Asian Journal of Research in Biochemistry

Volume 14, Issue 6, Page 232-240, 2024; Article no.AJRB.128756
ISSN: 2582-0516

In vitro Anti-venom Effect of *Catunaregam nilotica* (STAPF) Root Extracts against *Echis ocellatus* Phospholipase A_2 Activity

Idris Aliyu Kankara [a*], Akos Noel Ibrahim [a] and Emmanuel Onoja [a]

[a] Department of Science Laboratory Technology, Federal Polytechnic Kaura Namoda, Zamfara State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author IAK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ANI and EO managed the analyses of the study. Author EO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajrb/2024/v14i6344

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/128756

Original Research Article

Received: 13/12/2024
Accepted: 29/12/2024
Published: 30/12/2024

ABSTRACT

Aims: This study evaluates the inhibitory effect of the crude extract of *Catunaregam nilotica* (STAPF) and its fraction against *Echis ocellatus* phospholipase A_2 (PLA_2) activity using an *In vitro* approach.
Study Design: In-vitro Anti-venom Study.
Place and Duration of Study: Department of Science Laboratory Technology, Federal Polytechnic Kaura Namoda, Zamfara State, Nigeria, between September to December, 2024.

**Corresponding author: E-mail: aliyukankara@gmail.com;*

Cite as: *Kankara, Idris Aliyu, Akos Noel Ibrahim, and Emmanuel Onoja. 2024. "In Vitro Anti-Venom Effect of Catunaregam Nilotica (STAPF) Root Extracts Against Echis Ocellatus Phospholipase A2 Activity". Asian Journal of Research in Biochemistry 14 (6):232-40. https://doi.org/10.9734/ajrb/2024/v14i6344.*

Methodology: Crude extraction was performed through cold maceration and fractionated via solvent-solvent partitioning using solvents that increased polarity. The phytochemical contents were screened using standard methods. The inhibitory effect of the crude extracts and their fraction against PLA_2 activity was evaluated via the in-vitro acidimetry method.
Results: The phytochemical analysis revealed alkaloids, anthraquinones, flavonoids, phenols, saponins, steroids, tannins, terpenoids, and steroids in the crude extracts. The in-vitro inhibition assay results revealed that both the hexane and the ethyl acetate fractions significantly inhibited the activity of PLA_2 ($P<0.05$) compared with the venom controls in varying degrees of efficacy.
Conclusion: This study supports the potential of medicinal plants in developing effective anti-venom therapies. Further studies are recommended to isolate the active compound and elucidate its mechanism of action.

Keywords: In vitro; anti-venom; PLA2; Catunaregam nilotica; Echis ocellatus.

1. INTRODUCTION

Snakes belong to the Phylum *Chordata*, Order *Squamata*, Sub-order *Serpentes* and Class *Reptilia*. Linnean taxonomy places all modern snakes within the Sub-order *Serpentes*, part of the Order *Squamata* [1,2] and various families, including Colubridae, Boidae, Elapidae, Pythonidae, and Viperidae [3]. Venomous snakes are species that produce venom in their venom glands, and these groups of snakes have been classified into four families: *Viperidae, Atractaspididae, Elapidae* and *Colubridae* [4], *Naja nagricollis* and kraits of *Elapidae* family cause maximum envenomation [5,6]. Venomous snakes are prevalent on most continents except Antarctica, Ireland, New Zealand, and many small Atlantic and Central Pacific islands [7]. *Elapidae* families (African cobra, Asian kraits, African mambas, American coral snakes, Asian cobra, Australian and New Guinean venomous snakes, and sea snakes) and *Viperidae* (Asian pit vipers, American rattlesnakes and pit vipers, and old-world vipers) are medically important snakes [8]. These medically significant snakes can cause snakebite envenomation (SBEs), which can lead to symptoms such as pain, swelling, bleeding, severe tissue damage, local necrosis, haemorrhage and oedema at the bite site, tissue death, and neurological issues [5]. In severe cases, SBEs can cause loss of function in the affected limb and acute respiratory and renal failure, which can lead to death [7,9].

