RESEARCH ARTICLE

Abdulhafiz Lamiya [1]
Dayyabu Shehu [2]
Adamu Jibril Alhassan [2]
Maryam Dangambo [2]

In silico studies for the identification of lead phytocompounds as *Naja nigricollis* venom antidote from selected Nigerian anti-snake venom plants

Authors' addresses:
[1] Department of Science Laboratory Technology, Modibbo Adama University, Yola P. M. B. 2076, Adamawa State, Nigeria.
[2] Department of Biochemistry, Faculty of Basic Medical Sciences Bayero University, Kano P.M.B. 3011, Kano Sate, Nigeria.

Correspondence:
Abdulhafiz Lamiya
Department of Science Laboratory Technology, Modibbo Adama University, Yola P. M. B. 2076, Adamawa State, Nigeria.
Tel.: +234 8108183922
e-mail: alamiya@mau.edu.ng

Article info:
Received: 12 December 2024
Accepted: 8 May 2025

ABSTRACT

Several plants used in traditional setting of Nigeria for the treatment of snake bites have been subjected to preliminary snake venom neutralization activity validation and some phytocompounds have been identified and isolated in their extracts. This research sought to identify lead phytocompounds as *Naja nigricollis* venom antidote from compounds identified in these plants that can be channeled in to anti-venom discovery pipeline. Relevant science data bases that include "Google Scholar", "PubMed", "PubmedCentral", and "Science Direct" were searched for published works on anti-snake venom activities of Nigerian plants between the years of 2014-2024. Compounds isolated from such plants were downloaded from "PubChem" and subjected to molecular docking against the major venom proteins of *Naja nigricollis* three finger toxins (neurotoxin and cardiotoxin) and phospholipase A_2 using PyRx and Discovery Studio. The top three hit compounds for each of the toxins were then subjected to ADMET analysis using Swiss-ADME and PROTOX-II to identify lead compound with the best drug likeness and safety property. Lead compounds identified were cabenegrin A-I, cabenegrin A-II, and lupeol for neurotoxin, cardiotoxin and phospholipase A_2, respectively with their respective docking score as -5.7, -6.3 and -11.2 Kcal/mol, respectively. All the lead phytocompounds passed the Lipinski rule of five and have no probability of organ toxicity except for lupeol, which has a high probability of causing respiratory toxicity. The lead compounds identified in this study hold the potential of providing novel anti-snake venom. Thus, their activities can be validated through advanced techniques and channeled into the drug discovery pipeline.

Key words: anti-snake venom, phytocompounds, molecular docking, botanical therapeutics

Introduction

Snake envenomation is a significant public health challenge mostly affecting populations in the tropical and sub-tropical regions of the world, contributing significant morbidity and mortality (Afroz et al., 2024). Envenomation leads to diverse clinical manifestations ranging from local tissue damage to life-threatening systemic effects, including neurotoxicity (neuromuscular paralysis), haemotoxicity (haemorrhage and coagulopathy), and/or cytotoxicity (swelling, blistering, and tissue necrosis). These could be attributed to the effects of the various venom components (Offor et al., 2022). In a recent study Afroz et al. (2024), reported approximate annual global snakebites and envenomation of 5.4 and 1.8-2.7 million respectively, with 435 000 to 580 000 cases occurring across Africa. Globally, snakebite has led to 81 410-137 880 deaths and around three times as many individuals suffering from permanent disfigurement and/or disabilities, including limb amputations (Afroz et al., 2024).

Envenomation due to snake bite in sub-Saharan Africa has been attributed to two families of snakes: elapids and viperids (Offor et al., 2022). The families of elapids include cobras (*Naja* spp.) and mambas (*Dendroaspis* spp.), while the viperids family are adders (*Bitis* spp.) and saw-scaled/carpet vipers (*Echis* spp.) with each family causing a different type of toxicity due to differences in venom toxin constituents. Envenomation by Viperidae mainly induces myotoxicity and haemotoxicity, whereas the Elapidae leads to cytotoxicity, neurotoxicity, and cardiotoxicity. Offor et al. (2022), also reported that Viperidae venom proteome was dominated by snake venom metalloproteinases (SVMPs–41%), snake venom serine proteases (SVSPs–16%), and phospholipase A2 (PLA_2–17%) protein families, while three-finger toxins

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20252071

(3FTxs–66%) and PLA_2s (16%) dominated those of the Elapidae.

Despite the prevailing morbidity and mortality associated with snake bites, the only available antidote is an immunoglobulin-based antivenin, which is effective in the treatment of systemic alterations due to snake bites (Okafor & Onyike, 2021). However, several drawbacks are associated with the "life-saving" conventional antivenom include; availability, affordability and storage, these limit its accessibility by the affected population (Hassan et al., 2023). In addition some patients show an adverse immune reaction to the conventional antivenin (Hassan et al., 2023). It is imperative to note that conventional antivenin only neutralizes systemic alterations caused by the venom sparing local tissue damage, which results in various degrees of disfigurements or disabilities.

Bedeviled by the challenges associated with the conventional antivenin, most of the snake bite victims in such resource constrained regions of tropics and sub-tropics Nigeria inclusive resort to traditional healers who rely on the power in the diverse flora of these regions for anti-snake venom remedy. Various plants from the diverse vegetation of Nigeria that include: *Mucuna puriens, Anonna senegalenses, Parkia biglobosa, Piliostigma thonningi, Azadritcha indica, Crinnum jagus* among others (Isah et al., 2023) have been used by these traditional practitioners to treat snake bite envenomation of which many have been scientifically validated and their phytochemical constituents spectroscopically characterized. Natural products from these plants hold the potential of providing complements or alternative antivenom to the conventional antivenom. Therefore, the investigation and prioritization of these natural products to develop a novel antivenin becomes justifiable. This study carried out virtual screening of a library of phytocompounds isolated from Nigerian anti-snake venom plants against the major components of *Naja nigricollis* venom components with the aim of prioritizing phytocompounds that will be used in the development of novel antivenom.

