Comparing Traditional and Toxin-Oriented Approaches towards Antivenom Production against *Bitis arietans* Snake Venom

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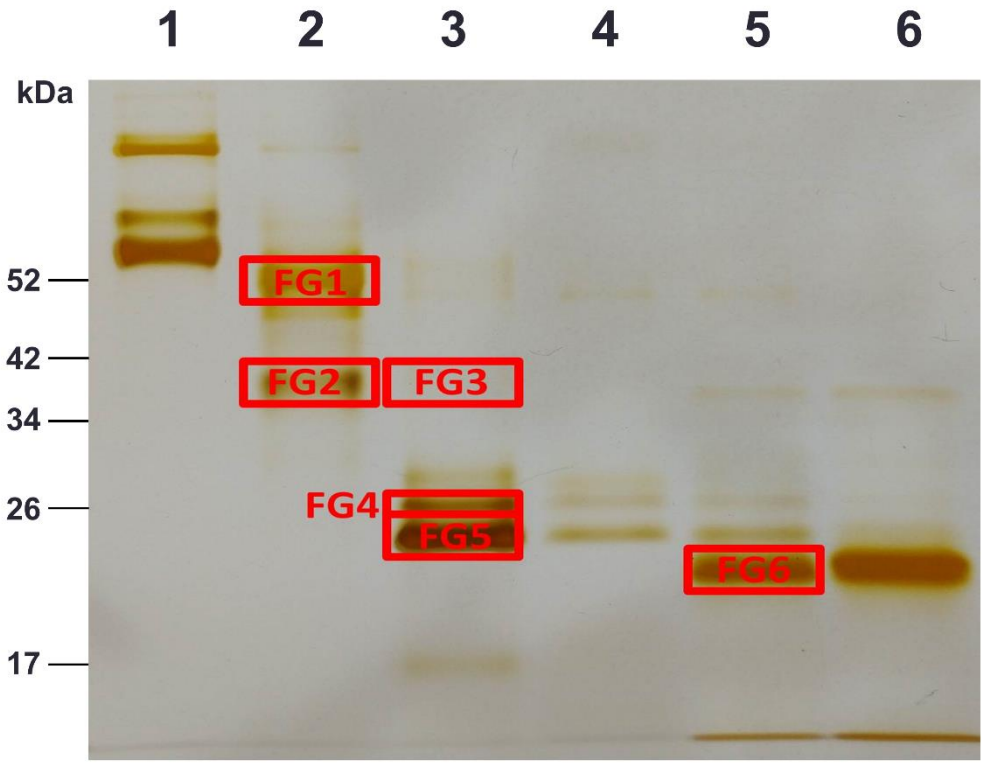

Supplementary Figure S1. Selection of protein bands from peaks 2, 3 and 5 from fractionating of *B. arietans* venom by size exclusion chromatography to protein identification on LC-MS/MS analysis. 5 µg of each peak were submitted to 12.5% SDS-PAGE, in non-reducing conditions. Selected bands (FG1 to FG6) were cut and submitted to in-gel trypsin digestion.

Supplementary Table S1. Identification of protein bands content by LC-MS/MS from fractions obtained from *B. arietans* venom af ter size exclusion chromatography.

Sample	Score	Accession	Description	OS
FG1	78	SLRB_BITHR	Snaclec rhinocetin subunit beta	*Bitis rhinoceros*
	49	A0A140DC05_BITAR	C-type lectin 1b	*Bitis arietans*

