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Venom proteomic analysis of 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 responsible for the highest number of deaths. Hydrophilic interaction liquid chromatography coupled with LC-MS/MS was used to analyze the crude venoms 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 venoms led to the identification of 85 and 79 proteins, respectively. Seventy-eight (78) proteins were common between the two snake species with a 91.8% similarity score. The identified proteins belong to 18 protein families in *E. ocellatus* and 14 protein families in *B. arietans*. Serine proteases (22.31%) and metalloproteinases (21.06%) were the dominant proteins in the venom of *B. arietans*; while metalloproteinases (34.84%), phospholipase A_2s (21.19%) and serine proteases (15.50%) represent the major toxins in the *E. ocellatus* venom. Other protein families such as three-finger toxins and cysteine-rich venom proteins were detected in low proportions. This study provides an insight into the venom proteomic analysis of the two Nigerian viper species, which could be useful in identifying the toxin families to be neutralized in case of envenomation.

1. Introduction

Venomous snakes pose severe health problems especially for the rural dwellers in the tropical regions of developing countries [1–3]. Despite the remarkable efforts for effective management of envenomings, the World Health Organization (WHO) incorporated snakebite in the list of neglected tropical diseases [4]. Thus, further and better efforts are required to address the life-threatening situation posed by this disease. Snakebites are a common health hazard in the Savanna region of West Africa [2–4]. In Nigeria, the *Viperidae*, notably *Bitis arietans* (Puff adder) and *Echis ocellatus* (Carpet viper) are associated with the highest incidence of morbidity and mortality [1,5–7] 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 than the hospital-based estimates [2,8,9]. The WHO reported about 5.4 million incidences of snakebites annually, with 2.7 million cases of envenomings, 138,000 deaths and 400,000 cases of disabilities [10]. In Nigeria, the incidence of snakebites leads to 10,000 deaths annually where envenomation by *E. ocellatus* was responsible for 66% of total cases [7,11]. Snakebite inflicts severe damage to different organs of the body, and envenomings by the *Viperidae* are characterized by life-threatening symptoms, primarily due to local tissue damage and bleeding [1].

The evolution of venomics and antivenomics using the '-omics' technologies has paved a way to obtain valuable insights into snake

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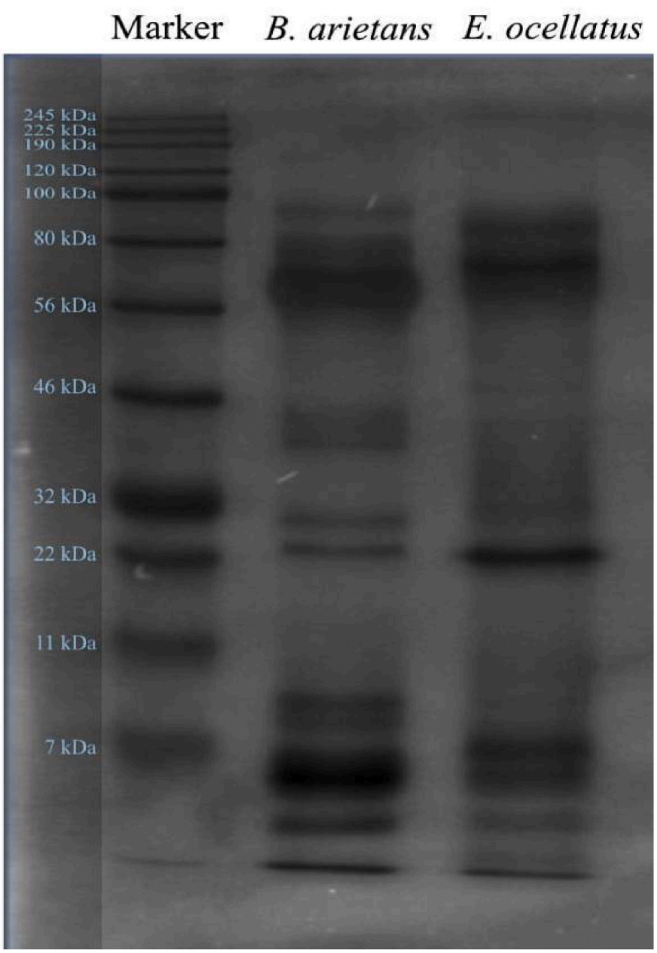

Fig. 1. One-dimensional SDS-PAGE profile of *B. arietans* and *E. ocellatus* venoms.

venom compositions, revealing the protein constituents and different types of toxic components [12,13]. In addition, the '-omics' approach has led to the rapid examination of the immune reactivity of antivenoms against the toxins found in snake venoms [2,14]. Due to the complex nature of the protein mixture in snake venoms, envenomation is characterized by varied pathological manifestations [2]. It is reported that geographical inhabitation of snake [15–18], diet type [19] and age [20] contribute to the variability of the venom composition. Hence, analyzing venom proteomes to gain an insight into snake venom compositions can help in understanding their biological and pathological effects [21]. 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 [22,23]. In the current study, the venom proteomes of *B. arietans* and *E. ocellatus* captured from different regions of Nigeria were analyzed with the view to understanding their toxin profile and protein identities.

2. Materials and method

2.1. Bitis arietans and Echis ocellatus venom samples

For this study, 42 male snakes (36 *E. ocellatus* and 6 *B. arietans*) were used. All protocols for snake handling were observed and carried out in accordance with the Ahmadu Bello University Committee on Animal Use and Care and in compliance with the revised ARRIVE guidelines 2.0. Adult snakes *B. arietans* and *E. ocellatus* were captured from different regions of the Southern and Northern parts of Nigeria. This sampling strategy justifies a generalized inference about the protein profile of these Nigerian vipers. The snakes were maintained at the serpentarium of the Department of Veterinary Pharmacology and Toxicology of Ahmadu Bello University. After 3 days, their venoms were manually extracted as described by Hill and Mackessy [24]. 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 following a method described previously [25]. Two milligrams (dry-weight) of the lyophilized crude venom (from each species) were solubilized in 50 μL 1 × 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 1 × 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 described previously [25]. 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 30 mM iodoacetamide (Sigma) in 50 mM TEAB at 25 °C and the mixture was kept in the dark for 30 min. After alkylation, two-fold dilutions of the samples were made with a 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 the magnetic particles were washed twice with 95% acetonitrile for 1 min. Thereafter, 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 the content was incubated overnight at 27 °C with constant shaking. Following the digestion, peptides were recovered with 1% trifluoroacetic acid (TFA); which were then incubated at room temperature for 3 min and analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

