Science World Journal Vol. 21(No 2) 2026 https://dx.doi.org/10.4314/swj.v21i2.18
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

IN VITRO ANTIOXIDANT AND ANTIVENOM ACTIVITIES OF AQUEOUS AND CHITOSAN ENCAPSULATED *ANNONA SENEGALENSIS* BARK EXTRACTS AGAINST NAJA NIGRICOLLIS VENOM

*[1]AZAKI Gideon Philip, [1]AUDU Isaac Sumaila, [1,5]ADAMU Makanta Salihu, [1]FADIPE Labake Ajoke, [1,2]SHABA Elijah Yanda, [3]AGBAI Uche Timothy & [4]JAMES Daniel

[1]Department of Chemistry, Federal University of Technology, Minna, Niger State, Nigeria
[2]Nanotechnology Research Group, Africa Centre of Excellence for Mycotoxin and Food Safety, Federal University of Technology, PMB 65, Minna, Niger State, Nigeria
[3]Department of Chemistry and Biochemistry, University of New Hampshire, New Hampshire, USA
[4]Wuhan Institute of Virology, University of Chinese Academy of Science, China
[5]Department of Chemistry and Biochemistry, SSBSR, Sharda University, Greater Noida, U.P., India

*Corresponding Author Email Address: azakigideon@gmail.com Phone: +2348160286039

ABSTRACT
Snake envenomation is a neglected tropical disease responsible for thousands of deaths and morbidities annually. One of the most important venomous snakes in Nigeria is *najanigricollis*. Its enzyme constituent of the venom has been linked to fatal complications after being envenomated. Therefore, this study aimed to compare the *in vitro* antioxidant and antivenom activities of aqueous crude extract and chitosan-encapsulated extract of *Annona senegalensis* bark. *In vitro* antioxidant tests, DPPH radical and ferric reducing power tests, and in vitro antivenom tests, including phospholipase A2 and proteinase inhibition tests, were carried out using standard procedures. The extracts exhibited high DPPH radical-scavenging and ferric-reducing power; however, the encapsulated extract demonstrated greater activity, with IC50 values of 20.45 µg/mL and 7.45 µg/mL against DPPH radical and FRAP, respectively, compared to the crude extract (50.36 µg/mL). and 20.69 µg/mL. Furthermore, the extracts had significant inhibitory effects on the venom enzymes, with the encapsulated extract showing greater inhibition. The encapsulated extract showed stronger proteinase inhibition (IC_{50} = 47.30 µg/mL) compared to the crude extract (IC_{50} = 45.04 µg/mL). In comparison, the crude extract exhibited stronger phospholipase A_2 inhibition (IC_{50} = 15.10 µg/mL) than the encapsulated extract (IC_{50} = 30.14 µg/mL). This is the first study to assess the antivenom potential of *A. senegalensis* bark extract against *N. nigricolli* encapsulated in chitosan. These findings suggest that chitosan encapsulation enhances the extract's antioxidant activity and improves selected antivenom properties, highlighting its potential as a lead candidate for further development of plant-based therapies for snakebite envenomation.

Keywords: *Naja nigricollis*, *Annona senegalensis*, Snake venom, Antioxidant, Phospholipase A2, Proteinases, Chitosan nanoparticles

