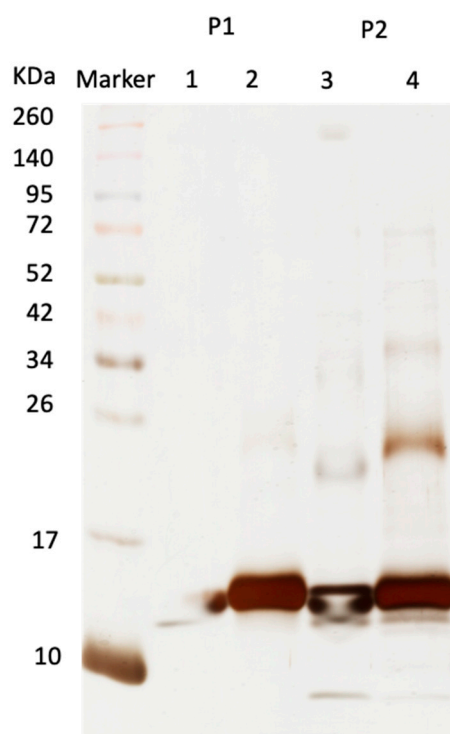


Supplementary data

Supplementary 1



Supplementary Figure S1: SDS-PAGE of protein bands from the fraction eluted in the G75 Superdex™ column named P1 and P2 (Figure 1b). Five µg of protein bands in SDS-PAGE gel stained with silver staining. The first fraction eluted from the G75 Superdex™ column named P1 exhibited dominant protein bands in between the molecular weight of 15 to 26 KDalton in both reducing and nonreducing conditions (lanes 1 and 2). The second elution named P2 (lines 3 and 4) showed protein bands at broader size, up to 72 KDa. P2 protein bands revealed two bands at MW 20KDa and 15KDa at nonreducing condition (lane 3), respectively. Meanwhile, protein bands for P2 fractions exhibited four bands with estimated MW were 72KDa, 40KDa, 23KDa and 15KDa. All bands in each fraction (P1 & P2) were cut and digested with trypsin before subjected to ESI-LC-MSMS for its protein identification. All LC-MSMS data obtained in the fractions were pooled and presented in supplementary tables 1a and 1b.