	50	**SLA_BITAR**	**Snaclec bitiscetin subunit alpha**	***Bitis arietans***
	32	**A0A0B6VJU7_PROFL**	**Beta-actin**	***Protobothrops flavoviridis***
	32	**A0A0F7Z857_CROAD**	**Poly(a)-specific ribonuclease PARN**	***Crotalus adamanteus***
	28	**A0A1I9KNN4_VIPAA**	**Serine proteinase SP-3**	***Vipera ammodytes ammodytes***
	28	**A0A0F7Z8B1_CROAD**	**E3 ubiquitin-protein ligase**	***Crotalus adamanteus***
	18	**A0A6B2FD68_9SAUR**	**C-type lectin domain-containing protein**	***Bothriechis nubestris***
	17	**A0A0K8RXM4_CROHD**	**Cyclin-dependent kinase 1-like**	***Crotalus horridus***
	131	**A0A194AM96_9SAUR**	**Serine proteinase 17**	***Agkistrodon piscivorus***
	42	**A0A194APC3_9SAUR**	**Serine proteinase 14b**	***Agkistrodon piscivorus***
	58	**A0A8A8C7M3_BOTJA**	**Thrombin-like enzyme**	***Bothrops jararaca***
	56	**VSPY_MACLB**	**Chymotrypsin-like protease VLCTLP**	***Macrovipera lebetina***
	66	**E9JG14_ECHCS**	**Serine protease (fragment)**	***Echis carinatus sochureki***
	61	**D5KRX9_ECHOC**	**Snake venom serine protease (fragment)**	***Echis ocellatus***
	116	**A0A6B7FPJ0_VIPAA**	**Serine proteinase SP-8**	***Vipera ammodytes ammodytes***
	81	**J3RZT5_CROAD**	**Keratin, type I cytoskeletal 19**	***Crotalus adamanteus***
	58	**SLRB_BITRH**	**Snaclec rhinocetin subunit beta**	***Bitis rhinoceros***
	50	**SLA_BITAR**	**Snaclec bitiscetin subunit alpha**	***Bitis arietans***
	57	**A0A2H4Z2W1_DABSI**	**RVV-X heavy chain**	***Dabola siamensis***
	52	**A0A0F7ZB82_CROAD**	**Ankyrin repeat and BTB/POZ domain-containing protein 2**	***Crotalus adamanteus***
	48	**A0A1I9KNL5_VIPAA**	**Serine proteinase SP-2**	***Vipera ammodytes ammodytes***

FG2	45	A0A0B8RV71_CROHD	Keratin, type II cytoskeletal cochleal-like	*Crotalus horridus*
	44	OXLA_DABRR	L-amino-acid oxidase	*Daboia russelii*
	43	A0A0F7ZAB1_CROAD	Keratin, type I cytoskeletal 18-like protein	*Crotalus adamanteus*
	36	A0A1W7RD94_AGKCO	Sister chromatid cohesion protein PDS5	*Agkistrodon contortrix contortrix*
	34	A0A0F7ZBJ1_CROAD	FAS-associated factor 1	*Crotalus adamanteus*
	33	A0A1W7REY6_AGKCO	Sister chromatid cohesion protein PDS5 B	*Agkistrodon contortrix contortrix*
	33	A0A0F7Z8R3_CROAD	Interferon-inducible GTPase 5-like	*Crotalus adamanteus*
	32	A0A0F7Z8B1_CROAD	E3 ubiquitin-protein ligase	*Crotalus adamanteus*
	29	A0A194APM7_9SAUR	C-type lectin 9d	*Agkistrodon piscivorus*
	28	A0A0F7ZEI3_CROAD	Protein c20orf29	*Crotalus adamanteus*
	27	A0A0F7Z857_CROAD	Poly(A)-specific ribonuclease PARN	*Crotalus adamanteus*
	26	A0A0F7ZE88_CROAD	Dermatan-sulfate epimerase	*Crotalus adamanteus*
	26	A0A1W7RF54_AGKCO	NudC domain-containing protein 2	*Agkistrodon contortrix contortrix*
	25	A0A0F7Z7G6_CROAD	PAT1 1-like protein	*Crotalus adamanteus*
	23	J3SFD0_CROAD	SURF1-like protein	*Crotalus adamanteus*
	20	A0A0F7Z8B2_CROAD	Exosome component 10 isoform 1	*Crotalus adamanteus*
	19	A0A0F7Z5N5_CROAD	Polycomb protein SCMH1 isoform X2	*Crotalus adamanteus*
	19	A0A0F7ZCD4_CROAD	Oxidation resistance protein 1 isoform X3	*Crotalus adamanteus*
	19	A0A0F7Z415_CROAD	Serine/threonine-protein kinase TBK1-like protein	*Crotalus adamanteus*
	18	A0A0F7ZEN3_CROAD	Ankyrin repeat domain-containing protein 12-like	*Crotalus adamanteus*