Materials and Methods

Ligand library generation and preparation

Relevant electronic data bases that include: PubMed (https://pubmed.ncbi.nlm.nih.gov/), Google Scholar (https://scholar.google.com/), PubmedCentral (https://www.ncbi.nlm.nih.gov/pmc/), and Science Direct (https://www.sciencedirect.com/) were searched using relevant key words such as "anti-snake venom plants in Nigeria", "antivenom phytocompounds isolated from Nigerian plants", phytocompounds with anti-venom activities", "anti-snake venom natural compounds reported in Nigerian studies", "natural compounds reported to inhibit snake venom activities". Abstracts and full-length papers were studied to identify phytocompounds identified and isolated from plants studied for anti-venom activities and published between the years of 2014 and 2024. Phytocompounds identified were compiled to form a library with the corresponding characteristics of each compound and source noted. The 3D SDF format structures of the compounds were then downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in PDB format. Structures of compounds not available in PubChem were sketched using Chem Draw software version 21 and converted to 3D SDF formats. The structures were then converted to PDB format using Open Babel embedded in PyRx software version 0.9.9 and saved. The canonical SMILES of the compounds were obtained using Swiss ADMET (http://www.swissadme.ch/). All the compounds were imported into Open Babel within the Python Prescription Virtual Screening Tool (PyRx) version 0.9.9 and subjected to energy minimization and then converted to PDBQT format ready for docking.

Target preparation

The 3D crystal structures of *Naja nigricollis* three finger toxins (neurotoxin and cardiotoxin) were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB) (https://www.rcsb.org) as 1IQ9 (neurotoxin) and 1CXN (cardiotoxin) as PDB files. These molecules were then prepared in Biovia Discovery Studio software version 2023 SP1. Water molecules and heteroatoms were removed, and polar hydrogen was added. Subsequently, the receptor grid file was generated to define the binding pocket for the ligands in PyRx.

The 3D structure of *Naja nigricollis* phospholipase A2 not available in protein data bank was modelled using SWISS-MODEL (https://swissmodel.expasy.org/). *N. nigricollis* phospholipase A2 amino acid sequence (GI: 85986) was retrieved from the National Center for Biotechnology Information (NCBI) database.

Template search with BLAST and HHBlits was performed against the SWISS-MODEL template library. The target sequence was searched with BLAST against the primary amino acid sequence contained in SMTL. A total of 292 templates were found. An initial HHBlits profile was built, followed by 1 iteration of HHBlits against NR20. The obtained profile was then searched against all profiles of the SMTL and a total of 315 templates were found. The model was created and the PDB format was downloaded and saved for further analysis. This protein was also prepared using Biovia Discovery Studio.

Virtual screening (Protein-ligand docking)

Structure-based virtual screening of the ligand library against the individual proteins one for each was performed

using Vina wizard tool compiled in PyRx. The search space encompassed the whole of the modelled crystal. The prepared ligands were docked into the binding pockets of the protein targets, respectively. The docking was run at an exhaustiveness of 8, and the 3 top-ranked compounds (compounds with the smallest binding energy in kcal/mol were exported to SWISS-ADME and PROTOX-II online tools for Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) analysis to eliminate compounds with predicted toxicity and/or poor bioavailability. Analysis of the results were performed based on Lipinski's rule of five (RO5) and Veber's rule. Finally, the docked poses of the best phytocompounds that passed the ADMET analysis were imported into Biovia Discovery Studio for visualization and ligand-receptor interaction analysis. All ligand-receptor complexes images were generated with Biovia Discovery Studio software version 2023 SP1.

Results

A total of twenty-five (25) phytocompounds were reported to be isolated from thirteen (13) Nigerian anti-snake venom plant materials (Table 1). The most common phytocompounds identified in the plants studied were lupeol and quercetin. Each of these phytochemicals was isolated from two different anti-snake venom plant materials. Lupeol, which is a triterpenoid, was reported to be isolated from *Cryptolepisob longifolia* roots and *Vernonia glaberrima* leaf (Table 1). Quercetin, which is a flavonoid, was isolated from

Table 1. *Anti-snake venom phytocompounds isolated from Nigerian medicinal plants.*

Phytocompounds	Plant name	Plant Part	Venom	Reference
γ-sitosterol	*Moringa oleifera*	leaf	*Bitis arietans*	(Ajisebiola et al., 2023)
Quercetin	*Moringa oleifera*	leaf	*Bitis arietans*	(Ajisebiola et al., 2023)
n-hexadecanoic acid	*Moringa oleifera*	leaf	*Bitis arietans*	(Ajisebiola et al., 2023)
n-pentacosane	*Moringa oleifera*	leaf	*Bitis arietans*	(Ajisebiola et al., 2023)
2-Hydrazino-8-hydroxy-4-phenylquinoline	*Moringa oleifera*	leaf	*Naja haje* and *Naja nigricollis*	(Adeyi et al., 2023)
β-Stigmasterol	*Pittosporum dasycaulon*	leaf	*Daboia russelii* and *Naja naja*	(Chakkinga et al., 2023)
[6,8,9-trimethyl-4-(2-phenylethyl)-3-oxabicyclo[3.3.1]non-6-en-1-yl]methanol	*Moringa oleifera*	leaf	*Echis ocellatus*	(Adeyi et al., 2022)
Flavan-3-ol (catechin)	*Neocarya macrophylla*	stem bark	*Naja nigricollis*	(Yusuf et al., 2020)
5-methylcoumarin-4-β-glucoside (5MC4BG)	*Vernonia glaberrima*	leaf	*Naja nigricollis*	(Yusuf et al., 2024)
Scopoletin	*Catuneragamnilotica*	root	*Naja nigricollis*	(Salihu et al., 2024)
2-(2-hydroxypropyl)-1,4-benzenediol	*Cissus multistriata*	leaf	*Naja nigricollis*	(Omale et al., 2017)
Microphyllose A	*Neocarya macrophylla*	fruit	*Naja nigricollis*	(Jega et al., 2021)
Hexadecanoic acid, ethyl ester	*Schumanniophyton magnificum*	leaf	-	(Joshua et al., 2020)
2',4'- dihydroxy-4-prenyloxychalcone	*Indigofera conferta*	aeriel	*Naja nigricollis*	(Isah et al., 2023)
Cabenegrins A-I (phenolic pterocarpans)	*Annona senegalensis*	leaf	*Echis ocellatus, Bitis arietans and Naja nigricollis*	(Gbolade, 2021)
Aristolochic acid (alkaloid)	*Aristolochia albida*	rhizome	*Naja nigricollis*	(Gbolade, 2021)
Resveratrol (phenolic compound)	*Crinum jagus* Dandy	bulb	Echis ocellatus, *Bitis arietans and Naja nigricollis*	(Gbolade, 2021)
Kaemferol	*Moringa oleifera*	leaf	*Bitis arietans*	(Ajisebiola et al., 2023)
Quercetin	*Neocarya macrophylla*	leaf	*Naja nigricollis*	(Yusuf et al., 2019)
Paroxypropione	*Moringa oleifera*	leaf	*Echis ocellatus*	(Adeyi et al., 2022)
Oleanylerucoate	*Cryptolepisoblongifolia*	Ariel part	*Naja nigricollis*	(Umar et al., 2014)
Lupeol	*Cryptolepisoblongifolia*	root	*Naja nigricollis*	(Umar et al., 2023)
Kolaviron	*Garcinia kola*	-	*Naja nigricollis*	(Okafor & Onyike, 2021)
Lupeol	*Vernonia glaberrima*	leaf	*Naja nigricollis*	(Yusuf et al., 2024)
Microphyllose B	*Neocarya macrophylla*	fruit	*Naja nigricollis*	(Jega et al., 2021)
Cabenegrins A-II (phenolic pterocarpans)	*Annona senegalensis*	leaf	*Echis ocellatus, Bitis arietans and Naja nigricollis*	(Gbolade, 2021)
2, 6, 8-Trimethylbicyclo[4, 2, 0]oct-2-ene-1,8-diol	*Cissus multistriata*	leaf	*Naja nigricollis*	(Omale et al., 2017)