INTRODUCTION

Snakebite envenomation (SBE) is a severe occupational and underdiagnosed community health issue that is prevalent worldwide, especially in developing nations in tropical and subtropical regions (Gutiérrez, 2021; Warrell & Williams, 2023). Snakebite envenomation is said to cause the death of more than 100,000 people each year, and a high proportion of the survivors are permanently disabled or deformed. Snakebites are common in the regions of Latin America, Asia, and Sub-Saharan Africa, where 15,000-20,000 snakebite cases are reported annually in Nigeria alone, which have led to deaths of approximately 2,000 individuals, and amputation of 1,700 to 2,000 individuals (Olorunnimbe, 2021). In the treatment of snake bites, specific serum-based antivenoms are primarily administered. However, they have certain weaknesses, including high production costs, inaccessibility in rural areas, allergic reactions, and a lack of uniformity across different snake species (Gamulin *et al.,* 2023). Consequently, there is a pressing need for good, more accessible antivenom remedies based on natural sources, including plant extracts. Ancient civilizations (including the Chinese, Native Americans, Tibetans, and Indian Ayurvedic practitioners) used herbal extracts from medicinal plants to treat illnesses associated with systemic poisoning (Puzari *et al.,* 2022). The indigenous people across the World often use herbal preparations to help with systemic poisoning cases (Liaqat *et al.,* 2022). Nevertheless, it was found that the transport of the desired concentration of phytochemicals to snake-bitten target organs is not entirely effective; therefore, this challenge, along with their insufficient solubility in biological fluids, hampers their delivery to the intended target site (Faria *et al.,* 2020). Nanotechnology has enabled superior formulations with improved pharmacokinetics, pharmacodynamics, and therapeutic efficacy. The use of traditional herbs in various nano-based formulations has also been shown to significantly improve properties, broaden applications, and enable precise delivery. Therefore, to facilitate their directed and selective delivery, it is crucial to develop new encapsulations of these phytochemicals in herbs (Kulabhusan *et al.,* 2020). Due to its biocompatibility and biodegradability in biological systems, chitosan, an organic molecule derived from chitin, has been used as a promising drug delivery vehicle. It is a great choice for encapsulating biologically active components due to its special properties, including low toxicity, controlled release, and absorption on mucosal membranes. Also, chitosan nanoparticles have shown potential to improve the stability and availability of the encapsulated agents (Kulabhusan *et al.,* 2020). One of the key medicinal plants in sub-Saharan Africa, traditionally used as a snakebite antidote, is

Annona senegalensis, which belongs to the family Annonaceae (Sookanging *et al.,* 2022). In Asian countries such as India and Myanmar, Annona senegalensis has been traditionally used to treat diarrhea, malaria, and respiratory issues (Detroja *et al.,* 2024). However, studies, particularly in terms of encapsulation of the bark extract of this plant in the chitosan nanoparticles in comparison to the venom of naja nigricollis, are still absent, although the potential of this plant remains.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The bark of *Annona senegalensis* was collected at Gwada, Nigeria (Latitude 13 N, Longitude 13 E) on the 17th November, 2025. The plant was identified by a plant taxonomist at the Department of Plant Biology, Federal University of Technology, Minna, Nigeria, under voucher number FUT MIN/SLS/PB-067-025, and the specimen was deposited in the university's herbarium.

Chemicals and Reagents

All the chemicals and reagents in this study were of analytical grade and purchased from the Central Drug House (CDH). India. The chemicals and reagents that were used in conducting this study are: methanol, distilled water, phosphate buffer, chitosan, acetic acid, phosphate-buffered saline, potassium hexacyanoferrate (III), ferric chloride, Folin reagent, sodium tripolyphosphate, potassium hexacyanoferrite (III), thiobarbituric acid (TCA), sodium acetate, sodium deoxycholate, trichloroacetic acid (TCA), among others.

Preparation of Chitosan Nanoparticles

To obtain a 1% w/v solution of chitosan, 1 g of chitosan that had a deacetylation degree of 90 was dissolved in 100 mL of 1% acetic acid. The solution was stirred using a magnetic stirrer at 37 °C until the chitosan was fully dissolved (a clear solution was obtained). Tripolyphosphate (TPP) solution (0.5% w/v) (5 mL) was then added gradually into the chitosan solution at a rate of 0.2 mL/min with constant stirring. This was followed by further stirring of the solution at room temperature (37 °C) to ensure homogeneity. Lastly, centrifugation was performed to separate the chitosan nanoparticles at 20,000 × g for 30 minutes at 4 °C (Abdullahi *et al.,* 2025; Farid *et al.,* 2024).

