

Supplementary material

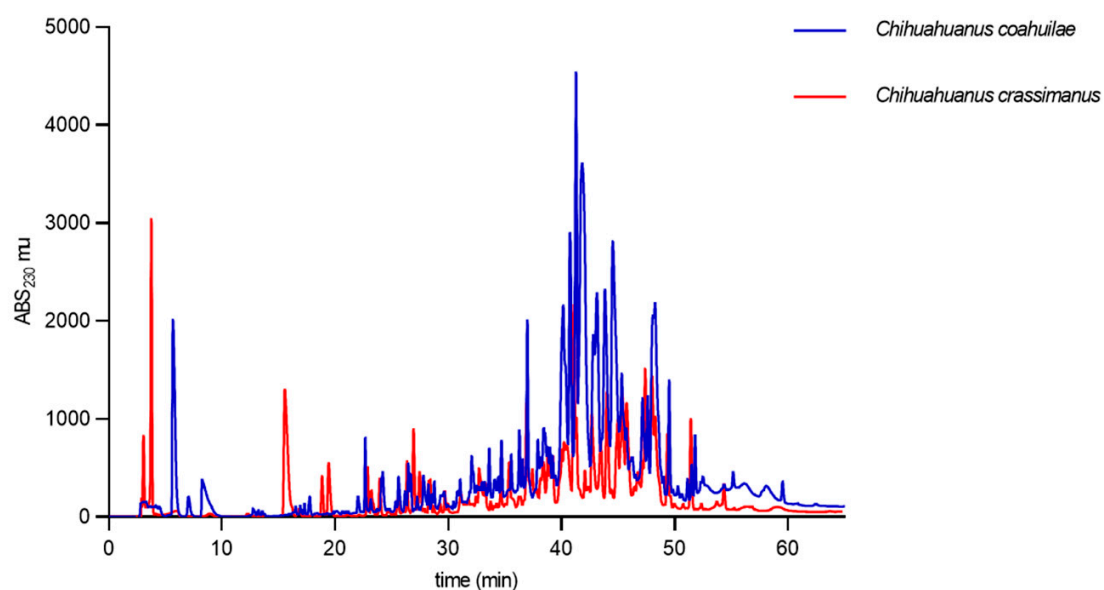


Figure S1. Overlapping HPLC profiles of two scorpion venoms. The venom of *Chihuahuanus coahuilae* and *Chihuahuanus crassimanus* scorpions were separated by RP-HPLC. The HPLC separation was performed using a C18 column in a gradient of 0 to 60%

Table S1. Comparative view of HPLC obtained fraction, molecular weight and effects of two different scorpion venoms

<i>Ch. coahuilae</i> venom					<i>Ch. crassimanus</i> venom					
Fraction	Retention time (min)	Molecular mass (Da)	Mouse (2 µg)	Cricket (10 µg)	Fraction	Retention time (min)	Molecular mass (Da)	Mouse (2 µg)	Cricket (10 µg)	Comments
35	36	2,977.0 3,066.1	-	-	22	37.5	3,066.7	-	-	KTx
39	40.7	8,302.3	Dead	-	26	41.3	8,302.6	-	-	NaTx
43	43.1	6,559.1 6,736.1	Dead	Dead	29	43.7	6,709.4 6,747.2	Dead	Paralyzed	Insect-Toxin / Contaminated fraction
44	43.6	6,643.7 6,616.4	-	Paralyzed	30	44.3	6,643	-	Paralyzed	Insect-Toxin / Contaminated fraction

Chihuahuanus coahuilae

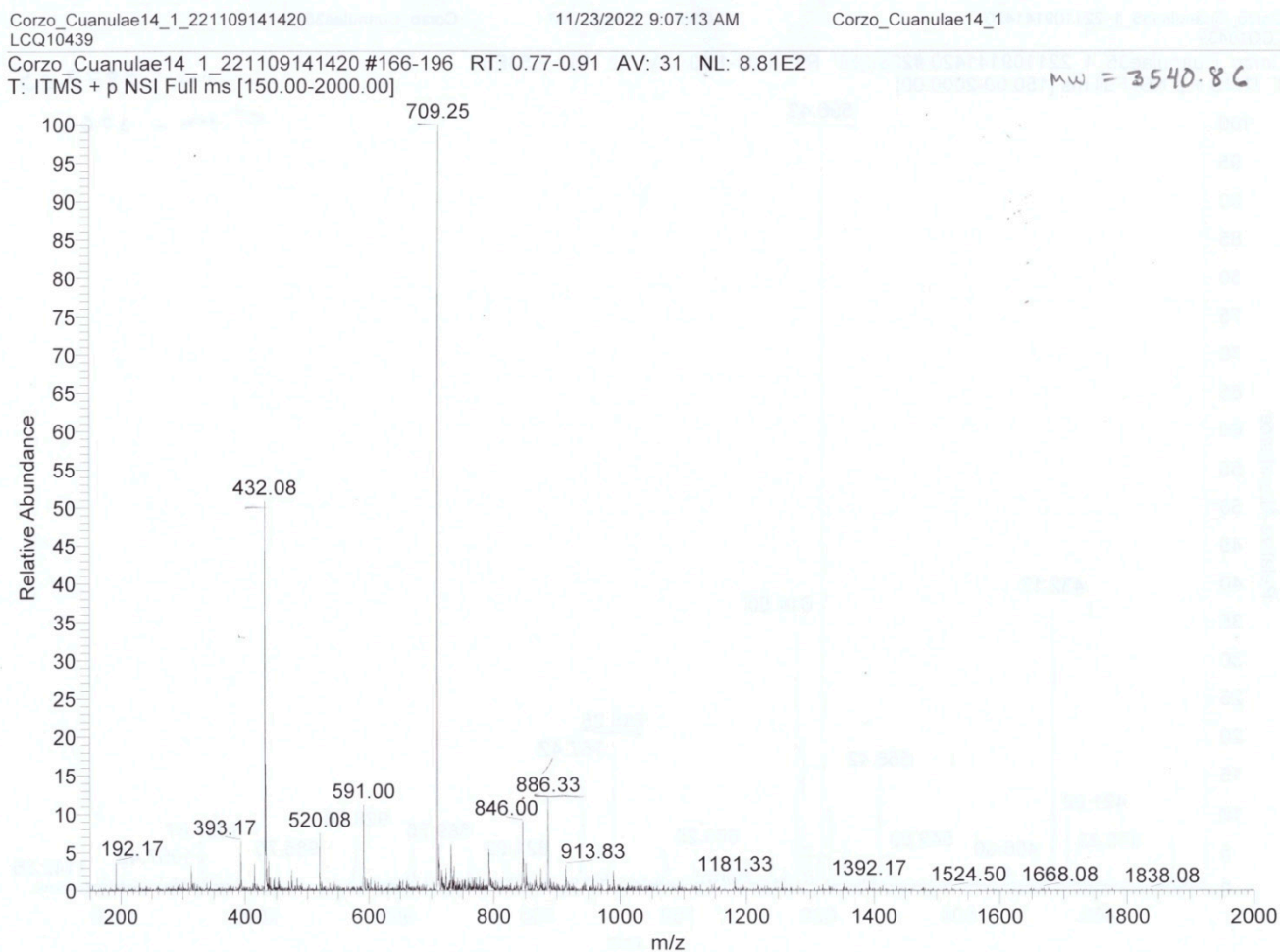


Figure S2. Mass spectrum fraction 14 *Chihuahuanus coahuilae*

Corzo_Cuanulae39_1_221109141420 #207-217 RT: 0.96-1.00 AV: 11 NL: 2.06E3
T: ITMS + p NSI Full ms [150.00-2000.00]