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20252071

the leaf of *Neocarya macrophylla*, which belongs to the Chrysobalanaceae family, and *Moringa oleifera* leaf (Table 1). Most of the phytocompounds (eight) including γ-sitosterol, quercetinn-hexadecanoic acid,n-pentacosane, 2-Hydrazino-8-hydroxy-4-phenylquinoline, [6,8,9-trimethyl 4(2phenylethyl) 3oxabicyclo[3.3.1]non-6-en-1-yl]methanol, kaemferol and paroxypropione were isolated from *Moringa oleifera* (Table 1). The activities of the plants were based on *in vivo* studies carried out in rat models with crude venoms from either the Viperidae or Elapidae families of the most common venomous snakes in Nigeria.

Table 2 summarizes molecular docking results. The phytocompounds docked into the binding pockets of the various venom proteins revealed varying binding scores recorded in Kcal/mol. Compounds with the lowest binding energy for each of the venom proteins were considered as the top hits. The three top hit compounds for each of the neurotoxin, cardiotoxin, and PLA_2 are presented in Table 2. Lupeol had a binding score of -5.9 Kcal/mol, microphyllose A, quercetin, and cabenegrin A-I each had binding scores of -5.7 Kcal/mol, and kaemferol with a docking score of -5.6 Kcal/mol were the top three hits for neurotoxin. In the case of cardiotoxin, the top three hit compounds were stigmasterol, lupeol and cabenegrin A-II, and 5-methylcoumarin-4-beta-glucoside with binding scores of -6.4, -6.3 and -6.1 Kcal/mol, respectively. Phospholipase A-II had lupeol, stigmasterol and gamma-sitosterol as the three top hit compounds with binding score of -11.2, -9.8 and -9.2 Kcal/mol, respectively. The interacting amino acids in the various venom proteins, their respective non-covalent bonds and bond lengths, are presented in Figures 1b, 2b and 3b.

Table 2. *Top three hit (in Kcal/mol) phytocompounds for each of the Phospholipase A2, Cardiotoxin (cytotoxin) and neurotoxin of N. nigricollis toxins.*

Phytocompound	Toxin proteins/ receptor	Binding score (Kcal/mol)
Lupeol	Neurotoxin	-5.9
Microphyllose A		-5.7
Quercetin		-5.7
Cabenegrin a-I		-5.7
Kaemferol		-5.6
Stigmasterol	Cardiotoxin (cytotoxin)	-6.4
Lupeol		-6.3
Cabenegrin A-II		-6.3
5-methylcoumarin-4-beta-glucoside		-6.1
Lupeol,	Phospholipase A_2	-11.2
Stigmasterol		-9.8
Gamma-Sitosterol		-9.2