Encapsulation of *A. senegalensis* bark extract in Chitosan Nanoparticles

The synthesized chitosan nanoparticles (3 g) were dissolved in 30 mL of 1% acetic acid and stirred on a magnetic stirrer to encapsulate the A. senegalensis bark extract within the nanoparticles. Subsequently, 15 mL of A. senegalensis bark extract (10 mg/ml) was introduced into the solution of chitosan nanoparticles. The blend was stirred for 1 hour to ensure complete extraction (Mady *et al.,* 2024).

In Vitro Antioxidant Activity of the Bark Extract Ferric Reducing Antioxidant Power (FRAP) Assay:

The antioxidant activities of the plant extracts were estimated using the ferric reducing antioxidant power assay, following the procedure described by Ibrahim *et al.* (2020). The crude extract, encapsulated extract, and ascorbic acid (control) were then prepared as stock solutions at 1000 ug/mL, from which different concentrations were prepared: 50, 100, 200, and 400 ug/mL. In these assays, a mixture of 1 mL of the extract, 1 mL of 0.2 M sodium phosphate buffer, and 1 mL of 1% potassium hexacyanoferrate(III) was prepared, with the ascorbic acid concentration adjusted as needed. The reaction mixtures were incubated at 50 °C for 20 min. Afterward, 1 ml of 10% TCA was added. The reaction mixtures were centrifuged at 3000 rpm for 10 minutes at room temperature. Then, 1 mL of each supernatant obtained was combined with 1 mL of distilled water, followed by the addition of 0.2 mL of 0.1% ferric chloride. The blank was prepared with the same extract as the samples, except that the extract was replaced with distilled water. The test mixtures were read at 700nm. The percentage of antioxidant activity was calculated using the formula below:

$$\% \text{ activity} = \frac{\text{A sample- A blank x 100}}{\text{A sample}} \quad (1)$$

In vitro venom-contained enzymes inhibitory assays

To determine the inhibitory effects of the crude extract and fractions of *A. senegalensis* bark extract on the venom enzymes, 0.5 mL of 0.25% of the venom was put into test tubes in the presence of 1 mL of the different concentrations of the crude extract and fractions (100, 50, 25, 12.5, 6.25 µg/mL, respectively) and incubated at 30 ^{o}C. An enzyme assay was done using the incubated venom.

Phospholipase A

The phospholipase A2 assay used was prepared according to the acidimetric method as reported by Abdullahi et al. (2025) and Salihu et al. (2024), with minor modifications. A suspension of lecithin was prepared containing 1 percent lecithin, 18 mM calcium chloride, and 8.1 mM sodium deoxycholate in equal parts. The pH of the suspension was adjusted to 8.0 by adding 1 M sodium hydroxide, and the egg yolk was stirred for 10 minutes to obtain a homogeneous mixture. To initiate hydrolysis, 0.1 mL of preincubated venom solution was added to 15 mL of egg yolk suspension. The pH was initially measured and then decreased by 1 unit, corresponding to 133 moles of fatty acid release. The activity of the enzyme was expressed as U/mol fatty acid released/minute. In the study, the crude and encapsulated venom extracts were pre-incubated at 37 °C.

Protease Inhibitory Assay

Protease test on crude venom was conducted as done by Hansiya *et al.* (2021). The reaction mixtures included 0.5% casein, 1.0 mL of Tris-HCl buffer (pH 8.0), and 0.5 mL of 0.25% preincubated venom, and were incubated at 37 °C for 4 hours. The reaction was stopped after four hours by adding 1 mL of 10% trichloroacetic acid (TCA), and the mixture was filtered. Protein estimation with L-tyrosine as the standard was performed on the filtrate (1.0 mL). In the experiment above, 1 unit of enzyme activity was defined as the quantity that produced 0.02 moles of tyrosine/hour under the given experimental conditions. The treatment of the control was similar to that of the samples, except that it included the venom incubated with neither of the two extracts of the free extract nor the encapsulated extract.

