FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 No. 7, July, 2025, pp 256 – 261
DOI: https://doi.org/10.33003/fjs-2025-0907-3707

IN VITRO ANTIOXIDANT AND ANTIVENOM ACTIVITIES OF AQUEOUS AND CHITOSAN-ENCAPSULATED *TAMARINDUS INDICA* SEED EXTRACTS AGAINST *NAJA NIGRICOLLIS* VENOM

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ABSTRACT

Snake envenomation is a neglected tropical disease responsible for thousands of mortalities and morbidities annually. Naja nigricollis is one of the venomous snakes of most importance in Nigeria. The enzymatic component of its venom has been associated with devastating complications following envenomation. Hence, this study aimed to evaluate the in vitro antioxidant and antivenom activities of aqueous crude extract and chitosan-encapsulated extract of Tamarindus indica seed. In vitro antioxidant assays; DPPH radical and ferric reducing power assays as well in vitro antivenom activities; phospholipase A2 and proteinases inhibition assays were determined using standard methods. The extracts showed significant DPPH radical-scavenging activities and ferric-reducing powers. However, the encapsulated extract exhibited higher activities with IC50 values of 19.62 µg/mL and 3.67 µg/mL against DPPH radical and FRAP, respectively than crude extract with IC50 values of 33.27 µg/mL and 17.56 µg/mL, respectively. Further, the extracts exerted significant inhibitory effects against the venom enzymes, with the encapsulated extract showing higher inhibitory effects. The IC50 values of the encapsulated extract against the enzymes were 34.58 µg/mL and 45.53 µg/mL against phospholipase A2 and proteinases, respectively, while free extract had IC50 values of 47.42 µg/mL and 62.01 µg/mL, respectively. This is the first study evaluating antivenom potential of chitosan-encapsulated T. indica seed against N. nigricollis. The encapsulated extract showed potential antivenom activities and could serve as a drug lead for the production of herbal therapy against Naja nigricollis venom in order to address antivenom limitations.

Keywords: Naja nigricollis, Tamarindus indica, Snake venom, Antioxidant, Phospholipase A2, Proteinases, Chitosan nanoparticles

INTRODUCTION

Snakebite envenomation (SBE) is a serious occupational and underreported public health problem that is widespread globally, particularly in developing countries in tropical and subtropical regions (Gutiérrez, 2021; Williams et al., 2023). The annual number of deaths from snakebite envenomation is estimated to be over 100,000, and a significant percentage of survivors suffer long-term disabilities or deformities. The regions of Latin America, Asia, and Sub-Saharan Africa have high rates of snakebite, with 15,000 to 20,000 snakebite cases reported in Nigeria each year, resulting in the deaths of about 2,000 people and the amputation of 1,700 to 2,000 people (Olorunnimbe, 2021). The main strategy for treating snake bites is the administration of a particular serum-based antivenom. However, current antivenom treatments have some drawbacks, such as high production costs, limited accessibility in remote locations, the possibility of allergic reactions, and inconsistent effectiveness against various snake species (Gamulin et al., 2023). As a result, there is an urgent need for effective and more widely available antivenom treatments derived from natural sources, such as plant extracts.

Ancient civilizations (the Chinese, native American, Tibetan, and Indian ayurvedic practitioners) used to use herbal extracts from medicinal plants and herbs as treatments for illnesses involving systemic poisoning (Puzari et al., 2022). Herbal preparations are frequently used as remedies for systemic poisoning situations by indigenous people throughout the World (Liaqat et al., 2022). However, the effective transport of the suggested concentration of phytochemicals to the intended target site is hampered by their poor solubility in biological fluids (Faria et al., 2020).

Formulations with improved pharmacokinetics, pharmacodynamics, and therapeutic results can now be developed due to nanotechnology. Traditional herbs have been used in a variety of nano-based formulations that have been shown to have greatly enhanced qualities and a wide range of uses with precise delivery. To enable their controlled and targeted distribution, it is therefore essential to create innovative encapsulations for these herbal phytochemicals (Kulabhusan et al., 2020). Because of its biocompatibility and degradability with biological systems, chitosan, an organic molecule derived from chitin, has received attention as a promising drug delivery vehicle. Its special qualities, such as its low toxicity, controlled release capabilities, and capacity to stick to mucosal membranes, make it an excellent choice for encapsulating biologically active components. Additionally, chitosan nanoparticles have demonstrated potential in enhancing the durability and accessibility of encapsulated agents (Kulabhusan *et al*., 2020).

