Venom Proteomic Analysis of Two Medically Important Nigerian Viper (*Echis Ocellatus* and *Bitis Arietans*) Snake Species

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Abstract

Snakebite envenoming remains a neglected tropical disease, which poses severe health hazard, especially for the rural inhabitants in Africa. In Nigeria, vipers are the most toxin-producing snakes that cause the highest number of deaths. Hydrophilic interaction liquid chromatography coupled with LC-MS/MS was used to analyze the crude venom extracts of *Echis ocellatus* (Carpet viper) and *Bitis arietans* (Puff adder) in order to understand their venom proteomic identities. Results obtained revealed that gel-free proteomic analysis of the crude venom extracts from *E. ocellatus* and *B. arietans* yielded the identification of 85 and 79 proteins, respectively. Seventy-nine (79) proteins were common between the two snake species with a 90.8% similarity score. The identified proteins belong to 12 protein families where serine proteases (22.31%) and metalloproteinases (21.06%) were the dominant proteins in the venom of *B. arietans*. Metalloproteinases (34.84%), phospholipase A₂s (25.69%) and serine proteases (17.25%) represents the major toxins in the *E. ocellatus* venom. Other protein families such as three-finger toxins and cysteine-rich venom proteins were also detected, albeit, in low proportions. This study represents the venom proteomic analysis of the two Nigerian viper species, which provides some valuable insights into the toxin families to be neutralized in case of envenomation. Data are available via ProteomeXchange with identifier PXD024638.

1. Introduction

Venomous snakes poses severe health problem especially for the rural dwellers in the tropical regions of developing countries^{1,2,3}. Despite the remarkable efforts for effective management of envenomings, the World Health Organization (WHO) incorporated snakebite envenoming in the list of neglected tropical diseases⁴. Given the inclusion of snakebite envenoming as a neglected tropical disease, better efforts are required to address the life-threatening situation posed by this disease. The scourge is predominant in the Savanna region of West Africa including other developing countries where over 95% of all reported cases occur^{3,2,4}. In Nigeria, the *viperidae*, notably *Bitis arietans* (Puff adder) and *Echi ocellatus* (Carpet viper) are associated with the highest number of morbidity and mortality^{5,6,7,1}, and are regarded as the most dangerous venomous snake species. The actual fatality index resulting from envenomings by these vipers remains unknown due to inaccurate epidemiological data^{1,2,8}, as most of the victims do not have access to health care facilities and therefore resort to ethno-medicinal means for treatments. Reports from community-based surveys suggest a higher envenomation incidence compared to the hospital-based estimates^{9,8,2}. As reported by the WHO, about 5.4 million snakebites occur annually, resulting in 2.7 million cases of envenomings, 138,000 deaths and 400,000 cases of disabilities¹⁰. In the sub-Saharan Africa, the incidence of snakebites was estimated at 314,000 cases, which resulted in over 6,000 amputations and 7,300 deaths⁴. In Nigeria, the incidence of snakebites leads to 10,000 deaths annually with envenomation by *E. ocellatus* responsible for 66% of total cases⁷. Snakebite occurs accidentally by infiltration of venom components, which are a mixture of toxic secretions into humans, through the fangs of the snake². The incidents are high during the rainy season when farming activities coincides with the snakes' breeding season². The victims are predominantly young males that are engaged in farming, plantation, agricultural harvests and herding activities^{11,6,12}. Snakebite envenoming inflicts severe damage to different organs of the body, depending on the nature of toxins in the venom. Envenomings by the *viperidae* snakes are characterized by life-threatening symptoms, primarily due to local tissue damage and bleeding¹.

The evolution of venomomics and antivenomics using the 'omics' technologies has paved a way to obtain valuable insights into snake venom compositions, revealing the protein constituents and different types of toxic components^{13,14}. In addition, the 'omics' approach has led to the rapid examination of the immune reactivity of antivenoms against the toxins in snake venoms^{2,15}. It is widely inferred that the pathological complications associated with snake envenomation is influenced by the geographical location of the snake^{16,17,18,19}, diet type²⁰, age²¹ and mutations in the venom-related genes. This in turn hinders progressive treatment of envenomed patients. Hence, analyzing venom proteomes to gain insight into snake venom variability can help in understanding their biological and pathological effects²², especially the viperids *B. arietans* and *E. ocellatus*. It will also help in making appropriate choice of antivenom to use in treatment. The venom gland transcriptomic of Nigerian *B. arietans* and *E. ocellatus* was reported previously^{23,24}. In the current study, we analyzed the venom proteomes of *B. arietans* and *E. ocellatus* captured from different regions of Nigeria with the view to understanding their toxin profile and protein identities. This contributes in enhancing the effectiveness of the treatment for envenoming by these Nigerian viper snakes.

2. Materials And Method

2.1. Bitis arietans and Echi ocellatus venom samples

All protocols for snake handling were observed and carried out in accordance with the Ahmadu Bello University Committee on Animal Use and Care with approval number ABUCAUC/2021/028, and in compliance with the revised ARRIVE guidelines 2.0. Adult snakes *B. arietans* and *E. ocellatus* were randomly captured from different regions of the Southern (Niger-Delta, South-East and South-West) and Northern (North-East, North-West and North-Central) Nigeria, during the raining season. Six (6) snakes (comprising of 3 snakes from each of the specie) were captured in each of the geo-political areas listed, making a total of 36 snakes. They were maintained at the serpentarium of the Department of Veterinary Pharmacology and Toxicology. After 3 days, their venoms were manually extracted as described by Hill and Mackessy²⁵. Venoms from snakes of the same species were combined. They were frozen at -80°C, lyophilized using a freeze-dryer, and stored at -20°C.

2.2. Chemicals and reagents

Magnetic bead-based hydrophilic interaction liquid chromatography (HILIC) particles were purchased from Sigma Aldrich (USA), whereas the protein standard marker and analytical-grade trypsin were obtained from Agilent Technologies (Santa Clara, CA, USA).

2.3. Venom protein extraction and pellet Solubilization

The venom proteins were extracted as described by Adamude et al³². Two milligrams (dry-weight) of the lyophilized crude venom (from each species) were solubilized in 50 µL 1X PBS (pH 7.4), vortexed for 10 min, and centrifuged at 15,700 × *g* for 5 min at 4°C. Acetone precipitation was performed on the supernatant by the addition of 600 µL cold acetone, and the samples were incubated at -20°C for 15 min. Precipitated samples were centrifuged at 15,700 × *g* for 15 min at 4°C. Protein pellets were air-dried and resuspended in 100 µL 1X PBS buffer (pH 7.4). Protein concentration of each sample was quantified using the RC DC Protein Assay Kit 11 (Bio-Rad Laboratories) and confirmed with the microvolume protein concentration determination method. Data was acquired on a Nanodrop Spectrophotometer 2000c (Thermo Fisher Scientific, USA).

2.4. One-dimensional SDS-PAGE

Aliquots of each sample (15 µg) were subjected to 12% SDS-PAGE under non-reducing conditions and ran for 90 min at 100 V. The gels were stained with Coomassie Brilliant Blue (G-250), and de-stained in 10% glacial acetic acid containing 1% glycerol. The resolved protein bands were visualized using a Molecular Imager PharosFX Plus System (Bio-Rad, California, USA).

