	*Daboia siamensis*	18.53	23	3
	WDKDIMLIK	1161.634	2	581.3193						
	TSTYIAPLSLPSSPPR	1686.906	2	843.9558						
	VYDYTDWIQSIIAGNTAATCPP	2456.144	3	819.3924						
	WDKDIMLIK	1177.629	2	589.3145						
7	RPEIQNEIVDLHNSLR	1933.025	3	645.0106	Cysteine-rich secretory protein Dr-CRPK (ACE73567.1)	CRISP	*Daboia russelii*	57.07	37	5
	RPEIQNEIVDLHNSLRR	2089.126	4	523.0367						
	WTAIIHEWHK	1320.685	3	440.9006						
	SVTPTASNMLK	1148.598	2	574.802						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	CILNHSPYNSR	1360.643	3	454.2163						
	DFVYGQGASPANAVVGHYTQIVWYK	2770.362	3	924.1251						
	MEWYPEAAANAER	1537.674	2	769.3407						
	KDFVYGQGASPANAVVGHYTQIVWYK	2898.457	4	725.3729						
7	QQCSSHWTDGSAVSYETVTK	2270.998	3	757.6691	C-type lectin-like protein subunit 8 / Dabocetin alpha subunit (ABA86561.1)	Snaclec	*Daboia siamensis*	12.02	40	3
	YHAWIGLR	1015.547	2	508.2772						
	FCTQQANGWHLASIESVEEANFVAQLASETLTK	3679.77	3	1227.267						
7	SAGQLYQESLGK	1280.648	2	640.8265	Secreted L-amino acid oxidase precursor (ACF70483.1)	LAAO	*Daboia russelii*	51.68	16	5
	NLLLETADYVIVCTTSR	1968.011	3	656.6766						
	VTVLEASERPGGR	1370.739	3	457.5813						
	LNEFVQETENGWYFIK	2016.97	2	1008.99						
	HIVIVGAGMSGLSAAYVLAGAGHK	2279.233	3	760.4131						
7	NIRNEDEQIR	1286.645	3	429.5502	Factor V activator RVV-V alpha (P18964.1)	SVSP	*Daboia russelii*	250.77	90	3
	NIRNEDEQIRVPR	1638.867	4	410.4701						
	VVGGDECNINEHPFLVALYTSTSSTIHCGGALINR	3801.832	4	951.2134						
	WCEPLYPWVPADSR	1775.821	3	592.6125						
	YFCLNTK	945.4499	2	473.2282						
	YFCLNTKFPNGLDKDIMLIR	2474.257	4	619.3189						
	TLCAGILK	875.5019	2	438.2542						
	VFDYNNWIQNIIAGNR	1936.966	2	968.9881						
	NEDEQIRVPR	1255.639	3	419.2153						
	NEDEQIR	903.4167	2	452.2076						
	EWVLTAAHCDR	1357.632	3	453.2149						
	EWVLTAAHCDRR	1513.733	3	505.2477						
	FPNGLDKDIMLIR	1531.83	2	766.4215						
	DTCHGDSGGPLICNGQIQGIVAGGSEPCGQHLKPAVYTK	4078.917	5	816.5848						
	GKYFCLNTK	1130.566	2	565.7844						
	LRRPVTYSTHIAPVSLPSR	2150.219	4	538.309						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	ISTTEDTYPDVPHCTNIFIVK	2450.191	3	817.408						
	HKWCEPLYPWVPADSR	2040.975	4	510.9995						
7	AAAICFR	808.4134	2	404.7094	Basic phospholipase A2 3 (P86368.1)	PLA_2	*Daboia russelii*	75.87	53	3
	SLLEFGMMILEETGK	1729.839	2	865.4223						
	CCFVHDCCYGNLPDCNPK	2315.876	3	772.6311						
	IYMLYPDFLCK	1462.711	2	731.8601						
	ICECDK	824.3277	2	412.6677						
	ICECDKAAAICFR	1613.723	3	538.5795						
7	NNAEIRLPEER	1340.692	3	447.5665	Serine beta-fibrinogenase-like protein precursor (ADP88560.1)	SVSP	*Daboia siamensis*	65.65	25	4
	VYDYTDWIQSIIAGNTAATCPP	2456.144	2	1228.576						
	WDKDIMLIK	1177.629	2	589.3184						
	TSTYIAPLSLPSSPPR	1686.906	2	843.9584						
	FFCLSNK	915.4393	2	458.2231						
7	AAAICFR	808.4134	2	404.7094	A Chain A, Phospholipase A2 VRV-PL-VIIIa (2O1N)	PLA_2	*Daboia russelii*	281.89	83	4
	QNLNTYSK	967.4843	2	484.2422						
	QNLNTYSKK	1095.579	2	548.2943						
	RVNGAIVCEK	1145.61	2	573.3033						
	SLEEFGKMILEETGK	1694.903	2	847.9536						
	VNGAIVCEK	989.5084	2	495.2549						
	CCFVHDCCYGNLPDCNPK	2315.876	3	772.6311						
	YMLYPDFLCK	1349.627	2	675.3178						
	GTSCENRICECDK	1628.646	3	543.5502						
	KYMLYPDFLCK	1477.722	2	739.3654						
	LAIPSYSSYGCYCGWGGK	2025.883	2	1013.449						
	ICECDK	824.3277	2	412.6677						
	ICECDKAAAICFR	1613.723	3	538.5795						
	YMLYPDFLCK	1365.622	2	683.3138						
7	QEAFSFFK	1003.488	2	502.2477	Basic phospholipase A2 Drk-b1 (A8CG89.1)	PLA_2	*Daboia russelii*	6.99	18	3
	IVCETYNR	1054.499	2	527.7499						
	LVEYSYSYR	1179.568	2	590.2875						
7	WDKDIMLIK	1177.629	2	589.3184	Serine alpha-	SVSP	*Daboia*	44.21	38	8

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	VIYRPLPEQSR	1357.759	3	453.257	fibrinogenase-like protein precursor (ADP88559.1)		*siamensis*			
	TLCAGVSGR	920.4618	2	460.7318						
	GAPSIYTK	836.4512	2	418.7264						
	EKFFCLSSK	1145.566	2	573.2868						
	ILPFVPHCANINIVPYTVCR	2383.241	3	795.0862						
	IMGWGSITSPK	1192.603	2	596.8041						
	LNKPVTYSTHIASLSLPSNPPR	2392.298	4	598.8304						
8	AAAICFR	808.4134	2	404.7104	A Chain A, Phospholipase A2 VRV-PL-VIIIa (2O1N)/ Daboxin P (C0HK16.1)	PLA_2	*Daboia russelii*	166.81	55	6
	YMLYPDFLCK	1365.622	2	683.3156						
	VNGAIVCEK	989.5084	2	495.2568						
	MILEETGK	920.4757	2	460.7403						
	LAIPSYSSYGCYCGWGGK	2025.883	2	1013.447						
	QNLNTYSK	967.4843	2	484.2433						
8	FCTEQANSGHLVSIK	1690.822	3	564.2788	P31 alpha subunit (ADK22829.1)	Snaclec	*Daboia limitis*	12.85	34	6
	MCQGLAK	807.3852	2	404.1958						
	IIYVNWK	935.5349	2	468.2717						
	DGIYVWIGLR	1191.652	2	596.3286						
	SEWTDGSK	909.3949	2	455.1998						
	SWADAEK	806.3679	2	403.6868						
	QEIAEINR	972.5109	2	486.7589						
	LALDIEIATYR	1277.71	2	639.36						
8	TSTYIAPLSLPSSPPR	1686.906	2	843.9562	Serine beta-fibrinogenase-like protein precursor (ADP88560.1)	SVSP	*Daboia siamensis*	53.36	16	3
	FFCLSNK	915.4393	2	458.2234						
	NNAEIRLPEER	1340.692	3	447.5685						
8	TLCAGVSGR	920.4618	2	460.7339	Serine alpha-fibrinogenase-like protein precursor (ADP88559.1)	SVSP	*Daboia siamensis*	18.52	12	3
	FFCLSSK	888.4284	2	444.7181						
	GAPSIYTK	836.4512	2	418.7281						
8	STTDLPSR	876.4421	2	438.7231	Secreted L-amino acid oxidase	LAAO	*Daboia russelii*	21.14	8	4
	SAGQLYQESLGK	1280.648	2	640.8274						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	YDTYSTK	877.3938	2	439.1998	precursor (ACF70483.1)					
	VTVLEASERPGGR	1370.739	3	457.5837						
	SLVNLGGSK	874.4993	2	437.7533						
	SISISVAR	832.4887	2	416.7478						
	SLNNQFASFIDKVR	1638.86	3	546.9589						
	SLNNQFASFIDK	1383.69	2	692.3499						
	SLDLDSIIAEVK	1302.715	2	651.8618						
	SKAEAESLYQSK	1340.669	2	670.8367						
	THNLEPYFESFINNLR	1993.977	3	665.332						
	YEELQITAGR	1179.6	2	590.3048						
	WELLQQVDTSTR	1475.749	2	738.3785						
	TNAENEFVTIKK	1393.732	3	465.2491						
	TNAENEFVTIK	1265.637	2	633.3232						
	TLLEGEESR	1033.516	2	517.2605						
	QISNLQQSISDAEQR	1716.851	2	858.93						
	FLEQQNQVLQTK	1475.785	2	738.3975						
	DVDGAYMTK	1015.44	2	508.2231						
	AQYEDIAQK	1065.521	2	533.2634						
	AEAESLYQSK	1125.542	2	563.2741						
	NKYEDEINKR	1308.654	3	436.8882						
	IEISEINR	973.5313	2	487.2696						
	NMQDMVEDYR	1332.519	2	666.7605						
	SGGGFSSGSAGIINYQR	1657.793	2	829.3993						
	GGGGGGYGSGGSSYGSGGGSYGSGGGGGGGR	2383.952	3	795.3204						
	SVGIVQVQGNR	1156.643	2	578.8259						
9	AAAICFR	808.4134	2	404.7102	A Chain A, Phospholipase A2 VRV-PL-VIIIa (2O1N)	PLA_2	*Daboia russelii*	152.21	79	10
	QNLNTYSK	967.4843	2	484.244						
	YMLYPDFLCK	1349.627	2	675.319						
	VNGAIVCEK	989.5084	2	495.2565						
	YKRVNGAIVCEK	1436.768	3	479.5931						
	YMLYPDFLCK	1365.622	2	683.3151						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	CCFVHDCCYGNLPDCNPK	2315.876	3	772.6309						
	KYMLYPDFLCK	1493.717	3	498.5787						
	ICECDKAAAICFR	1613.723	3	538.5809						
	LAIPSYSSYGCYCGWGGK	2025.883	2	1013.446						
	RVNGAIVCEK	1145.61	2	573.307						
	AAAICFRQNLNTYSK	1756.88	3	586.3031						
9	AAAICFR	808.4134	2	404.7102	Basic phospholipase A2 3 (P86368.1)	PLA_2	*Daboia russelii*	46.09	75	4
	SLLEFGMMILEETGK	1713.844	2	857.4279						
	VNGAIVCEQGTSCENR	1793.791	2	897.398						
	CCFVHDCCYGNLPDCNPK	2315.876	3	772.6309						
	LAVPFYSSYGCYCGWGGK	2071.904	2	1036.46						
	IYMLYPDFLCK	1462.711	2	731.8611						
	ICECDKAAAICFR	1613.723	3	538.5809						
	IYMLYPDFLCK	1478.706	2	739.8583						
9	FVSFVCK	886.4491	2	443.7286	P31 beta subunit (ADK22832.1)	Snaclec	*Daboia siamensis*	36.43	32	5
	GSHLASIHSSEEEAFVSK	1914.919	3	638.9811						
	EQHKGSHLASIHSSEEEAFVSK	2437.174	5	488.2402						
	RPYCTVMVLKPDR	1650.845	3	550.9528						
9	QQCSSHWTDGSAVSYETVTK	2270.998	3	757.6734	C-type lectin-like protein subunit 8 / Dabocetin alpha subunit (ABA86561.1)	Snaclec	*Daboia siamensis*	15.9	30	4
	SKYHAWIGLR	1230.674	3	410.896						
	YHAWIGLR	1015.547	2	508.2778						
	TWEDAEK	878.389	2	439.6974						
	CFGLNKETK	1096.546	2	548.7752						
9	MTFPIFR	911.4808	2	456.2443	Snaclec 7 / Dabocetin beta subunit (AAY63876.1)	Snaclec	*Daboia siamensis*	25.93	34	5
	WSDGVNLDYK	1196.558	2	598.7828						
	SSEEMDFVIR	1228.551	2	614.7802						
	TTDNQWLR	1033.506	2	517.2576						
	TWEDAEK	878.389	2	439.6974						
	FDFFWIGLR	1200.62	2	600.8155						
	TPADYVWIGLR	1290.684	2	645.8463						
9	WDYVNCAEHYR	1512.632	3	504.8828	P31 alpha subunit	Snaclec	*Daboia*	48.58	35	7