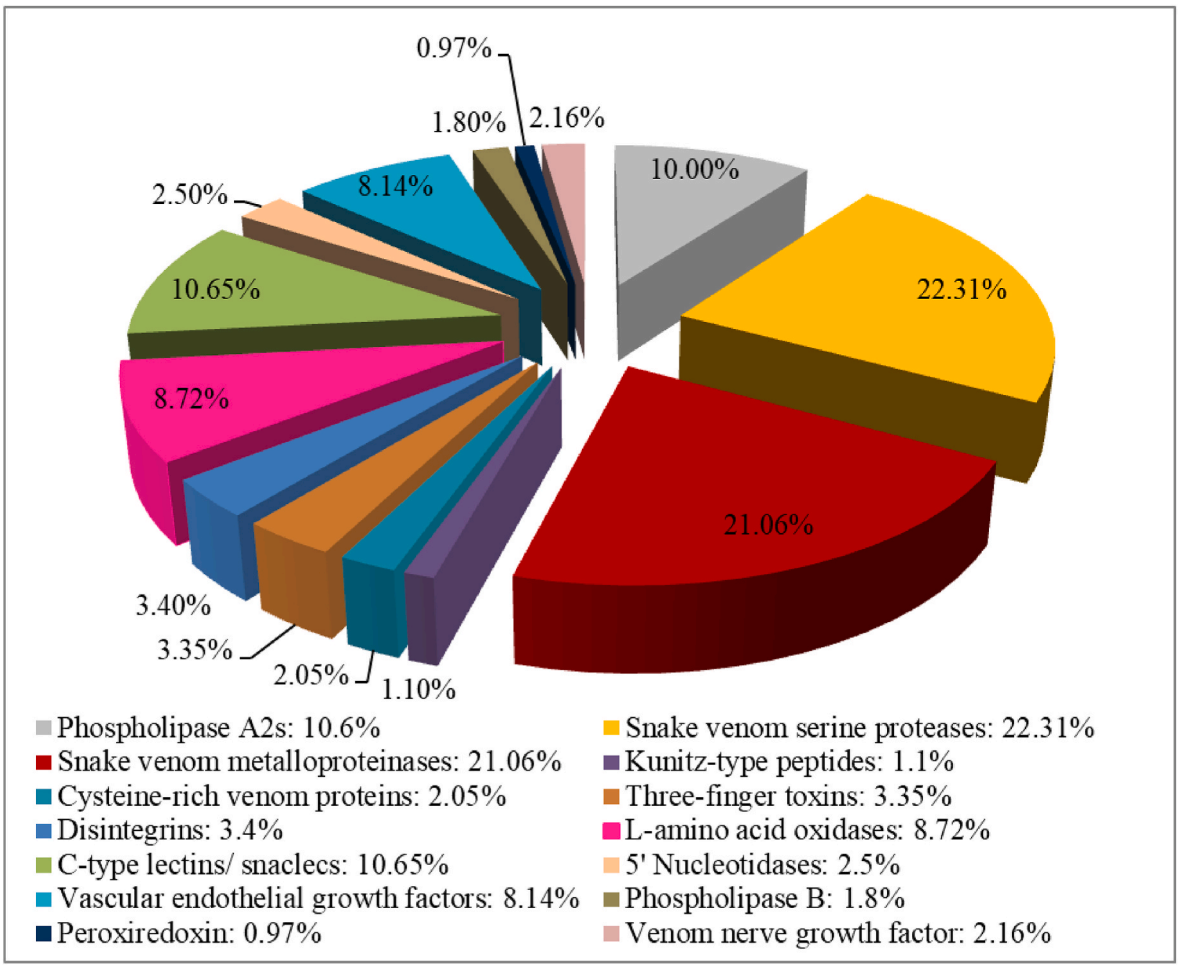

Fig. 2. Relative distribution of protein families in the venom proteome of *B. arietans*.

2.6. Characterization of venom proteins

Liquid chromatography was performed as described by Lomonte et al. [26] with slight modifications using an UltiMate™ 3000 RSLCnano System (Thermo Fisher Scientific, USA) equipped with a C_{18} trap column (5 mm × 300 μm; Thermo Fisher Scientific) and a CSH C_{18} analytical column (1.7 μm, 25 cm × 75 μm), 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 and ion transfer capillary set at 1.8 kV and 280 °C respectively. 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 at 120,000 resolutions over an *m/z* range of 350–1650 with an AGC target at 3E5 and maximum injection time of 40 ms. 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 s 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 ms. Data were acquired in centroid mode.

2.7. Data analysis

The mass spectra obtained were subjected to analysis and processing using Proteome Discoverer v1.4 software (Thermo Fisher Scientific, USA) and the Sequest and Amanda algorithms, respectively. Database interrogation was performed against a concatenated database created by concatenating all 'snake protein' entries, including the previously analyzed transcriptome data of the two snakes 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 relative abundances (expressed as percentage of the total venom proteins) of the protein families were obtained by the unique spectral count strategy for label-free quantitative proteomics [27]. For accurate quantitation, Abacus computational tool for spectral