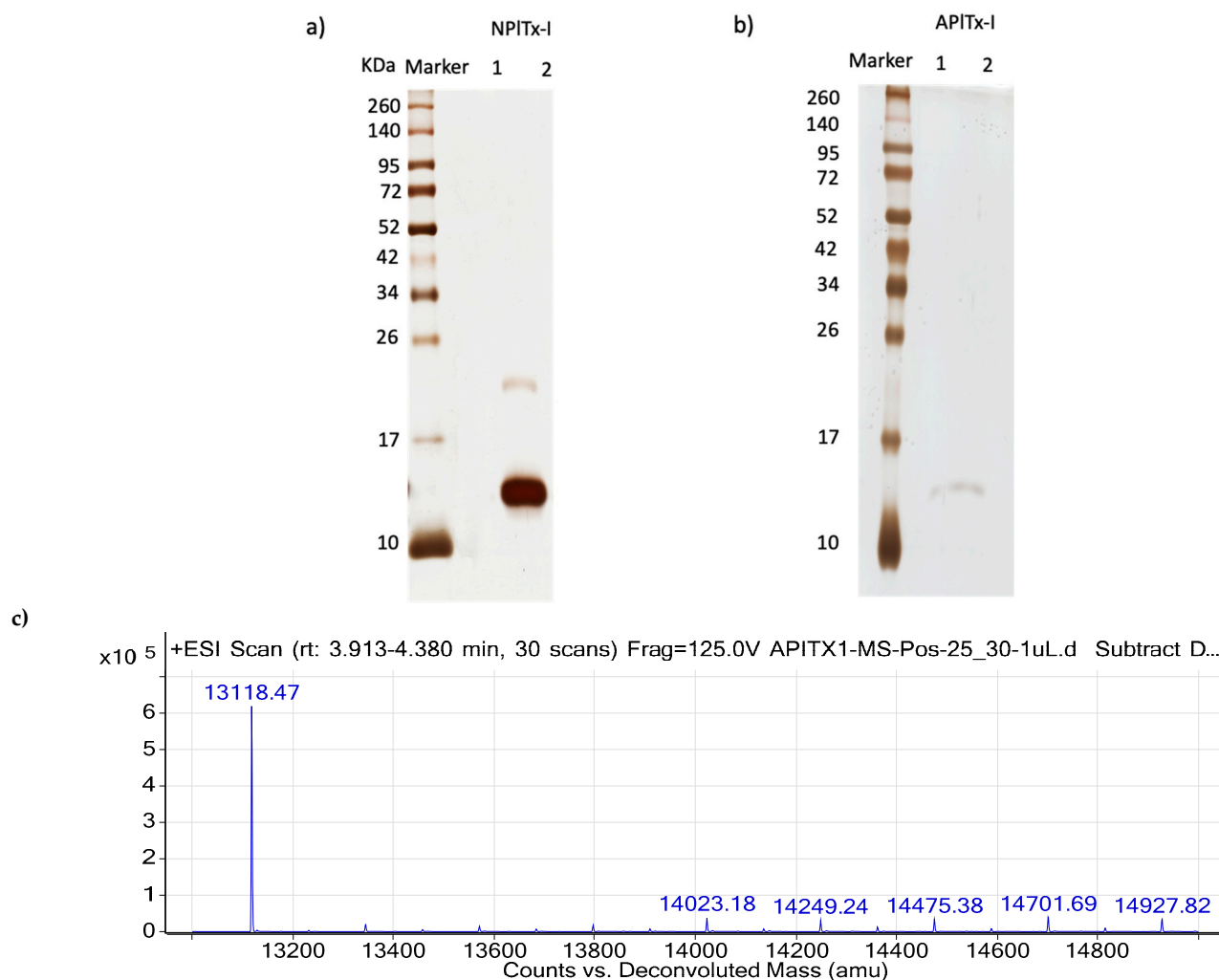
Supplemental Table S1a: Protein profile of fraction P1 eluted from G75 Superdex™ column. Protein gel bands were tryptic digested and identified by ESI-LC-MSMS. Most of the proteins detected in P1 protein bands are phospholipase A₂ and venom nerve growth factor from *Naja* species.

Fraction	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	Avg. Mass	Description	Origin of species
P1	Q9I900	255.4	78	36	5	16097	Acidic phospholipase A ₂ D	<i>Naja sputatrix</i>
	P00596	231.01	79	26	2	16271	Acidic phospholipase A ₂ 1	<i>Naja kaouthia</i>
	Q9I133	216.42	79	20	2	15949	Acidic phospholipase A ₂ 2	<i>Naja atra</i>
	Q6T179	210	52	23	2	14198	Acidic phospholipase A ₂ 4 (Fragment)	<i>Naja sagittifera</i>
	P01140	198.37	91	12	1	13023	Venom nerve growth factor	<i>Naja naja</i>
	P60043	188.05	48	17	3	14003	Basic phospholipase A ₂ 1 (Fragment)	<i>Naja sagittifera</i>
	P25498	185.72	66	14	3	13229	Acidic phospholipase A ₂ E	<i>Naja oxiana</i>
	P60044	174.18	50	15	1	14073	Acidic phospholipase A ₂ 2 (Fragment)	<i>Naja sagittifera</i>
	P60045	168.88	67	13	2	13969	Acidic phospholipase A ₂ 3 (Fragment)	<i>Naja sagittifera</i>
	Q92084	108.77	49	5	5	16189	Neutral phospholipase A ₂ muscarinic inhibitor	<i>Naja sputatrix</i>

Supplemental Table S1b: Protein profile of fraction P2 eluted from G75 Superdex™ column. Protein gel bands were tryptic digested and identified by ESI-LC-MSMS. Protein profile in P2 exhibited the presence of acidic and neutral phospholipase A₂, venom nerve growth factors and thaicobrin.