In Nigeria, the majority of snake species that are of medical importance belong to three families' viz., Viperidae (Vipers and Adder), *Elapidae* (Cobras and Mambas) and Colubridae (Boomslag). The saw-scaled or carpet viper (*Echis ocellatus*), Cobras (Naja spp.), and puff adders (Bitisarietans) have proved to be the most important causes of mortality and morbidity. Specifically, *Echis ocellantus* is the most common cause of morbidity and mortality in North-Eastern Nigeria [10].

Echis ocellatus venom comprises a diverse array of enzymatic families, including snake venom metalloproteinases (SVMPs), snake venom serine proteases (SVSPs), and snake venom phospholipase A2, which collectively constitute approximately 70% of the complete venom proteome [11]. These enzymes possess the capability to function either synergistically or independently, thus inducing local tissue injury, myotoxic effects and hemorrhagic manifestations through the degradation of the basement membrane of capillary vessels [12,6]. The hemorrhagic consequences associated with *Echis ocellatus* venom can lead to mortality as they cause bleeding from crucial organs by inflicting damage upon the vascular endothelium [10].

Phospholipases A_2 (PLA_2; EC 3.1.1.4), commonly found in snake venoms, play a key role in breaking down phospholipids and can induce biological effects such as oedema, platelet aggregation modulation and neurotoxic, anticoagulant, and myotoxic activities. Myotoxic PLA_2s bind to lipids or proteins on the plasma membrane, compromising its integrity through enzymatic hydrolysis or direct interaction with the membrane. This disruption leads to uncontrolled ion permeability, primarily Ca^{2+} influx, which triggers destructive processes like muscle hypercontraction, membrane damage and mitochondrial Ca^{2+} overload, which ultimately result in muscle cell necrosis [13].

PLA_2, abundant in viperid and crotalid venoms, plays a key role in developing myotoxicity following snakebites, which can manifest as local or systemic muscle damage. Their action can cause irreversible injuries, potentially leading to limb amputation. While effective against systemic

effects, anti-venoms have limited efficacy in preventing local tissue damage. This highlights the need for alternative inhibitors and complementary therapies to enhance conventional anti-venom treatment.

Anti-venom (ASV) represents the only approved pharmacological intervention employed to mitigate the effects of snake envenomation, thereby enhancing the immune response following a snake bite [14]. Nonetheless, ASV is associated with numerous limitations. It can precipitate acute anaphylaxis or anaphylactic reactions attributed to its heterologous nature, which may vary from mild to severe and manifest within one hour post-administration of the anti-venom [15]. Sometimes, it fails to confer adequate protection against local toxicities induced by snake venoms, such as haemorrhage, necrosis, and nephrotoxicity [16]. The procurement of ASVs is economically burdensome and necessitates optimal storage conditions [17], which may be inadequate in rural regions endemic to snake populations.

Plants are reputed to neutralize the toxic effects of snake venom, with many plants claimed to be antidotes for snake bites in traditional folk medicine [17]. These plants are used, single or in combination, as antidotes for snake envenomation by rural populations in Nigeria and many parts of the world [18]. Several reports indicate the extensive use of medicinal plants for treating snake bites globally, particularly in tropical and subtropical regions like Asia, Africa, and South America [19]. However, the literature reveals that only a few of these plants have been scientifically validated.

Catunaregam nilotica is a medicinal plant commonly found in Northern Nigeria, widely used in the treatment of bites from two of the most venomous snakes in the region, *Naja nigricollis* (black-necked spitting cobra) and *Echis ocellatus* (saw-scaled viper) as well as scorpion stings. Communities in Isa (Sokoto state), Zuru (Kebbi state) and Gumel (Jigawa state) have long utilized this plant for its broad range of traditional applications. The root and stem bark mainly treat various poisons, particularly snakebites. Additionally, the plant holds significant value in managing gonorrhoea and other sexually transmitted diseases (using the leaves), as a genital stimulant (using the root), and for its use of anti-dysenteric, anti-inflammatory, and anti-fertility properties (root and stem bark) [20].