An essential food in the tropics is *Tamarindus indica*, a member of the Fabaceae subfamily *Caesalpinioideae*. Almost every aspect of this versatile tree has some benefit, whether it is medicinal or nutritious (Sookying et al., 2022). *Tamarindus indica* has been utilised to cure snakebites in Asian nations including India and Myanmar (Detroja et al., 2024). Nevertheless, research especially examining the encapsulation of this plant's seed extract in chitosan nanoparticles against the venom of *Naja nigricollis* is still lacking, despite the plant's potential.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The whole parts *Tamarindus indica* were collected from Bosso, Minna, Niger state, Nigeria (Latitude 13° 4′ 48.84″ N, Longitude 13° 18′ 37.08″ E) on the 17th of September, 2024. The plant material was identified at the Department of Plant Biology, Federal University of Technology, Minna, Nigeria with voucher number FUT.MIN/SLS/PB-020-024 and the specimen was deposited at the herbarium unit of the university.

Chemicals and Reagents

The chemicals and reagents used in this study were of analytical grade and products of Central Drug House (CDH)-India. The chemicals and reagents employed in this study include methanol, distilled water, phosphate buffer, chitosan, acetic acid, phosphate-buffered saline, sodium tripolyphosphate, potassium hexacyanoferrate (III), ferric chloride, Folin's reagent, aluminum chloride, thiobarbituric acid, sodium acetate, sodium deoxycholate, trichloroacetic acid (TCA), among others.

Preparation of Chitosan Nanoparticles

A Chitosan solution concentration of 1% w/v was prepared by dissolving 1 g of chitosan with a deacetylation degree of 90 in 100 mL of 1% acetic acid. The mixture was stirred on a magnetic stirrer at a temperature of 37 °C until the chitosan had completely dissolved (indicated by the appearance of a clear solution). Then, 5 mL of tripolyphosphate (TPP) solution (0.5% w/v) was slowly added to the chitosan solution at the rate of 0.2 mL/min with continuous stirring. Afterwards, the solution was further stirred for 1 hour at room temperature (37 °C) to ensure homogeneity. Finally, the chitosan nanoparticles were separated by centrifugation at speed of 20,000 x g and a temperature of 4 °C for 30 minutes (Farid *et al*., 2024).

Encapsulation of *T. Indica* Seed Extract in Chitosan Nanoparticles

To encapsulate the *T. indica* seed extract in chitosan nanoparticles, 3 g of the synthesized chitosan nanoparticles was dissolved in 30 mL of 1% acetic acid and stirred on a magnetic stirrer until complete dissolution. Thereafter, 15 mL of *T. indica* seed extract (10 mg/mL) was added to the solution of chitosan nanoparticles. The mixture was stirred for 1 hour to ensure the extract was maximally entrapped (Mady *et al*., 2024).

In Vitro Antioxidant Activity of the Seed Extract

Ferric Reducing Antioxidant Power (FRAP) Assay

Estimation of antioxidant activities of the plant extracts via ferric reducing antioxidant power assay was conducted according to the method described by Ibrahim *et al*. (2020). Stock solutions of the free extract and encapsulated extract and ascorbic acid (control) (1000 µg/mL) were prepared, from which different concentrations of 50, 100, 200, and 400 µg/mL were prepared. In this assay, 1 mL of each of the extracts and ascorbic acid concentration was mixed with 1 mL of 0.2 M sodium phosphate buffer and 1 mL of 1% potassium hexacyanoferrate (III). The reaction mixtures were incubated at 50 °C for 20 minutes. Thereafter, 1 mL of 10% TCA was added. The reaction mixtures were then centrifuged at 3000 rpm for 10 minutes at room temperature. Then 1 mL of each supernatant obtained was mixed with 1 mL of distilled water and then 0.2 mL of 0.1% ferric chloride was added. The blank was prepared in the same extracts as samples except that the extracts were replaced with distilled water. The absorbance of the test mixtures was read at 700 nm. The percentage of antioxidant activity was calculated using the formula below.

$$\% \text{ activity} = \frac{Asample - Ablank}{Asample}\ x\ 100$$

In vitro venom-contained enzymes inhibitory assays

To assess the inhibitory effects of the crude extract and fractions of *T. indica* seed on venom enzymes, 0.5 mL of 0.25% of the venom was added to test tubes containing 1 mL of different concentrations of the crude extract and fractions (100, 50, 25, 12.5, 6.25 µg/mL, respectively) and incubated for 30 minutes. The incubated venom was used for the enzyme assays.