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	SWADAEK	806.3679	2	403.6872	(ADK22829.1)		*limitis*			
	SWADAEKFCTEQANSGHLVSIK	2478.172	4	620.2961						
	FCTEQANSGHLVSIK	1690.822	3	564.283						
	DGIYVWIGLR	1191.652	2	596.3272						
	IIYVNWK	935.5349	2	468.2731						
	SWEAAER	848.3897	2	424.6981						
	TWEAAER	862.4054	2	431.7057						
9	RPEIQNEIVDLHNSLR	1933.025	3	645.0107	Cysteine-rich seceretory protein Dr-CRPK (ACE73567.1)	CRISP	*Daboia russelii*	23.16	36	3
	MEWYPEAAANAER	1553.669	2	777.3383						
	SVTPTASNMLK	1148.598	2	574.8018						
	MEWYPEAAANAER	1537.674	2	769.3407						
	CILNHSPYNSR	1360.643	3	454.2185						
	DFVYGQGASPANAVVGHYTQIVWYK	2770.362	3	924.1258						
	WTAIIHEWHK	1320.685	3	440.9012						
	SVTPTASNMLK	1164.593	2	582.7985						
9	NNAEIRLPEER	1340.692	3	447.5692	Serine beta-fibrinogenase-like protein precursor (ADP88560.1)	SVSP	*Daboia siamensis*	55.26	27	4
	WDKDIMLIK	1177.629	2	589.3176						
	TSTYIAPLSLPSSPPR	1686.906	2	843.957						
	IYLGVHNLTLR	1298.758	3	433.5909						
	VYDYTDWIQSIIAGNTAATCPP	2456.144	2	1228.581						
9	WDKDIMLIK	1177.629	2	589.3176	Serine alpha-fibrinogenase-like protein precursor (ADP88559.1)	SVSP	*Daboia siamensis*	24.08	28	6
	VIYRPLPEQSR	1357.759	2	679.3837						
	TLCAGVSGR	920.4618	2	460.7338						
	FFCLSSK	888.4284	2	444.7185						
	EKFFCLSSK	1145.566	2	573.2874						
	IMGWGSITSPK	1176.608	2	588.8097						
	LNKPVTYSTHIASLSLPSNPPR	2392.298	4	598.8306						
9	RPVTYSTHIAPVSLPSR	1881.034	3	627.6837	RVV-V gamma-like protein precursor (ADP88558.1)	SVSP	*Daboia siamensis*	37.9	43	13
	NEDEQIRVPR	1255.639	3	419.2172						
	NIRNEDEQIR	1286.645	3	429.5512						
	NIRNEDEQIRVPR	1638.867	4	410.4716						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	WCEPLYPWVPADSR	1775.821	2	888.4154						
	YFCLNTK	945.4499	2	473.2289						
	TLCAGILK	875.5019	2	438.2552						
	FPNGLDKDIMLIR	1531.83	3	511.2819						
	EWVLTAAHCDR	1357.632	3	453.2156						
	LRRPVTYSTHIAPVSLPSR	2150.219	4	538.3108						
	FPNGLDKDIMLIR	1547.825	3	516.6139						
	ISTTEDTYPDVPHCTNIFIVK	2450.191	3	817.4052						
9	SAGQLYQESLGK	1280.648	2	640.8294	Secreted L-amino acid oxidase precursor (ACF70483.1)	LAAO	*Daboia russelii*	14.82	12	7
	VTVLEASERPGGR	1370.739	3	457.5837						
	VTVTYQTTQK	1168.621	2	584.8134						
	KFWEDDGIQGGK	1379.659	2	690.3328						
	EGWYANLGPMR	1293.604	2	647.3063						
	FWEDDGIQGGK	1251.564	2	626.2864						
	EGWYANLGPMRVPEK	1762.858	3	588.2905						
	SGGGFSSGSAGIINYQR	1657.793	2	829.3998						
	SGGGFSSGSAGIINYQRR	1813.894	3	605.3056						
	SKAEAESLYQSK	1340.669	2	670.8365						
	SLDLDSIIAEVK	1302.715	2	651.8627						
	SLNNQFASFIDK	1383.69	2	692.3508						
	SISISVAR	832.4887	2	416.75						
	SLVNLGGSK	874.4993	2	437.7522						
	SLVNLGGSKSISISVAR	1687.97	3	563.3297						
	NKLNDLEDALQQAK	1599.834	3	533.9517						
	NKYEDEINKR	1308.654	3	436.8877						
	QISNLQQSISDAEQR	1716.851	3	572.9567						
	NMQDMVEDYR	1332.519	2	666.7618						
	WELLQQVDTSTR	1475.749	2	738.3774						
	YEELQITAGR	1179.6	2	590.3057						
	TLLEGEESR	1033.516	2	517.2629						
	TNAENEFVTIKK	1393.732	3	465.2491						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	FLEQQNQVLQTK	1475.785	2	738.3991						
	FSSCGGGGGSFGAGGGFGSR	1765.735	2	883.3704						
	GGGGGGYGSGGSSYGSGGGSYGSGGGGGGGR	2383.952	2	1192.478						
	DVDGAYMTK	999.4452	2	500.2262						
	DYQELMNTK	1157.514	2	579.2599						
	AQYEDIAQK	1065.521	2	533.2634						
	LLRDYQELMNTK	1523.789	3	508.6014						
	LNDLEDALQQAK	1357.696	2	679.3509						
	IEISEINR	973.5313	2	487.27						
	LALDIEIATYR	1277.71	2	639.3604						
	AEAESLYQSK	1125.542	2	563.2735						
	LRSEIDNVK	1073.595	2	537.2995						
10	AAAICFR	808.4134	2	404.7107	A Chain A, Phospholipase A2 VRV-PL-VIIIa (2O1N)	PLA_2	*Daboia russelii*	167.38	64	9
	VNGAIVCEK	1365.622	2	683.3159						
	YMLYPDFLCK	989.5084	2	495.2563						
	YKRVNGAIVCEK	1349.627	2	675.318						
	ICECDKAAAICFR	1436.768	3	479.5926						
	LAIPSYSSYGCYCGWGGK	1613.723	2	807.3673						
	KYMLYPDFLCK	2025.883	2	1013.45						
	RVNGAIVCEK	1477.722	2	739.3629						
	QNLNTYSK	1145.61	2	573.3059						
	AAAICFR	967.4843	2	484.244						
10	IYMLYPDFLCK	808.4134	2	404.7107	Basic phospholipase A2 3 (P86368.1)	PLA_2	*Daboia russelii*	51.79	61	5
	RVNGAIVCEQGTSCENR	1478.706	2	739.8596						
	VNGAIVCEQGTSCENR	1949.892	3	650.6331						
	SLLEFGMMILEETGK	1793.791	2	897.3962						
	LAVPFYSSYGCYCGWGGK	1713.844	2	857.429						
	ICECDKAAAICFR	2071.904	2	1036.459						
	MEWSDR	1613.723	2	807.3673						
10	SMTCNFIAPVVCK	823.3403	2	412.1734	Snaclec coagulation factor X-activating	Snaclec	*Daboia siamensis*	8.24	51	5
	ALAEESYCLIMITHEK	1526.716	2	763.8632						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	KGSHLVSLHSR	1907.924	3	636.6457	enzyme light chain 1 (Q4PRD1)					
	VLDCPSGWLSYEQHCYK	1220.686	3	407.5664						
	GSHLASIHSSEEEAFVSK	2141.942	2	1071.477						
	FVSFVCK	1914.919	4	479.4869						
	RPYCTVMVLKPDR	886.4491	2	443.7286						
	SKYHAWIGLR	1650.845	3	550.9518						
	YHEWITLPCGDK	1230.674	3	410.8972						
	YHAWIGLR	1518.705	3	506.9063						
	YHEWITLPCGDKNPFICK	1015.547	2	508.2789						
	CFGLNKETK	2278.078	4	570.275						
	TWEDAEK	1096.546	2	548.7747						
	QQCSSHWTDGSAVSYETVTK	878.389	2	439.6955						
	TPADYVWIGLR	2270.998	3	757.6699						
10	AQYCISK	1290.684	2	645.8469	P68 alpha subunit (ADK22825.1)	Snaclec	*Daboia siamensis*	6.71	18	3
	WTDGSSVIYK	869.4186	2	435.2112						
	MTFPIFR	1155.568	2	578.2881						
10	SSEEMDFVIR	927.4757	2	464.2413	Snaclec 7 / Dabocetin beta subunit (AAY63876.1)	Snaclec	*Daboia siamensis*	65.84	34	5
	FDFFWIGLR	1228.551	2	614.7791						
	WSDGVNLDYK	1200.62	2	600.8149						
	TWEDAEK	1196.558	2	598.7842						
	WDYVNCAEHYR	878.389	2	439.6955						
10	SVGEANFVAQLASGFMQK	1512.632	2	756.82	P31 alpha subunit (ADK22829.1)	Snaclec	*Daboia limitis*	60.94	50	9
	SWADAEKFCTEQANSGHLVSIK	1883.932	3	628.6505						
	FCTEQANSGHLVSIK	2478.172	3	826.7239						
	DGIYVWIGLR	1690.822	2	845.9153						
	IIYVNWK	1191.652	2	596.329						
	IIYVNWKEGESK	935.5349	2	468.2722						
	SWADAEK	1465.769	3	489.26						
	SVGEANFVAQLASGFMQK	806.3679	2	403.6861						
	MWFNHK	1899.927	3	633.9827						
	FITHFWIGLR	862.4029	2	431.705						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	CEEPYPFVCK	1289.715	3	430.5773						
	RPEIQNEIVDLHNSLR	1328.565	2	664.7865						
10	MEWYPEAAANAER	1933.025	3	645.0111	Cysteine-rich seceretory protein Dr-CRPK (ACE73567.1)	CRISP	*Daboia russelii*	33.01	37	4
	RSVTPTASNMLK	1537.674	3	513.2303						
	SVTPTASNMLK	1304.699	3	435.5703						
	CILNHSPYNSR	1148.598	2	574.802						
	DFVYGQGASPANAVVGHYTQIVWYK	1360.643	3	454.2184						
	WTAIIHEWHK	2770.362	3	924.1285						
	KDFVYGQGASPANAVVGHYTQIVWYK	1320.685	3	440.9002						
	VYDYTDWIQSIIAGNTAATCPP	2898.457	4	725.3708						
10	NNAEIRLPEER	2456.144	2	1228.58	Serine beta-fibrinogenase-like protein precursor (ADP88560.1)	SVSP	*Daboia siamensis*	47.4	25	4
	WDKDIMLIK	1340.692	3	447.5697						
	TSTYIAPLSLPSSPPR	1161.634	2	581.3219						
	FFCLSNK	1686.906	3	562.9749						
	WDKDIMLIK	915.4393	2	458.2239						
10	TLCAGVSGR	1161.634	2	581.3219	Serine alpha-fibrinogenase-like protein precursor (ADP88559.1)	SVSP	*Daboia siamensis*	29.09	18	5
	EKFFCLSSK	920.4618	2	460.7324						
	FFCLSSK	1145.566	2	573.2863						
	IMGWGSITSPK	888.4284	2	444.7185						
	GAPSIYTK	1176.608	2	588.809						
	VTVTYQTTQK	836.4512	2	418.7275						
10	VTVLEASERPGGR	1168.621	2	584.8126	Secreted L-amino acid oxidase precursor (ACF70483.1)	LAAO	*Daboia russelii*	19.63	13	7
	SAGQLYQESLGK	1370.739	3	457.5835						
	FWEDDGIQGGK	1280.648	2	640.8275						
	IFLTCTK	1251.564	2	626.2866						
	KFWEDDGIQGGK	882.4754	2	441.7424						
	HDDIFAYEK	1379.659	2	690.3333						
	QISNLQQSISDAEQR	1137.521	2	569.2638						
	NMQDMVEDYR	1716.851	3	572.9559						
	NKYEDEINKR	1332.519	2	666.7616						
	NMQDMVEDYR	1308.654	3	436.8878						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	TLLEGEESR	1316.525	2	658.7658						
	TNAENEFVTIKK	1033.516	2	517.26						
	SGGGFSSGSAGIINYQR	1393.732	2	697.3695						
	SKAEAESLYQSK	1657.793	2	829.4016						
	SLDLDSIIAEVK	1340.669	2	670.8352						
	SLNNQFASFIDK	1302.715	2	651.8634						
	SISISVAR	1383.69	2	692.3491						
	SLVNLGGSK	832.4887	2	416.7491						
	FLEQQNQVLQTK	874.4993	2	437.7524						
	GGGGGGYGSGGSSYGSGGGSYGSGGGGGGGR	1475.785	2	738.395						
	DYQELMNTK	2383.952	2	1192.476						
	AEAESLYQSK	1141.519	2	571.2647						
	AQYEDIAQK	1125.542	2	563.2717						
	DVDGAYMTK	1065.521	2	533.2627						
	WELLQQVDTSTR	999.4452	2	500.2244						
	IEISEINR	1475.749	2	738.3783						
	YEELQITAGR	973.5313	2	487.2695						
	LALDIEIATYR	1179.6	2	590.3052						
	NKLNDLEDALQQAK	1277.71	2	639.3615						
	LRSEIDNVK	1599.834	2	800.4222						
	NKYEDEINK	1073.595	2	537.2985						
	LNDLEDALQQAKEDLAR	1152.553	2	576.7766						
11	GSHLASIHSSEEEAFVSK	1914.919	3	638.9801	P31 beta subunit (ADK22830.1)	Snaclec	*Daboia siamensis*	53.11	21	3
	IFWFNR	882.4621	2	441.7354						
	FVSFVCK	886.4491	2	443.7275						
11	MTFPIFR	927.4757	2	464.2425	Snaclec 7 / Dabocetin beta subunit (AAY63876.1)	Snaclec	*Daboia siamensis*	154.18	35	5
	WSDGVNLDYK	1196.558	2	598.7836						
	TWEDAEK	878.389	2	439.6964						
	TTDNQWLR	1033.506	2	517.2572						
	SSEEMDFVIR	1212.557	2	606.7833						
	FDFFWIGLR	1200.62	2	600.8148						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	KTWEDAEK	1006.484	2	503.7441						
11	SKYHAWIGLR	1230.674	3	410.8967	C-type lectin-like protein subunit 8 / Dabocetin alpha subunit (ABA86561.1)	Snaclec	*Daboia siamensis*	159.03	63	8
	QQCSSHWTDGSAVSYETVTK	2270.998	3	757.6724						
	YHAWIGLR	1015.547	2	508.2791						
	TWEDAEK	878.389	2	439.6964						
	FCTQQANGWHLASIESVEEANFVAQLASETLTK	3679.77	3	1227.263						
	CFGLNKETK	1096.546	2	548.7743						
	YHEWITLPCGDKNPFICK	2278.078	3	760.0335						
11	SAGQLYQESLGK	1280.648	2	640.8267	Secreted L-amino acid oxidase precursor (ACF70483.1)	LAAO	*Daboia russelii*	20.91	14	7
	VTVTYQTTQK	1168.621	2	584.8119						
	AVEELKR	844.4887	2	422.7465						
	IFLTCTK	882.4754	2	441.7424						
	VTVLEASERPGGR	1370.739	3	457.5828						
	AIEESVHFK	1059.547	2	530.2761						
	EGWYANLGPMR	1293.604	2	647.3074						
11	YFCLNTK	945.4499	2	473.229	RVV-V gamma-like protein precursor (ADP88558.1)	SVSP	*Daboia siamensis*	23.58	27	7
	FPNGLDKDIMLIR	1531.83	3	511.2832						
	NIRNEDEQIR	1286.645	3	429.5511						
	WCEPLYPWVPADSR	1775.821	2	888.4178						
	LRRPVTYSTHIAPVSLPSR	2150.219	4	538.311						
11	AAAICFR	808.4134	2	404.7099	Basic phospholipase A2 3 (P86368.1)	PLA_2	*Daboia russelii*	73.11	62	6
	SLLEFGMMILEETGK	1713.844	2	857.4265						
	RVNGAIVCEQGTSCENR	1949.892	3	650.6321						
	NLNTYSK	839.4258	2	420.2146						
	VNGAIVCEQGTSCENR	1793.791	2	897.3959						
	IYMLYPDFLCK	1462.711	2	731.8632						
	LAVPFYSSYGCYCGWGGK	2071.904	2	1036.456						
11	AAAICFR	808.4134	2	404.7099	A Chain A, Phospholipase A2 VRV-PL-VIIIa (2O1N)	PLA_2	*Daboia russelii*	120.51	56	7
	VNGAIVCEK	989.5084	2	495.2556						
	YMLYPDFLCK	1349.627	2	675.3185						
	LAIPSYSSYGCYCGWGGK	2025.883	2	1013.446						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	KYMLYPDFLCK	1477.722	3	493.2472						
	QNLNTYSK	967.4843	2	484.2437						
11	TWEDAEK	878.389	2	439.6964	Snaclec 6 (Q4PRC7.1)	Snaclec	*Daboia siamensis*	13.85	33	3
	KGSHLVSLHSR	1220.686	3	407.568						
	MEWSDR	823.3403	2	412.1734						
	FVVNLISENLEYPATWIGLGNMWK	2794.427	3	932.1551						
	TWEAAER	862.4054	2	431.7044						
	KCFVLEK	923.5019	2	462.2533						
11	MWFNHK	862.4029	2	431.7052	Snaclec coagulation factor X-activating enzyme light chain 2 (ADJ67473.1)	Snaclec	*Daboia siamensis*	5.99	15	3
	CEEPYPFVCK	1328.565	2	664.7861						
11	SVGIVQVQGNR	1156.643	2	578.826	RVV-X heavy chain (AUF41652.1)	SVMP	*Daboia siamensis*	26.94	10	5
	GSYYGYCR	1025.415	2	513.2094						
	DQLQQNGQPCQNNR	1699.757	2	850.3783						
	DSCFQENLK	1140.499	2	570.7549						
	IPCAPQDVK	1027.524	2	514.2637						
	LFCLNNSPGNK	1263.615	2	632.3115						
	KIPCAPQDVK	1155.619	2	578.3114						
11	WDYVNCAEHYR	1512.632	3	504.884	P31 alpha subunit (ADK22829.1)	Snaclec	*Daboia limitis*	145.27	41	6
	WTYFHK	881.4305	2	441.2185						
	SVGEANFVAQLASGFMQK	1883.932	3	628.6524						
	FCTEQANSGHLVSIK	1690.822	3	564.2799						
	IIYVNWK	935.5349	2	468.2715						
	SWADAEK	806.3679	2	403.6858						
11	WDKDIMLIK	1161.634	2	581.3232	Serine beta-fibrinogenase-like protein precursor (ADP88560.1)	SVSP	*Daboia siamensis*	104.66	25	4
	TSTYIAPLSLPSSPPR	1686.906	2	843.9587						
	FFCLSNK	915.4393	2	458.2239						
	NNAEIRLPEER	1340.692	3	447.5699						
	VYDYTDWIQSIIAGNTAATCPP	2456.144	3	819.3889						
12	AAAICFR	808.4134	2	404.7098	A Chain A,	PLA_2	*Daboia*	102.97	57	8