$m/z = 8302.31$

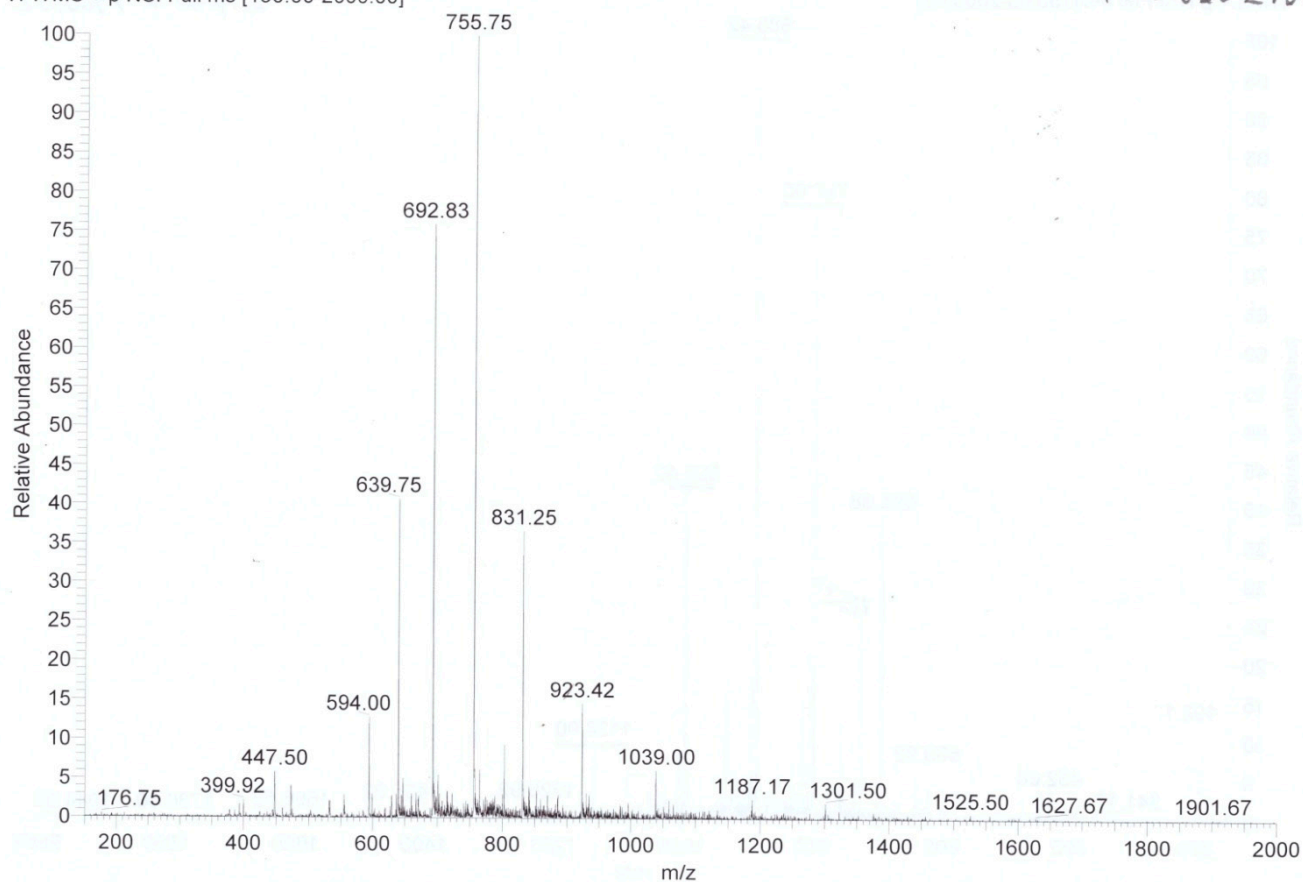


Figure S3. Mass spectrum fraction 39 *Chihuahuanus coahuilae*

Corzo_Cuanulae43_1_221109141420
LCQ10439

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Corzo_Cuanulae43_1

1

Corzo_Cuanulae43_1_221109141420 #255-319 RT: 1.16-1.46 AV: 65 NL: 5.47E2
T: ITMS + p NSI Full ms [150.00-2000.00]

~ MW = 6559.05
O MW = 6736.17

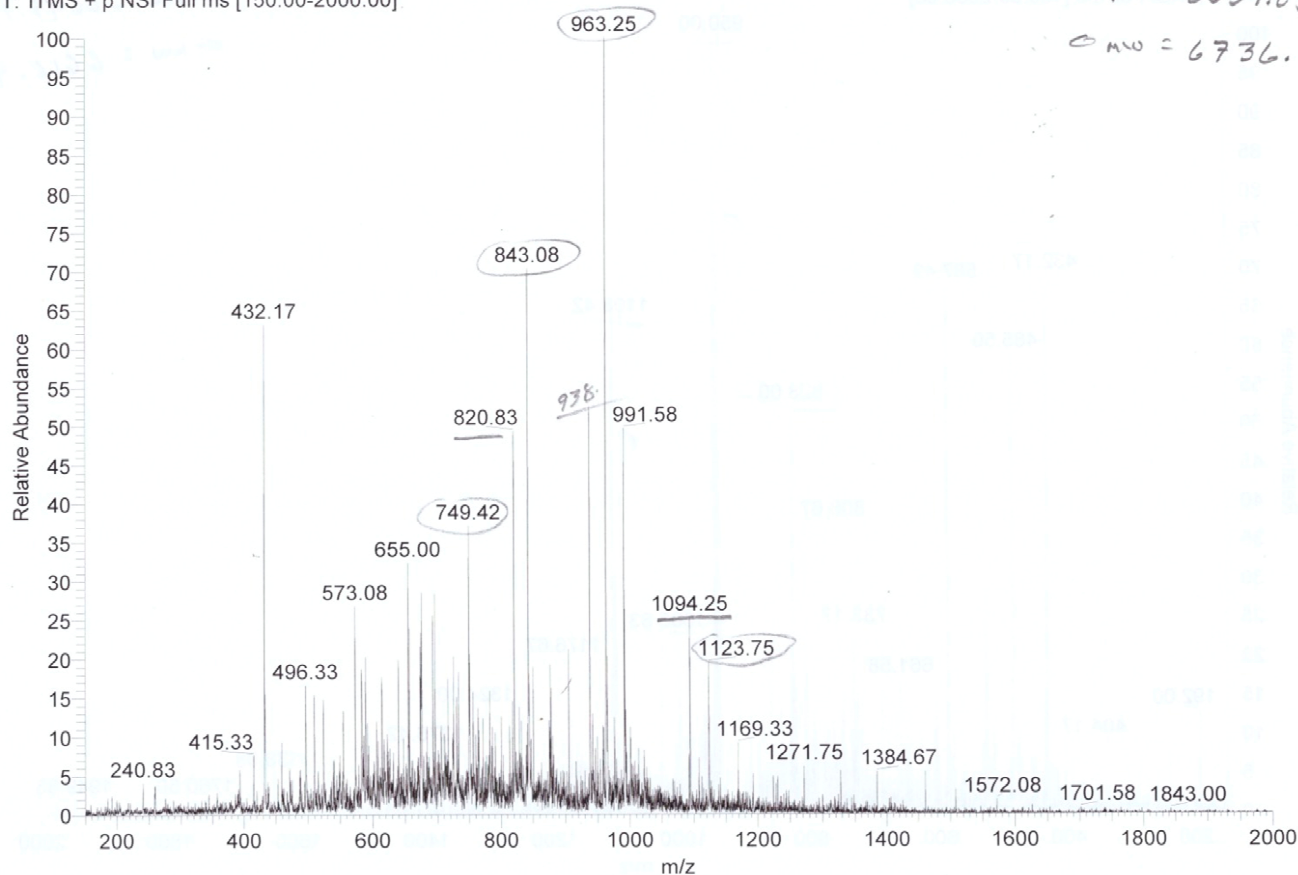


Figure S4. Mass spectrum fraction 43 *Chihuahuanus coahuilae*

Corzo_Cuanulae44_1_221109141420
LCQ10439

11/23/2022 10:00:54 AM

Corzo_Cuanulae44_1

Corzo_Cuanulae44_1_221109141420 #191-209 RT: 0.88-0.96 AV: 19 NL: 1.25E2
T: ITMS + p NSI Full ms [150.00-2000.00]