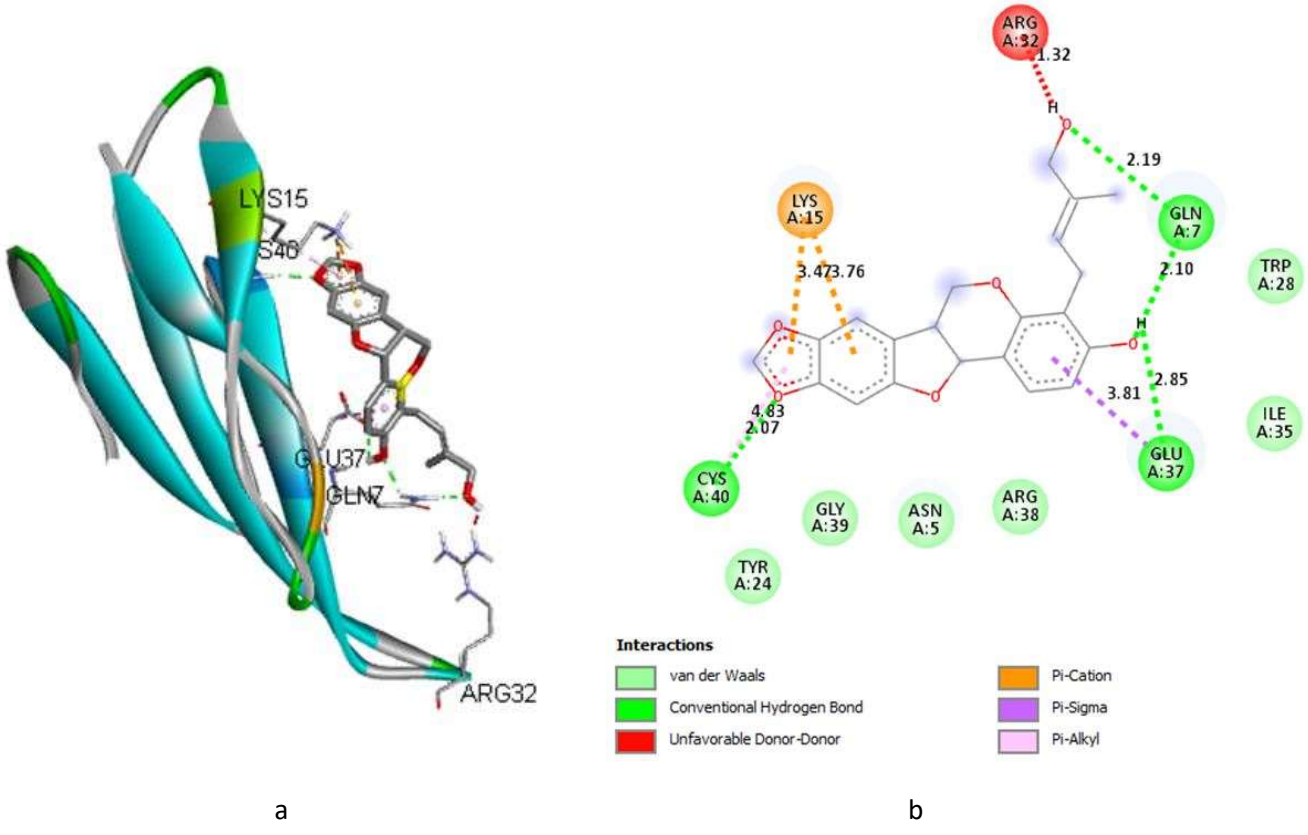
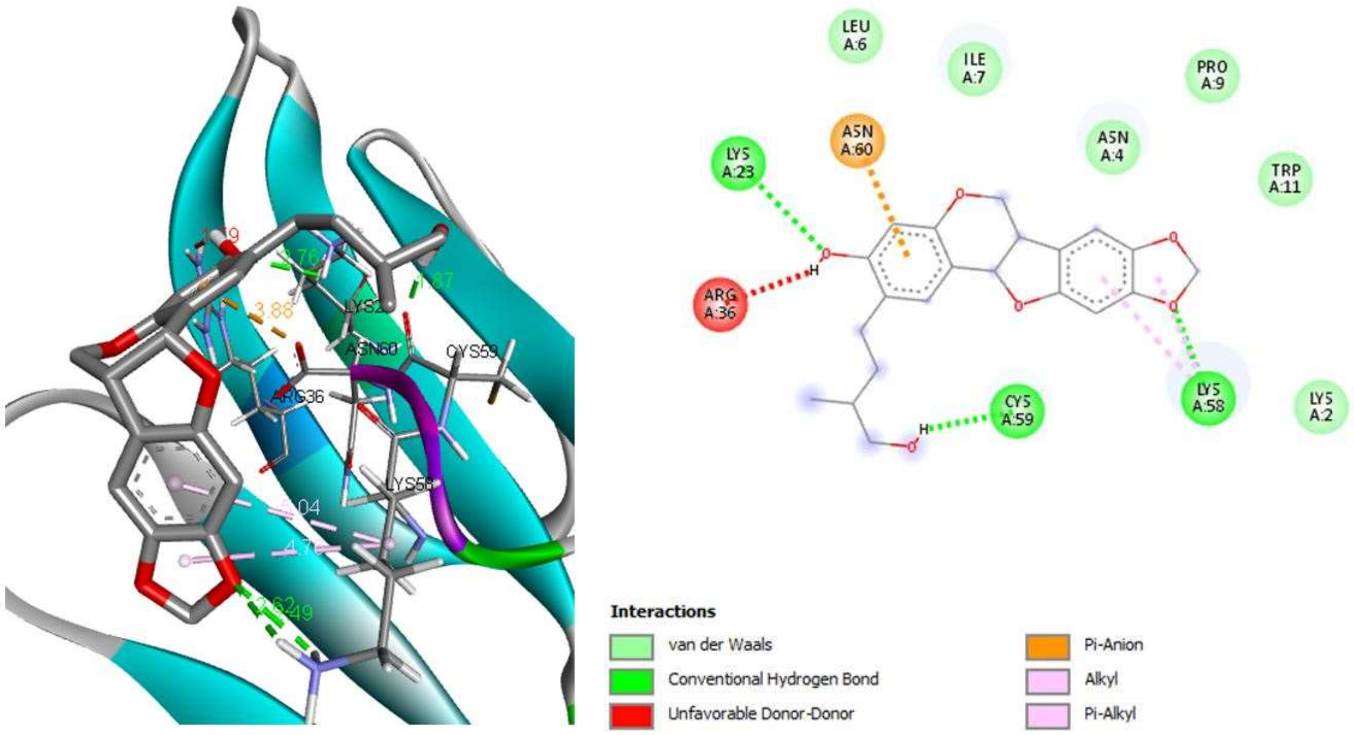


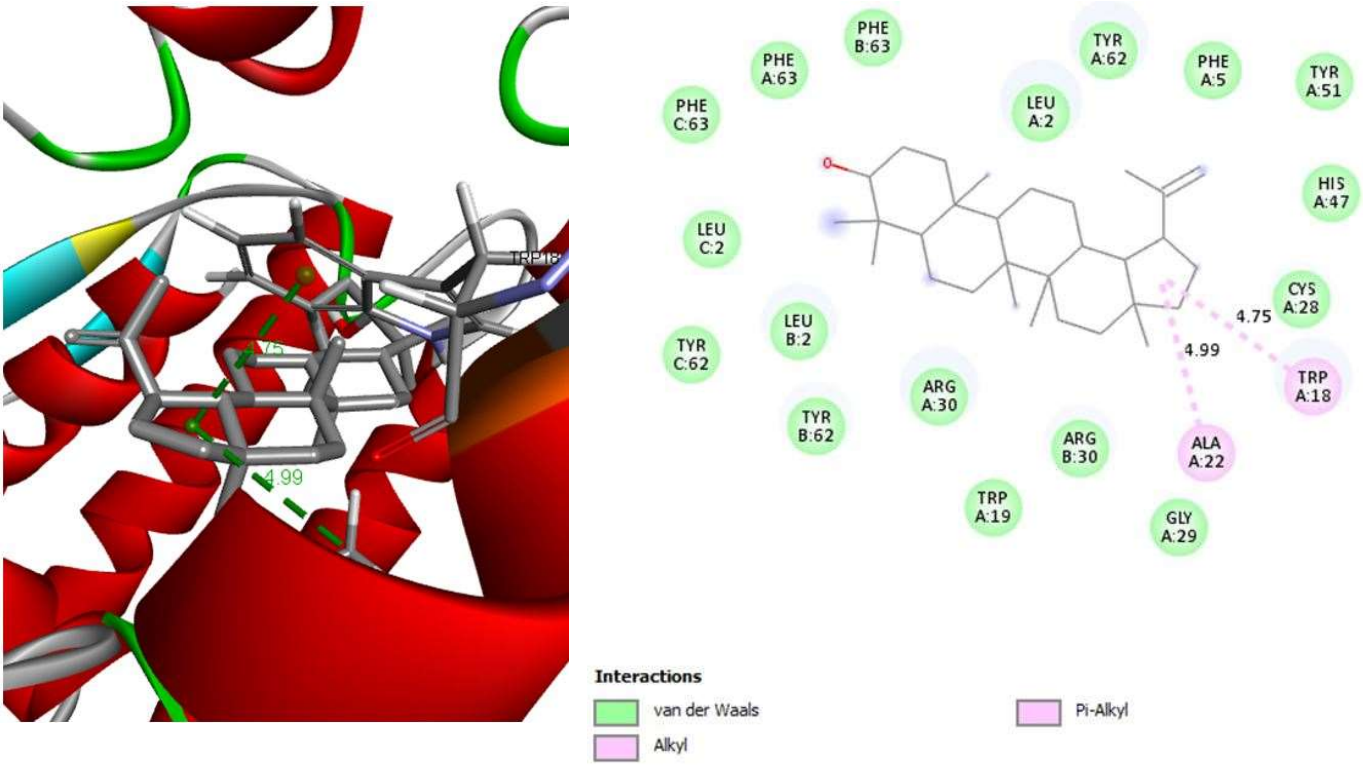
Figure 1. *a) 3D docking pose of cabenegrin A-I and neurotoxin; b) 2D docking pose of neurotoxin and cabenegrin A-I, specifying interaction bonding forces.*