2.5. Hydrophilic Interaction Liquid Chromatography (HILIC) and Trypsin digestion

HILIC was carried out as described³². Fifty micrograms (50 µg) of protein from each venom sample was suspended in a final concentration of 50 mM triethylammonium bicarbonate (TEAB; Sigma). The proteins were reduced with a final concentration of 10 mM Dithiothreitol (DTT; Sigma) in 50 mM TEAB for 40 min at 56°C. Samples were cooled to room temperature and alkylated with a final concentration of 30 mM iodoacetamide (Sigma) in 50 mM TEAB at 25°C and kept in the dark for 30 min. After alkylation, two-fold dilutions of the samples were made with binding buffer (100 mM ammonium acetate, 30% acetonitrile, pH 4.5). The protein solution was added to pre-equilibrated MagResyn® HILIC magnetic particles (Resyn Biosciences) prepared according to the manufacturer's instructions and incubated overnight at 4°C. After binding, the supernatant was removed and magnetic particles washed twice with 95% acetonitrile for 1 min. For digestion, the magnetic particles were suspended in 50 mM ammonium formate (pH 8.0) containing trypsin (Promega) to a final enzyme:protein ratio of 1:10 and incubated overnight at 27°C with constant shaking. Following digestion, peptides were recovered with 1 % trifluoroacetic acid (TFA) and incubated at room temperature for 3 min and analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

2.6. Characterization of venom proteins

Liquid chromatography was performed as described previously¹⁴ with slight modification using a UltiMate™ 3000 RSLCnano System (Thermo Fisher Scientific, USA) equipped with a C₁₈ trap column (5 mm × 300 µm; Thermo Fisher Scientific) and a CSH C₁₈ analytical column (1.7 µm, 25 cm x 75 µm; Waters), with a linear gradient of 0.1% trifluoroacetic acid in water (solution A) and acetonitrile (solution B). The samples were loaded on the trap column and the flow rate was set at 250 nL/min and the gradient was generated as follows: 5–35% solution B for 60 min and 35–50% solution B for 60–75 min. Chromatography was performed at 40°C, and the outflow was delivered to the mass spectrometer through a stainless-steel nano-bore emitter. LC-MS analysis was performed on a

Fusion mass spectrometer (Thermo Scientific, San Jose, CA) equipped with a nanospray ion source (Nanospray Flex™ Ion Sources, Thermo Fisher Scientific) coupled to a Dionex Ultimate 3000 RSLC nano-HPLC system. Peptides recovered from the on-bead HILIC digestion for each venom sample was introduced through a stainless-steel emitter. Data were collected in positive mode with a spray voltage set to 1.8 kV and ion transfer capillary set to 280°C. Spectra were internally calibrated using polysiloxane ions at m/z 445.12003 and 371.10024. The first MS scan was performed using the orbitrap detector set to a resolution of 120,000 resolutions over an m/z range of 350–1650 with an AGC target at $3E5$ and maximum injection time of 40 milliseconds. Data were acquired in profile mode. The second MS scan was performed using monoisotopic precursor selection for the ion with charges of +2 to +7 with error tolerance set at ± 10 ppm. Precursor ions were excluded for 60 seconds after fragmentation. Precursor ions were selected for fragmentation in the HCD mode using the quadrupole mass analyzer with HCD energy set to 30%. Fragment ions were detected in the orbitrap mass analyzer at a resolution of 30000. The AGC target was set to $5E4$ and the maximum injection time was set to 80 milliseconds. Data were acquired in centroid mode.

2.7. Data analysis

Data was analyzed following the protocol described previously³². The mass spectra obtained were subjected to Proteome Discoverer v1.4 software (Thermo Fisher Scientific, USA) and processed using the Sequest and Amanda algorithms. Database interrogation was performed against a concatenated database created by concatenating all 'snake protein' entries in the Uniprot-Serpentes database with the cRAP contaminant database (<https://www.thegpm.org/crap/>). The precursor mass tolerance was set to 10 ppm and fragment mass tolerance was set to 0.02 Da. Peptide validation was performed using the Target-Decoy PSM validator node. The search results were subjected to Scaffold Q+ version 4.10.0 for further validation (www.proteomesoftware.com). Peptide identification, by peptide-spectrum match approach against UniProt-Serpentes database, and subsequent assembly, matching, and identification of protein sequences were performed using X! Tandem and Sequest search engines. The search engines were incorporated into Scaffold Q+, version 4.10.0 at 99% protein threshold, 95% peptide threshold, and two-peptide minimum criterion. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE²⁶ with identifier PXD024638.

3. Results And Discussion

3.1. One-dimensional SDS-PAGE of the crude snake venoms

A fraction (15 µg) of each crude venom extract was size fractionated on a one dimensional SDS polyacrylamide gel and visualized using Coomassie Brilliant Blue R-250 dye (Fig. 1). The protein banding patterns observed for the two venoms extract were distinctly different and clearly separated in a molecular weight range of 7 kDa, 22 kDa and 100 kDa (Fig. 1). The distinct banding pattern on the 1D gel correlated with the LC-MS/MS analysis which showed that *B. arietans* had serine proteases and metalloproteinases as the major venom proteins (Fig. 3), while the venom of *E. ocellatus* was composed of three major proteins; phospholipase A₂s, serine proteases and metalloproteinases (Fig. 4). In Nigeria, the highest number of mortality is associated with envenoming by *E. ocellatus*⁷. Our findings indicated that *E. ocellatus* produced more toxins due to the high amount of phospholipase A₂s compared to the amount found in the venom of *B. arietans*, this could be a possible reason for the higher mortality rates associated with envenoming by *E. ocellatus*. In related reports^{27,28,2}, high rates of lethality are caused by viper envenoming.

3.2. Snake venom proteomic characterization using LC-MS/MS

A total of 79 proteins were detected in the venom of *B. arietans* whereas 85 proteins were detected in the venom of *E. ocellatus* (Table 1) when the tryptic digested crude venom extract was analysed using LC-MS/MS. A total of 79 proteins were common in the venom of the two snake species (Fig. 2). In addition, only one uncommon protein was identified in the venom of *B. arietans* whereas 7 uncommon proteins were identified in the venom of *E. ocellatus* (Fig. 2), with a protein similarity index of 90.8%. All the proteins detected (in the molecular mass range of 4–93 kDa) (Table 2) belonged to 12 protein families (Table 3). The most abundant proteins in the venom of *B. arietans* were serine proteases (22%) and metalloproteases (21%) (Fig. 3), followed by C-type lectins/ snakecs (10.65%), phospholipase A₂ (10.6%), vascular endothelial growth factors (10.3%) and L-amino acid oxidases (8.72%). Three-finger toxins (4.32%), disintegrins (3.4%), 5'-nucleotidases (2.5%), and cysteine-rich venom proteins (2.05%) were distributed in much lower abundance. Kunitz-type peptides and phospholipase B were the least abundant protein families (< 1%) identified in *B. arietans* (Fig. 3). On the other hand, the most abundant proteins identified in the venom extract of *E. ocellatus* include metalloproteinases (34.84%),

phospholipase A₂ (25.69%), and serine proteases (17.25%) (Fig. 4). C-type lectins/ snakelecs (3.95%), three-finger toxins (3.73%), vascular endothelial growth factors (3.3%) and cysteine-rich venom proteins (2.9%) were found in much lower abundance. Disintegrins, 5'-nucleotidases, L-amino acid oxidases, and Kunitz-type peptides families were very low in abundance (< 3 %), while phospholipase B was virtually absent (0.6%) as shown in Fig. 4.