- MW = 6643.7
= MW = 6616.49

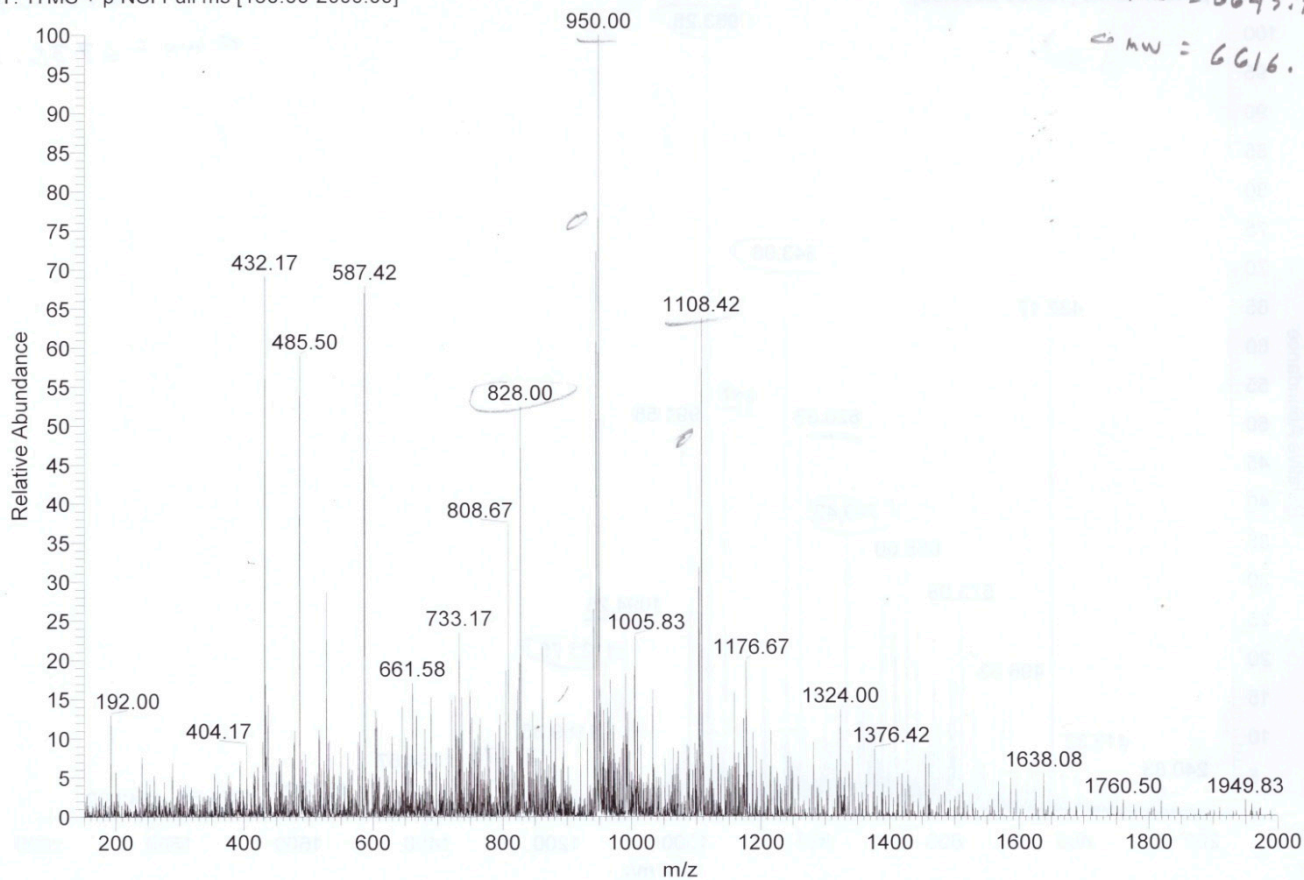


Figure S5. Mass spectrum fraction 44 *Chihuahuanus coahuilae*

Corzo_Cuanulae45_1_221109141420 #174-191 RT: 0.80-0.88 AV: 18 NL: 4.95E4
T: ITMS + p NSI Full ms [150.00-2000.00]