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20252071



a b

Figure 2. *a) 3D docking pose of cabenegrin A-II and cardiotoxin; b) 2D docking pose of cardiotoxin and cabenegrin A-II indicating interaction/bonding forces.*



a b

Figure 3. *a) 3D docking pose of lupeol and PLA_2; b) 3D docking pose of lupeol and PLA2 specifying interaction/bonding forces.*

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20252071

ADMET analysis of the three top hit compounds of the three venom proteins are presented in Table 3. Cabenegrin A-I is the most druggable and safe compound among the top hits of neurotoxin, having relatively best oral bioavailability, drug likeness (zero violation of Lipinski and Veber's rule) and lacking any organ toxicity at oral LD_{50} of 2 500 mg/Kg body weight. These attributes are similar to those of cabenegrin A-II. Whereas in the case of top hits of phospholipase A_2 inhibitors, they all violated one Lipinski rule of five (log P >4.15), have low oral bioavailability, and a high probability of respiratory, end point toxicities. However, lupeol was relatively better with LD_{50} of 2 000 mg/Kg body weight.

The docking poses of the top hit compounds for every venom protein are shown in Figures 1-3. The different types of non-covalent interactions, such as hydrogen, electrostatic, and Van der Waals, distance bond length/distance, and amino acids participating in the interactions are displayed in the figures (Figures 1-3).

Discussion

Several Nigerian plants that have been used in the management or treatment of snake bite in the traditional setting have been validated by preliminary studies with some extracts spectroscopically characterized (Hassan et al., 2023). Identification of active substances from these plant extracts is invaluable in the discovery of novel anti-snake venom. A systematic approach that integrates various scientific disciplines is involved in modern drug design and development (Prabu, 2019). In this study, twenty-three (23) phytocompounds were compiled from fifteen (15) Nigerian anti-snake venom plants published between the years of 2014-2024 (Table 1). This depicts the lag between the validation of traditional anti-snake venom plant and the discovery of novel anti-snake venom from those plants, similar to the observation made by Gbolade (2021). The current study can therefore be considered as an attempt to shift the barrier towards the discovery of novel anti-snake venom from the Nigerian plants. Phytocompounds have different physicochemical properties that amount to their anti-venom activities. These can either be through inhibition of enzymatic activity, receptor blocking, configurational changes in the proteome structure of venom proteins, protein precipitation, enzyme activation, enzyme chelation, antioxidant, protein folding, and/or non-specific interaction. One more of these processes can lead to the formation of stable complex and ultimately venom neutralization (Gbolade, 2021). Top hit compounds were identified from the compound library through virtual screening. For each of the

Table 3. *ADMET analysis result of all the top three hit compounds of the three venom proteins.*

Phytocompound	Number of Lipinski rule of 5 and Veber' s ule violation(s)	Lipophilicity (logP)	Water Solubility value/class	Polar surface area PSA	GI absorption	Toxicity class	LD_{50} (mg/Kg b.wt)	High probabilityof Organ toxicity	High probability of end points Toxicity
Lupeol	1violation: MLOGP>4.15	6.92	-8.64/poor	20.23 Å²	low	4	2000	Respiratory	Blood brain barrier
Microphyllose A	4	-5.09	soluble	371.57A²	-	6	7000	Neurotoxicity, nephrotoxicity, respirator	Immunotoxicity
Quercetin	0	-0.56	-3.16/soluble	131.36Å²	high	3	159	Respiratory	
Cabenegrin A-I	0	1.98	-4.33/moderate	77.38 Å²	High	5	2500	-	Immunotoxicity
Kaemferol	0	-0.03	-3.31/soluble	111.13Å²	high	5	3919	Respiratory	-
Stigmasterol	1 violation: MLOGP>4.15	6.62	-7.46/poor	20.23 Å²	low	4	890	Respiratory	Immunotoxicity, blood brain barrier
Gamma-sitosterol	1 violation: MLOGP>4.15	6.73	-7.90/poor	20.23 Å²	low	4	890	Respiratory	Immunotoxicity
Cabenegrin A-II	0	2.06	-4.31/moderate	77.38 Å²	high	5	2500	-	Immunotoxicity
5-methylcoumarin-4-beta-glucoside	0	-0.72	soluble	129.59Å²	high	4	1469	Nephrotoxicity, cardiotoxicity	Mutagenicity

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20252071

N. nigricollis neurotoxin, cardiotoxin, and PLA_2 that served as the receptor molecule for the screening, the top three hits for each of the receptor molecules were identified and presented in Table 2. This selection was based on compounds that have the highest binding affinity (low binding energy). Binding affinity depicts the strength of interaction between ligand and receptor and it is inferred from binding energy with a unit of Kcal/mol. The binding energy is estimated from various interactions that include hydrogen bonds, van der Waals forces, hydrophobic and electrostatic interactions, as well as solvation effects. The numerical value derived from these interactions as binding energy was used to rank the ligand library as well as ligand poses according to Sharma et al. (2023). Ligands with more negative binding energies (lower) were considered to have higher binding affinities, thus stronger interaction with targets. Phytocompounds with higher binding affinity are generally more likely to exhibit better biological activity stemming from greater thermodynamic stability of the ligand-target complex and selectivity for their targets over other potential off targets (Angelina et al., 2014). The top three hit phytocompounds of each of the three venom proteins (Table 2) have potentials of being selective to their respective venom proteins and also form stable complex, a good characteristic of a drug candidate. Thus, these compounds have a tendency to exhibit better anti-snake venom activity in the drug discovery pipeline.