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	RVNGAIVCEK	1145.61	2	573.308	Phospholipase A2 VRV-PL-VIIIa (2O1N)		*russelii*			
	VNGAIVCEK	1365.622	2	683.3166						
	YMLYPDFLCK	989.5084	2	495.2566						
	YKRVNGAIVCEK	1349.627	2	675.3207						
	ICECDKAAAICFR	1477.722	2	739.3641						
	LAIPSYSSYGCYCGWGGK	1613.723	2	807.366						
	KYMLYPDFLCK	2025.883	2	1013.447						
12	RVNGAIVCEQGTSCENR	808.4134	2	404.7098	Basic phospholipase A2 3 (P86368.1)	PLA_2	*Daboia russelii*	99.69	67	6
	SLLEFGMMILEETGK	1949.892	3	650.6331						
	NLNTYSK	1713.844	2	857.4264						
	VNGAIVCEQGTSCENR	839.4258	2	420.2148						
	IYMLYPDFLCK	1793.791	2	897.3974						
	ICECDKAAAICFR	1478.706	2	739.852						
	LAVPFYSSYGCYCGWGGK	1613.723	2	807.366						
	FGSVWIGLNDPWHNCNWEWSDNAR	2071.904	3	691.3084						
12	EQHKGSHLASIHSSEEEAFVSK	2960.296	3	987.4395	P31 beta subunit (ADK22832.1)	Snaclec	*Daboia siamensis*	38.17	39	5
	FVSFVCK	2437.174	4	610.0476						
	GSHLASIHSSEEEAFVSK	886.4491	2	443.7281						
	SGDAETFCTEQANSGHLVSIESVEEAEFVAQLISENIK	1914.919	3	638.9802						
12	WTDGSSVIYK	4138.94	4	1035.495	P68 alpha subunit (ADK22825.1)	Snaclec	*Daboia siamensis*	17.04	56	6
	TWFNLSCGDDYPFVCK	1155.568	2	578.2884						
	TPADYVWIGLR	2008.857	2	1004.937						
	AQYCISK	1290.684	2	645.8452						
	NCFGLEK	869.4186	2	435.2117						
	SSEEMDFVIR	867.4029	2	434.2065						
12	VFTEEMNWADAEK	1569.689	2	785.3492	Snaclec 3 (Q4PRD0.1)	Snaclec	*Daboia siamensis*	18	26	2
	GSHLLSLHNIAEADFVLKK	2092.155	4	523.7951						
12	WSDGVNLDYK	1212.557	2	606.7834	Snaclec 7 / Dabocetin beta subunit	Snaclec	*Daboia siamensis*	66.31	34	5
	TWEDAEK	1196.558	2	598.784						
	TTDNQWLR	878.389	2	439.6972						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	FDFFWIGLR	1033.506	2	517.2563	(AAY63876.1)					
	MTFPIFR	1200.62	2	600.8144						
	WDYVNCAEHYR	927.4757	2	464.2427						
12	SWADAEKFCTEQANSGHLVSIK	1512.632	2	756.8203	P31 alpha subunit (ADK22829.1)	Snaclec	*Daboia limitis*	29.08	47	8
	SWADAEK	2478.172	3	826.7244						
	SVGEANFVAQLASGFMQK	806.3679	2	403.6871						
	FCTEQANSGHLVSIK	1883.932	2	942.473						
	DGIYVWIGLR	1690.822	3	564.2816						
	IIYVNWK	1191.652	2	596.3323						
	WTYFHK	935.5349	2	468.2722						
	SWEAAER	881.4305	2	441.2177						
	FITHFWIGLR	848.3897	2	424.698						
	MWFNHK	1289.715	3	430.577						
	RPVTYSTHIAPVSLPSR	862.4029	2	431.7052						
12	NIRNEDEQIRVPR	1881.034	3	627.6837	Factor V activator RVV-V alpha (P18964.1)	SVSP	*Daboia russelii*	64.8	56	14
	NIRNEDEQIR	1638.867	3	546.9599						
	WCEPLYPWVPADSR	1286.645	3	429.5518						
	YFCLNTK	1775.821	2	888.4152						
	TLCAGILK	945.4499	2	473.2295						
	NEDEQIRVPR	875.5019	2	438.2542						
	EWVLTAAHCDR	1255.639	3	419.2176						
	FPNGLDKDIMLIR	1357.632	3	453.2157						
	GKYFCLNTK	1547.825	3	516.6153						
	LRRPVTYSTHIAPVSLPSR	1531.83	3	511.2833						
	DTCHGDSGGPLICNGQIQGIVAGGSEPCGQHLKPAVYTK	2150.219	3	717.4118						
	RSVTPTASNMLK	4078.917	5	816.5883						
12	RPEIQNEIVDLHNSLRR	1304.699	3	435.5703	Cysteine-rich seceretory protein Dr-CRPK (ACE73567.1)	CRISP	*Daboia russelii*	52.97	36	4
	RPEIQNEIVDLHNSLR	2089.126	4	523.0391						
	WTAIIHEWHK	1933.025	3	645.0123						
	SVTPTASNMLK	1320.685	3	440.8998						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	CILNHSPYNSR	1148.598	2	574.8016						
	DFVYGQGASPANAVVGHYTQIVWYK	1360.643	3	454.2181						
	MEWYPEAAANAER	2770.362	3	924.1319						
	NNAEIRLPEER	1537.674	2	769.3424						
12	WDKDIMLIK	1340.692	3	447.5695	Serine beta-fibrinogenase-like protein precursor (ADP88560.1)	SVSP	*Daboia siamensis*	49.32	23	3
	VYDYTDWIQSIIAGNTAATCPP	1161.634	2	581.322						
	TSTYIAPLSLPSSPPR	2456.144	3	819.3883						
12	TLCAGVSGR	1161.634	2	581.322	Serine alpha-fibrinogenase-like protein precursor (ADP88559.1)	SVSP	*Daboia siamensis*	26.62	26	6
	FFCLSSK	920.4618	2	460.7336						
	EKFFCLSSK	888.4284	2	444.7186						
	GAPSIYTK	1145.566	2	573.2864						
	LNKPVTYSTHIASLSLPSNPPR	836.4512	2	418.7288						
	IMGWGSITSPK	2392.298	4	598.831						
	SAGQLYQESLGK	1176.608	2	588.8118						
12	RITFKPPLPPK	1280.648	2	640.8276	Secreted L-amino acid oxidase precursor (ACF70483.1)	LAAO	*Daboia russelii*	62.06	42	19
	NLLLETADYVIVCTTSR	1293.804	3	431.9403						
	VTVTYQTTQK	1968.011	3	656.6773						
	VTVLEASERPGGR	1168.621	2	584.813						
	FDEIVGGMDQLPTSMYR	1370.739	3	457.5835						
	EGWYANLGPMR	1958.899	2	979.9517						
	FWEDDGIQGGK	1293.604	2	647.307						
	EDDYEEFLEIAK	1251.564	2	626.2853						
	AIEESVHFK	1500.674	2	750.8425						
	DLQTFCYPSIIQK	1059.547	2	530.2773						
	LNEFVQETENGWYFIK	1612.804	2	806.9069						
	IFLTCTK	2016.97	2	1008.993						
	KFWEDDGIQGGK	882.4754	2	441.7428						
	KDPGLLKYPVKPSEAGK	1379.659	2	690.3317						
	HIVIVGAGMSGLSAAYVLAGAGHK	1827.037	3	609.6829						
	HDDIFAYEK	2279.233	3	760.4157						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	YPVKPSEAGK	1137.521	2	569.264						
	DPGLLKYPVKPSEAGK	1075.578	2	538.2924						
	SKAEAESLYQSKYEELQITAGR	1698.943	3	566.9859						
	SKAEAESLYQSK	2501.252	4	626.0693						
	SGGGFSSGSAGIINYQRR	1340.669	2	670.8353						
	SLVNLGGSKSISISVAR	1657.793	2	829.4019						
	SLVNLGGSK	1687.97	2	844.4912						
	SISISVAR	874.4993	2	437.755						
	SLNNQFASFIDK	832.4887	2	416.7486						
	SLDLDSIIAEVKAQYEDIAQK	1383.69	2	692.3507						
	SLDLDSIIAEVK	2349.218	3	783.7452						
	NMQDMVEDYR	1302.715	2	651.8618						
	NSKIEISELNR	1332.519	2	666.7623						
	NKYEDEINKR	1302.701	2	651.8562						
	QISNLQQSISDAEQR	1308.654	3	436.8881						
	WELLQQVDTSTR	1716.851	3	572.9562						
	YEELQITAGR	1475.749	2	738.3794						
	THNLEPYFESFINNLRR	1179.6	2	590.3048						
	NKLNDLEDALQQAKEDLAR	1993.977	3	665.3329						
	TNAENEFVTIKK	2184.126	3	728.7134						
	TLLEGEESR	1393.732	3	465.2492						
	NKLNDLEDALQQAK	1033.516	2	517.261						
	FLEQQNQVLQTK	1599.834	2	800.4205						
	GGGGGGYGSGGSSYGSGGGSYGSGGGGGGGR	1475.785	2	738.3974						
	FSSCGGGGGSFGAGGGFGSR	2383.952	2	1192.478						
	DYQELMNTK	1765.735	2	883.3691						
	AQYEDIAQK	1141.519	2	571.2654						
	AEAESLYQSKYEELQITAGR	1065.521	2	533.2645						
	AEAESLYQSK	2286.125	3	762.7161						
	DVDGAYMTK	1125.542	2	563.2737						
	LNDLEDALQQAKEDLAR	999.4452	2	500.2253						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	LNDLEDALQQAK	1941.988	3	648.0022						
	LLRDYQELMNTK	1357.696	2	679.352						
	IEISEINR	1523.789	3	508.6014						
	LALDIEIATYR	973.5313	2	487.2697						
13	AAAICFR	808.4134	2	404.7097	A Chain A, Phospholipase A2 VRV-PL-VIIIa (2O1N)/ Daboxin P (C0HK16.1)	PLA_2	*Daboia russelii*	41.71	56	7