Data Analysis

Statistical analysis using One-way ANOVA and independent T-test was performed to determine the p-value below which values would be significant (Statistical Package for the Social Sciences, version 22.0, SPSS Inc., Chicago, IL, USA) for each *in vitro* antioxidant and

Science World Journal Vol. 21(No 2) 2026
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University
https://dx.doi.org/10.4314/swj.v21i2.18

antivenom activity. The data were presented as the mean standard error of five replicates.

RESULTS

The DPPH-scavenging potentials of crude and encapsulated extracts of the *A. senegalensis* bark are depicted in Figure 1. The antioxidant potentials of the two extracts were significant at the highest concentration tested (100 µg/mL), following a concentration-dependent pattern, with the best antioxidant activity recorded at that concentration (93.5 %). Compared with the crude extract (79.90%), the antioxidant potential of the encapsulated extract is significantly higher ($p < 0.05$). In addition, the encapsulated extract exhibited activity much higher than that of ascorbic acid (85.02 %). Nevertheless, the crude extract exhibited lower antioxidant activity than ascorbic acid ($p < 0.05$). IC_{50} of crude extract, ascorbic acid, and encapsulated extract were estimated to be 50.36 µg/mL, 34.04 µg/mL, and 20.45 µg/mL, respectively.

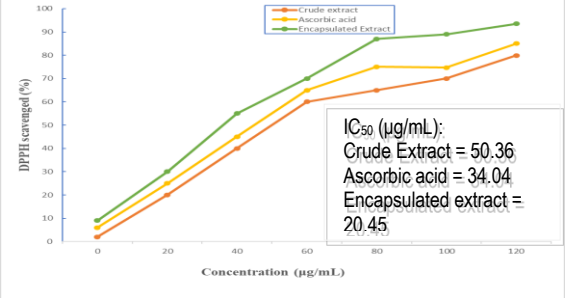

Figure 1: DPPH Radical-Scavenging Potentials of Crude and Encapsulated Extracts of *A. senegalensis* bark

The crude extract and the encapsulated extract of *A. senegalensis* bark exhibited moderate ferric-reducing capacities, respectively (Figure 2). The ferric-reducing capacities, as with the DPPH-scavenging potentials, were observed to be concentration-dependent; the higher the concentration, the greater the antioxidant activity. The encapsulated extract reduced ferric ion (Fe^{+3}) to ferrous (Fe^{+2}) by 90.08 % (with an IC_{50} value of 7.45 µg/mL), which was noticeably more than the crude extract (70.45 %) with an IC_{50} value of 20.69 µg/mL at the highest test concentration (100 µg/mL). Besides, the ferric-reducing capacity of the observed encapsulated extract was significantly higher compared with that of ascorbic acid (86.02%), where the IC50 was 15.24 µg/mL.

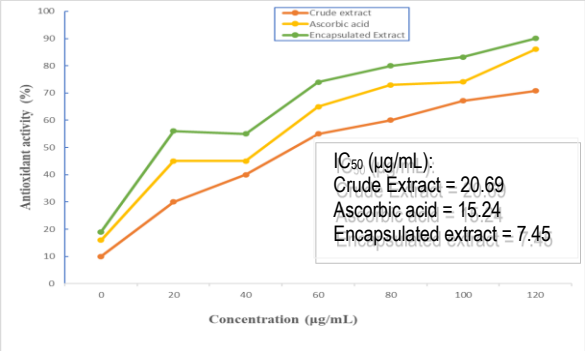

Figure 2: Ferric Reducing Antioxidant Powers of Crude and Encapsulated Extracts of *A. senegalensis* bark

Figure 3 shows the inhibitory effects of crude and encapsulated extracts of *A. senegalensis* bark against phospholipase A2. The extracts were observed to play a major role in inhibiting the activity of N. nigricollis venom phospholipase A2 in a concentration-dependent manner. The highest test concentration of 100 µg/mL gave the greatest inhibitory potentials of the extracts. However, the encapsulated extract demonstrated a greater inhibitory effect on the enzyme, with a % inhibition of 98.03 (IC50 of 30.14 µg/mL) compared with the crude extract (80.10%) and an IC50 of 50.15 µg/mL.