Table 1
Protein identification in the venoms of *B. arietans* and *E. ocellatus*

Protein/peptide detected				
S/no.	<i>Echis ocellatus</i>	Originating specie	<i>Bitis arietans</i>	Originating specie
1	Acidic phospholipase A2	<i>Echis ocellatus</i>	Acidic phospholipase A2	<i>Echis ocellatus</i>
2	Acidic phospholipase A2	<i>Naja mossambica</i>	Acidic phospholipase A2	<i>Naja mossambica</i>
3	Alpha-fibrinogenase	<i>Macrovipera lebetina</i>	Alpha-fibrinogenase	<i>Macrovipera lebetina</i>
4	Alpha-fibrinogenase-like	<i>Daboia siamensis</i>	Alpha-fibrinogenase-like	<i>Daboia siamensis</i>
5	Beta-fibrinogenase brevinase	<i>Gloydius blomhoffii</i>	Beta-fibrinogenase brevinase	<i>Gloydius blomhoffii</i>
6	C-type lectin 1	<i>Bitis gabonica</i>	C-type lectin 1	<i>Bitis gabonica</i>
7	Cationic trypsin	<i>Bos taurus</i>	Cationic trypsin	<i>Bos taurus</i>
8	Coagulation factor X-activating enzyme heavy chain	<i>Daboia siamensis</i>	Coagulation factor X-activating enzyme heavy chain	<i>Daboia siamensis</i>
9	Coagulation factor X-activating enzyme heavy chain	<i>Macrovipera lebetina</i>	Coagulation factor X-activating enzyme heavy chain	<i>Macrovipera lebetina</i>
10	Cysteine-rich venom protein	<i>Trimeresurus stejnegeri</i>	Cysteine-rich venom protein	<i>Trimeresurus stejnegeri</i>
11	Cytotoxin 1	<i>Naja mossambica</i>	Cytotoxin 1	<i>Naja mossambica</i>
12	Disintegrin EO5A	<i>Echis ocellatus</i>	Disintegrin EO5A	<i>Echis ocellatus</i>
13	Disintegrin bitistatin	<i>Bitis arietans</i>	Disintegrin bitistatin	<i>Bitis arietans</i>
14	Glutaminy-peptide cyclotransferase	<i>Boiga dendrophila</i>	Kunitz-type serine protease inhibitor bitisilin-1	<i>Bitis gabonica</i>
15	Hemoglobin subunit beta	<i>Erythrolamprus miliaris</i>	Kunitz-type serine protease inhibitor bitisilin-3	<i>Bitis gabonica</i>
16	Kunitz-type serine protease inhibitor bitisilin-1	<i>Bitis gabonica</i>	L-amino-acid oxidase	<i>Bitis gabonica</i>
17	Kunitz-type serine protease inhibitor bitisilin-3	<i>Bitis gabonica</i>	L-amino-acid oxidase	<i>Echis ocellatus</i>
18	L-amino-acid oxidase	<i>Bitis gabonica</i>	L-amino-acid oxidase	<i>Pseudechis australis</i>
19	L-amino-acid oxidase	<i>Echis ocellatus</i>	Long neurotoxin 1	<i>Naja anchietae</i>
20	L-amino-acid oxidase	<i>Pseudechis australis</i>	Peroxiredoxin-4	<i>Crotalus atrox</i>
21	Long neurotoxin 1	<i>Naja anchietae</i>	Phospholipase A2 homolog	<i>Echis ocellatus</i>
22	Peroxiredoxin-4	<i>Crotalus atrox</i>	Phospholipase-B 81	<i>Drysdalia coronoides</i>
23	Phospholipase A2 homolog	<i>Echis ocellatus</i>	Serine protease harobin	<i>Hydrophis hardwickii</i>
24	Phospholipase A2 inhibitor 1	<i>Protobothrops flavoviridis</i>	Serine protease sp-Eoc49	<i>Echis ocellatus</i>
25	Phospholipase-B 81	<i>Drysdalia coronoides</i>	Short neurotoxin 1	<i>Naja nivea</i>
26	Secretory phospholipase A2 receptor	<i>Pongo abelii</i>	Snaclec 2	<i>Bitis gabonica</i>

Protein/peptide detected				
27	Serine protease harobin	<i>Hydrophis hardwickii</i>	Snaclec 3	<i>Bitis gabonica</i>
28	Serine protease sp-Eoc49	<i>Echis ocellatus</i>	Snaclec agglucetin subunit beta-1	<i>Deinagkistrodon acutus</i>
29	Short neurotoxin 1	<i>Naja nivea</i>	Snaclec bitiscetin subunit alpha	<i>Bitis arietans</i>
30	Snaclec 2	<i>Bitis gabonica</i>	Snaclec bitiscetin subunit beta	<i>Bitis arietans</i>
31	Snaclec 3	<i>Bitis gabonica</i>	Snaclec clone 2100755	<i>Deinagkistrodon acutus</i>
32	Snaclec CTL-Eoc124	<i>Echis ocellatus</i>	Snake venom 5'-nucleotidase	<i>Crotalus adamanteus</i>
33	Snaclec CTL-Eoc125	<i>Echis ocellatus</i>	Snake venom metalloproteinase ACLH	<i>Agkistrodon contortrix laticinctus</i>
34	Snaclec agglucetin subunit beta-1	<i>Deinagkistrodon acutus</i>	Snake venom metalloproteinase kistomin	<i>Calloselasma rhodostoma</i>
35	Snaclec bitiscetin subunit alpha	<i>Bitis arietans</i>	Snake venom metalloproteinase lebetase-4	<i>Macrovipera lebetina</i>
36	Snaclec bitiscetin subunit beta	<i>Bitis arietans</i>	Snake venom metalloproteinase-disintegrin-like mocarhagin	<i>Naja mossambica</i>
37	Snaclec clone 2100755	<i>Deinagkistrodon acutus</i>	Snake venom serine protease BthaTL	<i>Bothrops alternatus</i>
38	Snaclec convulxin subunit beta	<i>Crotalus durissus terrificus</i>	Snake venom serine protease HS114	<i>Bothrops jararaca</i>
39	Snake venom 5'-nucleotidase	<i>Crotalus adamanteus</i>	Snake venom serine protease NaSP	<i>Naja atra</i>
40	Snake venom metalloproteinase ACLH	<i>Agkistrodon contortrix laticinctus</i>	Snake venom serine protease	<i>Philodryas olfersii</i>
41	Snake venom metalloproteinase kistomin	<i>Calloselasma rhodostoma</i>	Snake venom vascular endothelial growth factor toxin barietin	<i>Bitis arietans</i>
42	Snake venom metalloproteinase lebetase-4	<i>Macrovipera lebetina</i>	Thrombin-like enzyme cerastocytin	<i>Cerastes cerastes</i>
43	Snake venom metalloproteinase-disintegrin-like mocarhagin	<i>Naja mossambica</i>	Vascular endothelial growth factor A	<i>Bitis gabonica</i>
44	Snake venom serine protease BthaTL	<i>Bothrops alternatus</i>	Venom nerve growth factor 2	<i>Naja sputatrix</i>
45	Snake venom serine protease HS114	<i>Bothrops jararaca</i>	Weak neurotoxin 7	<i>Naja naja</i>
46	Snake venom serine protease NaSP	<i>Naja atra</i>	Weak toxin CM-2a	<i>Naja annulifera</i>
47	Snake venom serine protease	<i>Philodryas olfersii</i>	Zinc metalloproteinase homolog-disintegrin albolatin	<i>Trimeresurus albolabris</i>
48	Snake venom vascular endothelial growth factor toxin barietin	<i>Bitis arietans</i>	Zinc metalloproteinase leucurolysin-B	<i>Bothrops leucurus</i>
49	Thrombin-like enzyme cerastocytin	<i>Cerastes cerastes</i>	Zinc metalloproteinase-disintegrin BA-5A	<i>Bitis arietans</i>
50	Venom nerve growth factor 2	<i>Naja sputatrix</i>	Zinc metalloproteinase-disintegrin B1atH1	<i>Bothriechis lateralis</i>