MW = 2103.97

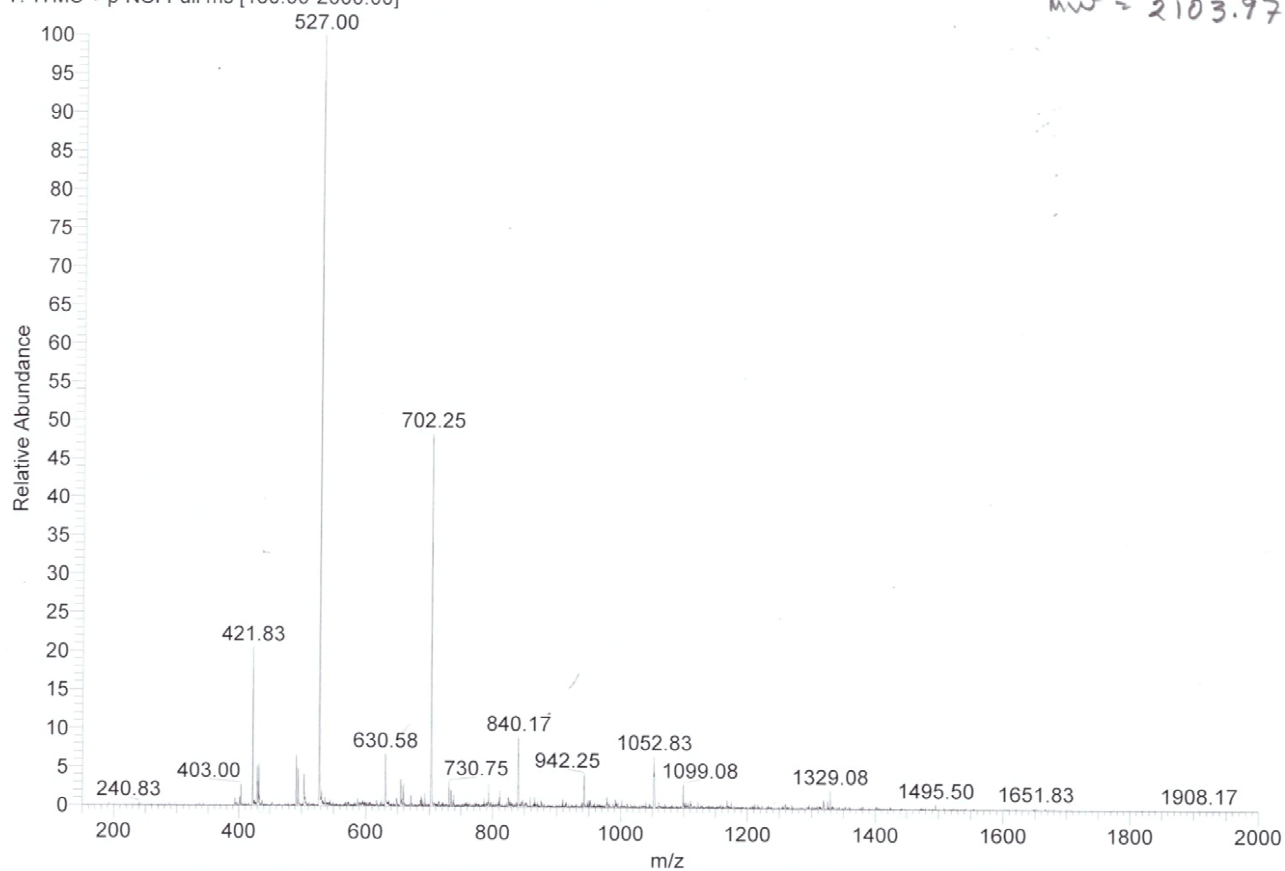


Figure S6. Mass spectrum fraction 45 *Chihuahuanus coahuilae*

Corzo_Coonolae50_1_221109141420
LCQ10439

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Corzo_Coonolae50_1

Corzo_Coonolae50_1_221109141420 #214-237 RT: 0.98-1.08 AV: 24 NL: 3.75E3
T: ITMS + p NSI Full ms [150.00-2000.00]

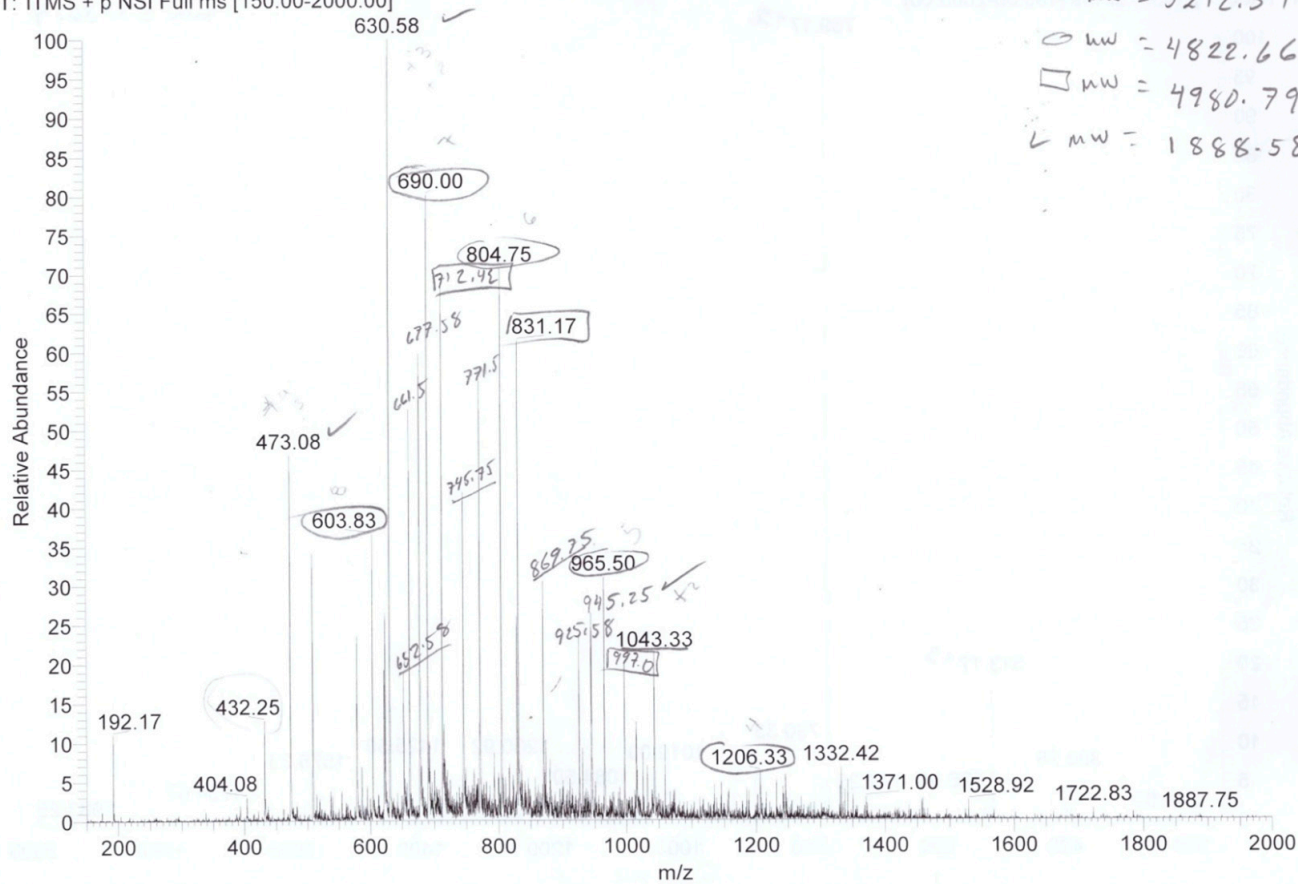


Figure S7. Mass spectrum fraction 50 *Chihuahuanus coahuilae*

Corzo_Coonolae51_1_221109141420 #235-253 RT: 1.08-1.16 AV: 19 NL: 3.45E3
T: ITMS + p NSI Full ms [150.00-2000.00]