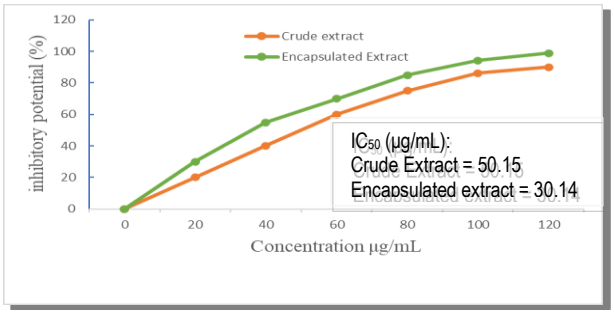

Figure 3: Inhibitory Potentials of Crude and Encapsulated Extracts of *A. senegalensis* bark Against Venom Phospholipase A2

Figure 4 demonstrates the inhibitory activity of the crude and encapsulated extracts of *A. senegalensis* bark against the action of protease present in the venom of *N. nigricollis*. It was also noted in the same study that both extracts displayed a relative inhibitory activity against the enzyme. The inhibitory effects of the extract were also concentration-dependent, with maximum inhibition at the highest test concentration of 100 µg/mL. The inhibitory effect of the encapsulated extract (96.08% with an IC_{50} value of 15.10 ug/mL) was far better than that of the crude extract (79.56%). However, the crude and encapsulated extracts had significant inhibitory effects.

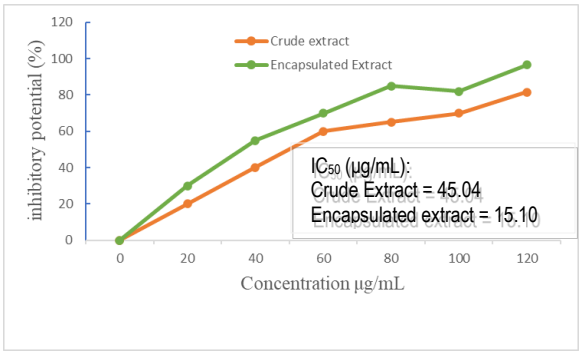

Figure 4: Inhibitory Potentials of Crude and Encapsulated Extracts of *A. senegalensis* bark

DISCUSSION

The crude and encapsulated extracts of *A. senegalensis* bark

exhibited significant DPPH-scavenging activity, with the encapsulated extract showing greater scavenging potential than the crude extract, as reflected in the % DPPH scavenged and IC_{50} values. Even though little to no research has been carried out on the encapsulated extract of A. senegalensis bark, Farooq et al. (2022) reported that the butanol extract of *A. senegalensis* bark elicited appreciable DPPH-scavenging activity. Although a different solvent (aqueous) was employed in our study compared to this study, A. senegalensis bark extracts with polar solvents have been shown to exhibit higher antioxidant activity due to higher concentrations of phenolic compounds, including phenols and flavonoids. A commonly used technique for assessing a compound's antioxidant activity is the DPPH assay (Odewade *et al.,* 2023). A purple-colored DPPH solution turns into the reduced form of the DPPH radical (DPPH-H) when it is combined with a solution of a compound that may donate a hydrogen atom. Hence, the DPPH test assesses the hydrogen-donating properties of compounds (Ibrahim *et al.,* 2020). As such, the appreciable antioxidant activities exhibited by crude and encapsulated extracts of *A. senegalensis* bark suggest their efficient hydrogen-donating potentials. Additionally, IC_{50} has been used to assess a compound's antioxidant potency. Low IC_{50} values indicate strong antioxidant activity, whereas high IC50 values indicate weak antioxidant activity (Tariq *et al.,* 2022). Thus, the encapsulated extract has a lower IC50 than the crude extract, making it a stronger antioxidant.