	16	**A0A0F7Z988_CROAD**	**Creatine kinase**	***Crotalus adamanteus***
	16	**A0A1W7RHL5_AGKCO**	**Endoplasmic reticulum-Golgi intermediate compartment protein 2-like protein**	***Agkistrodon contortrix contortrix***
	14	**A0A0F7Z6K8_CROAD**	**Dynamin_N domain-containing protein**	***Crotalus adamanteus***
	13	**T1E5M6_CROHD**	**Acetyl-CoA C_myristoyltransferase**	***Crotalus horridus***
FG3	**73**	**VSPC_TRIGA**	**Snake venom serine protease 2C**	***Trimeresurus gramineus***
	68	**J3RZT5_CROAD**	**Keratin, type I cytoskeletal 19**	***Crotalus adamanteus***
	61	**A0A194AM96_9SAUR**	**Serine proteinase 17**	***Agkistrodon piscivorus***
	54	**D5KRX9_ECHOC**	**Snake venom serine protease (fragment)**	***Echis ocellatus***
	52	**D8MIA2_BITRH**	**Snake venom serine protease (fragment)**	***Bitis rhinoceros***
	47	**A0A6B7FPJ0_VIPAA**	**Serine proteinase SP-8**	***Vipera ammodytes ammodytes***
	42	**A0A194APM7_SAUR**	**C-type lectin 9d**	***Agkistrodon piscivorus***
	33	**A0A0F7ZAB1_CROAD**	**Keratin, type I cytoskeletal 18-like protein**	***Crotalus adamanteus***
	29	**A0A5A4WNG2_BITAR**	**Bitiscetin-3 subunit alpha**	***Bitis arietans***
	24	**A0A1W7RC08_AGKCO**	**Ubiquitin-like conjugating enzyme ATG3-like protein**	***Agkistrodon contortrix contortrix***
	23	**A0A0K8S0L2_CROHD**	**Iron-sulfur cluster assembly 1, mitochondrial-like protein**	***Crotalus horridus***
	23	**A0A0F7Z8B1_CROAD**	**E3 ubiquitin-protein ligase**	***Crotalus adamanteus***
	22	**A0A0F7ZEI3_CROAD**	**Protein C20orf29**	***Crotalus adamanteus***
	22	**A0A0F7Z7A4_CROAD**	**Protein phosphatase 1 regulatory subunit 12B-like**	***Crotalus adamanteus***
	22	**A0A1W7RF54_AGKCO**	**NudC domain-containing protein 2**	***Agkistrodon contortrix contortrix***

	21	**A0A0F7ZBJ1_CROAD**	**FAS-associated factor 1**	***Crotalus adamanteus***
	21	**A0A0F7Z958_CROAD**	**Guanine nucleotide-binding protein subunit alpha-11**	***Crotalus adamanteus***
	20	**A0A0K8RWZ3_CROHD**	**Proteasome-associated protein ECM29-like**	***Crotalus horridus***
	19	**A0A0F7Z3D0_CROAD**	**Ubiquitin C**	***Crotalus adamanteus***
	19	**A0A0F7Z434_CROAD**	**RAD21-like protein**	***Crotalus adamanteus***
	19	**A0A2H4Z2W1_DABSI**	**RVV-X heavy chain**	***Daboia siamensis***
	18	**A0A6B2FB67_BOTNI**	**U6 snRNA-associated Sm-like protein LSm8**	***Bothriechis nigroviridis***
FG4	**37**	**I7JA31_BITRH**	**C-type lectin-like protein 4**	***Bitis rhinoceros***
	33	**A0A0C5DKL1_MACLB**	**C-type lectin-like protein 3A**	***Macrovipera lebetina***
	33	**A0A140DC06_BITAR**	**C-type lectin 2**	***Bitis arietans***
	33	**A0A1B3AXS3_BITAR**	**C-type lectin-like protein**	***Bitis arietans***
	33	**A0A6B7FMP6_VIPAA**	**C-type lectin-like Snaclec-9**	***Vipera ammodytes ammodytes***
	33	**SLAE_MACLB**	**Snaclec A-14**	***Macrovipera lebetina***
	33	**SLAF_MACLB**	**Snaclec a15**	***Macrovipera lebetina***
	33	**SLA_DABPA**	**Snaclec VP12**	***Daboia palaestinae***
	33	**SLA2_BITGA**	**Snaclec 2**	***Bitis gabonica***
	33	**I7JX23_BITRH**	**C-type lectin-like protein 3**	***Bitis rhinoceros***
	33	**K9JBV0_DABSI**	**P68 alpha subunit**	***Daboia siamensis***
	33	**K9JDF2_DABRR**	**P68 alpha subnunit**	***Daboia russelii limitis***
	27	**A0A0C5DKK6_MACLB**	**C-type lectin-like protein 2A**	***Macrovipera lebetina***
	27	**SLMA_MACLB**	**Snaclec macrovipecetin subunit alpha**	***Macrovipera lebetina***