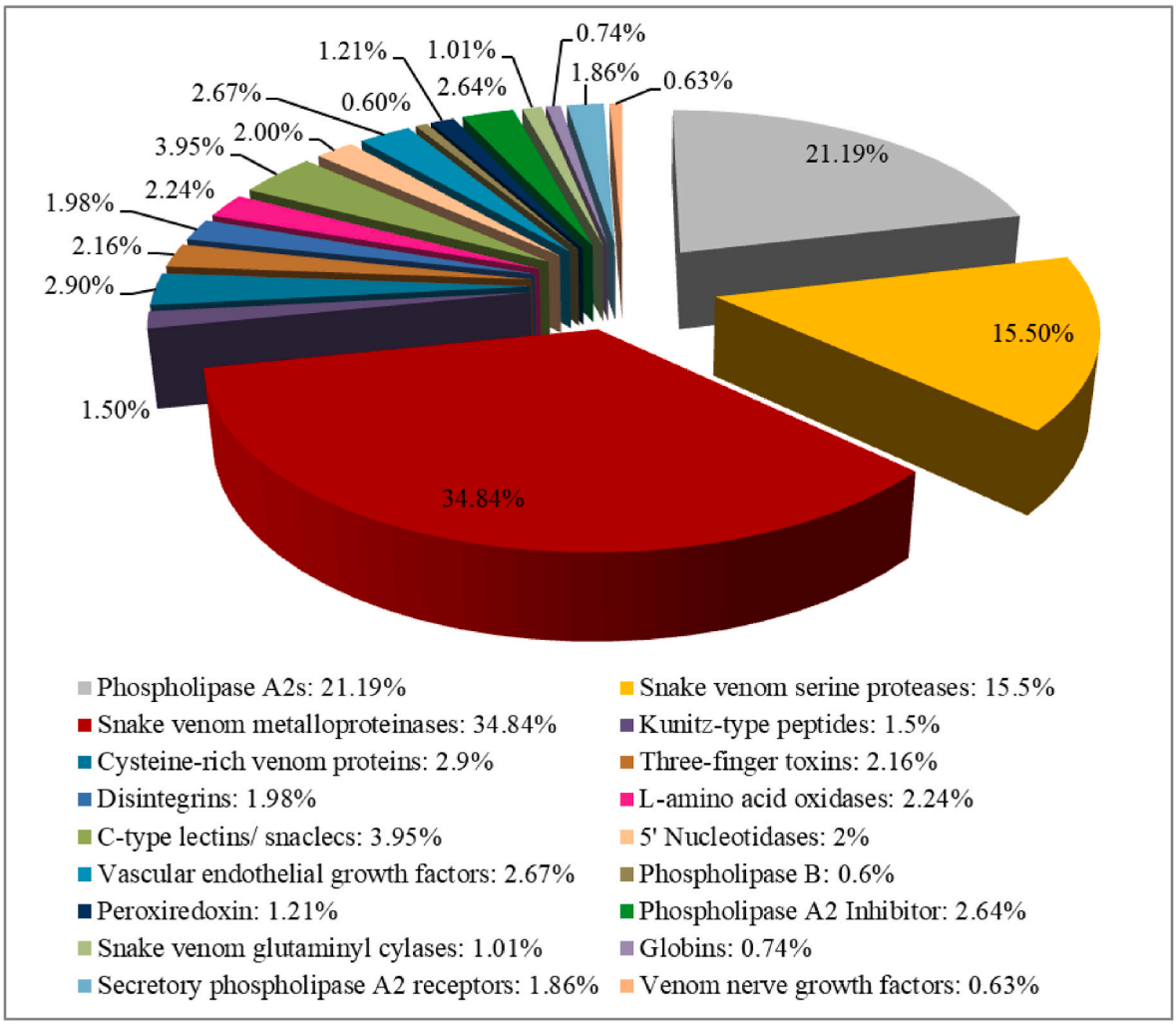

Fig. 3. Relative distribution of protein families in the venom proteome of *E. ocellatus*.

counts was used to implement a distributed Normalized Spectral Abundance Factor (dNSAF), to accurately account for peptides shared across multiple proteins [28,29]. The mass spectrometric data has been deposited into the ProteomeXchange Consortium via the PRIDE [30] with an 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 was size-fractionated on a one-dimensional SDS polyacrylamide gel and visualized using Coomassie Brilliant Blue R-250 dye (Fig. 1). The protein bands observed for the two venoms were distinctly different and clearly separated within the molecular weight ranges of 7 kDa, 22 kDa and 100 kDa (Fig. 1). The distinct pattern on the 1D gel correlated with the LC-MS/MS analysis which showed that *B. arietans* had serine proteases and metalloproteinases as the most abundant venom proteins (Fig. 2), while the venom of *E. ocellatus* was composed of three major protein families; i.e. metalloproteinases, phospholipase A_2s, and serine proteases (Fig. 3).

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

Annotation was against the comprehensive UniProt Serpentes database where all curated data are available, including the venom gland transcriptome dataset of Nigerian *E. ocellatus* and *B. arietans*. 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 digested crude venom was analyzed using LC-MS/MS. Table 2 summarizes the difference and similarities of the protein and protein families. These vipers shared a total of 78 proteins and 14 protein families in common. In addition, *E. ocellatus* had 4 protein families namely; phospholipase A_2 inhibitors, snake venom glutaminyl cyclases, secretary phospholipase A_2 receptors and globin exclusive to its venom (Table 2). Overall, the protein similarity index was found to be 91.8%. Thus, all the proteins detected (in the molecular mass range of 4–93 kDa) (Table 3) belonged to 14 protein families in *B. arietans* and 18 protein families in *E. ocellatus* (Table 2). The most abundant proteins in the venom of *B. arietans* were serine proteases (22%) and metalloproteases (21%) (Fig. 2), followed by C-type lectins/snaclecs (10.65%), phospholipase A_2 (10.6%), L-amino acid oxidases (8.72%) and vascular endothelial growth factors (8.14%). Three-finger toxins (3.35%), disintegrins (3.4%), 5′-nucleotidases (2.5%), venom nerve growth factor (2.16%) and cysteine-rich venom proteins (2.05%) were found in relatively low abundance. Kunitz-type peptides, peroxiredoxin and phospholipase B were the least abundant protein families (<1%) identified in *B. arietans* (Fig. 2). On the other hand, the most abundant proteins identified in the venom of *E. ocellatus* include metalloproteinases (34.84%), phospholipase A_2 (21.19%), and serine proteases (15.05%) (Fig. 3). C-type lectins/snaclecs (3.95%), three-finger toxins (2.16%), vascular endothelial growth factors (2.67%), cysteine-rich venom proteins (2.9%) and phospholipase A_2 inhibitors (2.64%) were found in much lower