This study revealed that the crude and encapsulated extracts of A. senegalensis bark significantly reduced Fe3+ to Fe2+, indicating strong antioxidant activity. In addition, the extracts exhibited low IC50 values, further indicating their strong reducing power. Similarly, Farooq *et al.* (2022) reported that the butanol extract of A. senegalensis bark exhibits strong ferric-reducing power. An essential method for evaluating the antioxidant activity of plant extracts is the reducing power assay, which measures electron-donating capacity. Reducing power and antioxidant capacity are positively correlated (Gulcin & Alwasel, 2025). As a result, the significant ferric-reducing power of the extracts suggests they are potent antioxidants. The low IC_{50} values of the extracts further support their potential antioxidant properties, with the encapsulated extract being more potent, with a lower IC50 than the crude extract.

In this study, both crude and encapsulated extracts effectively inhibit the activity of phospholipase A2 in the venom of N. nigricollis, with the encapsulated extract exhibiting greater inhibitory activity and a lower IC50 than the crude extract. The finding reported for the free extract agrees with that of Ushanandini *et al.* (2006), who also reported that bark extract of A. senegalensis inhibits the activity of phospholipase A2 in *V. russelli* venom. Although different snake venoms were employed in our study rather than in this study, phospholipase A2 from these venoms catalyzes the same fundamental reaction (Sampat *et al.,* 2023). Phospholipase A2 (PLA2) is a ubiquitous enzyme that is a significant component of snake venoms. This enzyme hydrolyzes glycerophospholipids at the sn-2 position of the glycerol backbone, yielding lysophospholipids and fatty acids. The PLA2 proteins found in snake venom have a wide range of biological actions, including antiplatelet, hemorrhagic, neurotoxic, myotoxic, and anticoagulant effects (Fernandes *et al.,* 2024). Hence, the significant inhibitory effects of the crude and encapsulated extracts on phospholipase A2 imply that these extracts could alleviate the pathophysiological manifestations of N. nigricollis envenomation mediated by phospholipase A2.

The activity of proteinases contained in the venom of *N. nigricollis* was significantly inhibited by the extracts, with the encapsulated extract exerting a higher inhibitory effect. The extract's significant inhibitory effects led to a low IC_{50} value. The bark extract of *A. senegalensis* bark has also been reported to significantly inhibit *V. russelli* venom proteinases activity (Ushanandini *et al.,* 2006). Many biological functions, including digestion, blood coagulation, the immune system, and inflammation, are affected by proteinases, which catalyze the cleavage of covalent peptide bonds in proteins (Jia *et al.,* 2025). Venom serine proteinases (VSPs) disrupt the control and regulation of important biological processes in fibrinolysis, blood platelet activation, and the blood coagulation cascade (Swenson *et al.,* 2021; Vidal *et al.,* 2024). It can therefore be inferred that the extract's inhibitory effects could prevent or mitigate the resulting biological complications associated with proteinases following *N. nigricollis* envenomation.

The higher antioxidant and antivenom activities of the encapsulated extract than those of the crude extract are not surprising, as encapsulation shields sensitive phytochemicals from light, heat, and oxygen, thereby preventing oxidative and chemical degradation and enhancing biological activities (Popescu *et al.,* 2023).

Conclusion

The antioxidant and antivenom activities of the crude and encapsulated extract showed significant inhibition of venom enzymes. However, higher activity was observed than in the crude extract. This finding presents the encapsulated extract as a potential lead for the development of herbal antivenom therapy against the venom of *N. nigricollis*. To better understand the mechanism of action, future research should focus on *in vivo* antivenom evaluation of the encapsulated extract of *A. senegalensis* bark against *N. nigricollis* venom.

Acknowledgement

The authors acknowledge all affiliated institutions that provided facilities, equipment, and reagents for this research.

Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research authorship, and/or publication of this article.