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Kaura Namoda, Nigeria, within the Biochemistry Research Laboratory in the Department of Science Laboratory, under the School of Science, Federal Polytechnic Kaura Namoda, Zamfara State, Nigeria.

2.2 Standard Snake Venom Antiserum (Antivenin)

The polyvalent snake venom antiserum known as African Snake Venom Antiserum (Asna Anti-venom C), designated with Batch Number AF10/10 and manufactured in October 2021 with an expiration date set for December 2025, was obtained from Bharat Serums and Vaccines Ltd, India. This antiserum served as a benchmark for evaluating the comparative efficacy of the botanical extract.

2.3 Collection of Snake Venom

Lyophilized venom of *Echis ocellatus* was procured from the pharmacology department at Ahmadu Bello University, Zaria, Nigeria.

2.4 Preparation of Venom

The lyophilized venom was reconstituted in 0.9 % saline and kept at 4°C. The venom concentration was expressed in terms of dry weight (mg/ml) [21].

2.5 Collection and Authentication of the Plant Material

The fresh root barks of *Catunaregam nilotica* were collected from Gabake village in the Birnin Magaji Local Government Area of Zamfara State, Nigeria. The collected specimens were subsequently transported in polyethene bags to the biochemistry laboratory at Kebbi State University of Science and Technology, Aliero. Professor Dramendra Singh authenticated the sample at the herbarium unit of the university, and voucher number Ksusta/PSB/H/Voucher No. 234A was assigned. The roots were meticulously washed and air-dried under shade until they reached a constant weight.

2.6 Preparation of Crude Extract of *Catunaregam nilotica* Root Bark

The extract was prepared following the methodology established by Jimoh et al. [22].

The harvested root was cleaned using distilled water, air-dried in a shaded environment and ground into a fine powder using pestle and mortar. The root powder of *Catunaregam nilotica* (1kg) was immersed in 2.5 L of methanol (95%). The resultant mixture was maintained at ambient temperature for 72 hours and filtered twice, first employing a muslin cloth followed by a Whatman filter paper No.1. The resulting filtrate was evaporated to dryness at a controlled temperature of 45°C using a rotary evaporator.

2.7 Solvent-fractionation of Crude Extract *Catunaregam nilotica* Root Bark

The crude methanol extract derived from the root bark of *Catunaregam nilotica* was subjected to fractionation through liquid-liquid extraction using n-hexane, ethyl acetate, and n-butanol sequentially according to their increasing polarity. The crude extract, 100 grams, was reconstituted in 200 millilitres of distilled water within a 500-millilitre separating funnel and subjected to vigorous shaking. Subsequently, the resulting solution was partitioned with an equivalent volume of n-hexane and agitated intensely. After allowing the components to settle, the lower section of the separating funnel was opened to facilitate the removal of the aqueous layer. The residual contents within the separating funnel were transferred into a clean vessel designated for the n-hexane fraction. An equal volume of n-hexane was introduced, followed by further shaking and separation. This process was repeated continuously until the extraction yielded negligible amounts of extract in the n-hexane layer. An analogous procedure was executed for both ethyl acetate and n-butanol. The resulting fractions underwent concentration to dryness via a rotary evaporator, culminating in the acquisition of n-hexane (n-HF), ethyl acetate (EAF), n-butanol (n-BF), and aqueous fractions (AF) [23,24];. The fractions were concentrated to dryness, and the resultant residues were maintained in a refrigerator within an airtight container for subsequent analysis. Before utilization, each fraction was reconstituted in either distilled water or a 1% solution of Tween80 (polysorbate) and quantified in terms of dry weight (mg/ml).

2.8 Qualitative Phytochemical Screening

5g of crude extracts were dissolved in 40 ml of distilled water and subjected to phytochemical screening through established methodologies [25,26,27].