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

Protein/peptide detected				
S/no.	*Echis ocellatus*	Originating species	*Bitis arietans*	Originating species
1	Acidic phospholipase A_2	*Echis ocellatus*	Acidic phospholipase A_2	*Echis ocellatus*
2	Acidic phospholipase A_2	*Naja mossambica*	Acidic phospholipase A_2	*Naja mossambica*
3	Alpha-fibrinogenase	*Macrovipera lebetina*	Alpha-fibrinogenase	*Macrovipera lebetina*
4	Alpha-fibrinogenase-like	*Daboia siamensis*	Alpha-fibrinogenase-like	*Daboia siamensis*
5	Beta-fibrinogenase brevinase	*Gloydius blomhoffii*	Beta-fibrinogenase brevinase	*Gloydius blomhoffii*
6	C-type lectin 1	*Bitis gabonica*	C-type lectin 1	*Bitis gabonica*
7	Cationic trypsin	*Bos taurus*	Cationic trypsin	*Bos taurus*
8	Coagulation factor X-activating enzyme heavy chain	*Daboia siamensis*	Coagulation factor X-activating enzyme heavy chain	*Daboia siamensis*
9	Coagulation factor X-activating enzyme heavy chain	*Macrovipera lebetina*	Coagulation factor X-activating enzyme heavy chain	*Macrovipera lebetina*
10	Cysteine-rich venom protein	*Trimeresurus stejnegeri*	Cysteine-rich venom protein	*Trimeresurus stejnegeri*
11	Cytotoxin 1	*Naja mossambica*	Cytotoxin 1	*Naja mossambica*
12	Disintegrin EO5A	*Echis ocellatus*	Disintegrin EO5A	*Echis ocellatus*
13	Disintegrin bitistatin	*Bitis arietans*	Disintegrin bitistatin	*Bitis arietans*
14	Glutaminyl-peptide cyclotransferase	*Boiga dendrophila*	Kunitz-type serine protease inhibitor bitisilin-1	*Bitis gabonica*
15	Hemoglobin subunit beta	*Erythrolamprus miliaris*	Kunitz-type serine protease inhibitor bitisilin-3	*Bitis gabonica*
16	Kunitz-type serine protease inhibitor bitisilin-1	*Bitis gabonica*	L-amino-acid oxidase	*Bitis gabonica*
17	Kunitz-type serine protease inhibitor bitisilin-3	*Bitis gabonica*	L-amino-acid oxidase	*Echis ocellatus*
18	L-amino-acid oxidase	*Bitis gabonica*	L-amino-acid oxidase	*Pseudechis australis*
19	L-amino-acid oxidase	*Echis ocellatus*	Long neurotoxin 1	*Naja anchietae*
20	L-amino-acid oxidase	*Pseudechis australis*	Peroxiredoxin-4	*Crotalus atrox*
21	Long neurotoxin 1	*Naja anchietae*	Phospholipase A_2 homolog	*Echis ocellatus*
22	Peroxiredoxin-4	*Crotalus atrox*	Phospholipase-B 81	*Drysdalia coronoides*
23	Phospholipase A_2 homolog	*Echis ocellatus*	Serine protease harobin	*Hydrophis hardwickii*
24	Phospholipase A_2 inhibitor 1	*Protobothrops flavoviridis*	Serine protease sp-Eoc49	*Echis ocellatus*
25	Phospholipase-B 81	*Drysdalia coronoides*	Short neurotoxin 1	*Naja nivea*
26	Secretory phospholipase A_2 receptor	*Pongo abelii*	Snaclec 2	*Bitis gabonica*
27	Serine protease harobin	*Hydrophis hardwickii*	Snaclec 3	*Bitis gabonica*
28	Serine protease sp-Eoc49	*Echis ocellatus*	Snaclec agglucetin subunit beta-1	*Deinagkistrodon acutus*
29	Short neurotoxin 1	*Naja nivea*	Snaclec bitiscetin subunit alpha	*Bitis arietans*
30	Snaclec 2	*Bitis gabonica*	Snaclec bitiscetin subunit beta	*Bitis arietans*
31	Snaclec 3	*Bitis gabonica*	Snaclec clone 2100755	*Deinagkistrodon acutus*
32	Snaclec CTL-Eoc124	*Echis ocellatus*	Snake venom 5′-nucleotidase	*Crotalus adamanteus*
33	Snaclec CTL-Eoc125	*Echis ocellatus*	Snake venom metalloproteinase ACLH	*Agkistrodon contortrix laticinctus*
34	Snaclec agglucetin subunit beta-1	*Deinagkistrodon acutus*	Snake venom metalloproteinase kistomin	*Calloselasma rhodostoma*
35	Snaclec bitiscetin subunit alpha	*Bitis arietans*	Snake venom metalloproteinase lebetase-4	*Macrovipera lebetina*
36	Snaclec bitiscetin subunit beta	*Bitis arietans*	Snake venom metalloproteinase-disintegrin-like mocarhagin	*Naja mossambica*
37	Snaclec clone 2100755	*Deinagkistrodon acutus*	Snake venom serine protease BthaTL	*Bothrops alternatus*
38	Snaclec convulxin subunit beta	*Crotalus durissus terrificus*	Snake venom serine protease HS114	*Bothrops jararaca*
39	Snake venom 5′-nucleotidase	*Crotalus adamanteus*	Snake venom serine protease NaSP	*Naja atra*
40	Snake venom metalloproteinase ACLH	*Agkistrodon contortrix laticinctus*	Snake venom serine protease	*Philodryas olfersii*
41	Snake venom metalloproteinase kistomin	*Calloselasma rhodostoma*	Snake venom vascular endothelial growth factor toxin barietin	*Bitis arietans*
42	Snake venom metalloproteinase lebetase-4	*Macrovipera lebetina*	Thrombin-like enzyme cerastocytin	*Cerastes cerastes*
43	Snake venom metalloproteinase-disintegrin-like mocarhagin	*Naja mossambica*	Vascular endothelial growth factor A	*Bitis gabonica*
44	Snake venom serine protease BthaTL	*Bothrops alternatus*	Venom nerve growth factor 2	*Naja sputatrix*
45	Snake venom serine protease HS114	*Bothrops jararaca*	Weak neurotoxin 7	*Naja naja*
46	Snake venom serine protease NaSP	*Naja atra*	Weak toxin CM-2a	*Naja annulifera*
47	Snake venom serine protease	*Philodryas olfersii*	Zinc metalloproteinase homolog-disintegrin albolatin	*Trimeresurus albolabris*
48	Snake venom vascular endothelial growth factor toxin barietin	*Bitis arietans*	Zinc metalloproteinase leucurolysin-B	*Bothrops leucurus*
49	Thrombin-like enzyme cerastocytin	*Cerastes cerastes*	Zinc metalloproteinase-disintegrin BA-5A	*Bitis arietans*
50	Venom nerve growth factor 2	*Naja sputatrix*	Zinc metalloproteinase-disintegrin BlatH1	*Bothriechis lateralis*
51	Weak neurotoxin 7	*Naja naja*	Zinc metalloproteinase-disintegrin bilitoxin-1	*Agkistrodon bilineatus*
52	Weak toxin CM-2a	*Naja annulifera*	Zinc metalloproteinase-disintegrin-like BfMP	*Bungarus fasciatus*
53	Zinc metalloproteinase homolog-disintegrin albolatin	*Trimeresurus albolabris*	Zinc metalloproteinase-disintegrin-like EoMP06	*Echis ocellatus*
54	Zinc metalloproteinase leucurolysin-B	*Bothrops leucurus*	Zinc metalloproteinase-disintegrin-like EoVMP2	*Echis ocellatus*
55	Zinc metalloproteinase-disintegrin BA-5A	*Bitis arietans*	Zinc metalloproteinase-disintegrin-like Eoc1	*Echis ocellatus*
56	Zinc metalloproteinase-disintegrin BlatH1	*Bothriechis lateralis*	Zinc metalloproteinase-disintegrin-like HF3	*Bothrops jararaca*
57	Zinc metalloproteinase-disintegrin bilitoxin-1	*Agkistrodon bilineatus*	Zinc metalloproteinase-disintegrin-like MTP9	*Drysdalia coronoides*
58	Zinc metalloproteinase-disintegrin-like BfMP	*Bungarus fasciatus*	Zinc metalloproteinase-disintegrin-like NaMP	*Naja atra*
59	Zinc metalloproteinase-disintegrin-like EoMP06	*Echis ocellatus*	Zinc metalloproteinase-disintegrin-like	*Cerberus rynchops*
60	Zinc metalloproteinase-disintegrin-like EoVMP2	*Echis ocellatus*	Zinc metalloproteinase-disintegrin-like TSV-DM	*Trimeresurus stejnegeri*
61	Zinc metalloproteinase-disintegrin-like Eoc1	*Echis ocellatus*	Zinc metalloproteinase-disintegrin-like VLAIP-A	*Macrovipera lebetina*
62	Zinc metalloproteinase-disintegrin-like HF3	*Bothrops jararaca*	Zinc metalloproteinase-disintegrin-like VLAIP-B	*Macrovipera lebetina*
63	Zinc metalloproteinase-disintegrin-like MTP9	*Drysdalia coronoides*	Zinc metalloproteinase-disintegrin-like acurhagin	*Deinagkistrodon acutus*
64	Zinc metalloproteinase-disintegrin-like NaMP	*Naja atra*	Zinc metalloproteinase-disintegrin-like atrolysin-A	*Crotalus atrox*