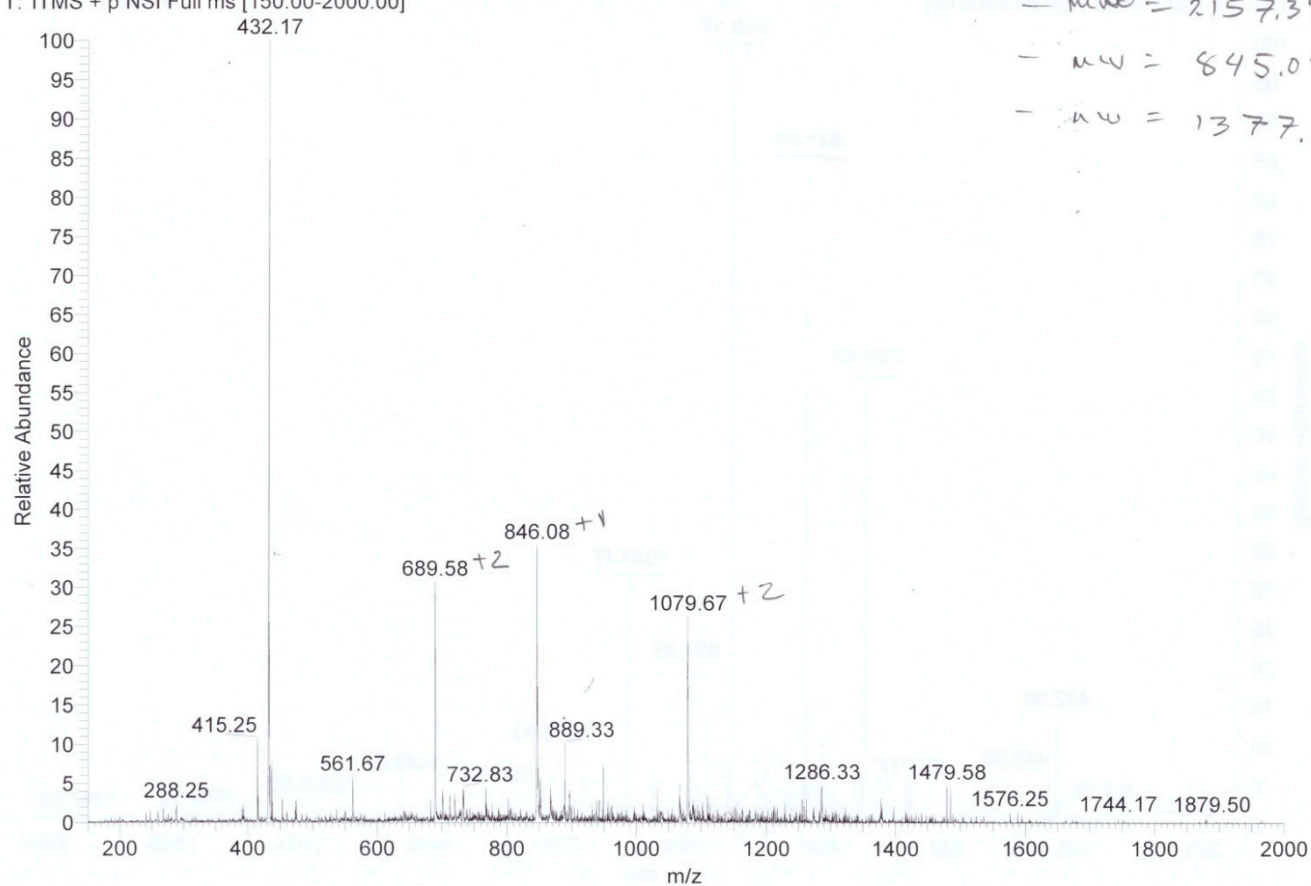


Figure S8. Mass spectrum fraction 51 *Chihuahuanus coahuilae*

Corzo_Coonolae54_1_221129085353
LCQ10439

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Corzo_Coonolae54_1

Corzo_Coonolae54_1_221129085353 #219-250 RT: 1.00-1.14 AV: 32 NL: 6.83E3
T: ITMS + p NSI Full ms [150.00-2000.00]

MW = 1364.17

1.752 ug

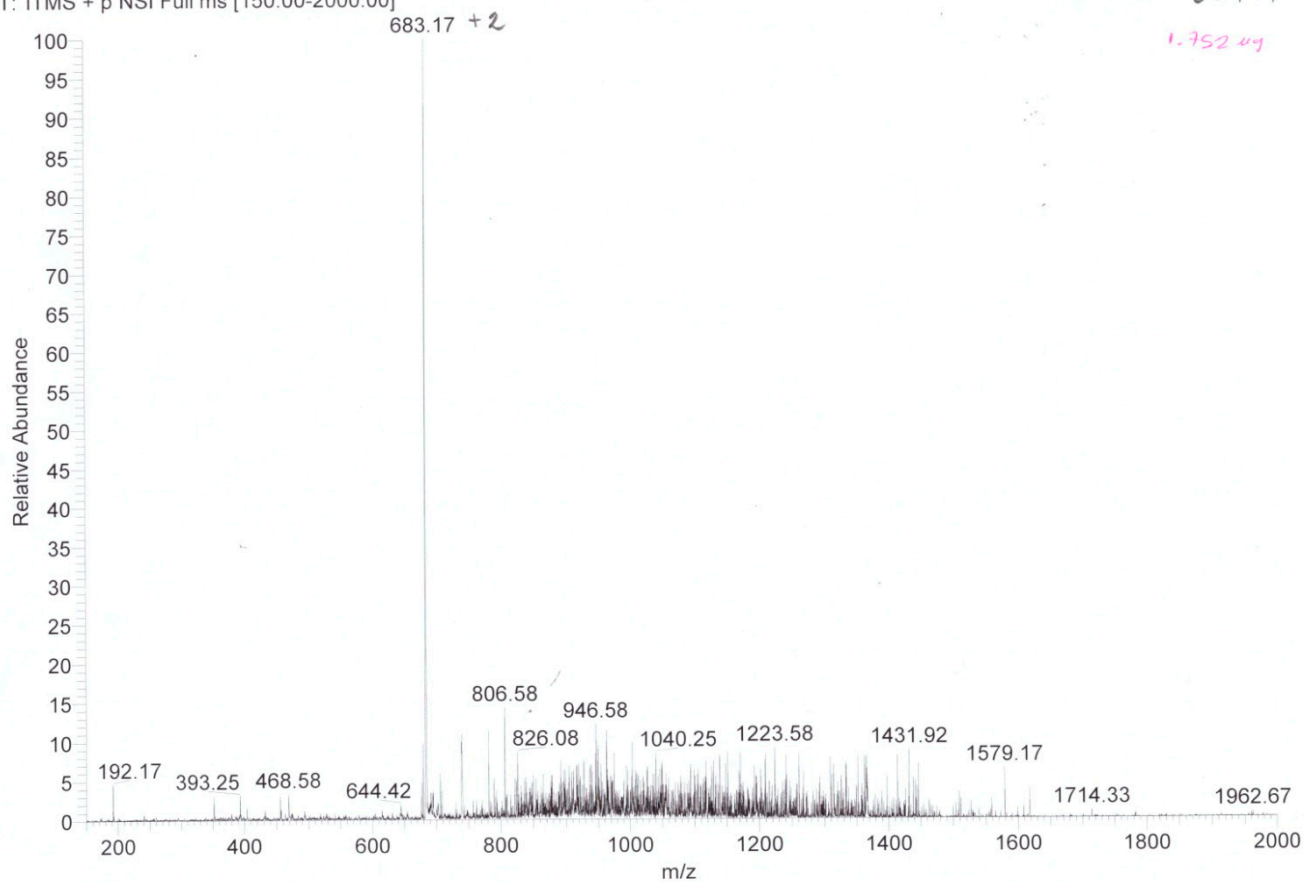


Figure S9. Mass spectrum fraction 54 *Chihuahuanus coahuilae*

Corzo_Coonolae59_1_221109141420 #200-222 RT: 0.92-1.02 AV: 23 NL: 1.33E4
T: ITMS + p NSI Full ms [150.00-2000.00]