Protein/peptide detected				
51	Weak neurotoxin 7	<i>Naja naja</i>	Zinc metalloproteinase-disintegrin bilitoxin-1	<i>Agkistrodon bilineatus</i>
52	Weak toxin CM-2a	<i>Naja annulifera</i>	Zinc metalloproteinase-disintegrin-like BfMP	<i>Bungarus fasciatus</i>
53	Zinc metalloproteinase homolog-disintegrin albolatin	<i>Trimeresurus albolabris</i>	Zinc metalloproteinase-disintegrin-like EoMP06	<i>Echis ocellatus</i>
54	Zinc metalloproteinase leucurolysin-B	<i>Bothrops leucurus</i>	Zinc metalloproteinase-disintegrin-like EoVMP2	<i>Echis ocellatus</i>
55	Zinc metalloproteinase-disintegrin BA-5A	<i>Bitis arietans</i>	Zinc metalloproteinase-disintegrin-like Eoc1	<i>Echis ocellatus</i>
56	Zinc metalloproteinase-disintegrin B1atH1	<i>Bothriechis lateralis</i>	Zinc metalloproteinase-disintegrin-like HF3	<i>Bothrops jararaca</i>
57	Zinc metalloproteinase-disintegrin bilitoxin-1	<i>Agkistrodon bilineatus</i>	Zinc metalloproteinase-disintegrin-like MTP9	<i>Drysdalia coronoides</i>
58	Zinc metalloproteinase-disintegrin-like BfMP	<i>Bungarus fasciatus</i>	Zinc metalloproteinase-disintegrin-like NaMP	<i>Naja atra</i>
59	Zinc metalloproteinase-disintegrin-like EoMP06	<i>Echis ocellatus</i>	Zinc metalloproteinase-disintegrin-like	<i>Cerberus rynchops</i>
60	Zinc metalloproteinase-disintegrin-like EoVMP2	<i>Echis ocellatus</i>	Zinc metalloproteinase-disintegrin-like TSV-DM	<i>Trimeresurus stejnegeri</i>
61	Zinc metalloproteinase-disintegrin-like Eoc1	<i>Echis ocellatus</i>	Zinc metalloproteinase-disintegrin-like VLAIP-A	<i>Macrovipera lebetina</i>
62	Zinc metalloproteinase-disintegrin-like HF3	<i>Bothrops jararaca</i>	Zinc metalloproteinase-disintegrin-like VLAIP-B	<i>Macrovipera lebetina</i>
63	Zinc metalloproteinase-disintegrin-like MTP9	<i>Drysdalia coronoides</i>	Zinc metalloproteinase-disintegrin-like acurhagin	<i>Deinagkistrodon acutus</i>
64	Zinc metalloproteinase-disintegrin-like NaMP	<i>Naja atra</i>	Zinc metalloproteinase-disintegrin-like atrolysin-A	<i>Crotalus atrox</i>
65	Zinc metalloproteinase-disintegrin-like	<i>Cerberus rynchops</i>	Zinc metalloproteinase-disintegrin-like batroxstatin-1	<i>Bothrops atrox</i>
66	Zinc metalloproteinase-disintegrin-like TSV-DM	<i>Trimeresurus stejnegeri</i>	Zinc metalloproteinase-disintegrin-like batroxstatin-3	<i>Bothrops atrox</i>
67	Zinc metalloproteinase-disintegrin-like VLAIP-A	<i>Macrovipera lebetina</i>	Zinc metalloproteinase-disintegrin-like berythracivase	<i>Bothrops erythromelas</i>
68	Zinc metalloproteinase-disintegrin-like VLAIP-B	<i>Macrovipera lebetina</i>	Zinc metalloproteinase-disintegrin-like bothrojarin-2	<i>Bothrops jararaca</i>
69	Zinc metalloproteinase-disintegrin-like acurhagin	<i>Deinagkistrodon acutus</i>	Zinc metalloproteinase-disintegrin-like brevilysin H2a	<i>Gloydus brevicaudus</i>
70	Zinc metalloproteinase-disintegrin-like atrolysin-A	<i>Crotalus atrox</i>	Zinc metalloproteinase-disintegrin-like cobrin	<i>Naja kaouthia</i>
71	Zinc metalloproteinase-disintegrin-like batroxstatin-1	<i>Bothrops atrox</i>	Zinc metalloproteinase-disintegrin-like daborhagin-K	<i>Daboia russelii</i>
72	Zinc metalloproteinase-disintegrin-like batroxstatin-3	<i>Bothrops atrox</i>	Zinc metalloproteinase-disintegrin-like stejnihagin-A	<i>Trimeresurus stejnegeri</i>
73	Zinc metalloproteinase-disintegrin-like berythracivase	<i>Bothrops erythromelas</i>	Zinc metalloproteinase-disintegrin-like stejnihagin-B	<i>Trimeresurus stejnegeri</i>
74	Zinc metalloproteinase-disintegrin-like bothrojarin-2	<i>Bothrops jararaca</i>	Zinc metalloproteinase/disintegrin	<i>Protobothrops mucrosquamatus</i>