Fraction	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	Avg. Mass	Description	Origin of species
P2	Q9I133	231.54	63	23	1	15949	Acidic phospholipase A ₂ 2	<i>Naja atra</i>
	P01140	211.23	97	16	2	13023	Venom nerve growth factor	<i>Naja naja</i>
	P01140	200.75	95	12	11	13023	Venom nerve growth factor	<i>Naja naja</i>
	Q6T179	200.22	52	17	1	14198	Acidic phospholipase A ₂ 4 (Fragment)	<i>Naja sagittifera</i>
	A4FS04	192.21	76	13	2	13188	Acidic phospholipase A ₂ natratxin	<i>Naja atra</i>
	P25498	187.51	66	13	4	13229	Acidic phospholipase A ₂ E	<i>Naja oxiana</i>
	P82885	184.38	69	10	5	12038	Thaicobrin	<i>Naja kaouthia</i>
	P60044	180.37	60	13	1	14073	Acidic phospholipase A ₂ 2 (Fragment)	<i>Naja sagittifera</i>
	Q92084	154.38	68	7	2	16189	Neutral phospholipase A ₂ muscarinic inhibitor	<i>Naja sputatrix</i>
	P61898	149.37	94	10	1	13064	Venom nerve growth factor	<i>Naja atra</i>

Supplementary 2



Supplementary Figure S2: Protein bands of two fractions collected from the C18 reverse phase column separated in SDS-PAGE gel and stained with silver staining. The first peak eluted from the C18 column named NPITx-I were evaluated in Glycine SDS-PAGE both in nonreducing (lane 1) and reducing (lane 2) (a). NPITx-I showed a dominant band at MW 13KDa and another low express band at 20KDa. The second elution named APITx-I showed low expression of a protein band at a lower migration of 13KDa (b). Intact protein analysis using accurate mass LC-MS showed that APITx-II has a molecular weight of 13,118.47 Da suggested different isomers or consisted of PLA2 fragment due to the mass differences of ~2KDa with A2-EPTX-Nsm1a, which been originated from NPITx-I. All protein bands from NPITx-I and APITx-II were subjected to in-gel tryptic digestion, and protein identification was performed by ESI-LC-MSMS (Supplemental table 2a and 2b).

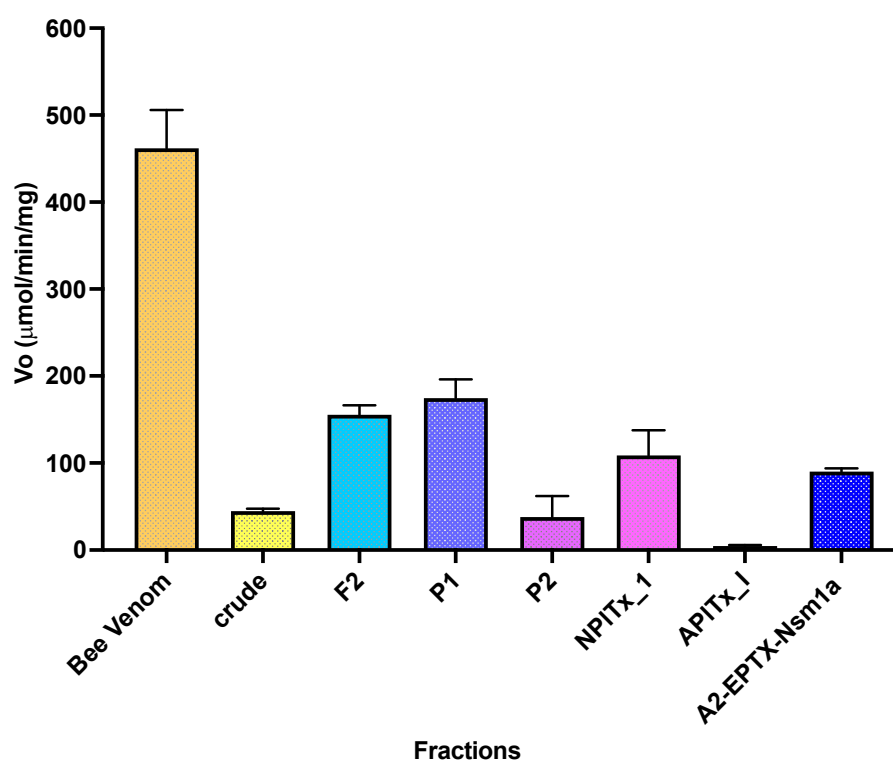
Supplemental Table S2a: Protein profile of fraction NPITx-I eluted from C18 reverse-phase column. Protein gel bands were tryptic digested and identified by ESI-LC-MSMS. Protein profiles of NPITx-I indicated similarity with phospholipase A₂ from *Naja* species.