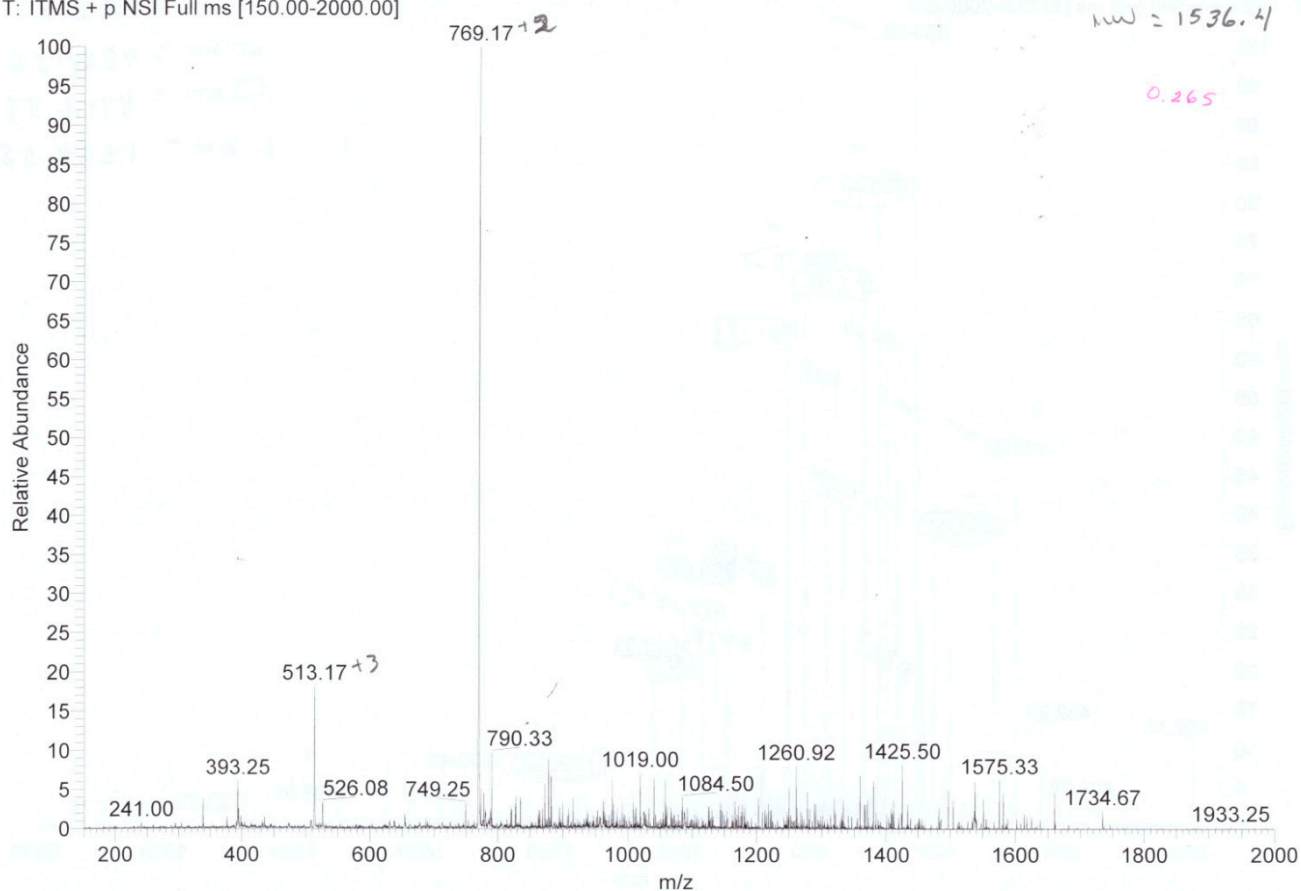


Figure S10. Mass spectrum fraction 59 *Chihuahuanus coahuilae*

Chihuahuanus crassimanus

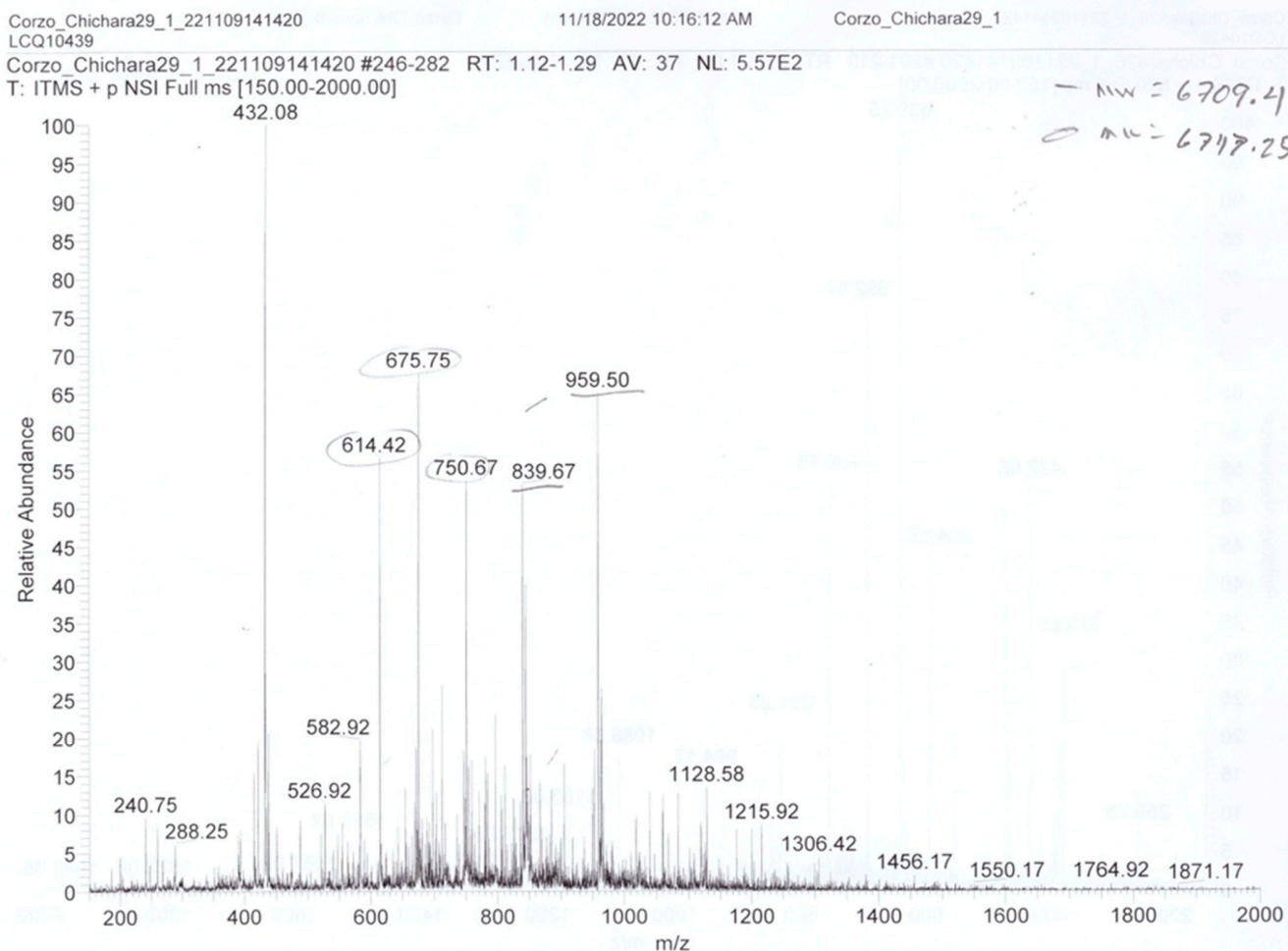


Figure S11. Mass spectrum fraction 29 *Chihuahuanus crassimanus*

Corzo_Chichara30_1_221109141420
LCQ10439

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Corzo_Chichara30_1

Corzo_Chichara30_1_221109141420 #210-223 RT: 0.97-1.03 AV: 14 NL: 1.85E3
T: ITMS + p NSI Full ms [150.00-2000.00]