2.9 Phospholipase A_2 Assay

The acidimetric assay for PLA_2 enzymes, described by Tan and Tan [28] and reported by Sani et al. [19], was adopted in this study. Constant volumes of substrates comprising Calcium chloride (18 mM), Tween eighty (1%), and egg yolk (2 mg/ml) were mixed and stirred for 10 min to get homogenous egg yolk suspension. The pH of the mixture was adjusted to 8.0 using NaOH (1 M) and HCL (1N). Snake venom (0.1mg/ml) was added to the above mixture (15 ml) to initiate the process of hydrolysis, and saline was used as a control. For venom enzyme inhibition assay, *Echis ocellatus* venom (0.1mg/ml) was pre-incubated with crude extract and its fractions (0.1mg/ml) for 30 minutes at 37°C to neutralize the hydrolytic action of PLA_2. A decrease in the pH of the suspension was noted after two minutes with the help of a pH meter. A pH decreases by one (1) unit corresponding to 133 µmole of fatty acid released. Enzyme activity was expressed as µmole of fatty acid released per minute [29].

The inhibitory activity by the extract and its fractions of the root of *C. nilotica* against the PLA_2 was calculated and expressed in terms of percentage activity using the following relationships.

$$\text{Enzyme activity} = \frac{\mu mol\ of\ FA\ released}{time\ taken\ in\ minutes}$$

$$\% \text{ Activity} = \frac{Enzyme\ activity\ of\ the\ test}{Enzyme\ activity\ of\ the\ control} \times 100$$

Where: FA = Fatty acid

$$\% \text{ Inhibition} = \frac{Enzyme\ activity\ of\ the\ control - Enzyme\ activity\ of\ the\ test}{Enzyme\ activity\ of\ the\ control} \times 100$$

3. RESULTS AND DISCUSSION

50.6g of black crude extract was obtained after 72h of extraction with methanol. It was then labelled as a crude extract.

3.1 Phytochemical Composition of the Crude Extract and its Fractions

Table 1 presents the qualitative phytochemical compositions of the crude extract. Alkaloids, anthraquinones, Flavonoids, Phenols, Saponins, Steroids, Tannins and Terpenoids were detected in the crude extract.

Table 1. Quantitative phytochemical of crude methanol extract of *Catunaregam nilotica* root bark

Test	Crude Extract
Alkaloids	+
Anthraquinones	+
Flavonoids	+
Phenols	+
Saponins	+
Steroids	+
Tannins	+
Anthracyanins	-
Glycosides	+

3.2 *In vitro* Anti-venom Effects

Table 2 presents the *In vitro* detoxifying effects of different fractions of *Catunaregam nilotica* root extracts on the PLA_2 enzyme activity of *Echis ocellatus* venom. The n-hexane fraction exhibited the most significant inhibitory effect among the fractions, with a 75.04% reduction in PLA_2 activity. The ethyl acetate fraction also showed a notable reduction in inhibiting the enzyme by 56.49%, indicating moderate detoxifying potential. The n-butanol fraction showed 52.06% inhibition of PLA_2 activity, while the aqueous fraction had the lowest effect, with only 29.56% inhibition. This makes it the least effective of the tested extracts. In comparison, ASV was highly effective, nearly completely neutralizing the enzyme's activity with 97.92% inhibition.

3.3 Discussion

The potential of phytochemical compounds in developing new drugs against deadly toxins is a significant area of research. Phytochemicals are known to counteract snake venom toxins through multiple mechanisms, including enzyme inhibition, deactivation of venom proteins, adjuvant effects, and chelation activities [30,31,19]. The phytochemical screening of the crude extract and its fractions revealed a promising array of bioactive compounds, including alkaloids, tannins, saponins, flavonoids, steroids, and terpenoids, each known for their antioxidant properties [32]. These findings align with research by (1) and Hassan et al. [20], highlighting the anti-venom potential of *Catunaregam nolitca*.