REFERENCES

Abdullahi, A., Ibrahim, Y. O., Muhammad, F. M., Umar, M. B., Abdulsalam, H., Odu, M., Ndamitso, M., & Busari, M. B. (2025). *In vitro* Antioxidant and Antivenom Activities of Aqueous and Chitosan-Encapsulated *Tamaridus Indica* Seed Extracts Against *NajaNigrocolis* Venom. *FUDMA Journal of Sciences*, 9(7), 256 – 261.

Detroja, K., Sharma, K., Mishra, S. K., & George, J. J. (2024). Phytochemical profiling of *Tamarindus indica:* a medicinal plant for snakebite. In Herbal Formulations, Phytochemistry and Pharmacognosy (pp. 413–421). Elsevier.

Faria, W. C. S., da Silva, A. A., Veggi, N., Kawashita, N. H., de França Lemes, S. A., de Barros, W. M., & Bragagnolo, N. (2020). Acute and subacute oral toxicity assessment of dry encapsulated and non-encapsulated green coffee fruit extracts. *Journal of Food and Drug Analysis*, 28(2), 337–352.

Farid, A., Mohamed, A., Ahmed, A., Mehanny, F., &Safwat, G.

(2024). Preparation of bee venom-loaded chitosan nanoparticles for treatment of streptozotocin-induced diabetes in male Sprague Dawley rats—Beni-Suef *University Journal of Basic and Applied Sciences,* 13(1), 97–115.

Farooq, S., Munir, R., Imtiaz, K., Sehar, S., Khurshid, A., Yunus, N., & Fang, N. (2022). Phytochemical investigation and antioxidant activities of tamarind (*Tamarindus indica L.*). *Notulae Botanicae Horti Agrobotanici Cluj*-Napoca, 50(4), 12892-12892.

Fernandes, D. A., Gomes, B. A., Mendonça, S. C., de Castro Pinheiro, C., Sanchez, E. O. F., Leitão, S. G., & Leitão, G. G. (2024). Alkaloids from *Siparuna* (*Siparunaceae*) are predicted as inhibitors of proteolysis and plasma coagulation caused by snake venom and potentially counteract phospholipase A2 activity of *Bothrops jararaca*—Journal *of Ethnopharmacology,* 332, 118349.

Gamulin, E., MateljakLukačević, S., Halassy, B., &Kurtović, T. (2023). Snake antivenoms: toward a better understanding of the route of administration. *Toxins,* 15(6), 398-415.

Gulcin, I., & Alwasel, S. H. (2025). Fe^{3+} Reducing Power as the Most Common Assay for Understanding the Biological Functions of Antioxidants. *Processes*, 13(5), 1296–1317.

Gutiérrez, J. M. (2021). Snakebite envenomation in Central America: epidemiology, pathophysiology, and treatment. In Handbook of Venoms and Toxins of Reptiles (pp. 543–558). *CRC Press.*

Hansiya, V. S., & Geetha, N. (2021). In vitro anti-venom potential of various solvent-based leaf extracts of *Andrographis serpyllifolia*(Rottler ex Vahl) Wight against *Najanaja* and *Daboia russelli—Journal of Ethnopharmacology,* 269, 113687.

Ibrahim, Y., Busari, M., Yusuf, R., & Hamzah, R. (2020). In vitro antioxidant activities of ethanol, ethyl acetate, and n-hexane extracts of *Mangifera indica* leaves. *Tanzania Journal of Science*, 46(3), 628–635.

Jia, Y., García, A., & Reyes, E. (2025). Single-Chlpain Variable Fragments: Targeting Snake Venom Phospholipase A2 and Serine Protease. *Toxins,* 17(2), 55–68.

Kulabhusan, P. K., Agrawal, S., Jeevanandam, J., & Danquah, M. K. (2020). Nanoformulated herbal drug delivery as an efficient antidote against systemic poisons. *Poisonous Plants and Phytochemicals in Drug Discovery*, 269–294.