Figure 1-3 shows the docking poses for the respective lead compounds, cabenegrin A-I, cabenegrin A-II, and lupeol, viz, with their respective protein receptors. Cabenegrin A-I was able to interact with the neurotoxin through four conventional hydrogen bonding with GLN7, GLU37, and CYS40, pi-alkyl with CYS40, pi-sigma with GLU37, pi-cation with LYS15, and Van der Waals forces that entail the complimentarily of the phytocompound and the binding pocket of the neurotoxin protein. Cabenegrin A-II interacted with cardiotoxin through two hydrogen bonding with LYS23 and LYS58, one pi-anion with ASN60, one pi-alkyl interaction with LYS58 and Van der Waals forces. Lupeol interacted with PLA_2 through one alkyl interaction with ALA2 and one pi-alkyl interaction with TRP18, and Van der Waals forces. Hydrogen bonds are strong and directional interactions that occur between a hydrogen atom covalently bonded to an electronegative atom and another electronegative atom, which can significantly stabilize ligand-receptor complex and are often critical for specificity. They are very important in orienting ligands correctly within the binding site of a receptor (Medimagh et al., 2023). This bond was observed in the interactions between cabenegrin A-I and A-II and polar amino acid residues of neurotoxin and cardiotin (Figure 1b and 2b) but absent in the interaction of lupeol and PLA_2 (Figure 3b). The absence of a hydrogen bond in the lupeol-PLA_2 interaction was probably due to non-polar/hydrophic nature of lupeol. The presence hydrogen bond between the ligands and receptors contributed significantly to the binding affinity observed. Alkyl interactions are the hydrophobic interactions between nonpolar alkyl groups, are important for the overall binding affinity, especially in the hydrophobic pockets of the receptors. They help to minimize the exposure of hydrophobic surfaces to water, thus contributing to the stability of the ligand-receptor complex. Alkyl interactions are interactions between non-polar alkyl groups of ligands and hydrophobic residues of receptors. It was found in the interaction of lupeol and ALA22 of the PLA_2 residue due to the hydrophobic nature of lupeol and the presence of non-polar/hydrophobic amino acids (ALA) in the binding pocket of PLA_2. This type of interaction was not observed in both cabenegrin A-I and A-II interactions with their receptors. According to Shanshan et al. (2013), Pi-alkyl interactions occur between the pi electrons of aromatic rings and alkyl groups. These interactions can enhance the binding affinity by providing additional stabilization through non-covalent interactions and are particularly relevant in the context of aromatic ligands binding to aromatic amino acids in the receptor binding pocket. This type of interaction was observed in all the interactions between cabenegrin A-I and A-II, as well as lupeol with their respective receptors, probably due to the presence of either aromatic ring or alkyl groups in the ligands and/or receptors. Zhou et al. (2012) explained that Pi-sigma interactions are often observed in systems where aromatic ligands interact with aliphatic residues in the binding pocket of the receptor. This was observed only in the interaction of the aromatic ring of cabenegrin A-I and the aliphatic chain of GLU37 of the neurotoxin. Pi-anion interaction observed in the interaction of the pi electrons of the aromatic ring of cabenegrin A-II and the anionic residue (ASN60) of cardiotoxin was due to the presence of pi electrons of the aromatic system of cabenegrin A-II and the negatively charged residue of cardiotoxin. They are particularly important for stabilizing charged ligands in the binding site of a receptor (Mahadevi & Sastry, 2016). Pi-cation interaction observed between cabenegrin A-I and neurotoxin due to attraction between pi electrons of the aromatic system of cabenegrin A-I and the positively charged residue (LYS15) of neurotoxin. This interaction is particularly significant in biological systems where it can play a role in the binding of a ligand to a receptor that contains positively charged amino acid residues, thus enhancing the specificity and strength of the overall interaction. Van der Waals interactions are weak, non-specific interactions that occur between all atoms regardless of their polarity. It arises from the transient dipole moments created when electron clouds around atoms fluctuate. Thus,

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20252071

all the atoms in the receptor binding sites participate in this type of interaction. While individually weak, collectively, they can contribute significantly to the stability of the ligand receptor complex (Lybrand, 2005). Van der Waals interaction was observed in the interactions of all the lead phytocompounds and their respective receptors, thus validating the ligand-receptor complementarity and compatibility, influencing binding affinity, specificity, and overall stability of the molecular complex. Each type of interaction mentioned above played a unique but significant role in the ligand-receptor interaction, binding energy, and their possible ligand-receptor complex stability, and ultimately determines the efficacy of a ligand in modulating receptor activity (Majewski et al., 2019). This interaction information will be very important in optimizing the lead compounds' designs and improving the specificity of their drug candidacy.