Phospholipase A_2 Inhibitory Assay

Phospholipase A_2 assay was determined according to the acidimetric method as described by Salihu *et al*. (2024) with slight modifications. Briefly, a lecithin suspension was prepared with 1% lecithin, 18 mM calcium chloride, and 8.1 mM sodium deoxycholate in equal proportions. The pH of the suspension was adjusted to 8.0 with 1 M sodium hydroxide, and egg yolk were stirred for ten minutes to ensure a homogenous mixture. An amount of 0.1 mL of preincubated venom solution was added to 15 mL of egg yolk suspension to initiate the hydrolysis. The initial decrease in pH was measured. A decrease of 1 pH unit corresponded to 133 µmoles of fatty acid release. Enzyme activity was expressed as µmoles of fatty acid released/minute. For the inhibition studies, venom was preincubated with the free extract and encapsulated extract for 30 minutes at 37°C.

Protease Inhibitory Assay

Protease assay of crude venom was performed according to the method as described by Hansiya *et al*. (2021). The reaction mixtures were composed of 0.5% casein, 1.0 mL of Tris–HCl buffer (pH 8.0), 0.5 mL of 0.25% preincubated venom for each of the concentrations of free extract and encapsulated extract respectively and the reaction mixtures were incubated for four hours at 37°C. At the end of four hours, the reaction was stopped by adding 1 mL of 10% trichloroacetic acid (TCA) and filtered. The filtrate (1.0 mL) was used for protein estimation using L-tyrosine as a standard. In the above investigation, one unit of enzyme activity was defined as the amount that yielded 0.02 µmole of tyrosine/hour under experimental conditions described. The control was treated in the same way as the samples only that it contained venom incubated with neither the free extract nor the encapsulated extract.

Data Analysis

The values obtained from each *in vitro* antioxidant and antivenom assays were subjected to statistical analysis using One-way ANOVA and independent T-test respectively (Statistical Package for Social Sciences, version 22.0, SPSS Inc., Chicago, IL, USA). p value less than 0.05 was considered significant. The data were expressed as mean ± standard error mean of five replicates.

RESULTS AND DISCUSSION

Figure 1 shows the DPPH-scavenging potentials of the crude and encapsulated extracts of *T. indica* seed respectively. The two extracts exhibited significant antioxidant potentials in a concentration-dependent manner, with maximum activity being recorded at the highest test concentration of 100 µg/mL. However, the antioxidant activity of the encapsulated extract (87.72%) significantly is higher ($p < 0.05$) than that of the crude extract (80.09%). Furthermore, the activity of the

encapsulated extract was also observed to be significantly higher than that of ascorbic acid (84.00%). However, the antioxidant activity of free extract was significantly lower ($p < 0.05$) than that of the ascorbic acid. The IC_{50} values of ascorbic acid, crude extract, and encapsulated extract were calculated to be 26.32 µg/mL, 33.27 µg/mL, and 19.62 µg/mL, respectively.

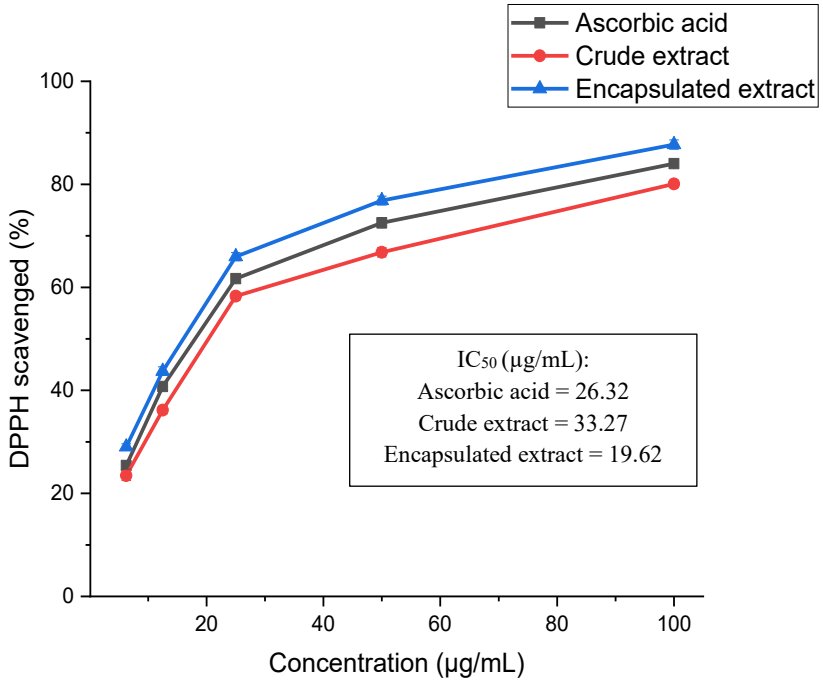

Figure 1: DPPH Radical-Scavenging Potentials of Crude and Encapsulated Extracts of *T. indica* Seed