(*continued on next page*)

Table 1 (*continued*)

Protein/peptide detected				
S/no.	*Echis ocellatus*	Originating species	*Bitis arietans*	Originating species
65	Zinc metalloproteinase-disintegrin-like	*Cerberus rynchops*	Zinc metalloproteinase-disintegrin-like batroxstatin-1	*Bothrops atrox*
66	Zinc metalloproteinase-disintegrin-like TSV-DM	*Trimeresurus stejnegeri*	Zinc metalloproteinase-disintegrin-like batroxstatin-3	*Bothrops atrox*
67	Zinc metalloproteinase-disintegrin-like VLAIP-A	*Macrovipera lebetina*	Zinc metalloproteinase-disintegrin-like berythractivase	*Bothrops erythromelas*
68	Zinc metalloproteinase-disintegrin-like VLAIP-B	*Macrovipera lebetina*	Zinc metalloproteinase-disintegrin-like bothrojarin-2	*Bothrops jararaca*
69	Zinc metalloproteinase-disintegrin-like acurhagin	*Deinagkistrodon acutus*	Zinc metalloproteinase-disintegrin-like brevilysin H2a	*Gloydius brevicaudus*
70	Zinc metalloproteinase-disintegrin-like atrolysin-A	*Crotalus atrox*	Zinc metalloproteinase-disintegrin-like cobrin	*Naja kaouthia*
71	Zinc metalloproteinase-disintegrin-like batroxstatin-1	*Bothrops atrox*	Zinc metalloproteinase-disintegrin-like daborhagin-K	*Daboia russelii*
72	Zinc metalloproteinase-disintegrin-like batroxstatin-3	*Bothrops atrox*	Zinc metalloproteinase-disintegrin-like stejnihagin-A	*Trimeresurus stejnegeri*
73	Zinc metalloproteinase-disintegrin-like berythractivase	*Bothrops erythromelas*	Zinc metalloproteinase-disintegrin-like stejnihagin-B	*Trimeresurus stejnegeri*
74	Zinc metalloproteinase-disintegrin-like bothrojarin-2	*Bothrops jararaca*	Zinc metalloproteinase/disintegrin	*Protobothrops mucrosquamatus*
75	Zinc metalloproteinase-disintegrin-like brevilysin H2a	*Gloydius brevicaudus*	Zinc metalloproteinase/disintegrin	*Trimeresurus gramineus*
76	Zinc metalloproteinase-disintegrin-like cobrin	*Naja kaouthia*	Zinc metalloproteinase/disintegrin	*Calloselasma rhodostoma*
77	Zinc metalloproteinase-disintegrin-like daborhagin-K	*Daboia russelii*	Metalloproteinase/disintegrin	*Echis ocellatus*
78	Zinc metalloproteinase-disintegrin-like stejnihagin-A	*Trimeresurus stejnegeri*	Zinc metalloproteinase/disintegrin	*Macrovipera lebetina*
79	Zinc metalloproteinase-disintegrin-like stejnihagin-B	*Trimeresurus stejnegeri*	Zinc metalloproteinase/disintegrin PMMP-1	*Protobothrops mucrosquamatus*
80	Zinc metalloproteinase/disintegrin	*Protobothrops mucrosquamatus*		
81	Zinc metalloproteinase/disintegrin	*Trimeresurus gramineus*		
82	Zinc metalloproteinase/disintegrin	*Calloselasma rhodostoma*		
83	Metalloproteinase/disintegrin	*Echis ocellatus*		
84	Zinc metalloproteinase/disintegrin	*Macrovipera lebetina*		
85	Zinc metalloproteinase/disintegrin PMMP-1	*Protobothrops mucrosquamatus*		

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.

abundance. Disintegrins, 5′-nucleotidases, L-amino acid oxidases, snake venom glutaminyl cyclases, secretary phospholipase A_2 receptors and Kunitz-type peptides families were very low in abundance (<3%), while phospholipase B, globin and venom nerve growth factor were virtually absent with percentage abundance of <1%, as shown in Fig. 3.

3.3. Relative distribution of the protein families

Increase in the accuracy of spectral count quantitation emerged following the advancements in bioinformatics tools [31]. As a general rule in spectral count, the number of spectra associated with a peptide shared by different proteins is used for computing spectral count of each protein with similar peptide. This has a limitation of introducing inaccuracy in the estimation of protein abundances, in that if one or more peptides are shared between high and low-abundant proteins, the proportion of the low-abundant protein tends to be overestimated [32]. This limitation was circumvented by implementing the distributed Normalized Spectral Abundance Factor (dNSAF), where shared spectrum count is distributed based on the relative proportions of unique spectra assigned to the proteins containing the shared peptides [28]. Thus, Abacus software was used so as to extract and adjust the spectral counts from MS/MS data sets to accurately account for peptides shared across multiple proteins, through normalization steps [29]. In principle, the composition of the analyzed venoms indicated that metalloproteinases (SVMPs), serine proteinases (SVSPs), and phospholipases A_2 (PLA_2s) constituted the major proportions of the venom proteomes, which is consistent with the report of previous studies on viper venomics [16,33,34]. 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 PLA_2s, SVSPs and SVMPs in *B. arietans* were 4.3%, 19.5% and 38.5% respectively [34]. More so, PLA_2s, and SVMPs were the major toxin families, with varied proportions in the venoms of *Echis* species [35]. In the venoms of these vipers, indigenous to Nigeria, the percentages were higher; with PLA_2s, SVSPs and SVMPs having 10.6%, 22.31%, and 21.06% respectively in *B. arietans* while in *E. ocellatus*, 21.19%, 15.50% and 34.84% were recorded in PLA_2s, SVSPs and SVMPs respectively (Figs. 2 and 3). These variations are attributed to factors such as snake's gender, age [20], geographical origin [15–18] and diet [19]. It is believed that mutations in the venom-related genes may affect the venom composition [25]. Fifteen secondary protein families (Table 4) 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, phospholipase B, peroxiredoxin, phospholipase A_2 inhibitors, snake venom glutaminyl cyclases, globin, secretory phospholipase A_2 receptors, and venom nerve growth factors, with a distribution range between ~1% and 4% (Figs. 2 and 3).