Phytochemical compounds neutralize snake venom toxicity by inhibiting critical enzymes such as PLA_2, proteases, and hyaluronidase. For instance, alkaloids modulate neurotoxins through interactions with voltage-gated ion channels, potentially reducing pain and muscle spasms [33]. Tannins and polyphenolic compounds, such as epigallocatechin gallate, exhibit strong inhibitory effects on PLA_2 activity, binding venom proteins and mitigating enzymatic toxicity [34,13].

PLA_2 enzymes in *Echis ocellatus* venom are particularly harmful, causing blood vessel damage, haemorrhage, hypovolemic shock, and organ failure [6]. Degrading membrane phospholipids, these enzymes trigger inflammation, oxidative stress, and necrosis [35,36,37]. The potent inhibition of PLA_2 activity observed in this study suggests that the bioactive compounds in n-HF target these enzymes, potentially preventing severe envenomation symptoms.

The results demonstrated significant differences in inhibitory effects among the fractions. The n-HF fraction exhibited the highest inhibitory activity, followed by EAF and n-BF, while AF showed the least efficacy when compared to antiserum (ASV). This hierarchy highlights n-HF as the most promising fraction, containing potent bioactive compounds capable of neutralizing PLA_2 and mitigating inflammatory and hemorrhagic effects [38].

Table 2. *In vitro* detoxifying effects of *Catunaregam nilotica* (STAPF) root extracts against *Echis ocellatus* phospholipase A_2 (PLA_2) enzyme

Treatment	Venom phospholipase A_2 Activity (µmol/l)	% (Inhibition)
Venom control	134.99 ± 0.01^{f}	0
Venom +ASV	2.81 ± 0.21^{a}	97.92
Ethyl acetate fraction	58.74 ± 0.44^{c}	56.49
n-Hexane fraction	33.69 ± 2.52^{b}	75.04
n-Butanol fraction	64.73 ± 0.09^{d}	52.06
Aqueous fraction	95.06 ± 0.04^{e}	29.56

PLA_2 activity is presented as Mean ± SEM (n = 4). Mean activity carrying different superscripts is significantly (P=.05) different

These findings are supported by previous studies showing that plant-based compounds such as rutin, quercetin, and naringenin effectively inhibit venom enzymes, improve survival, and restore hemostatic balance in envenomation cases [39,40]. Similarly, saponins and steroids from *Andrographis paniculata* have shown robust anti-snake venom activity by reducing oxidative stress and inhibiting enzymatic functions [41,3]. Tannins are particularly notable for their antioxidant, anti-inflammatory, and wound-healing properties [42]. Studies have demonstrated their ability to inhibit venom enzymes, neutralize free radicals, and promote tissue repair [43,44]. Alkaloids, on the other hand, provide neuroprotective effects by modulating ion channel activity, reducing the impact of neurotoxins, and alleviating symptoms such as pain and muscle spasms [45,46,47].

4. CONCLUSION

The potent PLA_2 inhibition observed with n-HF highlights its promise as a candidate for anti-venom therapy. Future studies should focus on in vivo validation to confirm these phytochemicals' therapeutic efficacy and safety in neutralizing snake venom toxicity. Further studies are recommended to isolate the active compound and elucidate its mechanism of action.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies ChatGPT have been used during the editing of manuscripts.