Fraction	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	Avg. Mass	Description	Origin of species
NPITx-I	Q9I900	204.12	71	20	1	16097	Acidic phospholipase A ₂ D	<i>Naja sputatrix</i>
	P00596	192.23	80	20	2	16271	Acidic phospholipase A ₂ 1	<i>Naja kaouthia</i>
	Q9I133	184.5	63	14	1	15949	Acidic phospholipase A ₂ 2	<i>Naja atra</i>
	Q6T179	162.87	52	13	1	14198	Acidic phospholipase A ₂ 4 (Fragment)	<i>Naja sagittifera</i>
	P60043	149.35	38	10	1	14003	Basic phospholipase A ₂ 1 (Fragment)	<i>Naja sagittifera</i>
	P25498	146.69	54	10	2	13229	Acidic phospholipase A ₂ E	<i>Naja oxiana</i>
	P60045	137.27	44	9	1	13969	Acidic phospholipase A ₂ 3 (Fragment)	<i>Naja sagittifera</i>
	P60044	133.62	50	8	1	14073	Acidic phospholipase A ₂ 2 (Fragment)	<i>Naja sagittifera</i>
	P00601	127.06	47	11	1	13360	Acidic phospholipase A ₂ DE-III	<i>Naja melanoleuca</i>
	Q92084	118.58	68	8	3	16189	Neutral phospholipase A ₂ muscarinic inhibitor	<i>Naja sputatrix</i>

Supplemental Table S2b: Protein profile of fraction APITx-I eluted from C18 reverse-phase column. Protein gel bands were tryptic digested and identified by ESI-LC-MSMS. Protein profiles of APITx-I indicated 49% similarity with neutral phospholipase A₂ originated from *Naja sputatrix*.

Fraction	Accession	-10lgP	Coverage (%)	#Peptides	#Unique	Avg. Mass	Description	Origin of species
APITx-I	Q92084	95.93	49	5	0	16189	Neutral phospholipase A ₂ muscarinic inhibitor	<i>Naja sputatrix</i>
	Q92085	95.6	49	5	1	16175	Neutral phospholipase A ₂ B	<i>Naja sputatrix</i>

Supplementary 3



Supplementary Figure S3: Confirmation of PLA₂ activity in crude and its fractions that identified with the presence of phospholipase obtained from ESI-LC-MS/MS. A similar amount of each selected fraction and crude *N. sumatrana* venom (0.045 μg/ml) indicated hydrolysis of heptanoyl phosphatidylcholine, indicating the presence of secretory PLA₂. Fraction 2 (F2) isolated from G50 Sephadex™ column, fraction 1 (P1) eluted from G75 Superdex™ column, and semi-purified PLA₂, NPITx-I isolated from reverse phase C18 column showed higher secretory PLA₂ activity as compared to fraction P2. This activity suggested due to other toxins such as venom nerve growth factor and thaicobrin (Supplemental table 1b), which probably more dominant in the P2. In addition, the activity of secretory PLA₂ in APITx-I demolished at the same concentration. The assay was performed in triplicate, and bee venom was used as the positive control.