The crude and encapsulated extracts of *T. indica* seed respectively showed appreciable ferric-reducing capacities (figure 2). Similar to the DPPH-scavenging potentials, the ferric-reducing capacities of the two extracts were observed to be concentration-dependent; the higher the concentration, the higher the antioxidant activity. The encapsulated extract reduced ferric ion (Fe^{+3}) to ferrous (Fe^{+2}) by 98.83% (with an IC_{50} value of 3.67 µg/mL) which was significantly higher than that of the crude extract (87%), with an IC_{50} value of 17.56 µg/mL, both at the highest test concentration (100 µg/mL). Additionally, the observed ferric-reducing capacity of encapsulated extract was significantly higher than that of ascorbic acid (91.15%), with an IC_{50} value of 13.00 µg/mL).

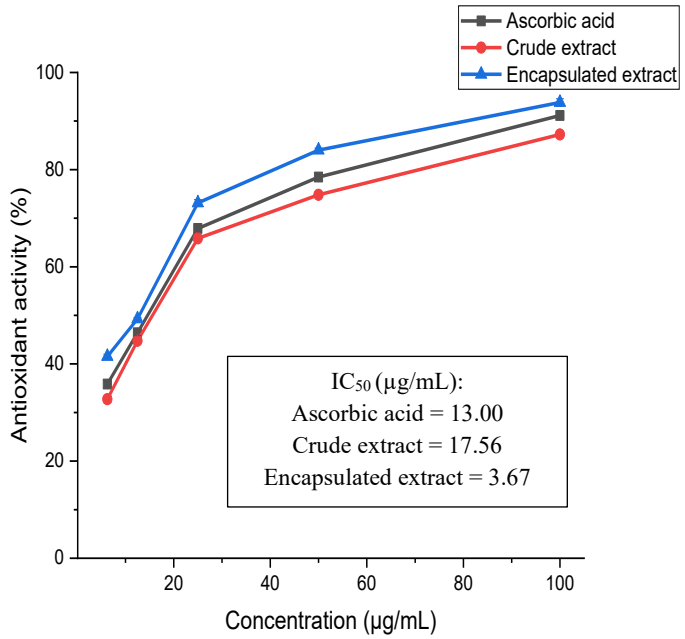

Figure 2: Ferric Reducing Antioxidant Powers of Crude and Encapsulated Extracts of *T. indica* Seed

Figure 3 depicts the inhibitory effects of crude and encapsulated extracts of *T. indica* seed on phospholipase A_2. The extracts were found to significantly inhibit the activity of *N. nigricollis*' venom phospholipase A2 in a concentration-dependent fashion. The maximum inhibitory potentials of the extracts were obtained at the highest test concentration of 100 µg/mL. However, the encapsulated extract exerted a higher inhibitory effect against the enzyme, with %inhibition of 86.32% (with an IC_{50} value of 34.58 µg/mL) than the crude extract (78.40%), with an IC_{50} value of 47.42 µg/mL.