Liaqat, A., Mallhi, T. H., Khan, Y. H., Khokhar, A., Chaman, S., & Ali, M. (2022). Anti-snake venom properties of medicinal plants: a comprehensive systematic review of literature. *Brazilian Journal of Pharmaceutical Sciences*, 58, e191124.

Mady, M. S., Sobhy, Y., Orabi, A., Sharaky, M., Mina, S. A., & Abo-Zeid, Y. (2024). Preparation and characterization of nano-emulsion formulations of Asparagus Densiflorus root and aerial parts extracts: Evaluation of in-vitro antibacterial and anticancer activities of nano-emulsion versus pure plant extract. *Drug Development and Industrial Pharmacy,* 50(7), 658–670.

Odewade, J. O., & Odewade, L. O. (2023). Evaluation of antibacterial and antioxidant properties of leaf extract of Chrysophyllum albidum against selected enteric bacterial pathogens. F*udma Journal of Sciences*, 7(2), 131–140.

Olorunnibe, M. (2021). Nigeria records 200,000 cases of snake bites and 2,000 deaths annually: Vanguard's News, 20 September, p. 64.

Popescu, L., Cojocari, D.,Gihendov-Mosanu, A., Lung, I., Soran, M.I., Opris, O., … &Sturza, R. (2023). The effect of aromatic plant extracts encapsulated in alginate on the bioactivity, textural characteristics, and shelf life of yogurt. *Antioxidants,* 12(4), 893-916.

Puzari, U., Fernandes, P.A., & Mukherjee, A.K. (2022). Pharmacological re-assessment of traditional medicinal plants-derived inhibitors as antidotes against snakebite envenoming: A critical review—Journal *of Ethnopharmacology*, 292, 115208.

Salihu, M., Hassan, L. G., Faruq, U. Z., & Yusuf, A. J. (2024). Deciphering the interactions of *scopoletin* and scopolin from *Catunaregam nilotica* roots against *Najanigricollis* phospholipase A2 enzyme. *Toxicon*, 243, 107732.

Sampat, G. H., Hiremath, K., Dodakallanawar, J., Patil, V. S., Harish, D. R., Biradar, P., … & Roy, S. (2023). Unraveling snake venom phospholipase A2: an overview of its structure, pharmacology, and inhibitors. *Pharmacological Reports*, 75(6), 1454–1473.

Sookanging, S., Duangjai, A., Saokaew, S., &Phisalprapa, P. (2022). Botanical aspects, phytochemicals, and toxicity of *Tamarindus indica* leaf and a systematic review of antioxidant capacities of T. indica leaf extracts—Frontiers in Nutrition, 9, 977015.

Swenson, S. D., Stack, S., &Markl, F. S. (2021). Thrombin- like serine proteinases in reptile venoms. In Handbook of venoms and toxins of reptiles (pp. 351–362). CRC Press.

Tariq, S., Umbreen, H., Noreen, R., Petitbois, C., Aftab, K., Alasmary, F. A., ... &Mazid, M. A. (2022). [Retracted] Comparative Analysis of Antioxidant Activity of Indigenously Produced *Moringa Oleifera* Seeds Extracts: bioMed *Research International*, 2022(1), 4987929.

Ushanandini, S., Nagaraju, S., Harish Kumar, K., Vedavathi, M., Machiah, D. K., Kemparaju, K., & Girish, K. S. (2006). The anti-snake venom properties of *Tamarindus indica* (Leguminosae) seed extract. *Phytotherapy Research*: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 20(10), 851–858.

Vidal, J. F., Schwartz, M. F., Garay, A. V., Valadares, N. F., Bueno, R. V., Monteiro, A. C. L., ... & Barbosa, J. A. R. (2024). Exploring the Diversity and Function of Serine Proteases in Toxicofera Reptile Venoms: A Comprehensive Overview. *Toxins*, 16(10), 428–441.

Warrell, D. A., & Williams, D. J. (2023). Clinical aspects of snakebite envenoming and its treatment in low-resource settings. *The Lancet,* 401(10385), 1382–1398.