FG5	**54**	**SLA_BITAR**	**Snaclec bitiscetin subunit alpha**	***Bitis arietans***
	52	**SLB6_MACLB**	**Snaclec B6 (fragment)**	***Macrovipera lebetina***
	49	**A0A0C5DKL1_MACLB**	**C-type lectin-like protein 3A**	***Macrovipera lebetina***
	46	**J3RZT5_CROAD**	**Keratin, type I cytoskeletal 19**	***Crotalus adamanteus***
	40	**A0A1W7RE48_AGKCO**	**Ribonuclease P protein subunit p38-like protein**	***Agkistrodon contortrix contortrix***
	38	**A0A0B8RV71_CROHD**	**Keratin, type II cytoskeletal cochleal-like**	***Crotalus horridus***
	34	**A0A0F7ZAB1_CROAD**	**Keratin, type I cytoskeletal 18-like protein**	***Crotalus adamanteus***
	31	**A0A0F7Z805_CROAD**	**MBT domain-containing protein 1 isoform**	***Crotalus adamanteus***
	29	**SLB1_MACLB**	**Snaclec B1**	***Macrovipera lebetina***
	29	**A0A0F7Z8B1_CROAD**	**E3 ubiquitin-protein ligase**	***Crotalus adamanteus***
	29	**A0A194APM7_9SAUR**	**C-type lectin 9d**	***Agkistrodon piscivorus***
	28	**A0A1W7RD94_AGKCO**	**Sister chromatid cohesion protein PDS5**	***Agkistrodon contortrix contortrix***
	26	**A0A1W7REY6_AGKCO**	**Sister chromatid cohesion protein PDS5 B**	***Agkistrodon contortrix contortrix***
	26	**SL114_ECHCA**	**Snaclec carinactivase-1 regulatory subunit 14 kDa chain (fragment)**	***Echis carinatus***
	25	**A0A0C5DKK6_MACBL**	**C-type lectin-like protein 2A**	***Macrovipera lebetina***
	24	**A0A1W7RC08_AGKCO**	**Ubiquitin-like-conjugating enzyme ATG3-like protein**	***Agkistrodon contortrix contortrix***
	23	**A0A0F7Z6K1_CROAD**	**Phosphatidate phosphatase**	***Crotalus adamanteus***
	22	**A0A0K8S0L2_CROHD**	**Iron-sulfur cluster assembly 1, mitochondrial-like protein**	***Crotalus horridus***
	20	**A0A0F7ZC99_CROAD**	**Charged multivesicular body protein**	***Crotalus adamanteus***

	18	**A0A1W7RDB4_AGKCO**	**Sialic acid synthase-like protein**	***Agkistrodon contortrix contortrix***
	17	**A0A1W7RF54_AGKCO**	**NudC domain-containing protein 2**	***Agkistrodon contortrix contortrix***
	16	**A0A1W7RHL5_AGKCO**	**Endoplasmic reticulum-Golgi intermediate compartment protein-2 like protein**	***Agkistrodon contortrix contortrix***
	15	**T1E4Y0_CROHD**	**Secernin-3-like protein**	***Crotalus horridus***
	15	**A0A0B8RW51_CROHD**	**Cytochrome P450 2j5-like protein**	***Crotalus horridus***
	15	**A0A0K8RXU5_CROHD**	**Tubulin-glutamate carboxypeptidase**	***Crotalus horridus***
	13	**A0A194AQ76_SISMB**	**C-type lectin 15**	***Sistrurus miliarius barbouri***
FG6	**54**	**J3RZT5_CROAD**	**Keratin, type I cytoskeletal 19**	***Crotalus adamanteus***
	52	**A0A194APM7_9SAUR**	**C-type lectin 9d**	***Agkistrodon piscivorus***
	49	**A0A0B8RV71_CROHD**	**Keratin, type II cytoskeletal cochleal-like**	***Crotalus horridus***
	35	**A0A1W7REY6_AGKCO**	**Sister chromatid cohesion protein PDS5 B**	***Agkistrodon contortrix contortrix***
	32	**A0A0F72BJ1_CROAD**	**FAS-associated factor 1**	***Crotalus adamanteus***
	30	**A0A0F7ZAB1_CROAD**	**Keratin, type I cytoskeletal 18-like protein**	***Crotalus adamanteus***
	29	**VM2_BITAR**	**Disintegrin bitistatin**	***Bitis arietans***

FG1 to FG6 were cut from the gel and subjected to in-gel digestion with trypsin. Analysis was performed using an Easy-nLC Proxeon nanoHPLC system coupled to an LTQ-Orbitrap Velos. The results obtained were compared with the "*Bitis*" and "Snakes" database downloaded from Uniprot (Taxid: 8570).