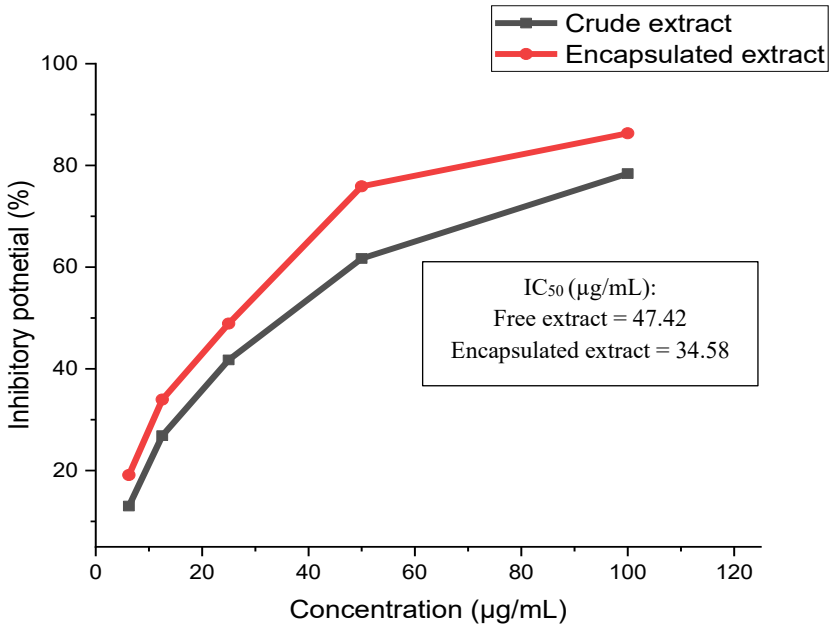

Figure 3: Inhibitory Potentials of Crude and Encapsulated Extracts of *T. indica* Seed Against Venom Phospholipase A2

The inhibitory effects of crude and encapsulated extracts of *T. indica* seed against the activity of protease contained in the venom of *N. nigricollis* is shown in figure 4. Therein, it was observed that both extracts showed appreciable inhibitory effects against the enzyme. Also, the inhibitory effects of the extract were found to be concentration-dependent, with highest inhibition being observed at 100 µg/mL (the highest test concentration). Though the crude and encapsulated extracts showed significant inhibitory effects, the inhibitory effect exerted by the encapsulated extract (78.16% [with an IC_{50} value of 45.53 µg/mL) was significantly higher than that of the crude extract (68.88%), with an IC_{50} value of 62.01 µg/mL.

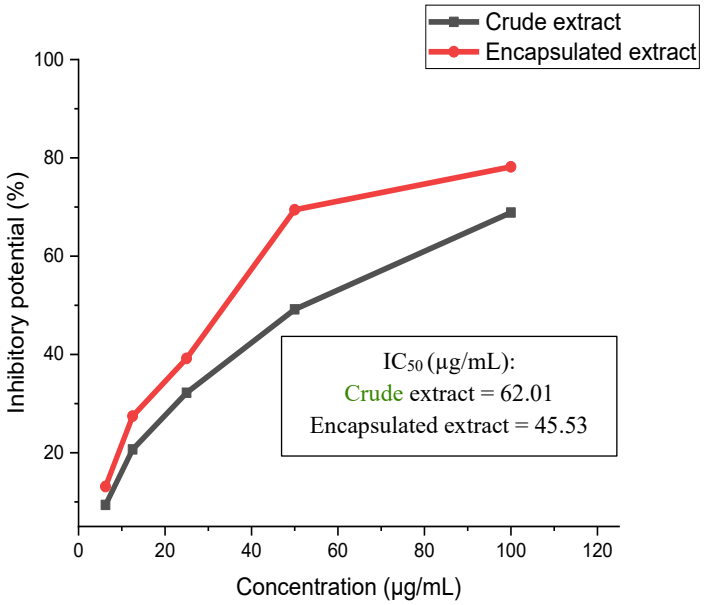

Figure 4: Inhibitory Potentials of Crude and Encapsulated Extracts of *T. indica* Seed Against Venom protease

Discussion

The crude and encapsulated extracts of *T. indica* seed showed significant DPPH-scavenging activities, with the encapsulated extract exerting more 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 *T. indica* seed, Farooq *et al*., (2022) reported that butanol extract of *T. indica* seed elicited appreciable DPPH-scavenging activity. Though a different solvent (aqueous) was employed in our study compared to this study, extracts of *T. indica* seed extracted with polar solvents have proven to have higher antioxidant activities due to the presence of 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 free and encapsulated extracts of *T. indica* seed denote their efficient hydrogen-donating potentials. Additionally, IC_{50} has been employed in showing how potent a compound as an antioxidant. Low IC_{50} values correspond to strong antioxidant activity while high IC_{50} values correspond to low weak antioxidant activity (Tariq *et al*., 2022). Thus, the lower IC_{50} value of the encapsulated extract than the free extract makes it a stronger antioxidant.

This study revealed that free extract and encapsulated extract of *T. indica* seed significantly reduced Fe^{+3} to Fe^{2+}, implying strong antioxidant activities. In addition, the extracts presented low IC_{50} values further revealing their strong reducing powers. Similarly, Farooq *et al*. (2022) reported the butanol extract of *T. indica* seed to possess strong ferric-reducing power. An essential method for evaluating the antioxidant activity of plant extracts is reducing power, which is a measure of electron-donating capacity. Reducing power and antioxidant capacity have been found to be positively correlated (Gulcin & Alwasel, 2025). As a result, the significant ferric-reducing powers exhibited by the extracts suggest them to be potent antioxidants. The low IC_{50} values of the extracts further affirm their potential antioxidant properties, with the encapsulated extract being a more potent antioxidant with a lower IC_{50} value than the crude extract.