Casewell et al. [22] showed a transcriptomic analysis of venom gland where snake venom metalloproteinases and serine proteases were the major toxin genes transcribed in Nigerian *B. arietans*, and were also found to be the most abundant toxin family secreted in the venom [22]. This is in tandem with our findings that SVSPs and SVMPs were the

Table 2
Summary of the proteins identified, presenting the similarities and differences of proteins and protein families.

Protein family	Number of proteins		
	B. arietans	*E. ocellatus*	Common to both venoms
Snake venom metalloproteinases	0	0	37
Snake venom Serine proteases	0	0	13
Phospholipase A_2s	0	0	3
Kunitz peptides	0	0	2
Cysteine-rich venom protein	0	0	1
Disintegrins	0	0	2
L-amino acid oxidases	0	0	3
Three-finger toxins	0	0	5
C-type lectins/Snaclecs	0	3	7
5′ Nucleotidases	0	0	1
Vascular endothelial growth factors	1	0	1
Phospholipase B	0	0	1
Peroxiredoxin	0	0	1
Phospholipase A_2 inhibitor	0	1	0
Snake venom glutaminyl cyclases	0	1	0
Globin	0	1	0
Secretory phospholipase A_2 receptor	0	1	0
Venom nerve growth factor	0	0	1
Total	**1**	**7**	**78**

The proteins identified were classified into 14 and 18 protein families in *B. arietans* and *E. ocellatus* respectively, with 78 proteins common to both venoms. There were 1 and 7 protein(s) exclusive to the venom of *B. arietans* and *E. ocellatus* respectively. *E. ocellatus* had additional 4 protein families (Phospholipase A_2 inhibitor, Snake venom glutaminyl cyclases, Globin and Secretary phospholipase A_2 receptor) exclusive to its venom.

dominant proteins in the venom of *B. arietans*. Similarly, snake venom metalloproteinases, phospholipase A_2 and serine proteases constituted the most abundant toxin genes transcribed in the venom glands of *E. ocellatus* from Nigeria [23]. The abundance of these 3 toxin families (PLA_2s, SVSPs and SVMPs) in the venom gland transcriptomic analyses correlates with the findings of this study. In a parallel comparison, the transcriptomic analyses showed that C-type lectins and L-amino oxidases were present in high abundances but in this study, they are present in low abundances 3.95% and 2.24% respectively in the venom proteome (Fig. 3). This disparity may be due to the fact that toxins are known to be transcribed at the highest level in the venom gland [22].

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 [36,37]. Envenomation by the viper primarily gives rise to local effects and bleeding [38,39]. Snake venom metalloproteinases (SVMPs) are zinc-dependent proteinase toxins that are responsible for many of the generally known pathological phenotypes in envenomation by the viper snake species [16,40–42] and play a key role in coagulopathies commonly associated with viper envenoming [40,41,43]. Similarly, SVMPs provoke a manifold of clinical manifestations, including hemorrhagic, pro-coagulant, anticoagulant, fibrinolytic, apoptotic, and antiplatelet activities [44,45]. Thus, SVMPs are believed to evolve from ADAM (a Disintegrin and Metalloproteinase) proteins, precisely ADAM28, characterized by metalloproteinase, disintegrin-like and cysteine-rich domains [46], which induce hemorrhagic activity by rupturing the capillary vessels, resulting to extravasation in envenomed victims [47]. This occurs through cleavage 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 [40,48]. 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 [49,50]. The venom of the viper snakes contained the three related structural classes of SVMPs, protein-type I, II and III. The P–I class has only a metalloproteinase domain, P-II class consists of a metalloproteinase and disintegrin domain, whereas the P-III class has a metalloproteinase domain, a disintegrin-like domain and a cysteine-rich domain. Among the protein-type I snake venom metalloproteinases, kistomin impairs platelet function by binding and cleaving of both platelet membrane glycoprotein Ib and glycoprotein VI [51]. ACLH exerts fibrinogenolytic and fibrinolytic effects [52], acurhagin preferentially cleaves Alpha chain of fibrinogen, followed by Beta chain, with minimal effects on the gamma chains [53]. Bilitoxin-1 represents protein-type II hemorrhagic toxin isolated from the venom of these snakes [54]. The catalytic activity of protein-type III hemorrhagic leucurolysin-B on extracellular matrix proteins leads to loss of capillary integrity, resulting in hemorrhage with alterations in platelet function [55]. Atrolysin A is a potent inhibitor of platelet aggregation, the region of the disintegrin-like domain is primarily responsible for blocking platelet aggregation [56]. Berythractivase contains metalloproteinase, disintegrin-like and cysteine-rich domains. Structurally, berythractivase is similar to snake-venom haemorrhagic metalloproteinases and functionally similar to group A prothrombin activators [57].

Snake venom serine proteinases (SVSPs) are multifunctional enzymes that induce toxicity in envenomed victims by affecting the hemostatic system. They alter blood coagulation processes, fibrinolysis and platelet aggregation [35,36,58].

In this study, the venom of *E. ocellatus* was found to be dominated by Phospholipases A_2 (PLA_2s) which constituted 21% of the venom proteome. Our data correlates with the previous studies in which PLA_2s were described as one of the commonest toxins in the venom of the front-fanged snakes [59,60]. Although PLA_2s were present in both venoms, it was found to be lower (10.6%) in the venom of *B. arietans* (Fig. 2). These toxins had molecular masses of 13 and 16 kDa (Table 3) and are important cause of myotoxic effect of viper envenomation [61,62], which often leads to severe necrosis [63]. In addition, PLA_2s from venoms of *Viperidae* snake family induce membranotropic effects [64,65].

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 toxins were detected, albeit, at lower abundances in the venom of *E. ocellatus* (Fig. 3) compared to that of *B. arietans* (Fig. 2). The toxin 5′-nucleotidases inhibit the aggregation of platelet through the release of adenosine from adenosine monophosphate which binds to receptors on the platelets [66], this shows that they act in synergy with hemorrhagic toxins to elicit anticoagulant effect observed in snake bite envenoming [67,68].

L-amino acid oxidases (LAAOs) in snake venoms catalyze oxidative deamination of amino acids, leading to the release of hydrogen peroxide [69], which provokes toxicity by impacting on platelet aggregation and inducing hemorrhage that ultimately leads to apoptosis of vascular endothelial cells [70,71].

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 Ca^{2+} and cyclic nucleotide-gated channels [72]. The C-type lectins/snaclecs (CTLs) are calcium-dependent non-enzymatic proteins that bind carbohydrates, mainly galactose [37] and play important roles in the agglutination of erythrocytes and aggregation of platelet [73].