Protein/peptide detected				
75	Zinc metalloproteinase-disintegrin-like brevilysin H2a	<i>Gloydius brevicaudus</i>	Zinc metalloproteinase/disintegrin	<i>Trimeresurus gramineus</i>
76	Zinc metalloproteinase-disintegrin-like cobrin	<i>Naja kaouthia</i>	Zinc metalloproteinase/disintegrin	<i>Calloselasma rhodostoma</i>
77	Zinc metalloproteinase-disintegrin-like daborhagin-K	<i>Daboia russelii</i>	metalloproteinase/disintegrin	<i>Echis ocellatus</i>
78	Zinc metalloproteinase-disintegrin-like stejnihagin-A	<i>Trimeresurus stejnegeri</i>	Zinc metalloproteinase/disintegrin	<i>Macrovipera lebetina</i>
79	Zinc metalloproteinase-disintegrin-like stejnihagin-B	<i>Trimeresurus stejnegeri</i>	Zinc metalloproteinase/disintegrin PMMP-1	<i>Protobothrops mucrosquamatus</i>
80	Zinc metalloproteinase/disintegrin	<i>Protobothrops mucrosquamatus</i>		
81	Zinc metalloproteinase/disintegrin	<i>Trimeresurus gramineus</i>		
82	Zinc metalloproteinase/disintegrin	<i>Calloselasma rhodostoma</i>		
83	metalloproteinase/disintegrin	<i>Echis ocellatus</i>		
84	Zinc metalloproteinase/disintegrin	<i>Macrovipera lebetina</i>		
85	Zinc metalloproteinase/disintegrin PMMP-1	<i>Protobothrops mucrosquamatus</i>		

Proteins were identified using Multi-Dimensional Protein Identification Technology (MuDPIT) incorporated on Scaffold Proteome software version 4.10.0 at 99% protein threshold, 95% peptide threshold, 0.5% false discovery rate (FDR), and two-peptide minimum criterion. Peptides and proteins were searched against the UniprotKB- Serpentes-database.

Table 2
Characterization of proteins identified in the venom extracts of *B. arietans* and *E. ocellatus*

S/No.	Protein/ Toxin detected	Protein accession number	Mol. mass (kDa)	<i>B. arietans</i>				<i>E. ocellatus</i>			
				AA (%)	NET	TUSC	EUP	AA (%)	NET	TUSC	EUP
1	Acidic phospholipase A2	PA2A5_ECHOC	16	83	32	12	8	61	439	79	38
2	Acidic phospholipase A2	PA2A1_NAJMO	13	39	19	1	1	39	9	1	1
3	Alpha-fibrinogenase	VSPA_MACLB	29	8.5	29	0	0	8.1	20	0	0
4	Alpha-fibrinogenase-like	VSPAF_DABSI	28	3.5	28	0	0	0.8	20	1	1
5	Beta-fibrinogenase brevinase	VSPB_GLOBL	26	8.6	54	0	0	16	76	2	1
6	C-type lectin 1	LEC1_BITGA	19	16	4	2	2	8.2	2	1	1
7	Cationic trypsin	TRY1_BOVIN	26	16	5	3	3	8.5	2	1	1
8	Coagulation factor X-activating enzyme heavy chain	VM3CX_DABSI	70	8.1	33	0	0	21	43	9	4
9	Coagulation factor X-activating enzyme heavy chain	VM3CX_MACLB	69	8.5	14	0	0	18	54	4	3
10	Cysteine-rich venom protein	CRVP_TRIST	26	4.3	1	0	0	13	14	0	0
11	Cytotoxin 1	3SA1_NAJMO	7	47	9	0	0	33	30	0	0
12	Disintegrin EO5A	DID5A_ECHOC	12	9.6	7	0	0	36	31	0	0
13	Disintegrin bitistatin	VM2_BITAR	9	88	80	11	6	27	6	12	6
14	Glutaminyl-peptide cyclotransferase	QPCT_BOIDE	42	0.0	0	0	0	17	12	1	1
15	Hemoglobin subunit beta	HBB_ERYML	16	0.0	0	0	0	6.2	3	1	1
16	Kunitz-type serine protease inhibitor bitisilin-1	VKT1_BITGA	10	18	5	2	2	10	2	2	2
17	Kunitz-type serine protease inhibitor bitisilin-3	VKT3_BITGA	17	42	12	5	4	17	7	2	2
18	L-amino-acid oxidase	OXLA_BITGA	7	12	4	0	0	20	7	1	1
19	L-amino-acid oxidase	OXLA_ECHOC	57	34	58	1	1	63	109	1	1
20	L-amino-acid oxidase	OXLA_PSEAU	59	1.4	2	0	0	6.2	8	0	0
21	Long neurotoxin 1	3L21_NAJAC	8	90	60	6	2	93	64	11	5
22	Peroxiredoxin-4	PRDX4_CROAT	4	58	4	2	2	31	2	1	1
23	Phospholipase A2 homolog	PA2HS_ECHOC	16	60	42	19	13	78	312	75	46
24	Phospholipase A2 inhibitor 1	PLI1_PROFL	22	0.0	0	0	0	8.5	20	1	1
25	Phospholipase-B 81	PLB_DRYCN	64	19	121	14	11	20	73	14	11
26	Secretory phospholipase A2 receptor	PLA2R_PONAB	18	0.0	0	0	0	1.2	3	1	1
27	Serine protease harobin	VSPHA_HYDHA	29	7.9	21	3	1	7.9	21	4	3
28	Serine protease sp-Eoc49	VSP_ECHOC	28	24	27	4	4	49	51	18	13

				<i>B. arientans</i>				<i>E. ocellatus</i>			
29	Short neurotoxin 1	3S11_NAJNI	7	61	8	0	0	66	17	2	2
30	Snaclec 2	SL2_BITGA	18	35	26	6	5	31	18	7	6
31	Snaclec 3	SL3_BITGA	18	18	7	3	2	24	9	4	2
32	Snaclec CTL-Eoc124	SL124_ECHOC	17	0.0	0	0	0	65	33	14	9
33	Snaclec CTL-Eoc125	SL125_ECHOC	18	0.0	0	0	0	47	11	6	6
34	Snaclec agglucetin subunit beta-1	SLB1_DEIAC	17	9.6	2	0	0	9.6	4	0	0
35	Snaclec bitiscetin subunit alpha	SLA_BITAR	15	96	124	0	0	91	82	0	0
36	Snaclec bitiscetin subunit beta	SLB_BITAR	15	60	71	27	13	50	60	18	7
37	Snaclec clone 2100755	SL_DEIAC	18	4.5	4	27	13	14	5	18	7
38	Snaclec convulxin subunit beta	SLB_CRODU	17	0.0	0	0	0	11	4	0	0
39	Snake venom 5'-nucleotidase	V5NTD_CROAD	65	39	43	11	5	30	52	5	3
40	Snake venom metalloproteinase ACLH	VM1AH_AGKCL	46	5.2	3	0	0	7.1	7	0	0
41	Snake venom metalloproteinase kistomin	VM1K_CALRH	47	5.3	10	0	0	2.9	4	0	0
42	Snake venom metalloproteinase lebetase-4	VM1L4_MACLB	24	12	4	0	0	25	17	1	1
43	Snake venom metalloproteinase-disintegrin-like mocarhagin	VM3M1_NAJMO	68	2.0	30	4	2	4.4	18	5	2
44	Snake venom serine protease BthaTL	VSPTL_BOTAL	26	5.6	7	0	0	5.6	6	0	0
45	Snake venom serine protease HS114	VSP14_BOTJA	28	5.4	1	0	0	5.4	3	0	0
46	Snake venom serine protease NaSP	VSP1_NAJAT	31	7.4	3	0	0	7.4	2	0	0
47	Snake venom serine protease	VSP_PHIOL	28	15	6	1	1	14	3	1	1
48	Snake venom vascular endothelial growth factor toxin barietin	TXVE_BITAR	17	55	302	30	15	59	107	24	13
49	Thrombin-like enzyme cerastocytin	VSP_P_CERCE	28	13	67	4	3	13	48	3	3
50	Vascular endothelial growth factor A	VEGFA_BITGA	22	9.9	3	2	2	0.0	0	0	0
51	Venom nerve growth factor 2	NGFV2_NAJSP	27	13	10	0	0	18	10	0	0
52	Weak neurotoxin 7	3N027_NAJNA	8	5	2	1	1	12	9	0	0
53	Weak toxin CM-2a	3SOK2_NAJHA	7	46	1	2	1	46	3	3	2
54	Zinc metalloproteinase homolog-disintegrin albolatin	VM2AL_TRIAB	54	5.4	13	2	1	12	13	1	1
55	Zinc metalloproteinase leucurolysin-B	VM3LB_BOTLC	36	13	8	1	1	18	26	1	1