mw = 6643

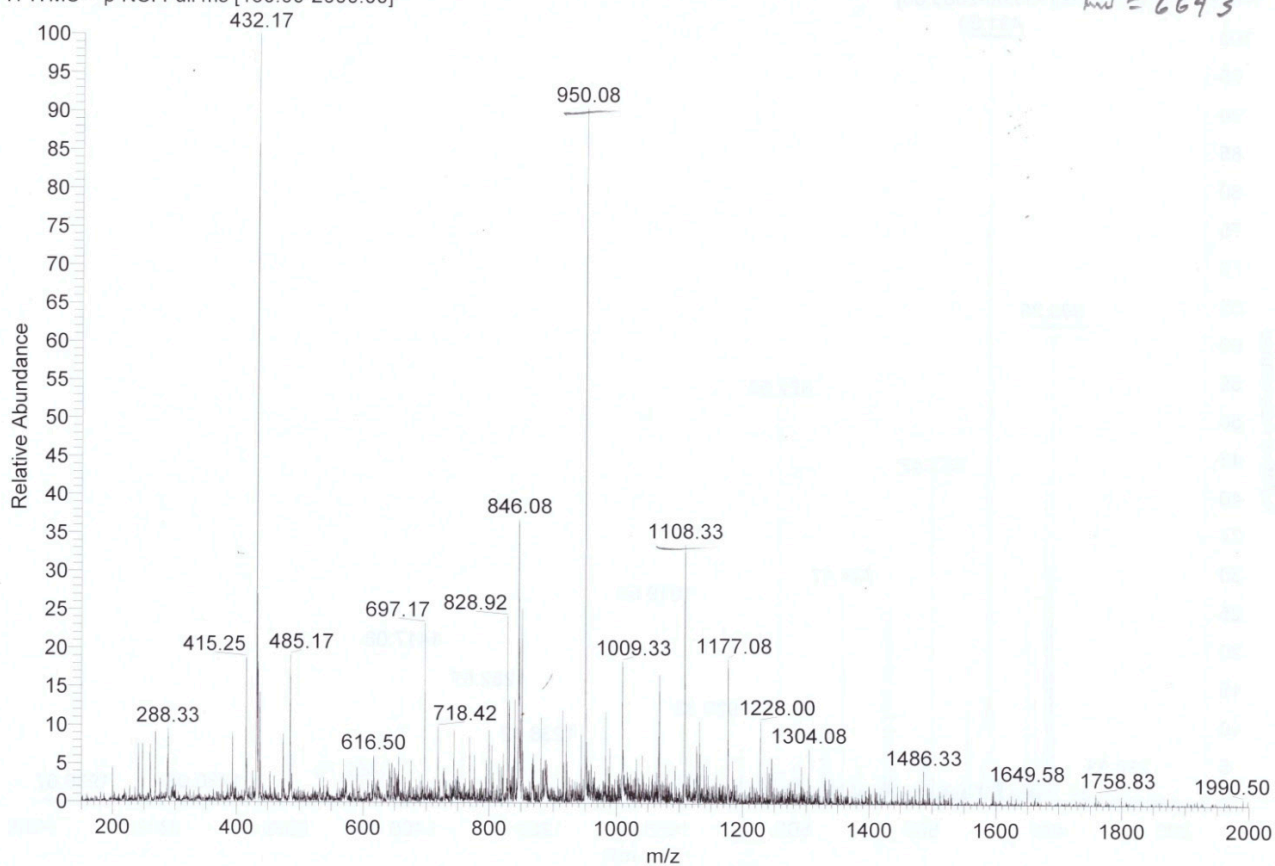


Figure S12. Mass spectrum fraction 30 *Chihuahuanus crassimanus*

Corzo_Chichara36_1_221109141420 #162-192 RT: 0.75-0.88 AV: 31 NL: 3.38E3
T: ITMS + p NSI Full ms [150.00-2000.00]

$m/z = 2133.34$

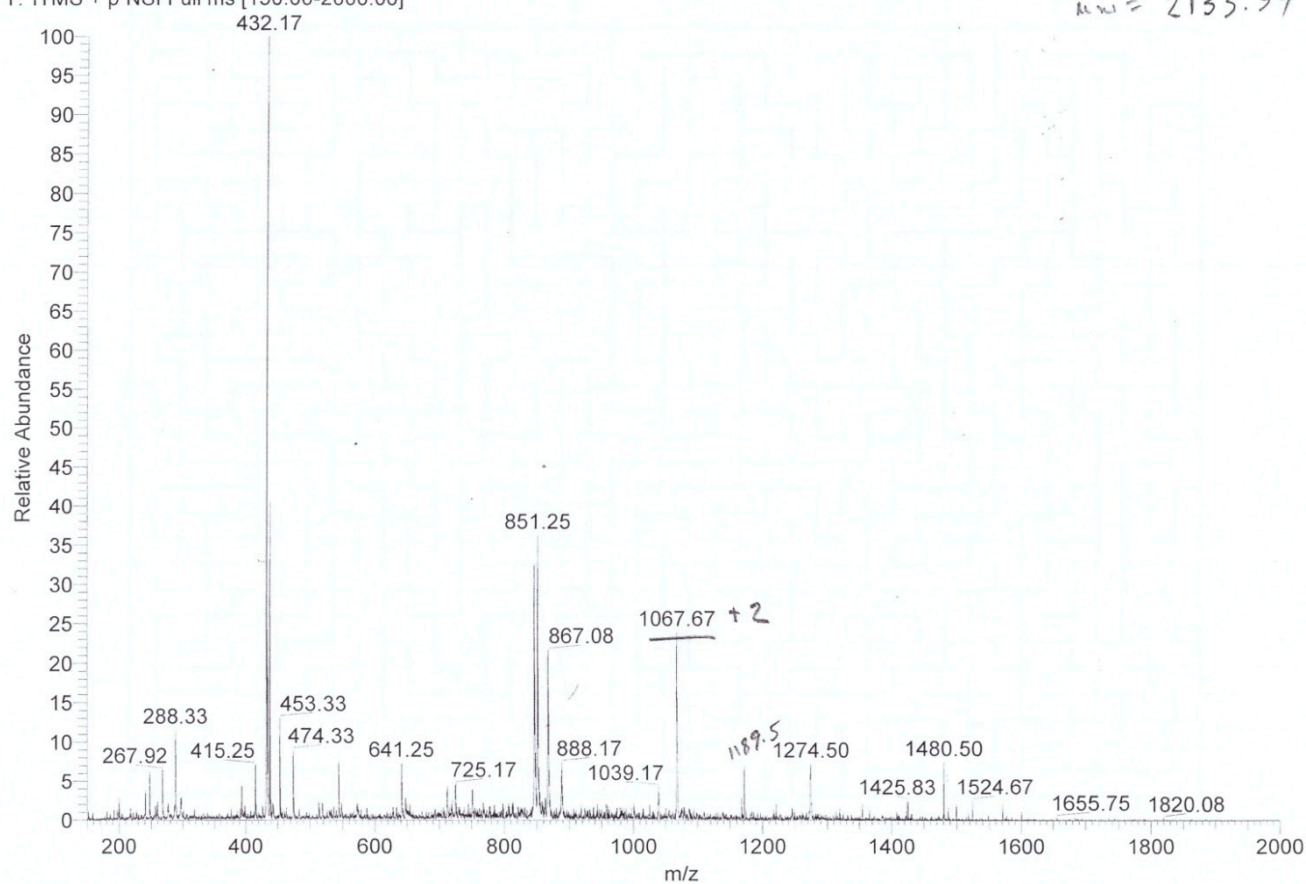


Figure S13. Mass spectrum fraction 36 *Chihuahuanus crassimanus*

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C0HK83_Pandinus_imperator_KTx_2.9      VDACYEACMHMHMSDDCI EACKNPVPP----- 28
P0C182_C_noxius_KTx_1.11              -----TFIDVDCTVSKECWAPCKAAGVD-RGKCMGKKCKCYV-- 37
Q9TXD1_C_noxius_KTx_2.4                -----TIINEKCFATSQCWTPCKKAIGSL-QSKCMNGKCKCYNG- 38
P08815_C_noxius_KTx_2.1                -----TIINVKCTSPKQCSKPKELYGSSAGAKCMNGKCKCYNN- 39
P85529_Centruroides_suffusus_KTx_2.13  -----IFINVKCSSPQQCLKPCKAAGFISAGGKINGKCKCYP-- 38
O46028_C_noxius_KTx_10.1               -----AVCVYRTCDKDK-RRGYR-SGKINNACKCYPY- 32
P0DJ31_Vaejovis_mexicanus_smithi_KTx_23.1 -----AAAI SCVGSPECPPCKR-AQGCK-NGKCMNRKCKCYC- 36
P0DJ32_Vaejovis_mexicanus_smithi_KTx_23.2 -----AAAI SCVGSKECLPKCK-AQGCK-SGKCMNRKCKCYC- 35
C0HK82_Pandinus_imperator_KTx_24.1     -----VAKCSTSECGHACQ-QAGCR-NSGCRYGSCICVGC- 33
P55928_Pandinus_imperator_KTx_7.2      -----TISCTNEKQCYPHCKKETGYP-NAKCMNRKCKCFGR- 35
Q10726_Pandinus_imperator_KTx_6.1      -----LVKCRGTSDCGRPCQQQTGCP-NSKCMNRKCKCYGC- 35
P58498_Pandinus_imperator_KTx_6.4      -----IEAIRCGGSRDCYRCPQKRTGCP-NAKCMNRKCKCYGC- 38

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Figure S14. KTxs. Sequences were obtained from Kalium database <https://kaliumdb.org/>, which is a collection of natural peptides acting on potassium channels mostly originating from the venoms of animals like scorpions, snakes, spiders and sea anemones. The alignment was made with the program Clustal Omega <https://www.ebi.ac.uk/Tools/msa/clustalo/>.