	QNLNTYSK	967.4843	2	484.2448						
	YMLYPDFLCK	1365.622	2	683.3164						
	VNGAIVCEK	989.5084	2	495.2568						
	MILEETGK	936.4707	2	468.738						
	KYMLYPDFLCK	1493.717	3	498.5803						
	LAIPSYSSYGCYCGWGGK	2025.883	2	1013.452						
13	AAAICFR	808.4134	2	404.7097	Basic phospholipase A2 3 (P86368.1)	PLA_2	*Daboia russelii*	47.8	56	5
	SLLEFGMMILEETGK	1729.839	2	865.4237						
	RVNGAIVCEQGTSCENR	1949.892	3	650.6325						
	VNGAIVCEQGTSCENR	1793.791	2	897.3986						
	IYMLYPDFLCK	1478.706	2	739.8569						
	LAVPFYSSYGCYCGWGGK	2071.904	2	1036.454						
13	SKYHAWIGLR	1230.674	3	410.896	C-type lectin-like protein subunit 8 / Dabocetin alpha subunit (ABA86561.1)	Snaclec	*Daboia siamensis*	46.55	38	7
	RQQCSSHWTDGSAVSYETVTK	2427.099	4	607.5294						
	QQCSSHWTDGSAVSYETVTK	2270.998	3	757.6722						
	YHAWIGLR	1015.547	2	508.2802						
	YHEWITLPCGDK	1518.705	3	506.9074						
	TWEDAEK	878.389	2	439.697						
	CFGLNKETK	1096.546	2	548.7755						
13	WTDGSSVIYK	1155.568	2	578.2903	P68 alpha subunit (ADK22825.1)	Snaclec	*Daboia siamensis*	9.03	22	4
	TPADYVWIGLR	1290.684	2	645.8484						
	AQYCISK	869.4186	2	435.2118						
	NCFGLEK	867.4029	2	434.2046						
13	SSEEMDFVIR	1228.551	2	614.7805	Snaclec 7 / Dabocetin beta subunit	Snaclec	*Daboia siamensis*	45.31	29	5
	WSDGVNLDYK	1196.558	2	598.7855						
	TTDNQWLR	1033.506	2	517.2576						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	TWEDAEK	878.389	2	439.697	(AAY63876.1)					
	MTFPIFR	927.4757	2	464.2439						
	KTWEDAEK	1006.484	2	503.7459						
	WTYFHK	881.4305	2	441.2188						
	SWADAEK	806.3679	2	403.6873						
	DGIYVWIGLR	1191.652	2	596.3308						
	IIYVNWK	935.5349	2	468.273						
	CEEPYPFVCK	1328.565	2	664.7875						
	SWEAAER	848.3897	2	424.6979						
13	RPVTYSTHIAPVSLPSR	1881.034	3	627.6849	RVV-V gamma-like protein precursor (ADP88558.1)	SVSP	*Daboia siamensis*	5.42	20	6
	NIRNEDEQIR	1286.645	3	429.5518						
	NIRNEDEQIRVPR	1638.867	4	410.4727						
	WCEPLYPWVPADSR	1775.821	2	888.4188						
	YFCLNTK	945.4499	2	473.2305						
	NEDEQIRVPR	1255.639	3	419.218						
13	SVTPTASNMLK	1148.598	2	574.8041	Secreted L-amino acid oxidase precursor (ACF70483.1)	LAAO	*Daboia russelii*	33.16	20	10
	CILNHSPYNSR	1360.643	3	454.2187						
	MEWYPEAAANAER	1537.674	2	769.3431						
	DGPGRDYSQYCSVIEDLK	2101.95	2	1051.478						
	LAADDFR	807.3995	2	404.2023						
14	AAAICFR	808.4134	2	404.7106	Basic phospholipase A2 3 (P86368.1)	PLA_2	*Daboia russelii*	131.36	82	6
	RVNGAIVCEQGTSCENR	1949.892	3	650.6334						
	SLLEFGMMILEETGK	1729.839	2	865.4252						
	IYMLYPDFLCK	1478.706	2	739.8537						
	VNGAIVCEQGTSCENR	1793.791	2	897.3978						
	CCFVHDCCYGNLPDCNPK	2315.876	3	772.629						
	ICECDKAAAICFR	1613.723	3	538.5788						
	LAVPFYSSYGCYCGWGGK	2071.904	2	1036.457						
	NLNTYSK	839.4258	2	420.215						
14	AAAICFR	808.4134	2	404.7106	Phospholipase A2 (AAZ53183.1)	PLA_2	*Daboia limitis*	120.66	66	8
	VNGAIVCEK	989.5084	2	495.2562						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	YMLYPDFLCK	1365.622	2	683.3141						
	CCFVHDCCYGNLPDCNPK	2315.876	3	772.629						
	ICECDKAAAICFR	1613.723	3	538.5788						
	LAIPSYSSYGCYCGWGGK	2025.883	2	1013.447						
	KYMLYPDFLCK	1477.722	2	739.3654						
	RVNGAIVCEK	1145.61	2	573.3059						
	YMLYPDFLCKGELK	1792.865	3	598.2984						
	QNLNTYSK	967.4843	2	484.2439						
	NPLSSYSNYGCYCGWGGK	2069.848	2	1035.428						
	VAATCFR	824.4083	2	412.7068						
14	QQCSSHWTDGSAVSYETVTK	2270.998	3	757.6708	C-type lectin-like protein subunit 8 / Dabocetin alpha subunit (ACN22046.1)	Snaclec	*Daboia siamensis*	32.85	58	6
	SKYHAWIGLR	1230.674	3	410.8962						
	YHEWITLPCGDK	1518.705	2	759.856						
	FCTQQANGWHLASIESVEEANFVAQLASETLTK	3679.77	4	920.7031						
	RQQCSSHWTDGSAVSYETVTK	2427.099	4	607.5292						
14	NCFGLEK	867.4029	2	434.2052	P68 alpha subunit (ADK22825.1)	Snaclec	*Daboia siamensis*	16.22	32	5
	TWFNLSCGDDYPFVCK	2008.857	2	1004.937						
	TPADYVWIGLR	1290.684	2	645.8469						
	AQYCISK	869.4186	2	435.2114						
	WTDGSSVIYK	1155.568	2	578.2879						
14	MTFPIFR	911.4808	2	456.2446	Snaclec 7 / Dabocetin beta subunit (AAY63876.1)	Snaclec	*Daboia siamensis*	35.62	34	5
	WSDGVNLDYK	1196.558	2	598.7851						
	SSEEMDFVIR	1228.551	2	614.7786						
	TTDNQWLR	1033.506	2	517.2584						
	FDFFWIGLR	1200.62	2	600.8147						
14	WDYVNCAEHYR	1512.632	2	756.8194	P31 alpha subunit (ADK22829.1)	Snaclec	*Daboia limitis*	19.23	47	7
	WTYFHK	881.4305	2	441.218						
	SWADAEKFCTEQANSGHLVSIK	2478.172	3	826.7261						
	FCTEQANSGHLVSIK	1690.822	2	845.9156						
	DGIYVWIGLR	1191.652	2	596.3323						
	IIYVNWK	935.5349	2	468.2712						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	SVGEANFVAQLASGFMQK	1883.932	2	942.4694						
14	MWFNHK	862.4029	2	431.7044	Snaclec coagulation factor X-activating enzyme light chain 2 (AAW69869.1)	Snaclec	*Daboia siamensis*	20.26	37	6
	SWEAAER	848.3897	2	424.6973						
	FITHFWIGLR	1289.715	3	430.5767						
	DKEQECSSEWSDGSSVSYDNLGK	2607.079	3	869.6975						
	IKDKEQECSSEWSDGSSVSYDNLGK	2848.258	3	950.0916						
	CEEPYPFVCK	1328.565	2	664.7868						
	TWEAAER	862.4054	2	431.7038						
	EQECSSEWSDGSSVSYDK	2079.808	2	1040.408						
	KCFVLEK	923.5019	2	462.2536						
14	RPEIQNEIVDLHNSLR	1933.025	3	645.0136	Cysteine-rich seceretory protein Dr-CRPK (ACE73567.1)	CRISP	*Daboia russelii*	25.24	36	4
	RPEIQNEIVDLHNSLRR	2089.126	4	523.0384						
	MEWYPEAAANAER	1553.669	2	777.339						
	WTAIIHEWHK	1320.685	3	440.8995						
	SVTPTASNMLK	1148.598	2	574.8						
	CILNHSPYNSR	1360.643	2	680.8247						
	DFVYGQGASPANAVVGHYTQIVWYK	2770.362	3	924.127						
14	NNAEIRLPEER	1340.692	3	447.569	Serine beta-fibrinogenase-like protein precursor (ADP88560.1)	SVSP	*Daboia siamensis*	51.45	17	3
	WDKDIMLIK	1161.634	2	581.3216						
	TSTYIAPLSLPSSPPR	1686.906	2	843.958						
	FFCLSNK	915.4393	2	458.2237						
14	WDKDIMLIK	1161.634	2	581.3216	Serine alpha-fibrinogenase-like protein precursor (ADP88559.1)	SVSP	*Daboia siamensis*	20.29	23	5
	TLCAGVSGR	920.4618	2	460.733						
	EKFFCLSSK	1145.566	2	573.2883						
	FFCLSSK	888.4284	2	444.7185						
	LNKPVTYSTHIASLSLPSNPPR	2392.298	3	798.1058						
	IMGWGSITSPK	1176.608	2	588.8102						
14	RPVTYSTHIAPVSLPSR	1881.034	3	627.6834	RVV-V gamma-like protein precursor (P86531.1)	SVSP	*Daboia siamensis*	52.15	41	11
	ISTTEDTYPDVPHCTNIFIVK	2450.191	3	817.4053						
	NIRNEDEQIRVPR	1638.867	3	546.9605						
	NIRNEDEQIR	1286.645	3	429.5513						