				<i>B. arientans</i>				<i>E. ocellatus</i>			
56	Zinc metalloproteinase-disintegrin BA-5A	VM25A_BITAR	59	13	10	6	5	31	96	28	21
57	Zinc metalloproteinase-disintegrin BlatH1	VM2H1_BOTLA	54	4.1	10	0	0	2.5	4	0	0
58	Zinc metalloproteinase-disintegrin bilitoxin-1	VM2B1_AGKBI	32	13	8	3	2	10	6	3	2
59	Zinc metalloproteinase-disintegrin-like BfMP	VM3_BUNFA	68	1.3	4	0	0	3.8	7	0	0
60	Zinc metalloproteinase-disintegrin-like EoMP06	VM3E6_ECHOC	58	17	21	6	4	92	165	28	17
61	Zinc metalloproteinase-disintegrin-like EoVMP2	VM3E2_ECHOC	69	19	20	6	5	55	241	117	66
62	Zinc metalloproteinase-disintegrin-like Eoc1	VM3E1_ECHOC	69	22	59	6	8	57	201	68	36
63	Zinc metalloproteinase-disintegrin-like HF3	VM3H3_BOTJA	68	9.6	14	0	0	13	30	0	0
64	Zinc metalloproteinase-disintegrin-like MTP9	VM39_DRYCN	68	1.3	4	0	0	5.4	8	0	0
65	Zinc metalloproteinase-disintegrin-like NaMP	VM3_NAJAT	69	3	5	1	1	1.6	2	1	1
66	Zinc metalloproteinase-disintegrin-like	VM3_CERRY	69	5.7	9	0	0	6.2	4	0	0
67	Zinc metalloproteinase-disintegrin-like TSV-DM	VM3TM_TRIST	69	8.2	12	0	0	6.8	7	0	0
68	Zinc metalloproteinase-disintegrin-like VLAIP-A	VM3VA_MACLB	69	19	58	7	6	22	92	10	7
69	Zinc metalloproteinase-disintegrin-like VLAIP-B	VM3VB_MACLB	69	14	37	2	2	11	38	2	1
70	Zinc metalloproteinase-disintegrin-like acurhagin	VM3AH_DEIAC	69	9.7	14	1	1	5.4	9	1	1
71	Zinc metalloproteinase-disintegrin-like atrolysin-A	VM3AA_CROAT	47	4.3	4	1	1	7.6	12	3	3
72	Zinc metalloproteinase-disintegrin-like batroxstatin-1	VM31_BOTAT	46	1.9	4	0	0	5.7	3	1	1
73	Zinc metalloproteinase-disintegrin-like batroxstatin-3	VM33_BOTAT	69	8	7	0	0	4.3	11	1	1
74	Zinc metalloproteinase-disintegrin-like berythracivase	VM3BE_BOTER	69	4.9	9	0	0	9	14	3	1
75	Zinc metalloproteinase-disintegrin-like bothrojarin-2	VM3B2_BOTJA	24	7.8	2	0	0	14	20	4	4
76	Zinc metalloproteinase-disintegrin-like brevilysin H2a	VM32A_GLOBR	47	6.4	6	0	0	11	29	0	0
77	Zinc metalloproteinase-disintegrin-like cobrin	VM3_NAJKA	68	4.7	2	1	1	8.2	5	2	2
78	Zinc metalloproteinase-disintegrin-like daborhagin-K	VM3DK_DABRR	70	7.3	5	2	2	15	34	5	5

				<i>B. arientans</i>				<i>E. ocellatus</i>			
79	Zinc metalloproteinase-disintegrin-like stejnihagin-A	VM3SA_TRIST	68	7.7	13	0	0	7.2	11	2	1
80	Zinc metalloproteinase-disintegrin-like stejnihagin-B	VM3SB_TRIST	68	7	11	0	0	8.3	13	0	0
81	Zinc metalloproteinase/disintegrin	VM2P3_PROMU	46	9.2	8	0	0	2.9	1	0	0
82	Zinc metalloproteinase/disintegrin	VM3G1_TRIGA	48	2.1	3	0	0	7.4	22	0	0
83	Zinc metalloproteinase/disintegrin	VM2RH_CALRH	54	12	16	0	0	6.3	13	2	1
84	Metalloproteinase/disintegrin	VM2OC_ECHOC	55	11	22	10	4	39	216	45	29
85	Zinc metalloproteinase/disintegrin	VM2L2_MACLB	53	13	17	0	0	17	2	4	2
86	Zinc metalloproteinase/disintegrin PMMP-1	VM2P1_PROMU	53	7.9	13	0	0	9.8	11	1	1

The characterization of proteins was achieved using Sequest and X!Tandem incorporated Scaffold Proteome Software 4.10.0: 2019/100

Abbreviation: Mol. mass: molecular mass, %AA: percentage of amino acids, NET: Number of estimated enzymatic cleavage, EUP: Exclusive unique Peptide, TUPC: Total Unique Protein Count.