Also, three-finger toxins (3FTXs) are non-enzymatic three-finger fold structure that is both neurotoxic and cytotoxic in nature [74]. 3FTXs are commonly found in the venoms of snakes from the Elapids family [16], they bind at the postsynaptic neuromuscular junctions, thereby inducing flaccid paralysis in victims [75]. Their biological activity in the venoms of *Viperidae* snake family needs to be explored [64]. However, their

Table 3
Characteristics of the proteins identified in the venom extracts of *B. arietans* and *E. ocellatus*.

				B. arietans				E. ocellatus			
S/No.	Protein/Toxin detected	Protein accession number	Mol. mass (kDa)	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
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	VSPP_CERCE	28	13	67	4	3	13	48	3	3
50	Venom nerve growth factor 2	NGFV2_NAJSP	27	13	10	0	0	18	10	0	0
51	Weak neurotoxin 7	3NO27_NAJNA	8	5	2	1	1	12	9	0	0
52	Weak toxin CM-2a	3SOK2_NAJHA	7	46	1	2	1	46	3	3	2
53	Zinc metalloproteinase homolog-disintegrin albolatin	VM2AL_TRIAB	54	5.4	13	2	1	12	13	1	1
54	Zinc metalloproteinase leucurolysin-B	VM3LB_BOTLC	36	13	8	1	1	18	26	1	1
55	Zinc metalloproteinase-disintegrin BA-5A	VM25A_BITAR	59	13	10	6	5	31	96	28	21
56	Zinc metalloproteinase-disintegrin BlatH1	VM2H1_BOTLA	54	4.1	10	0	0	2.5	4	0	0
57	Zinc metalloproteinase-disintegrin bilitoxin-1	VM2B1_AGKBI	32	13	8	3	2	10	6	3	2
58	Zinc metalloproteinase-disintegrin-like BfMP	VM3_BUNFA	68	1.3	4	0	0	3.8	7	0	0
59	Zinc metalloproteinase-disintegrin-like EoMP06	VM3E6_ECHOC	58	17	21	6	4	92	165	28	17
60	Zinc metalloproteinase-disintegrin-like EoVMP2	VM3E2_ECHOC	69	19	20	6	5	55	241	117	66
61	Zinc metalloproteinase-disintegrin-like Eoc1	VM3E1_ECHOC	69	22	59	6	8	57	201	68	36
62	Zinc metalloproteinase-disintegrin-like HF3	VM3H3_BOTJA	68	9.6	14	0	0	13	30	0	0
63	Zinc metalloproteinase-disintegrin-like MTP9	VM39_DRYCN	68	1.3	4	0	0	5.4	8	0	0
64	Zinc metalloproteinase-disintegrin-like NaMP	VM3_NAJAT	69	3	5	1	1	1.6	2	1	1
65	Zinc metalloproteinase-disintegrin-like	VM3_CERRY	69	5.7	9	0	0	6.2	4	0	0
66	Zinc metalloproteinase-disintegrin-like TSV-DM	VM3TM_TRIST	69	8.2	12	0	0	6.8	7	0	0
67	Zinc metalloproteinase-disintegrin-like VLAIP-A	VM3VA_MACLB	69	19	58	7	6	22	92	10	7
68	Zinc metalloproteinase-disintegrin-like VLAIP-B	VM3VB_MACLB	69	14	37	2	2	11	38	2	1
69	Zinc metalloproteinase-disintegrin-like acurhagin	VM3AH_DEIAC	69	9.7	14	1	1	5.4	9	1	1

(*continued on next page*)

Table 3 (*continued*)

S/No.	Protein/Toxin detected	Protein accession number	Mol. mass (kDa)	B. arietans				E. ocellatus			
				AA (%)	NET	TUSC	EUP	AA (%)	NET	TUSC	EUP
70	Zinc metalloproteinase-disintegrin-like atrolysin-A	VM3AA_CROAT	47	4.3	4	1	1	7.6	12	3	3
71	Zinc metalloproteinase-disintegrin-like batroxstatin-1	VM31_BOTAT	46	1.9	4	0	0	5.7	3	1	1
72	Zinc metalloproteinase-disintegrin-like batroxstatin-3	VM33_BOTAT	69	8	7	0	0	4.3	11	1	1
73	Zinc metalloproteinase-disintegrin-like berythractivase	VM3BE_BOTER	69	4.9	9	0	0	9	14	3	1
74	Zinc metalloproteinase-disintegrin-like bothrojarin-2	VM3B2_BOTJA	24	7.8	2	0	0	14	20	4	4
75	Zinc metalloproteinase-disintegrin-like brevilysin H2a	VM32A_GLOBR	47	6.4	6	0	0	11	29	0	0
76	Zinc metalloproteinase-disintegrin-like cobrin	VM3_NAJKA	68	4.7	2	1	1	8.2	5	2	2
77	Zinc metalloproteinase-disintegrin-like daborhagin-K	VM3DK_DABRR	70	7.3	5	2	2	15	34	5	5
78	Zinc metalloproteinase-disintegrin-like stejnihagin-A	VM3SA_TRIST	68	7.7	13	0	0	7.2	11	2	1
79	Zinc metalloproteinase-disintegrin-like stejnihagin-B	VM3SB_TRIST	68	7	11	0	0	8.3	13	0	0
80	Zinc metalloproteinase/disintegrin	VM2P3_PROMU	46	9.2	8	0	0	2.9	1	0	0
81	Zinc metalloproteinase/disintegrin	VM3G1_TRIGA	48	2.1	3	0	0	7.4	22	0	0
82	Zinc metalloproteinase/disintegrin	VM2RH_CALRH	54	12	16	0	0	6.3	13	2	1
83	Metalloproteinase/disintegrin	VM2OC_ECHOC	55	11	22	10	4	39	216	45	29
84	Zinc metalloproteinase/disintegrin	VM2L2_MACLB	53	13	17	0	0	17	2	4	2
85	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.

relative proportion was found to be 3.35% and 2.16% in the venoms of *B. arietans* (Fig. 2) and *E. ocellatus* (Fig. 3) respectively.

Disintegrins are venom toxins that were first detected in viper snakes [76], and are potent inhibitor of platelet aggregation, hence, antagonize the clotting of blood [77], 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 victims [78]. In the case of kunitz-type peptides, a very low percentage (1%) in the venom of these vipers (Figs. 2 and 3) was obtained with molecular mass range of 10–17 kDa (Table 3). Phospholipase B (PLB) was among the least abundant proteins with 1.8% (Figs. 2) and 0.6% (Fig. 3) in the venoms of *B. arietans* and *E. ocellatus* respectively. In snake venomics analysis, much interest has not been given to PLB probably because the enzyme is not commonly identified in snake venoms [79], therefore, there are limited data on this 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 [79,80], which is in accord with our findings.