HPLC Fraction	Sequence	MH+	z	m/z	Protein (Accession No.)	Family	Homology with	Sequest score	Coverage %	Unique Peptides
	NEDEQIRVPR	1255.639	3	419.2179						
	LRRPVTYSTHIAPVSLPSR	2150.219	4	538.3112						
	WCEPLYPWVPADSR	1775.821	2	888.4138						
	YFCLNTK	945.4499	2	473.229						
	TLCAGILK	875.5019	2	438.2542						
	EWVLTAAHCDR	1357.632	3	453.2158						
	FPNGLDKDIMLIR	1531.83	3	511.2842						
14	YPGSPGSYAVR	1153.564	2	577.2866	Glutaminyl-peptide cyclotransferases (AFE84762.1)	GPC	*Daboia russelii*	12.62	13	4
	WSPSDSLYGSR	1254.575	2	627.7911						
	TFSNIISTLNPLAK	1518.853	2	759.9338						
	LIFFDGEEAFVR	1442.731	2	721.8718						
14	SHDNALLFTDMR	1419.669	2	710.337	RVV-X heavy chain (AUF41652.1)	SVMP	*Daboia siamensis*	175.86	28	3
	NQCISLFGSR	1181.573	2	591.2901						
	SVGIVQVQGNR	1156.643	2	578.8257						
	FDLNTLGITFLAGMCQAYR	2191.067	2	1096.043						
	GMVDPGTKCEDGK	1393.609	3	465.2059						
	ASQLVATSAQFNK	1364.717	3	455.5774						
	DSCFQENLK	1140.499	2	570.7545						
	DQLQQNGQPCQNNR	1699.757	2	850.3788						
	CILYPPLR	1031.571	2	516.2904						
	LFCLNNSPGNK	1263.615	2	632.3115						
	KSHDNALLFTDMR	1547.763	2	774.3858						
	GYCYNGDCPIMR	1505.597	2	753.3051						
	KASQLVATSAQFNK	1492.812	3	498.2777						
	KIPCAPQDVK	1155.619	2	578.3107						
	IPCAPQDVK	1027.524	2	514.2657						
	GYCYNGDCPIMR	1521.592	2	761.2998						
	TAVIMAHELSHNLGMYHDGK	2224.064	4	556.7708						
	LFCLNNSPR	1120.557	2	560.7815						