Table 3
Classification of the detected venom proteomes of *B. arietans* and *E. ocellatus* based on families

S/No.	Protein detected	Protein families
1	Acidic phospholipase A ₂	Phospholipase A ₂ s
2	Acidic phospholipase A ₂	
3	Phospholipase A ₂ homolog	
4	Phospholipase A ₂ inhibitor 1	
5	Secretory phospholipase A2 receptor	
6	Phospholipase-B 81	Phospholipase B
7	Alpha-fibrinogenase	Snake venom Serine proteases
8	Alpha-fibrinogenase-like	
9	Beta-fibrinogenase brevinase	
10	Cationic trypsin	
11	Coagulation factor X-activating enzyme heavy chain	
12	Coagulation factor X-activating enzyme heavy chain	
13	Serine protease harobin	
14	Serine protease sp-Eoc49	
15	Snake venom serine protease BthaTL	
16	Snake venom serine protease HS114	
17	Snake venom serine protease NaSP	
18	Snake venom serine protease	
19	Thrombin-like enzyme cerastocytin	
20	Glutaminy-peptide cyclotransferase	
21	Hemoglobin subunit beta	
22	Kunitz-type serine protease inhibitor bitisilin-1	Kunitz peptides
23	Kunitz-type serine protease inhibitor bitisilin-3	
24	Snake venom metalloproteinase ACLH	Snake venom Metalloproteinases
25	Snake venom metalloproteinase kistomin	
26	Snake venom metalloproteinase lebetase-4	
27	Snake venom metalloproteinase-disintegrin-like mocarhagin	
28	Zinc metalloproteinase homolog-disintegrin albolatin	
29	Zinc metalloproteinase leucurolysin-B	
30	Zinc metalloproteinase-disintegrin BA-5A	
31	Zinc metalloproteinase-disintegrin Blath1	
32	Zinc metalloproteinase-disintegrin bilitoxin-1	
33	Zinc metalloproteinase-disintegrin-like BfMP	
34	Zinc metalloproteinase-disintegrin-like EoMP06	

S/No.	Protein detected	Protein families
35	Zinc metalloproteinase-disintegrin-like EoVMP2	
36	Zinc metalloproteinase-disintegrin-like Eoc1	
37	Zinc metalloproteinase-disintegrin-like HF3	
38	Zinc metalloproteinase-disintegrin-like MTP9	
39	Zinc metalloproteinase-disintegrin-like NaMP	
40	Zinc metalloproteinase-disintegrin-like	
41	Zinc metalloproteinase-disintegrin-like TSV-DM	
42	Zinc metalloproteinase-disintegrin-like VLAIP-A	
43	Zinc metalloproteinase-disintegrin-like VLAIP-B	
44	Zinc metalloproteinase-disintegrin-like acurhagin	
45	Zinc metalloproteinase-disintegrin-like atrolysin-A	
46	Zinc metalloproteinase-disintegrin-like batroxstatin-1	
47	Zinc metalloproteinase-disintegrin-like batroxstatin-3	
48	Zinc metalloproteinase-disintegrin-like berythracivase	
49	Zinc metalloproteinase-disintegrin-like bothrojarin-2	
50	Zinc metalloproteinase-disintegrin-like brevilysin	
51	Zinc metalloproteinase-disintegrin-like cobrin	
52	Zinc metalloproteinase-disintegrin-like daborhagin-K	
53	Zinc metalloproteinase-disintegrin-like stejnihagin-A	
54	Zinc metalloproteinase-disintegrin-like stejnihagin-B	
55	Zinc metalloproteinase/disintegrin	
56	Zinc metalloproteinase/disintegrin	
57	Zinc metalloproteinase/disintegrin	
58	Metalloproteinase/disintegrin	
59	Zinc metalloproteinase/disintegrin	
60	Zinc metalloproteinase/disintegrin PMMP-1	
61	Cysteine-rich venom protein	Cysteine-rich venom protein
62	Disintegrin EO5A	Disintegrins
63	Disintegrin bitistatin	
64	L-amino-acid oxidase	L-amino acid oxidases
65	L-amino-acid oxidase	
66	L-amino-acid oxidase	
67	Long neurotoxin 1	Three-finger toxins
68	Short neurotoxin 1	
69	Weak toxin CM-2a	
70	Peroxiredoxin-4	

S/No.	Protein detected	Protein families
71	Cytotoxin 1	C-type lectins/ Snaclecs
72	Snaclec 2	
73	Snaclec 3	
74	Snaclec CTL-Eoc124	
75	Snaclec CTL-Eoc125	
76	Snaclec agglucetin subunit beta-1	
77	Snaclec bitiscetin subunit alpha	
78	Snaclec bitiscetin subunit beta	
79	Snaclec clone 2100755	
80	Snaclec convulxin subunit beta	
81	C-type lectin 1	
82	Snake venom 5'-nucleotidase	5' Nucleotidases
83	Snake venom vascular endothelial growth factor toxin barietin	Vascular endothelial growth factors
84	Vascular endothelial growth factor A	
85	Venom nerve growth factor 2	

3.3. Relative distribution of the protein families

In principle, the composition of the analyzed venoms indicated that metalloproteinases (SVMPs), serine proteinases (SVSPs), and phospholipases A₂ (PLA₂s) constituted the major proportions of the venom proteomes, which is consistent with the report of previous studies of viper venomomics^{17,29,30}. However, a deep insight into the proportion of these major protein families revealed a significant variation among species from different regions of the world; the protein composition of the venoms from vipers indigenous to Gaboon in West Africa showed that the percentages of PLA₂s, SVSPs and SVMPs in *B. arietans* were 4.3%, 19.5% and 38.5% respectively³⁰. More so, PLA₂s, and SVMPs were the major toxin families, with varied proportions in the venoms of *Echis* species³¹. In the venoms of these true vipers, indigenous to Nigeria, the percentages are higher with PLA₂s, SVSPs and SVMPs having 10.6%, 22.31%, and 21.06% respectively in *B. arietans* while in *E. ocellatus*, 25.69%, 17.25% and 34.84% were recorded in PLA₂s, SVSPs and SVMPs respectively (Figs. 3 and 4). These variations are attributed to factors such as snake's gender, age²¹, geographical origin^{18,19} and diet²⁰. It is believed that mutations in the venom-related genes may impact the venom composition³². Nine secondary protein families were detected, namely, kunitz-type peptides, cysteine-rich venom proteins, three-finger toxins, disintegrins, L-amino acid oxidases, C-type lectins/ snaclecs, 5'nucleotidases, vascular endothelial growth factors and phospholipase B with a distribution range between 1–4% (Figs. 3 and 4).

In a previous study, venom gland transcriptomic analysis showed that snake venom metalloproteinases and serine proteases represent majority of the toxin genes transcribed in Nigerian *B. arietans*, and they are also the most abundant toxin family secreted in the venom²³. This is in tandem with our findings that SVSPs and SVMPs were the dominant proteins in the venom of *B. arietans*. Similarly, snake venom metalloproteinases, phospholipase A₂ and serine proteases constituted the most abundant toxin genes transcribed in the venom glands of *E. ocellatus* from Nigeria²⁴. The high abundance of these 3 toxin families (PLA₂s, SVSPs and SVMPs) in the venom gland transcriptomic analyses correlates with this present report that these toxins represent the highest in proteomic abundance. In a parallel comparison, transcriptomic analyses revealed that C-type lectins and L-amino oxidases were also present in high abundances but they are present in low abundances 3.95% and 2.24% respectively in the venom proteome (Fig. 4). This disparity is due to the fact that toxins are known to be transcribed at the highest level in the venom gland²³.