In this study, both crude and encapsulated extracts effectively inhibit the activity of phospholipase A_2 contained in the venom of *N. nigricollis*, though the encapsulated extract exerted higher inhibitory effects, resulting in a lower IC_{50} value than the crude extract. The finding reported for the free extract agrees with that of Ushanandini *et al*. (2006) who also reported seed extract of *T. indica* seed to inhibit the activity of phospholipase A2 of *V. russelli* venom. Though different snake venom was employed in our study as compared to this study, phospholipase A2 from different snake venoms catalyze the same fundamental type of reaction (Sampat *et al*., 2023). Phospholipase A_2 (PLA_2) 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 protein PLA2s found in snake venom has a wide range of biological actions, such as antiplatelet, hemorrhagic, neurotoxic, myotoxic, and anticoagulant effects (Fernandes et al., 2024). Hence, the significant inhibitory effects exerted on phospholipase A2 by the free and encapsulated extracts imply that the extracts could alleviate the pathophysiological presentations of *N. nigricollis* envenomation resulting from phospholipase A_2.

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 significant inhibitory effects of the extract resulted in a low IC_{50} value. The seed extract of *T. indica* 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 impacted by proteinases, which catalyse the breaking 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 inhibitory effects of the extract could prevent or mitigate the resultant biological complications associated with proteinases following *N. nigricollis* envenomation.

The higher antioxidant and antivenom activities of the encapsulated extract than the crude extract are not surprising as encapsulation shields sensitive compounds phytochemicals from light, heat, and oxygen thus preventing oxidative and chemical degradation of these compounds resulting in enhanced biological activities (Liliana *et al*., 2023).

CONCLUSION

The crude extract and encapsulated extract showed considerable antioxidant and antivenom activities through venom enzymes inhibition. However, the encapsulated extract exhibited higher antioxidant and antivenom activities when compared to the crude extract. As such, the encapsulated extract could serve as a promising lead in the development of herbal antivenom therapy against *N. nigricollis* venom. Further studies should be focused on the *in vivo* antivenom evaluation of the encapsulated extract of *T. indica* to better understand its mechanism of action against *N. nigricollis* venom.

ACKNOWLEDGEMENT

The authors acknowledged Federal University of Technology, Minna Institution-based Research committee for funding this research, with grant identification code TETFUND/FUTMINNA/2024/032.

REFERENCES

Detroja, K., Sharma, K., Mishra, S. K., & Georrge, 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.

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.

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 the 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., Mateljak Lukačević, S., Halassy, B., & Kurtović, T. (2023). Snake antivenoms—toward better understanding of the administration route. *Toxins*, *15*(6), 398.

Gulcin, İ., & Alwasel, S. H. (2025). Fe3+ Reducing Power as the Most Common Assay for Understanding the Biological Functions of Antioxidants. *Processes*, *13*(5), 1296.

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 Naja naja 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., Garcia, A., & Reyes, E. (2025). Single-Chain Variable Fragments: Targeting Snake Venom Phospholipase A2 and Serine Protease. *Toxins*, *17*(2), 55.

Kotha, R. R., Tareq, F. S., Yildiz, E., & Luthria, D. L. (2022). Oxidative stress and antioxidants—A critical review on in vitro antioxidant assays. *Antioxidants*, *11*(12), 2388.

Kulabhusan, P. K., Agrawal, S., Jeevanandam, J., & Danquah, M. K. (2020). Nanoformulated herbal drug delivery as efficient antidotes 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. *Fudma Journal of Sciences*, *7*(2), 131-140.

Olorunnibe, M. (2021). Nigeria records 200,000 cases of snake bites, 2,000 deaths annually. *Vanguards news*, 20 September, p. 64.

Popescu, L., Cojocari, D., Ghendov-Mosanu, A., Lung, I., Soran, M. L., Opriş, 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.

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 Naja nigricollis phospholipase A2 enzyme. *Toxicon*, *243*, 107732.

Sampat, G. H., Hiremath, K., Dodakallanavar, 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.

Sookying, 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 Antioxidants 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.

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.