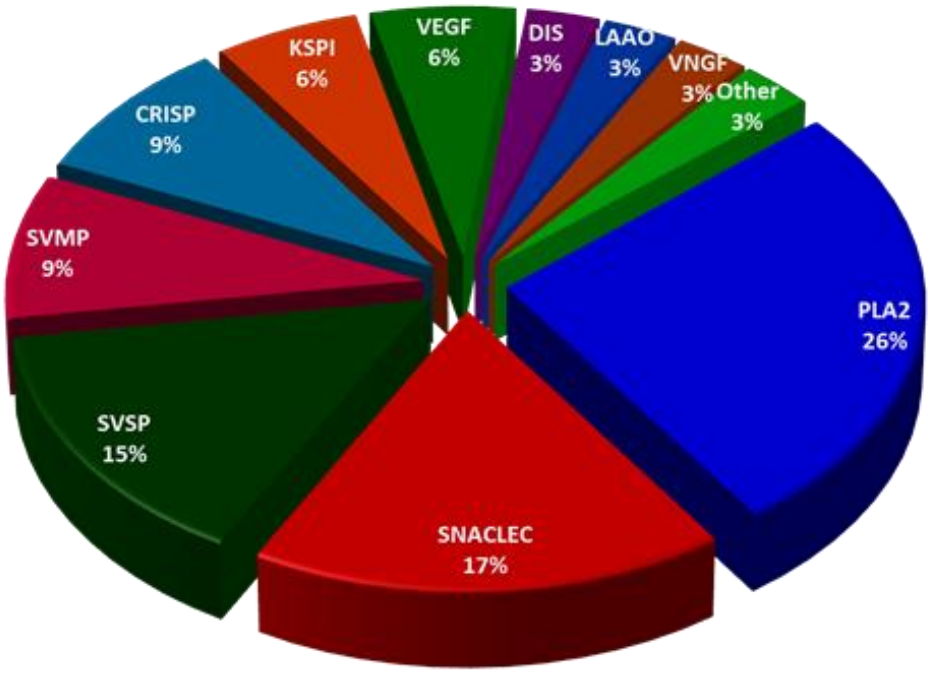


Figure 3.3: Relative distribution of various protein families in *Daboia russelii* venom (PLA_2: phospholipase A_2, SNACLEC: snake venom C-type lectin, SVSP: snake venom serine protease, DIS: disintegrin, KSPI: kunitz-type serinne protease inhibitor, SVMP: snake venom metalloprotease, VEGF: vascular endothelial growth factor, CRISP: cysteine rich secretory peptide, LAAO: L-amino acid oxidase, VNGF: vascular nerve growth factor)

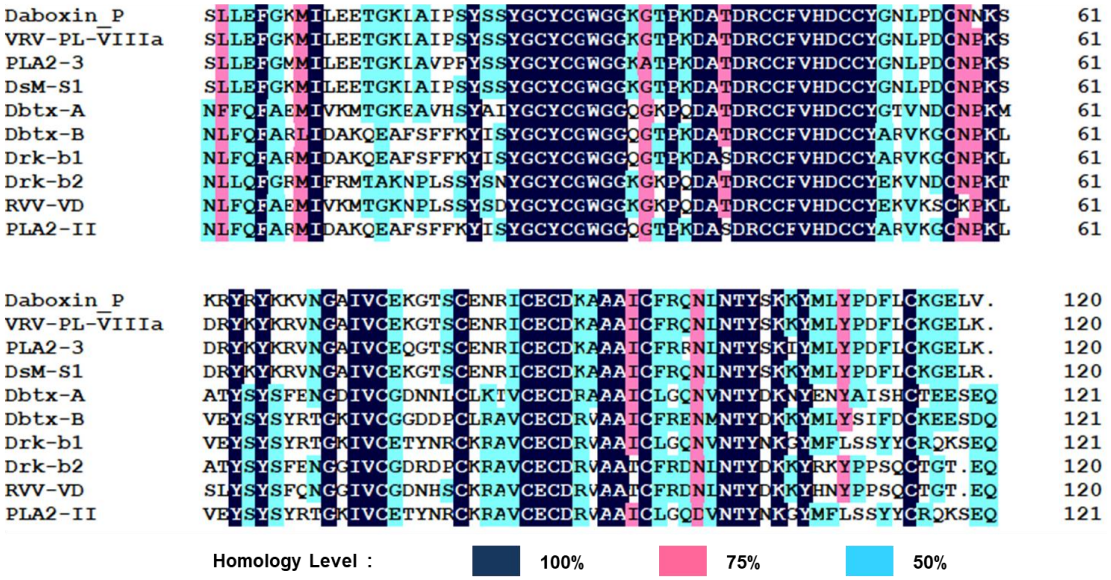


Figure 3.4: Multiple sequence alignment of identified phospholipase A_2 enzymes: The amino acid sequences of the PLA_2 enzymes were retrieved from NCBI database and aligned using DNAMAN 4.1.5.1 software

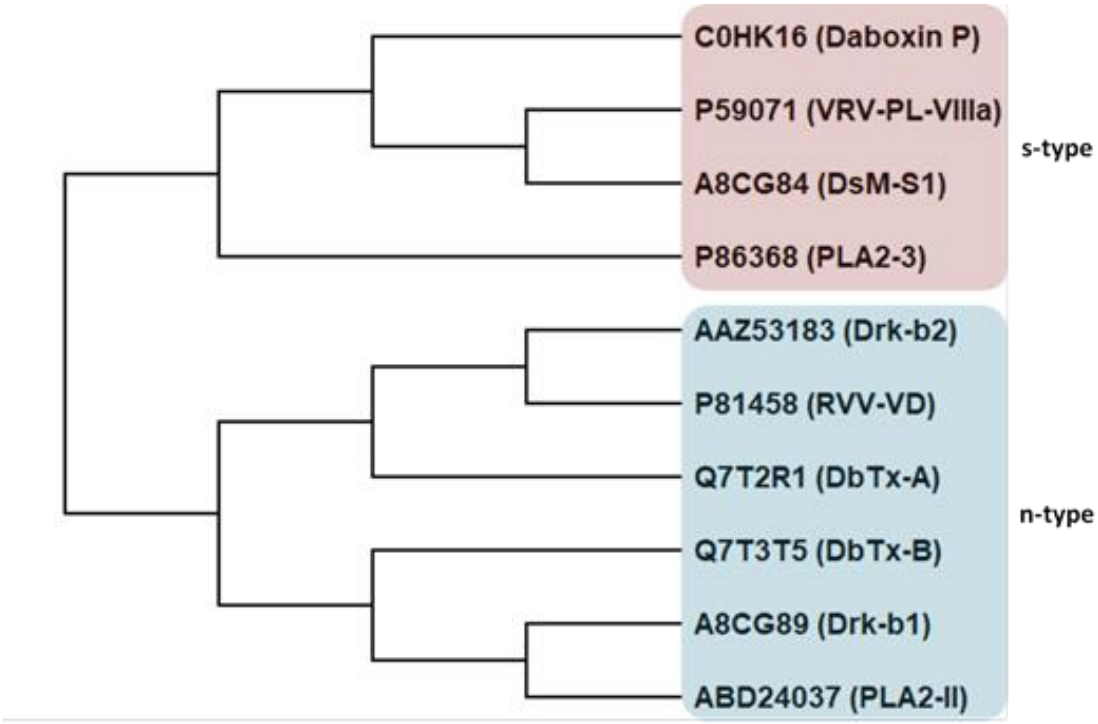


Figure 3.5: Phylogenetic tree of identified phospholipase A_2 enzymes: The amino acid sequences of the PLA_2 enzymes retrieved from NCBI database were aligned using ClustalW and phylogenetic tree was constructed using Neighbour joining algorithm with MEGA11 software

3.2.3 Partial biochemical characterization of crude *Daboia russelii* venom:

3.2.3.1 Coagulation assays:

Crude *Daboia russelii* venom exhibited dose-dependent procoagulant activity when tested in goat PPP with respect to the NCT (Fig 3.6A). With increase in dose from 0.01µg/ml, 0.1µg/ml, 1µg/ml and 10µg/ml, recalcification time was 90±2.6, 60±2.6, 45±2.6 and 30 sec respectively, while the NCT was 180±8 sec. Prothrombin time at concentrations 0.01µg/ml, 0.1µg/ml, 1µg/ml and 10µg/ml were 19±0.3, 15±0.8, 5±0 and 2±0 respectively, whereas the NCT was 22±2 sec. Moreover, at concentrations of 0.01µg/ml, 0.1µg/ml, 1µg/ml and 10µg/ml, activated partial thromboplastin time was 13±1.1, 9±1.1, 2±0 and 2±0 respectively, and the NCT was 18±3.

3.2.3.2 Phospholipase A_2 (PLA_2) activity:

Crude *Daboia russelii* venom exhibited PLA_2 activity in a dose-dependent manner. At concentrations of 0.01µg/ml, 0.1µg/ml, 1µg/ml and 10µg/ml, the PLA_2 activity was found to be 0.25±0.05, 0.5±0.18, 1.66±0.29 and 3.2±0.09 U respectively (Figure 3.6B).