ACKNOWLEDGEMENT

This research work was fully sponsored by the Tertiary Education Trust Fund (TETFund) through Institution-Based Research (IBR).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Alejandro S. "Diapsids III: Snakes" Father Sanchez's Web Site of West Indian Natural History: 2007. Avaliable:http://www.kingsnake.com/Westindian/metazoa12.html.
2. Database. *The Reptile.* 2023. Avaliable:http://www.reptile-database.org/db-info/SpeciesStat.html
3. Gutiérrez J, Calvete J, Habib A, Harrison R, Williams D, Warrell D. Snakebite envenoming. Nature Reviews Disease Primers. 2017;3(17063):1-10. DOI:10.1038/nrdp.2017.63
4. WHO. Guidelines for the management of snakebites. India: Regional Office for South-East Asia: 2016.
5. Ajisebiola B. Adeniji O, James A, Ajayi B, Adeyi A. *Naja nigricollis* venom altered reproductive and neurological functions via modulation of pro-inflammatory cytokines and oxidative damage in male rats. Metabolism Open. 2022;14(100188):1-9. Avaliable:https://www.sciencedirect.com/journal/metabolism-open
6. Nicola M, Pontara A, Kass G, Kramer N, Avella I, Pampena R. Vipers of major clinical relevance in Europe: Taxonomy, venom composition, toxicology and clinical management of human bites. Toxicology. 2021;453:152724. DOI:10.1016/j.tox.2021.152724
7. Guimarães CL, Moreira-Dill LS, Fernandes R, Costa T, Hage-Melim LI, Marcussi S, Stábeli RG. Biodiversity as a source of bioactive compounds against snakebites. Current Medicinal Chemistry. 2014:1-50.
8. Monteiro W, Bernal J, Bisneto P, Sachett J, Silva I, Lacerda M. *Bothrops atrox*, the most important snake involved in human envenomings in the Amazon: How venomics contributes to snake knowledge. Biology and Clinical Toxinology. Toxicon. 2020;(6):100037. Avaliable:https://doi.org/10.1016/j.toxcx.2020.100037
9. Shah M, Imam T, Bala A, Tukur Z. Survey of snakes bites among snake endemic communities in north eastern Nigeria. Snake Venom and Ecolog. 2022;1-15. Avaliable:http://dx.doi.org/10.5772/intechopen.105419
10. Oliveira AL, Viegas M, da Silva S, Soares A, Fernandes M, Fernandes F. The chemistry of snake venom and its medicinal potential. NATuRe Review. 2022:1-19. Avaliable:https://doi.org/10.1038/s41570-022-00393-7
11. Muhammad Y, Bala A, Magaji S, Doma A, Abubakar A, Goji S, Chedi B. Efficacy testing of commercially available anti-snake venoms against *Echis ocellatus* venom in northern Nigeria. Dutse Journal

of Pure and Applied Sciences (DUJOPAS). 2022;8(4a):116-128.
Avaliable:https://dx.doi.org/10.4314/dujopas.v8i4a.13