4. Snake venom phospholipase A_2 inhibitor

It is known that phospholipase A_2 is one of the venom toxins that cause the disorder associated with snakebite envenoming. Hence, inhibiting the lethal actions of snake venom Phospholipase A_2 presents a strategic target for therapeutic interference [81]. Interestingly, Phospholipase A_2 inhibitor, a potential antitoxin with molecular mass of 22 kDa was detected in the venom of *E. ocellatus*. Available literature reports that the blood plasma of numerous snake species are naturally composed of endogenous phospholipase A_2 inhibitors, generally known as snake blood phospholipase A_2 inhibitors (sbPLIs). Primarily, they neutralize deleterious effects of phospholipase A_2 [82,83]. It implies that sbPLIs exert self-protection role against the enzymes of their own venom, which eventually reach the circulatory system [82,83]. What seems new is the fact that phospholipase A_2 inhibitory protein was detected in the venom, revealing that the protein is not only expressed in the snake blood serum. Exploring this promising protein may serve as antidote to PLA_2-related disorders by giving rise to clinically relevant and specific antivenom that targets phospholipase A_2.

5. Conclusion

In addition to the detection of a venom-based antitoxin; venom phospholipase A_2 inhibitor, this study has provided a baseline information on the venom proteome of *E. ocellatus* and *B. arietans* indigenous to Nigeria, where *E. ocellatus* producing more of the toxins. In both snake venoms, their protein profile was found to be 91.8% similar and SVMPs, PLA_2s and SVSPs were the major toxin families. *E. ocellatus* had 34.84%, 21.19% and 15.50% while in *B. arietans*, 22.31%, 21.06% and 10.6% were recorded for SVSPs, SVMPs and PLA_2s 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 can serve as a guide in the use and design of a potential antivenom.

Authors' 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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Declaration of competing interest

The authors declare no competing interests.

Data availability

The mass spectrometry/proteomics data have been deposited into

Table 4
Classification of the detected venom proteomes of *B. arietans* and *E. ocellatus* based on families.

Proteins	Protein families
Acidic phospholipase A_2	Phospholipase A_2s
Acidic phospholipase A_2	
Phospholipase A_2 homolog	
Phospholipase-B 81	Phospholipase B
Alpha-fibrinogenase	Snake venom serine proteases
Alpha-fibrinogenase-like	
Beta-fibrinogenase brevinase	
Cationic trypsin	
Coagulation factor X-activating enzyme heavy chain	
Coagulation factor X-activating enzyme heavy chain	
Serine protease harobin	
Serine protease sp-Eoc49	
Snake venom serine protease BthaTL	
Snake venom serine protease HS114	
Snake venom serine protease NaSP	
Snake venom serine protease	
Thrombin-like enzyme cerastocytin	
Kunitz-type serine protease inhibitor bitisilin-1	Kunitz peptides
Kunitz-type serine protease inhibitor bitisilin-3	
Snake venom metalloproteinase ACLH	Snake venom metalloproteinases
Snake venom metalloproteinase kistomin	
Snake venom metalloproteinase lebetase-4	
Snake venom metalloproteinase-disintegrin-like mocarhagin	
Zinc metalloproteinase homolog-disintegrin albolatin	
Zinc metalloproteinase leucurolysin-B	
Zinc metalloproteinase-disintegrin BA-5A	
Zinc metalloproteinase-disintegrin BlatH1	
Zinc metalloproteinase-disintegrin bilitoxin-1	
Zinc metalloproteinase-disintegrin-like BfMP	
Zinc metalloproteinase-disintegrin-like EoMP06	
Zinc metalloproteinase-disintegrin-like EoVMP2	
Zinc metalloproteinase-disintegrin-like Eoc1	
Zinc metalloproteinase-disintegrin-like HF3	
Zinc metalloproteinase-disintegrin-like MTP9	
Zinc metalloproteinase-disintegrin-like NaMP	
Zinc metalloproteinase-disintegrin-like	
Zinc metalloproteinase-disintegrin-like TSV-DM	
Zinc metalloproteinase-disintegrin-like VLAIP-A	
Zinc metalloproteinase-disintegrin-like VLAIP-B	
Zinc metalloproteinase-disintegrin-like acurhagin	
Zinc metalloproteinase-disintegrin-like atrolysin-A	
Zinc metalloproteinase-disintegrin-like batroxstatin-1	
Zinc metalloproteinase-disintegrin-like batroxstatin-3	
Zinc metalloproteinase-disintegrin-like berythractivase	
Zinc metalloproteinase-disintegrin-like bothrojarin-2	
Zinc metalloproteinase-disintegrin-like brevilysin	
Zinc metalloproteinase-disintegrin-like cobrin	
Zinc metalloproteinase-disintegrin-like daborhagin-K	
Zinc metalloproteinase-disintegrin-like stejnihagin-A	
Zinc metalloproteinase-disintegrin-like stejnihagin-B	
Zinc metalloproteinase/disintegrin	
Zinc metalloproteinase/disintegrin	
Zinc metalloproteinase/disintegrin	
Metalloproteinase/disintegrin	
Zinc metalloproteinase/disintegrin	
Zinc metalloproteinase/disintegrin PMMP-1	
Cysteine-rich venom protein	Cysteine-rich venom protein
Disintegrin EO5A	Disintegrins
Disintegrin bitistatin	
L-amino-acid oxidase	L-amino acid oxidases
L-amino-acid oxidase	
L-amino-acid oxidase	
Long neurotoxin 1	Three-finger toxins
Short neurotoxin 1	
Weak toxin CM-2a	
Cytotoxin 1	
Snaclec 2	C-type lectins/Snaclecs
Snaclec 3	
Snaclec CTL-Eoc124	
Snaclec CTL-Eoc125	
Snaclec agglucetin subunit beta-1	
Snaclec bitiscetin subunit alpha	
Snaclec bitiscetin subunit beta	
Snaclec clone 2100755	
Snaclec convulxin subunit beta	
C-type lectin 1	
Snake venom 5′-nucleotidase	5′ Nucleotidases
Snake venom vascular endothelial growth factor toxin barietin	Vascular endothelial growth factors
Vascular endothelial growth factor A	
Peroxiredoxin-4	Peroxiredoxin
Phospholipase A_2 inhibitor 1	Phospholipase A_2 inhibitor
Snake venom glutaminyl-peptide cyclotransferase	Glutaminyl cyclases
Hemoglobin subunit beta	Globin
Secretory phospholipase A_2 receptor	Phospholipase A_2 receptor
Venom nerve growth factor 2	Venom nerve growth factor

the ProteomeXchange Consortium via the PRIDE[26] 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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