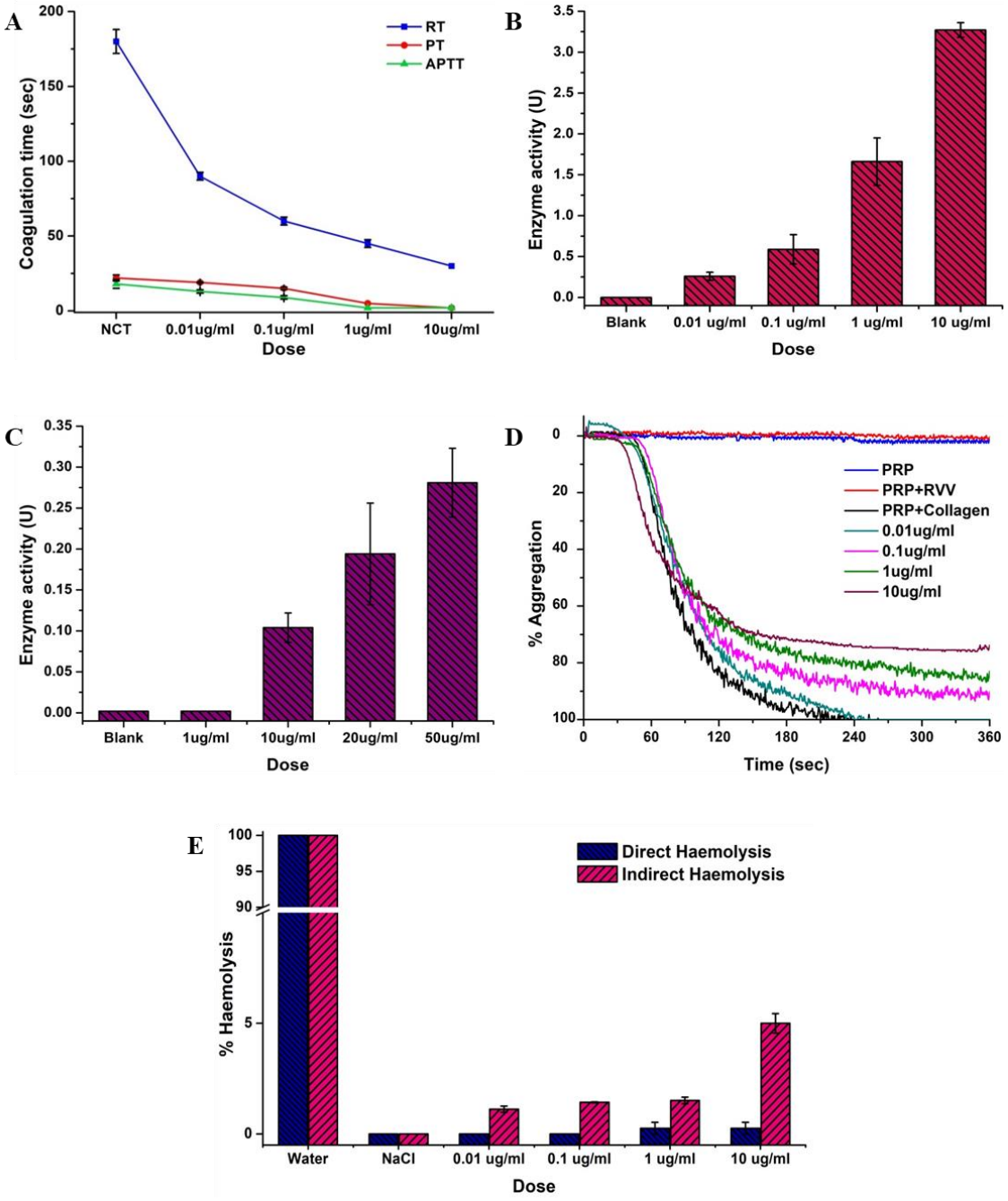


Figure 3.6: Partial characterization of *Daboia russelii* venom: (A) Coagulation assays (NCT- Normal Clotting Time, RT- Recalcification Time, APTT- Activated Partial Thromboplastin Time, PT- Prothrombin Time) **(B) Phospholipase A_2 activity (**PLA_2 activity was determined using turbidometric method) **(C) Caesinolytic activity (D) Platelet aggregation assay** (PRP- Platelet Rich Plasma, RVV- Russell's viper venom) **(E) Haemolytic assay** (Haemolytic activity of water and NaCl are taken as positive control and negative control respectively. Percentage haemolysis by water is considered as 100%) Results of A,B,C and E are mean ± SD of three independent experiments

3.2.3.3 Caesinolytic activity:

Caesinolytic activity of crude *Daboia russelii* venom was observed to increase in a dose-dependent manner. At concentrations of 10µg/ml, 20µg/ml and 50µg/ml caesinolytic activity was 0.104±0.02, 0.194±0.06 and 0.281±0.04 U respectively (Figure 3.6C). However, no activity was observed in lower concentrations such as 0.01 µg/ml, 0.1µg/ml and 1µg/ml (data not shown for 0.001 µg/ml and 0.1µg/ml)

3.2.3.4 Haemolytic activity:

Crude *Daboia russelii* venom exhibited slight direct haemolytic activity. At lower doses, 0.01µg/ml and 0.1µg/ml, no direct haemolysis of RBC was observed. Howevver, at both concentrations of 1µg/ml and 10µg/ml, 0.26±0.2% direct haemolytic activity was observed. Moreover, it exhibited indirect haemolytic activity in a dose dependent manner. At concentrations, 0.01µg/ml, 0.1µg/ml, 1µg/ml and 10µg/ml indirect haemolytic activity was observed to be 1.12±0.14, 1.43±0.02, 1.52±0.15, and 5±0.44 respectively (Figure 3.6).

3.2.3.5 Platelet aggregation assay:

The percentage aggregation of PRP with and without addition of the venom was 0% suggesting that the crude *Daboia russelii* venom did not initiate the aggregation of PRP. However, it inhibited platelet aggregation induced by collagen in a dose dependent manner (Figure 3.6D). It was observed that in presence of crude venom at concentrations 0.01, 0.1, 1 and 10 µg/ml of *Daboia russelii* venom, aggregation induced by 2µg/ml collagen was found to be 100%, 90%, 85% and 73% respectively.

3.3 DISCUSSION:

Advances in development of proteomics tools has made studies on venom proteomes more convenient, aiding toxicologists to better understand detailed pharmacological profiles and appreciate their intricacies. This knowledge can be crucial in understanding the signs and symptoms of envenomation triggered by these components as well as their mechanisms of action. Besides, comparative venom proteome profiles of snakes belonging to different geographical locations can enrich the current knowledge about venom variation and similarities [2,127]. In this chapter, using a combination of chromatographic and mass spectrometric techniques, we profiled the proteome of

Daboia russelii venom from Tanore, Rajshahi, Bangladesh (24˚43'27"N 88˚25'01.3"E). Moreover, this venom was partially characterized and its anti-platelet property was assessed.

The crude venom was subjected to SDS-PAGE and the electrophoretic separation exhibited the presence of various high and low molecular weight proteins in the range of ~150 – 15 kDa of varying intensities. Under reducing conditions, a total of 14 bands were observed with 7 prominent bands at ~100, 72, 60, 28, 25, 20 and 15 kDa; and 7 relatively faint bands at ~120, 95, 50, 40, 30, 26 and 18 kDa. However, under non-reducing conditions, apart from the bands observed in the reducing gel, a prominent band at 27 kDa and a faint band at 150 kDa were observed. The differences in bands in the reducing and non-reducing SDS-PAGE confirms the presence of enzymes and toxins belonging to various snake venom protein families like snake venom metalloproteases, snake venom serine proteases, L-amino acid oxidases, Snake C-type lectin like proteins, Phospholipase A_2 enzymes etc. Although most snake venom proteins exist as monomers, some of them are known to form complexes in the venom [3]. The differences in the electrophoretic profiles under reducing and non-reducing conditions suggest the presence of such proteins that exist as complexes in this venom.

Daboia russelii venom is known to be haemotoxic in nature [133]. In order to understand its role in manipulating the haemostatic system, the crude venom was partially characterized and it was found to exhibit procoagulant activity, phospholipase A_2 activity, indirect haemolytic activity and inhibition of collagen induced platelet aggregation. The most common clinical feature observed due to *Daboia russelii* envenomation is incoagulable blood. On contrary, in vitro coagulation assays performed during this study revealed the procoagulant nature of crude *Daboia russelii* venom from Bangladesh similar to its other counterparts of the subcontinent [1,113,130]. It hastened the clotting time of goat PPP in RT, PT and APTT assays suggesting the predominance of procoagulant components in the venom. Incoagulable blood occurs due to defibrination resulting from consumption of the components of the haemostatic system also known as consumption coagulopathy [134]. Once the patient's blood becomes defibrinated and incoagulable, abnormalities of platelet function leads to spontaneous systemic haemorrhage [81,135]. The proteolytic activity exhibited by the crude venom might be an indication of the presence of proteases while indirect haemolysis and PLA_2

activity suggests the presence of phospholipase A_2 enzymes. The effect of *Daboia russelii* venom on platelet aggregation was also studied. Although it did not have any direct effect on platelet aggregation, it inhibited collagen induced platelet aggregation. This suggests possible presence of snake venom protein families like Snaclecs and LAAO in the venom [136,137]. Presence of the protein families were subsequently confirmed by proteomic analysis.

A detailed exploration of the venom proteome by LC-MS/MS analysis revealed the presence of 37 different protein isoforms belonging to 11 different snake venom protein families (Table 2.1). Among the identified protein families, phospholipase A_2 (PLA_2) was found to be the most abundant. This is in agreement with the previously reported proteome profiles of *Daboia russelii* venom from the Indian subcontinent where PLA_2 was found to be the most abundant protein family. The previous studies reported that the relative abundance of PLA_2 was 22%, 23% 32.5% and 32.8% in the venom of *Daboia russelii* from West Bengal, Tamil Nadu, Maharashtra and Pakistan respectively [2,128–130]. In the present study, PLA_2 contributed to 26% of the proteome. This was closely followed by snake C-type lectin (snaclec) with 17% and snake venom serine protease (SVSP) with 15%. Snake venom metalloprotease (SVMP) and cysteine-rich secretory peptide (CRISP) each contributed 9% to the proteome. Disintegrins, kuntiz-type serine protease (KSPI) and vascular endothelial growth factor (VEGF) contributed 6% each while L-amino acid oxidase (LAAO), vascular nerve growth factor (VNGF) and glutaminyl-peptide cyclotransferase (GPC) were present in low abundance with only 3% each.

PLA_2 enzymes are known to induce a wide variety of pharmacological effects including coagulopathy, haemorrhage, haemolysis, myotoxicity, neurotoxicity, hypotension, cardiotoxicity, nephrotoxicity etc. in addition to their role in digestion [138]. In this proteome, 10 isoforms including an acidic PLA_2, daboiatoxin (Dbtx A Accession 2H4C and Dbtx B Accession Q7T3T5.1) and 8 basic PLA_2s VRV-PL-VIIIa (Accession1Q6V), basic phospholipase A_2 3 (Accession P86368.1), Daboxin P (Accession C0HK16), Drk-b1 (Accession A8CG89.1), Drk-b2 (Accession AAZ53183.1), DsM-S1 (Accession A8CG84.1), RVV-VD (Accession P81458.1) and phospholipase A_2-II (Accession ABD24037.1) were identified. Daboiatoxin is a presynaptic neurotoxin previously reported from the venom of *Daboia siamensis*, which

exists as heterodimer consisting of A and B chains (DbtxA and DbtxB respectively). DbtxA potentiates the lethality of DbtxB making Daboiatoxin the main lethal PLA_2 toxin of *Daboia siamensis* [139]. Although the presence of DbtxB has been reported earlier in *Daboia russelii* venom from the Indian sub-continent, this study reports presence of both Dbtx A and Dbtx B in *Daboia russelii* venom [87,128,130]. Drk-b1 and Drk-b2 are neurotoxic PLA_2s cloned from cDNA obtained from venom glands of Kolkata *Daboia russelii* whereas, RVV-VD is a strong anticoagulant PLA_2 [140,141]. These were reported earlier from the venom of *Daboia russelii* from Western India, Eastern India, Pakistan and Bangladesh. However, two acidic PLA_2s, Drk-a1 and Drk-a2, earlier detected from the venom of Bangladesh *Daboia russelii* was not detected in this proteome [87]. Additionally, four s-type PLA_2s, namely, VRV-PL-VIIIa, basic phospholipase A_2 3, Daboxin P and DsM-S1 were detected in this proteome. Daboxin P is the major PLA_2 enzyme present in the South Indian and Sri Lankan *Daboia russelii* venom [2,91,142]. It was also detected earlier in the venom of *Daboia russelii* from Burdwan, West Bengal [129].