12. Pereañez J, Núñez V, Patiño A, londoño MQ. Inhibitory effects of plant phenolic compounds on enzymatic and cytotoxic activities induced by a snake venom phospholipase A2. Vitae, Revista De La Facultad De Química Farmacéutica. 2011;295-304.
13. Mukherjee A. Species-specific and geographical variation in venom composition of two major cobras in Indian subcontinent: Impact on polyvalent anti-venom therapy. Toxicon. 2020; 20:(30434-7).
Avaliable:https://doi.org/10.1016/j.toxicon.2020.10.024.
14. Bhaumik S, Beri D, Lassi Z, Jagnoor J. Interventions for the management of snakebite envenoming: An overview of systematic reviews. Plos Neglected Tropical Diseases. 2020;10(14):e0008727.
Avaliable:https://doi.org/10.1371/journal.pntd.0008727
15. Roy B. A systematic review on snake venom and its therapeutic implication. Journal of Emerging Technologies and Innovative Research. JETIR. 2021;8(7): 477-487.
Avbaliable:http://www.jetir.org/
16. Liaqat A, Mallhi T, Habib Khan Y, Khokhar A, Chaman S, Ali M. Anti-snake venom properties of medicinal plants: A comprehensive systematic review of literature. Brazilian Journal of Pharmaceutical Sciences. 2022;58(e191124):1-16.
Avaliable:http://dx.doi.org/10.1590/s2175-97902022e191124
17. Omara T, Kagoya S, Openy A, Omute T, Ssebulime S, Kiplagat K, Bongomin C. Antivenin plants used for treatment of snakebites in Uganda: ethnobotanical reports and pharmacological evidences. Tropical Medicine and Health. 2020;48(6);1-16.
Avaliable:https://doi.org/10.1186/s41182-019-0187-0
18. Sani I, Rabiu A, Hassan S, Faruq U, Bello F. Lethality of *Naja nigricollis* reinhardt venom and anti-venom activity of *Azadirachta indic*a A. Juss. leaf extracts on albino rats. GSC Biological and Pharmaceutical Sciences. 2020;12(2):080-092.
Avaliable:https://doi.org/10.30574/gscbps.2020.12.2.0244
19. Hassan L, Faruq U, Yusuf A, Salihu M. *In vitro* Detoxifying effects of *Catunaregam nilotica* (STAPF) root extracts against *Naja nigricollis* phospholipase A2 (PLA2) Enzyme. Nile Journal of Engineering and Applied Science. 2023:1-8.
Avaliable:https://doi.org/10.5455/NJEAS.150317
20. Razi M, Asad M, Khan T, Chaudhary M, Ansari M, Arshad M, Najamus-Saqib Q. Antihaemorrhagic (Anti-venom) potentials of fagonia cretica against pakistani cobra venom (*Naja naja* karachiensis). Natural Products Research. 2011;25:1902-1907.
21. Jimoh A, Ilyas M, Sani Y, Bakare L, Adejoke S. Isolation of stigmasterol from the root bark of *Annona senegalensis* Pers. (Annonaceae). Nigerian Research Journal of Chemical Sciences, 2021:186-196.
Avaliable:http://www.unn.edu.ng/nigerian-research-journal-of-chemical-sciences/
22. Ahmed M, Arise R, Umaru I. Identification and biochemical characterization of anti-enteropooling compounds from *Annona senegalensis* root bark. Scientific African. 2022;15: e01128.
Avaliable:https://doi.org/10.1016/j.sciaf.2022.e01128
23. Harborne J. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis; 1973. (1st Edition ed.). London.: Chapman and Hall, UK.
24. Abubakar A, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purpose. Journal of Pharmacy and Bioallied Sciences. 2020;12(1): 1-10.
25. Trease G, Evans W. Trease and Evans Pharmacognosy; 1989(13th Edition ed.). London: Bailliere Tindall.
26. Sofowora A. Medicinal Plants and Traditional Medicine in Africa: 1993. (2nd Edition ed.). Ibadan: Spectrum Books, Ibadan, Nigeria.
27. Tan N, Tan C. Acidimetric assay for phospholipase A2 using egg yolk suspension as substrate. Analytical Biochemistry. 1988;170(2):282-288.
28. Yap M, Tan N, Fung S. Biochemical and toxinological characterization of *Naja sumatrana* (Equatorial spitting cobra) venom. Journal of Venom Animal Toxins Including Tropical Diseases. 2011;17(4):451–459.