Table S2. KTxs Sequence data was obtained from Kalium database (<http://kaliumdb.org>), which is a collection of natural peptides acting on potassium channels mostly originating from the venoms of animals like scorpions, snakes, spiders, and sea anemones.

Species	Toxin	Molecular weight (Da)
<i>Centruroides noxius</i>	KTx 1.11	4085.97
	KTx 2.1	4195.06
	KTx 2.4	4183.048
	KTx 10.1	3731.476
<i>Vaejovis mexicanus smithi</i>	KTx 23.1	3863.776
	KTx 23.2	3667.602
<i>Pandinus imperator</i>	KTx 6.1	3834.648
	KTx 6.4	4180.032
	KTx 7.2	4065.8637
	KTx 24.1	3334.7009
	KTx 2.9	3126.4445
<i>Centruroides suffusus</i>	KTx 2.13	4000.94

F1AWB0.1_Vejovine_Vaejovis_mexicanus	MNAKTLFVFLIGMLV-----TEQVEAGIWSIKNLASKAHNS---DIGQSLRNKAAGAINKFVADKIGVTPSQASMTLDEIVDAMYYD
AFH87944.1_CT1_Vaejovis_mexicanus	--MKTQFVILLIVAVVLLQLISHSEAFGLAMNVAKSVFGKRGRLR---NFDOLD-DTFEPE-----MSEADLKYLDLLR----
AFH87945.1_CT2_Vaejovis_mexicanus	--MKTQFVILLIVAVVLLQLIANSEAFSLTNWNAKSIKFGKRGRLR---NLDNLDODIFEPE-----MSEADLRYLDLLR----
P86344_Brotheas_amazonicus	-----IGDINSIGIQG-----
P86342_Brotheas_amazonicus	-----GFIGDINSIGIQG-----
R4JJN6_Pandinus_imperator	--MKTQFAILLIALVLFQLLSQSDAFLSTINGIKSLLGRRLN---ELDNLD-ELFDGE-----ISQADIDFLKELMS----
L0GCI6_Urodacus_yaschenkoi	--MKNQFVLLLLAIVFLQMFQSDAILSAIWSGIKSLFGKRGLE---NMOKFD-ELFDGD-----LSEADLDFLKLMLR----
C5J886_Opisthacanthus_cayaporum	--MKAQLCILLIALVLFQTFQSDAILSAIWSGIKSLFGKRGRLN---DLDDLD-ELFDGE-----ISQADVDLFLNELMR----
Urodacus_yaschenkoi_Uy17	-----ILSAIWSGIKGLL-----
L0GAZ8_Urodacus_yaschenkoi	--MKNQFAILLLAVVFLQLISQSDAIWSAIWSGIKGLLGRGLK---NADRLD-ELFDGD-----ISDADLDFLRELMLR----
P0DJ02_Heterometrus_petersii	--MKTQFAIFLITLVLQMFQSDAIFKAIWSGIKSLFGKRGLS---DLDDLD-ESFDGE-----VSQADIDFLKELMQ----
P0DME9_Heterometrus_petersii	--MKTQFAIFLITLVLQMFQSDAIFKAIWSGIKSLFGKRGLS---DLYDLD-EMFDGE-----ISQADIDFLKELMR----
P0DME8_Heterometrus_petersii	--MKTQFTVLLITLVLQMFQSDAIFKAIWSGIKSLFGKRGLS---DLSOLD-ELFDGE-----ITKADLDFLREIM----
Q8MTX2_Opisthacanthus_madagascariensis	--MKTQFAILLVALVLFQMFQSDAIFGAIWSGIKSLFGRRALNNDLDDGLD-ELFDGE-----ISQADVDLFLKELMR----
Q8MMJ7_Opisthacanthus_madagascariensis	--MKTQFAILLVALVLFQMFQSDAILGKIWEGIKSLFGKRGLS---DLDDLD-ELFDGE-----ISKADLDFLRELMLR----
P0DME6_Heterometrus_petersii	--MKTQFAILLITLVLQMFQSDAILGKIWEGIKSLFGKRGRLN---DLSOLD-ELFDGE-----ISEADVDLFLREIM----
A0A0C4G4L1_Heterometrus_spinifer	--MKTQFAILLITLVLQMFQSDAILGKIWEGIKSLFGKRGRLN---DLSOLD-ELFDGE-----ISKADLDFLREIM----
P0DME7_Heterometrus_petersii	--MKTQFAILLITLVLQMFQSDAILGKIWEGIKSLFGKRGRLN---DLSOLD-ELFDGE-----ISKADLDFLREIM----

Figure S15. AMPs Sequences found on CAMP R3 database- No molecular weight its reported.

Due to their wide range of functions and targets, rapid efficacy, and low potential for inducing resistance, antimicrobial peptides (AMPs) have emerged as promising candidates for new antibiotics, as highlighted by Huang et al. (2010) (Huang et al., 2010). AMPs primarily eliminate microorganisms by employing mechanisms that target their membranes and form pores, which inherently pose greater challenges for microbes to develop resistance against, as discussed by Hancock and Sahl (2006) (Hancock & Sahl, 2006). A considerable portion of the characterized scorpion venom Non-Disulfide-Bridged-Peptides (NDBPs) exhibit antimicrobial properties, thus classifying them as AMPs, as noted by Harrison et al. (2014) (Harrison et al., 2014). The precise reason for their presence in scorpion venom remains unclear. They may potentially contribute synergistically to venom activity or serve as a component of the venom gland's antimicrobial response, as suggested by Kuhn-Nentwig (2003) (Kuhn-Nentwig, 2003).

Table S3. AMPs. Scorpion-derived compounds with antibacterial activities

Specie	Antibacterial agent	Molecular weight	Reference
<i>U. yaschenkoi</i>	Uy234	1986.19	(Cesa-Luna et al., 2019)
	Uy17	1369.43	
	Uy192	1459.98	
<i>D. melici</i>	Red 1,4-benzoquinone: 3,5-dimethoxy-2-(methylthio) cyclohexa-2,5-diene-1,4-dione	168.15	(Carcamo-Noriega et al., 2019)
	Blue 1,4-benzoquinone: 5-methoxy-2,3-bis(methylthio)cyclohexa-2,5-diene-1,4-dione		
<i>Hadrurus gertschi</i>	Hadrurin	4435.3	(Torres-Larios et al., 2000)
<i>Opisthacanthus madagascariensis</i>	IsCTs	1501.9	(Dai et al., 2001)
<i>Vaejovis mexicanus</i>	vejovine	4873	(Hernández-Aponte et al., 2011)
<i>Pandinus imperator</i>	Scorpine	8350	(Conde et al., 2000)
	Pandinins 2	2612.11	(Corzo et al., 2001)