Snake venom proteins can be termed as major toxins based on their high abundance in the proteome and toxicity [142]. Cobrotoxin, for instance, is the major neurotoxic protein isolated from the venom of *Naja naja atra* [143]. Likewise, Daboiatoxin and Daboxin P are the major PLA_2s in the venom of *Daboia siamensis* from Myanmar and *Daboia russelii* from India respectively. Expression of only one chain of Daboiatoxin probably allows Daboxin P to be the most lethal component of *Daboia russelii* venom of the Indian sub-continent. And this variation in expression might be a result of differences in prey availability over time [86]. However, Bangladesh being geographically close to India and Myanmar, expression of both these lethal PLA_2s in the venom gland might be due to habitat and diet

Viperid PLA_2 enzymes can be classified as Asp49, Lys49, Ser49, Asn49 or Arg49 based on the amino acid present at the 49^{th} position, [144–147]. It has been reported that presence of Asp at the 49^{th} position is crucial for Ca^{2+} binding and, thus, for the catalytic activity and its substitution by other amino acid residues has rendered them with partial or complete loss of catalytic activity[148–150]. Again, PLA_2 enzymes have been classified into three types: N, S and H type based on the first amino acid residue in the N-terminal [151]. While the s-type PLA_2s are predominant in South Indian and Sri

Lankan *Daboia russelii* venom, the n-type PLA_2s are prevalent in the East Indian *Daboia russelii* and Burmese *Daboia siamensis* venom [87]. The *Daboia russelii* venom from Pakistan, however, which is considered as origin of the two radiation routes, expresses both the PLA_2 types [87]. Interestingly, in this study, multiple sequence alignment of the PLA_2s identified in the proteome of *Daboia russelii* from Bangladesh revealed the presence of both the s-type and n-type PLA_2s (Fig. 4). Furthermore, the phylogenetic analysis exhibited that these two types separated into different branches of the phylogram suggesting them to be evolutionarily distinct clades (Fig. 5). This similarity of the Bangladesh *Daboia russelii* venom with its Pakistani counterpart indicates their close evolutionary kinship. Besides, *Daboia russelii* from Bangladesh can be considered to be an early point in the dispersal route. Phospholipase A_2 enzymes have been reported to exist as monomers as well as complexes [3]. Presence of this family in 11 out of 14 RPHPLC fractions perhaps advocates the interaction of this enzyme with other proteins of the proteome in order to enrich toxicity of the interacting components.

Snaclecs were found to be the most abundant non-enzymatic protein family present in this proteome contributing to 17% with 8 isoforms. Snaclecs are large quaternary structures of disulfide linked homo or heterodimers; the molecular weight of the monomers range from 8 to 16 kDa [152]. They have been reported to manipulate blood coagulation cascade as well as the platelet aggregation pathway by binding to a wide range of coagulation factors, membrane receptors on platelets and other proteins critical in haemostasis [11,38,153]. Isoforms of both α and β subunits of dabocetin (Accession ABA86561.1 and AAY63876.1) were identified in this proteome. Dabocetin has been reported to induce inhibitory effect on ristocetin-induced platelet aggregation [154]. Similarly, α and β subunits of P31 (Accession ADK22829.1 and ADK22832.1 respectively), α subunit of P68 (AccessionADK22825.1) and both the light chains of coagulation factor X-activating enzyme were also identified (Accession Q4PRD1 and ADJ67473.1). These snaclecs were documented earlier in *Daboia russelii* venom from multiple locations of the Indian subcontinent as well as from the venom of *Daboia siamensis.* Moreover, in this study, a protein similar to coagulation factor X-activating enzyme light chain 1, Snaclec 6 (Accession Q4PRC7.1) was detected. This was identified earlier in the venom gland cDNA library of *Daboia siamensis* [155].

SVSPs were found to be the third most abundant protein family constituting 15% of the proteome with 5 isoforms. These enzymes disrupt the various aspects of the haemostatic system by degrading or selectively activating factors involved in the blood coagulation cascade or fibrinolysis [153]. The alpha and gamma isoproteins of the factor V activating enzyme (RVV-V) were identified (Accession P18964.1 and 3S9C respectively) in this proteome. RVV-V selectively activates the coagulation factor V to factor Va by a single cleavage at the Arg1545-Ser1546 bond [15,156]. RVV-V alpha has been previously reported from the venom proteome of *Daboia russelii* across the Indian sub-continent. However, RVV-V gamma was not detected in the earlier reported proteomes of *Daboia russelii* venom from South India, Western India, Sri Lanka and Bangladesh. Furthermore, two trypsin-like serine proteases, serine α-fibrinogenase like protein precursor (Accession ADP88559.1) and serine β-fibrinogenase like protein precursor (Accession ADP88560.1), which degrades the alpha and beta chain of fibrinogen respectively were identified. [157]. Both these serine protease precursors were previously detected in the venom samples of South India, West Bengal and Bangladesh [2,87,128,130,142,158]. Presence of these proteins belonging to SVSP family might be responsible for consumption coagulopathy leading to incoagulable blood and profuse bleeding observed during *Daboia russelii* envenomation.

CRISPs are non-enzymatic proteins known to target ion channels and mediate inflammatory responses in envenomed victims [4,159]. The proteome of *Daboia russelii* venom from Tanore, Bangladesh revealed the presence of three CRISPs, cysteine-rich secretory protein Dr-CRPK (Accession ACE73567.1), cysteine-rich secretory protein Dr-CRPB (Accession ACE73568.1) and cysteine-rich secretory protein (Accession ASU45033.1).

SVMP is an important enzymatic protein family of the viperidae venoms. These cause injuries at both local and systemic levels in the envenomed victims attributing to a wide range of pathophysiological implications such as haemorrhage, myonecrosis, dermonecrosis, edema, coagulopathy etc [4]. In this study, the proteome of *Daboia russelii* venom from Tanore, Bangladesh revealed the presence of 3 SVMPs, the heavy chain of coagulation factor X activating enzyme (RVV-X) (Accession AUF41652.1), daborhagin K (Accession B8K1W0.1) and *Daboia siamensis* apoptosis-inducing protease (DSAIP) (Accession AUF41660.1). RVV-X is a P-III metalloprotease with a

heavy chain and two light chains connected to each other by inter-chain disulphide bonds [16]. It converts coagulation factor X to Xa by cleaving at the Arg51-Ile52 of factor X leading to severe coagulopathy in victim [160]. Daborhagin K is a P-III metalloproteinase with hemorrhagic activity and proteolytic activity toward fibrinogen, fibronectin and collagen; which is predicted to be responsible for severe bleeding symptoms observed in *Daboia russelii* victims [97]. It has been previously identified from the venom of *Daboia russelii* from Eastern India and Bangladesh [87,97,129]. DSAIP, another P-III metalloproteinase was first detected in the transcriptome of *Daboia siamensis* from Myanmar [161]. However, its homolog VLAIP, known to proteolytically cleave fibrinogen, inhibit endothelial cell adhesion to extracellular matrix proteins and induce apoptosis of human endothelial cells, had been identified earlier in the venom of *Daboia russelii* from Sri Lanka and Pakistan [130,142,162].KSPIs are the low molecular weight toxins having around 60 amino acid residues with a conserved Kunitz-type motif [163]. Upon envenomation, these proteins are reported to inhibit trypsin and chymotrypsin along with potassium and calcium ion channels as well as exhibit diverse pharmacological effects on the victim like fibrinolysis and anticoagulation [164–166]. In this proteome, 2 isoforms of KSPI were identified having sequence similarity with KSPI (Accession AFD04724.1) and KSPI B5 (Accession A8Y7P5.1). KSPI B5, a chymotrypsin inhibitor first reported from Burmese *Daboia siamensis* was also detected in *Daboia russelii* venom from South India, Eastern India and Sri Lanka [167].

VEGFs are endothelial cell-specific mitogens, angiogenic inducers as well as mediators of vascular permeability [168]. They exert numerous effects by activating of two distinct endothelial receptor tyrosine kinases: KDR (kinase insert domain-containing receptor) and Flt-1 (fms-like tyrosine kinase-1). Snake venom VEGFs function by specific activation of KDR and contribute to venom dispersion and prey subjugation by inducing vascular permeability and hypotension [169]. VEGF constituted 6% of the proteome in this study with 2 isoforms, VEGF A isoform 1 (Accession ASU45062) and VR-1 precursor (Accession ACN22046), both of which were previously isolated from the venom of Indian *Daboia russelii* [169].

Disintegrins are a family of cysteine-rich, low molecular weight proteins with 40-100 amino acid residues in the molecular weight range of 4-14 kDa that are generated by

proteolytic cleavage of multidomain metalloproteases [170]. They are known to specifically inhibit integrin receptors, thus interfering with platelet activation, aggregation, blood clot formation and inhibiting cell-matrix and cell-cell adhesion [171,172]. In this study, a Disintegrin (Accession AUF41656.1) previously reported from *Daboia siamensis* venom was identified.

VNGFs are reported to cause apoptosis, vascular permeability and wound healing [173,174]. VNGF represented the lowest non-enzymatic family with 1 isoform, beta nerve growth factor (Accession ASU45040.1) which was earlier detected from the *Daboi russelii* venom of South India and Pakistan [87,130].

LAAOs catalyse the oxidation of L-amino acids releasing α-keto acid, ammonia and hydrogen peroxide. They are reported to affect the haemostatic system by various mechanisms including platelet aggregation activation and/or inhibition, haemorrhage of endothelial cells in addition to apoptosis of vascular endothelial cells induced by hydrogen peroxide [175]. In this proteome, secreted LAAO precursor (Accession ACF70483) was detected. This protein, isolated earlier from Eastern India *Daboia russelii* had shown to induce edema and inhibited ADP- and collagen-induced platelet aggregation [176].

Apart from the proteins belonging to the snake venom protein families, another enzyme, glutaminyl-peptide cyclotransferase (GPC) (Accession AFE84762.1) was detected in the proteome of *Daboia ruusselii* venom from Tanore, Rajshahi, Bangladesh. It is one of the enzymes crucial for protein post-translational modifications that are essential for structural stability, resistance to aminopeptidase degradation, and interaction of the proteins or peptides [177].

The present study unveils the proteome of *Daboia russelii* venom from Tanore, Rajshahi, Bangladesh which is comprised of different proteins and peptides belonging to various enzymatic and non-enzymatic families. The proteome profile shows similarities as well as differences with the previously reported proteomes of conspecific venoms from different locations of India, Pakistan, Sri Lanka and Bangladesh. The intra-specific similarities and differences indicate evolutionary relationships as well as geographical variation between *Daboia russelii* venom from different geographical locations. Such variation draws attention towards the major limitations of the present-day antivenom therapy such as paraspecific inefficacy and calls for “region-specific”

antivenoms to alleviate these challenges. The high abundance protein families present in this proteome are PLA_2, Snaclec, SVSP, SVMP and CRISP. Presence of a large number of proteins belonging to these families probably contributes to the haemotoxic nature of the venom. Besides, they might be responsible for the classical symptoms observed post *Daboia russelii* envenomation such as haemostatic disturbances leading to spontaneous bleeding and painful progressive swelling [133,178].

Large number of the SVMPs and SVSPs present in this venom proteome probably contributes to the observed in-vitro procoagulant activity. However, many proteases were identified; the proteolytic activity of the crude venom on casein was considerably low, which could possibly be due to non-specificity of these enzymes towards casein as substrate. PLA_2s are the most abundant protein family in this proteome. They are responsible for procoagulant and indirect haemolytic activity observed in the in-vitro assays. The PLA_2s along with snaclecs, SVSPs, disintegrins and LAAOs might be responsible for the anti-platelet activity observed. In the subsequent chapter, we shall seek to identify and characterize an anti-platelet protein from this venom.