29. Shivashankar S, Murali A, Sangeetha M. Molecular interaction of phytochemicals with snake venom: Phytochemicals of *Andrographis paniculata* inhibits phospholipase A2 of Russell's viper (*Daboia russelli*). Biocatalysis and agricultural biotechnology. 2019:1-10. Avaliable:https://doi.org/10.1016/j.bcab.2019.101058
30. Saravia-Otten P, Gutiérrez J, Hay Y, Cáceres A. Neutralization of toxic activities of Bothrops asper snake venom by ethnomedicinal plants used in Central America, with emphasis in Guatemala: a review. Ciencia, Tecnología y Salud. 2022;9(2):215-236.
31. Salihu M, Hassan L, Faruq U, Yusif A. Deciphering the interactions of scopoletin and scopolin from Catunaregam nilotica roots against *Naja nigricollis* phospholipase A2 enzyme. Toxicon. 2024;243(107732):1-9. DOI:https://doi.org/10.1016/j.toxicon.2024.107732
32. Bhambhani S, Kirtikumar R, Kondhare R, Kirtikumar R, Ashok K, Ashok G, Giri P. Diversity in chemical structures and biological properties of plant alkaloids. Molecules. 2021;58-72. DOI: 10.3390/MOLECULES26113374
33. HaiFeng G, Liang J, ChunMei L, Zhang J, HongLiang D. Study on inhibitory effects of persimmon tannins on activities of major enzymes from several snake venoms and its mechanism. Scientia Agricultura Sinica. 2008;41(3):910-917.
34. Williams H, Hayter P, Ravishankar D, Baines ALH, Croucher L, Wark C, Vaiyapuri S. Impact of *Naja nigricollis* venom on the production of methaemoglobin. Toxins. 2018;10(539):1-10. Avaliable:https://doi.org/10.3390/toxins10120539.
35. Tasoulis T, Pukala T, Isbister G. Abundance in Snake Venom Proteomes. Frontier. Pharmacology. 2022;12(768015): 1-13. Avaliable:https://doi.org/10.3389/fphar.2021.768015
36. Ledsgaard L, Wade J, Jenkins T, Boddum K, Oganesyan J, Harrison J, Karatt-Vellatt A. Discovery and optimization of a broadlyneutralizing human monoclonal antibody neutralizing human monoclonal antibody against long-chain α-neurotoxins from snakes. Nature Communications. 2023;14(682):1-9. Avaliable:https://doi.org/10.1038/s41467-023-36393-4
37. Elda E, Sánchez A, Rodríguez-Acosta. Inhibitors of snake venoms and development of new therapeutics. Immunopharmacology and Immunotoxicology: 2008. DOI: 10.1080/08923970802279019
38. Sachetto A, Miyamoto J, Tashima A, de Souza A, Santoro M. The bioflavonoids rutin and rutin succinate neutralise the toxins of B. jararaca venom and nhibit its lethality. Frontiers in Pharmacology. 2022;13(828269.):1-24. Avaliable:https://doi.org/10.3389/fphar.2022.828269
39. Srimathi R, Gurunathan J. Synergistic effect of flavonoids combined with anti-venom on neutralization of *Naja naja* venom. Asian Pacific Journal of Tropical Biomedicine. 2021;11(7):298-307. DOI: 10.4103/2221-1691.309665
40. Khan A. Identification of phytosaponins as novel biodynamic agents: an updated overview. Journal of Phytotherapy. 2012:1-8.
41. Sia F, Vejayan J, Jamuna A, Ambu S. Efficacy of tannins from *Mimosa pudica* and tannic acid in neutralizing. The Journal of Venomous Animals and Toxins including Tropical Diseases. 2011;17(1): 42-48.
42. Kuppusamy U, Das N. Protective effects of tannic acid and related natural compounds on Crotalus adamenteus subcutaneous poisoning in mice. Pharmacology Toxicology. 1993;72(4-5):290-5. DOI: 10.1111/j.1600-0773.1993.tb01652.x. PMID: 8372049.
43. Lindahl M, Tagesson C. Flavonoids as phospholipase A2 importance of their structure for selective inhibition of group II phospholipase A2. Inflammation. 1997;347-356.
44. Kaur R, Arora S. Alkaloids-Important Therapeutic Secondary Metabolites of Plant Origin. Journal of Critical Reviews. 2015;2(3):1-8.
45. Abdlmageed M, Elshiekh Y, Abdella A. Chemical composition and antibacterial activity of xeromphis nilotica bark extracts. World Journal of Biological Pharmaceutical and Health Sciences. 2020;3(1):054-059.
46. Andreimar M, Soares JA, Lourenço MV, Pereira AM, Pereira P. Neutralizing effects

of Brazilian plants against snake venoms. Drugs of The Future. 2004:15-26. DOI: 10.1358/DOF.2004.029.11.851973

47. Mathias SN, Abubakar K, October N, Abubakar M, Mshelia HE. Anti-venom potentials of friedelin isolated from hexane extract fraction of *Albizia chevalieri* hams (MIMOSACEAE). Ife Journal of Science. 2016;18(2): 473-483.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/128756