The selection of a drug candidate in pharmaceutical drug development is a complex process that not only considers efficacy, rather encompasses multiple factors bioavailability and safety inclusive (Deore et al., 2019). Bioavailability refers to the proportion of a drug that enters systemic circulation when introduced into the body, hence available for therapeutic action (Ruiz-Garcia et al., 2008). This is an important factor in drug candidate selection since it affects the efficacy, dosing regimen, formulation development, and route of administration (Wishart, 2007). Thus, the top three hit compounds for each of the venom proteins were screened in silico for bioavailability and safety base on Lipinski's rule of five (RO5) (Christiphor et al., 2019) and Veber's rule (Li et al., 2003) which evaluate whether or not a compound is likely to be membrane permeable and easily absorbed via passive diffusion in human intestine. Lipinski's rule of five helps to determine if a biologically active chemical/drug is likely to have chemical and physical properties to be orally bioavailable based on the following criteria: no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, molecular mass less than 500 Da, and partition coefficient not greater than 5. According to Lipinski's rule of five, a drug-like compound should not violate more than one of the rules of five. Veber's rule states that for a drug to be a good candidate, it should have polar surface area (TPSA) ≤ 140 and rotatable bond count (RB) ≤ 10 (Jega et al., 2021). Bioavailability and safety screening results revealed that the best candidates for the three finger toxin proteins (neurotoxin and cardiotoxin) ligands were cabenegrin A-I and cabenegrin A-II, respectively (Table 3). These candidates were found to have good oral bioavailability and drug likeness since none of the Linspki's or Veber's rules have been violated, and have no potential for organ toxicity at high probabilities observed. This implies that the compounds have the probability of being efficiently absorbed through the gastrointestinal tract and have a low potential for organ damage when used over a given period of time. The findings of this study corroborate previous reports of in vivo anti-snakevenom activity and safety of the different parts of *Annona senegalensis* (Hassan et al., 2023) from which these phytocompounds were isolated. Three-finger toxins are non-enzymatic proteins that constitute the dominant toxin (66-73.3%) of the proteome of Elapidae venom *N. nigricollis* inclusive (Okafor & Onyike, 2021). This toxin consists of cytotoxins, neurotoxins, and Muscarinic toxin-like protein in order of decreasing proportion (Offor et al., 2022). Identifying a potent drug candidate that can significantly inhibit the activity of this toxin group is a significant achievement towards the discovery of a novel drug specific to Elapidae envenomation. These compounds can be considered as hit compounds that can be subjected to *in vitro – in vivo* validation of their potency, pharmacokinetics, and safety, binding studies to elucidate the mechanism of action, and structural optimization for their improvement. When validated, these compounds will be invaluable in surmounting morbidities such as dermonecrotic associated with early oedema of the dermis, blistering, loss of skin appendages and reduction in cellularity as well as mortality due to *N. nigricollis* envenomation (Offor et al., 2022) either as a single drug, combination therapy or as a compliment or adjuvant to the contemporary and only antivenin as described in the work of (Yusuf et al., 2021).

All the top three hits of PLA_2 (lupeol, stitosterol and stigmasterol) failed one Lipinski rule (logP>5), and consequently had low gastrointestinal absorption. They also all had a high probability of respiratory toxicity and other endpoint toxicities that include immunotoxicity and blood-brain barrier impermeability. However, lupeol is considered to be a relatively better candidate for having a higher LD_{50} of 2 000 mg/kg b.wt compared to the 890 mg/kg body weight LD_{50} of sitosterol and stigmasterol. According to the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) (Morris-Schaffer & McCoy, 2021), substances with LD_{50}>2000 mg/Kg body weight are considered non-toxic. As such, lupeol stands to be the safer and most effective candidate, and consequently the lead phytocompound against *N. nigricollis* PLA_2. This phytocompound has been reported to be isolated from anti-snake venom plants that include *Vernonia glaberrima* leaf (Yusuf et al., 2024) and *Cryptolepis oblongifolia* (Umar et al., 2023) root. Thus, there is a need to optimize the structure of lupeol to improve its efficacy, safety, and pharmacokinetics.

Conclusion

Many Nigerian plants used in traditional snake envenomation treatment and management discovered in this

http://www.jbb.uni-plovdiv.bg DOI: 10.69085/jbb20252071

study have been scientifically validated and hold the potential of providing novel anti-snake venom from the array of phytocompounds they contain. The lead compounds cabenegrin A-I, cabenegrin A-II, isolated from Anonna senegaleses and lupeol, isolated from *Cryptolepis oblongifolia*, for neurotoxin, cardiotoxin, and phospholipase A2, respectively, reported in this study, hold the promise of being successful anti-snake venom candidate's vis-a-vis the in-silico docking and ADMET findings. Thus, the activities of these compounds should be validated in vitro and in vivo against the venom of the snake species in question for the advancement of the discovery process.

Acknowledgement

The authors are indeed grateful to Dr. Nasiru Abdullahi of the department of Biochemistry, Bayero University Kano for his kind recommendations on the choice of best approaches in the molecular docking and ADMET analysis process.

References

Adeyi AO, Jimoh AO, Ajisebiola BS, Adeyi OE, Metibemu DS, Okonji R E. 2023. Inhibition of phospholipase A2 from *Naja haje* and *Naja nigricollis* venoms by active fraction of *Moringa oleifera* leaves*: in vitro* and *in silico* methods. Toxin Rev., 42(3): 629-639.

Adeyi AO, Mustapha KK, Ajisebiola BS, Adeyi OE, Metibemu DS, Okonji RE. 2022. Inhibition of *Echis ocellatus* venom metalloprotease by flavonoid-rich ethyl acetate sub-fraction of *Moringa oleifera* (Lam.) leaves: *in vitro* and *in silico* approaches. Toxin Rev., 41(2): 476-486.

Afroz A, Siddiquea BN, Chowdhury HA, Jackson NW, Watt AD. 2024. Snakebite envenoming: A systematic review and meta-analysis of global morbidity and mortality. PLoS Neg. Trop. Diseases, 18(4): e0012080.

Ajisebiola BS, Bello FT, Osamudiamen PM, Oladele JO, Adeyi AO. 2023. Inhibition of metalloproteinase from *Bitis arietans* venom i*n vitro* by the ethyl acetate fraction of *Moringa oleifera* and its chemical profile. Revista Brasileira de Farmacognosia, 33(4): 822-830.

Angelina EL, Andujar SA, Tosso RD, Enriz RD, Peruchena ǸM. 2014. Non-covalent interactions in receptor-ligand complexes. A study based on the electron charge density. J. Phys. Organic Chem., 27(2): 128-134.

Chakkinga TR, Ibrahim JM, Nair AS, Thacheril SS. 2023. Exploring the potent inhibitor β-stigmasterol from *Pittosporum dasycaulon* Miq. leaves against snake venom phospholipase A2 protein through *in vitro* and molecular dynamics behavior approach. Toxin Rev., 42(1): 85-98.

Christiphor AL, Franco L, Beryl WD, Paul JF. 2019. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Del. Rev., 23(1-3): 3-25.

Deore AB, Dhumane JR, Wagh R, Sonawane R. 2019. The stages of drug discovery and development process. Asian J. Pharm. Res. Dev., 7(6): 62-67.

Emmanuel A, Ebinbin A, Amlabu W. 2014. Detoxification of *Echis ocellatus* venom-induced toxicity by *Annona senegalensis* Pers. J. Comp. Integ. Med., 11(2): 93-97.

Gbolade A. 2021. Nigerian medicinal plants with anti-snake venom activity. A review. J. Mal. Res. Phytomed., 4(1): 29-44

Hassan G, Salihu M, Yusuf AJ. 2023. Snake venom detoxification potential of selected Nigerian medicinal plants: a review. Caliphate J. Sci. Tech., 3121: 232-238.