3.4. Pathological mechanisms of the toxin families

Snake venom toxins are thought to act synergistically in exerting a wide range of biochemical and toxicological effects^{33,34}. Envenomation by the viper primarily gives rise to local effects and bleeding³⁵. Snake venom metalloproteinases (SVMPs) are zinc-dependent proteinase toxins that form the major component of viper venom¹⁷. They are the most lethal venom protein of snakes from the viper family^{29,36,37} which play a key role in coagulopathies commonly associated with viper envenoming^{38,29,36}. Similarly, SVMPs provoke a manifold of clinical manifestations, including hemorrhagic, pro-coagulant, anticoagulant, fibrinolytic, apoptotic, and antiplatelet activities^{39,40}. Thus, SVMPs are believed to evolve from ADAM (a Disintegrin and Metalloproteinase) proteins, precisely ADAM28, characterized by metalloproteinase, disintegrin-like and cysteine-rich domains⁴¹, which induce hemorrhagic activity by rupturing the capillary vessels, resulting to incoagulable blood in envenomed victims⁴². This occurs through cleaving of the basement membrane and adhesion proteins of the endothelial cells-matrix, causing the endothelial cells to detach and become thin, leading to the obstruction of the capillary walls and blood effusion^{29,43}. More so, SVMPs alters homeostasis by disrupting coagulation, through modulation of fibrinogenase and fibrolase that mediate the coagulation cascade which aids in eliciting their hemorrhagic role^{44,45}.

Snake venom serine proteinases (SVSPs) are hemotoxic affecting hemostatic system by provoking edema and hyperalgesia, which result in alteration of blood coagulation processes (pro-coagulant or anti-coagulant), fibrinolysis, platelet aggregation and lethal blood pressure^{31,33,46,45}.

Along with SVMPs and SVSPs as the major toxin families in venom of the vipers analyzed in this study, the venom of *E. ocellatus* were found to be dominated by Phospholipases A₂ (PLA₂s) which constituted 26% of the venom proteome. Our data correlates with the previous studies in which PLA₂s were described as one of the commonest toxin in the venom of the front-fanged snakes^{47,48,17}. Although PLA₂s were present in both venoms, it was in lower abundance (10.6%) in the venom of *B. arietans* (Fig. 3). These toxins had molecular masses of 13–22 kDa (Table 2) and are important causes of neurotoxic and myotoxic effects of viper envenomation^{49,50,51}. The myotoxic effect often leads to severe necrosis⁵¹, while respiratory paralysis seen in victims is caused by the neurotoxins². PLA₂s elicits its toxicity through the alteration of pre-synaptic terminals and the sensory nerve endings^{52,50,53,54}.

Other toxin families identified in the venom of these vipers were three-finger toxins, kunitz-type peptides, cysteine-rich secretary proteins, disintegrins, L-amino acid oxidases, C-type lectins, 5-nucleotidase, vascular endothelial growth factors and phospholipase B. These secondary toxins were detected, albeit, at lower abundances in the venom of *E. ocellatus* (Fig. 4) compared to that of *B. arietans* (Fig. 3). The toxin 5'-nucleotidases inhibit the aggregation of platelet through the release of adenosine from guanosine monophosphate and adenosine monophosphate which binds to receptors on the platelets⁵⁵, this shows that they act in synergy with hemorrhagic toxins to elicit anticoagulant effect observed in envenoming^{56,57}.

L-amino acid oxidases (LAAOs) in snake venoms catalyze oxidative deamination of amino acids, leading to the release of hydrogen peroxide⁵⁸, which provokes toxicity by impacting on platelet aggregation and inducing hemorrhage that ultimately leads to apoptosis of vascular endothelial cells^{59,60}.

Additionally, the cysteine-rich venom proteins (CRISPs) are toxin families whose exact function in snake venoms is not known, they are however, potential blockers of Ca²⁺ and cyclic nucleotide-gated channels⁶¹. The C-type lectins/ snaclecs (CTLs) are calcium-dependent non-enzymatic proteins that bind carbohydrates, mainly galactose³⁴ and play important roles in the agglutination of erythrocytes and aggregation of platelet⁶².

Also, three-finger toxins (3FTXs) are non-enzymatic three-finger fold structure that are neurotoxic in nature⁶³ and are commonly described in the venoms from snakes of the elapid family¹⁷. However, their relative proportion was found to be 4% and 3% in the venoms of *B. arietans* (Fig. 3) and *E. ocellatus* (Fig. 4) respectively. 3FTXs bind at the postsynaptic neuromuscular junctions, thereby inducing flaccid paralysis in victims⁶⁴ as well as respiratory paralysis².

In the case of kunitz-type peptides, a very low percentages (1%) in the venom of these vipers (Figs. 3 and 4) was obtained in the low molecular mass range of 10-17kDa (Table 2). This toxin family is reported to possess antifibrotic activity⁶⁵, and elicits toxicity by obstructing the ion-channels, induction of inflammation and interfering with blood coagulation processes⁶⁶.

Disintegrins are venom toxins that were first detected in viper snakes⁶⁷, and are potent inhibitor of platelet aggregation, hence, antagonize the clotting of blood⁶⁸, thereby contributing to lethality in envenomed victims.

Moreover, snake venom vascular endothelial growth factors were distributed in minute quantities notably in the venom of *E. ocellatus*. They possess vasculotoxin-like activity which makes snakebite site liable to rapid infiltration of venom into victims⁶⁹. Phospholipase B (PLB) was the least abundant protein with 1.8% (Fig. 3) and 0.6% (Fig. 4) in the venoms of *B. arietans* and *E. ocellatus* respectively. In snake venomomics analysis, much interest has not been given to PLB probably because the enzyme is not commonly identified in snake venoms⁷⁰, therefore, there are limited data on the enzyme, and hence, the exact pathological function of PLB in snake venom has not been explained. Several studies reported the presence of PLB in some viper venoms^{71,72,73}, which is in accord with our findings.

4. Conclusion

This report has provided baseline information describing the venom proteome of *E. ocellatus* and *B. arietans* indigenous to Nigeria, with *E. ocellatus* producing more of the toxins. In both snake venoms, there was 90.8% similarity in protein profile. SVMPs, PLA₂s and SVSPs were the toxin families. *E. ocellatus* had 34.84%, 25.69% and 17.25% respectively of these venom protein families, and for *B. arietans*, 22.31%, 21.06% and 10.6% were recorded for SVSPs, SVMPs and PLA₂s respectively. Going by these findings, one can say that although the envenomation by these snakes may present some common pathogenic signs due to some similarities in the venom composition, their venoms are very much different when the toxins are taken into consideration and as such, this should guide in the use and design of a potential antivenin.

Declarations

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Data availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE²⁶ partner repository with the dataset identifier PXD024638 and 10.6019/PXD024638, at <https://www.ebi.ac.uk/pride/archive/projects/PXD024638>.

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Author contributions

FAA, EJD, ABS: Conceptualization. ABS, AS, MSA: Supervision. EJD, FAA, GM, AK: Investigation. FAA, EJD: Data analysis. EJD: Writing original draft preparation. All authors critically reviewed and edited the final manuscript version.

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Competing interests

The authors declare no competing interests.

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Figures

Figure 1

One-dimensional SDS-PAGE profile of *B. arietans* and *E. ocellatus* venoms

Figure 2

Venn diagram presenting the common and exclusive proteins in the analyzed venom of *B. arietans* and *E. ocellatus* as revealed by mass spectrometry

Figure 3
Relative distribution of protein families in the venom proteome of *B. arietans*

Figure 4

Relative distribution of protein families in the venom proteome of *E. ocellatus*