Isah M, Murtala S, Abdullahi SM, Hamza AN, Tajuddeen N, Dauda ML, Mzozoyana V, Musa AM. 2023. A bioactive chalcone from the aerial parts of *Indigofera conferta* Gillet. Nat. Product Res., 37(21): 3631-3638.

Jega AY, Abdullahi MI, Musa AM, Kaita HA, Mzozoyana V, Emmanuel AA. 2021. Biochemical evaluation and molecular docking assessment of glucosamines from *Neocarya macrophylla* fruits against *Naja nigricollis* venom. Carb Res., 509: 108436.

Joshua PE, Anosike CJ, Asomadu RO, Ekpo DE, Uhuo EN, Nwodo OFC. 2020. Bioassay-guided fractionation, phospholipase A2-inhibitory activity and structure elucidation of compounds from leaves of *Schumanniophyton magnificum*. Pharma Bio., 58(1): 1069-1076.

Karaduman G, Kelleci ÇF. 2024. a Multivariate Interpolation Approach for Predicting Drug Ld50 Value. Ankara Universitesi Eczacilik Fakultesi Dergisi, 48(1): 20-33.

Li JJ, Holsworth DD, Hu LY. 2003. Molecular properties that influence the oral bioavailability of drug candidates. Chemtracts, 16(7): 439-442.

Lybrand TP. 2005. Protein-ligand interactions. Computational Approaches to Biochemical Reactivity, 363-374.

Mahadevi AS, Sastry GN. 2016. Cooperativity in Noncovalent Interactions. Chem. Rev., 116(5): 2775-2825.

Majewski M, Ruiz-Carmona S, Barril X. 2019. An investigation of structural stability in protein-ligand complexes reveals the balance between order and disorder. Comm. Chem., 2(1): 110.

Medimagh M, Issaoui N, Gatfaoui S, Kazachenko AS, Al Dossary OM, Kumar N, Marouani H, Bousiakoug LG. 2023. Investigations on the non-covalent interactions, drug likeness, molecular docking and chemical properties of 1,1,4,7,7-pentamethyldiethylenetriammonium trinitrate by density-functional theory. J. King Saud University - Science, 35(4): 102645.

Morris-Schaffer K, McCoy MJ. 2021. A review of the LD_{50} and its current role in hazard communication. ACS Chemical Health and Safety, 28(1): 25-33.

Offor BC, Muller B, Piater LA. 2022. A Review of the Proteomic Profiling of African *Viperidae* and *Elapidae* Snake Venoms and Their Antivenom Neutralisation. Toxins, 14(11): 723.

Okafo AI, Onyike E. 2021. Inhibition of key enzymes linked to snake venom induced local tissue damage by kolaviron. J Basic and Clinical Physi and Pharm., 32(6): 1121-1130.

Omale J, Idris F, Adejoh O, Paul A. 2017. FTIR and GC-MS analyses of phytochemicals from methanol leaf extract of *Cissus Multistriata* and physiological changes induced in male rats exposed to *Naja nigricollis* venom. Int. J. Chinese Med., 1(1): 24-31.

Prabu SL. 2019. Drug discovery: current state and future prospects. – In: Computer applications in drug discovery and development. (pp. 1-46. IGI Global).

Ruiz-Garcia A, Bermejo M, Moss A, Casabo VG. 2008. Pharmacokinetics in drug discovery. J. Pharmaceut. Sci., 97(2): 654-690.

Salihu M, Hassan LG, Faruq UZ, Yusuf AJ. 2024. Deciphering the interactions of scopoletin and scopolin from *Catunaregam nilotica* roots against *Naja nigricollis* phospholipase A2 enzyme. Toxicon, 243: 107732.

Santhosh MS, Hemshekhar M, Sunitha K, Thushara RM, Jnaneshwari S, Kemparaju K, Girish KS. 2013. Snake venom induced local toxicities: plant secondary metabolites as an auxiliary therapy. Mini-Rev. Med. Chem., 13(1): 106-123.

Shanshan L, Yuan X, Qiancheng S, Xian L, Jing L, Yadong C, Tao L, Cheng L, Xiaomin L, Mingyue Z, Hualiang J. 2013. Non-covalent interactions with aromatic rings: current understanding and implications for rational drug design. Current Pharmaceut. Design, 19(36): 6522–6533.

Umar S, Abubakar A, Ibrahim H, Sallau BA, Natasha O. 2014. Isolation of phospholipase A2 inhibitor from *Cryptolepis oblongifolia* (Meins) Schltr. J. Nat. Sci. Res., 4(4): 63–67.

Umar S, Ibrahim SI, Namadina MM, Ahmed A, Abdullahi BS. 2023. Inhibitory activity of lupeol from *Cryptolepis Oblongifolia* (Meins) Schltr on partially purified phospholiapase A2 venom of *Naja nigricollis* (Elapidae). Fudma J. Sci., 7(1): 79-83.

Wishart DS. 2007. Improving early drug discovery through ADME modelling: An overview. Drugs in R and D, 8(6): 349-362.

Yusuf AJ, Abdullahi MI, Musa AM, Haruna AK, Mzozoyana V, Abubakar H. 2019. Antisnake venom activity and isolation of quercetin from the leaf of *Neocarya macrophylla* (Sabine) Prance ex F. White (Malpighiales: Chrysobalanaceae). Brazilian J. Bio Sci., 6(13): 381-389.

Yusuf AJ, Abdullahi MI, Musa AM, Haruna AK, Mzozoyana V, Biambo AA, Abubakar H. 2020. A bioactive flavan-3-ol from the stem bark of *Neocarya macrophylla*. Scient. African, 7: e00273.

Yusuf AJ, Aleku GA, Bello UR, Liman, DU. 2021. Prospects and challenges of developing plant-derived snake antivenin natural products: a focus on West Africa. ChemMedChem, 16(24): 3635-3648.

Yusuf AJ, Bugaje AI, Sadiq M, Salihu M, Adamu HW, Abdulrahman M. 2024. Exploring the inhibitory potential of phytochemicals from *Vernonia glaberrima* leaves against snake venom toxins through computational simulation and experimental validation. Toxicon, 247: 107838.

Zhou P, Huang J, Tian F. 2012. Specific noncovalent interactions at protein-ligand interface: implications for rational drug design. Current Med Chem., 19(2): 226-238.