RASĀYAN *J. Chem.*
Vol. 19 | No. 2 | 851-857| April-June | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

BIOCHEMICAL PROPERTIES OF RUSSELL'S VIPER (*Daboia russelii*) VENOM AND ESTABLISHMENT OF AN *In-vitro* CELL LINE ASSAY TO DETERMINE TOXICITY

K. Sharma[1,2], M. Sharma[1], and G. Kumar[1,3,4,✉]

[1]School of Bioengineering and Biosciences, Lovely Professional University, Phagwara-144411, (Punjab), India

[2]Central Drugs Laboratory (CDL), Kasauli-173204, (Himachal Pradesh), India

[3]Amity Institute of Microbial Technology, Amity University Rajasthan, Jaipur-303002 (Rajasthan), India

[4]Amity Centre for Microbial Disease Diagnosis, Amity University Rajasthan, Jaipur-303002 (Rajasthan), India

✉Corresponding author: gauravkr01@gmail.com

ABSTRACT

Russell's viper (*Daboia russelii*) is one of the most medically important venomous snakes in India and is responsible for nearly 40% of mortality related to snakebites. The conventional approach for testing snake venom toxicity requires using animals, but animal research raises ethical concerns; therefore, alternatives have to be sought. The study was conducted to examine the biochemical profile of *Daboia russelii* venom and to develop an *in vitro* cellular-based toxicity assay using Vero cells. The venom yielded a protein fraction of 22.6 mg with a recovery of 90.4%. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) disclosed several protein bands, and Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) uncovered about 13 peaks. The increased clotting time (20 minutes) at a dose of 2 µg indicated that the venom had a strong anticoagulant effect. The cytotoxicity data showed a decrease in viability of Vero cells in a dose-related manner with an IC_{50} value of 42.39µg/ml. These results support the view that cell lines based on *in vitro* studies serve as a valuable, ethical option to determine venom toxicity as part of the production of anti-venom or laboratory research of venom pharmacology.

Keywords: Russel Viper (*Daboia russelii*); Vero Cell Lines; *In Vitro* Toxicity, Venom Toxicity, Cell-Based Assay

RASĀYAN *J. Chem.*, Vol. 19, No. 2, 2026

INTRODUCTION

There are about 3000 species of snakes worldwide, especially in the tropical and equatorial regions, with approximately 600 venomous species and more than 200 with medical relevance with regard to serious envenoming cases in humans.[1] Reportedly, India has about 250 snake species, 52 of which are venomous, causing nearly 15,000 deaths annually.[2] Krait (*Bungarus caeruleus*), Cobra *(Naja naja*), Saw-scaled (*Echis carinatus*), and Russell's viper (*Daboia russelli*) are four major venomous snakes that are mainly responsible for injuries and fatalities.[3] The only treatment available for snakebite envenomation is specific antivenom, which is obtained by immunising animals such as horses with snake venoms to generate hyperimmune serum lgG or its fragments that neutralise the toxic effect of the venom.[4] The potency of antivenom and venom's lethality (LD_{50}) is assessed in animal models, usually mice. This *in vivo* test is regarded as the gold standard to evaluate the venom's lethality and antivenom efficacy before clinical use and before the manufacturers and regulatory authorities conduct routine quality checks.[5] This has assessed antivenoms more reliant on expensive and laborious animal testing. Therefore, *in vitro* tests' development is essential to adhere to Russell and Burch's 3R concept and to conduct an ethical and reliable assessment of the toxicity of both venom and antivenom.[6] Early *in vitro* cytotoxicity studies evaluated impacts on cell viability to assess the toxicity of snake venom.[7] Vero cells were used in studies of the venoms of *Bothrops* and *Lachesis*[8], while different cancer cell lines were used to demonstrate the cytotoxicity of *Naja haje*[9], *Naja naja*10, *Naja oxiana*[11], and *Naja naja naja.*[7]Additionally, cell-based assays were also created for several *Bothrops* species[12,13], *Daboia russelii*[14], and *Macrovipera lebetina*

lebetina.[15] The current toxicity study is based on these findings. The MTT assay was used to create an *in vitro* approach that produced IC_{50} values and biochemical characterisation (Protein estimate, SDS-PAGE, RP-HPLC, hemolytic and anticoagulant assays). The presented method provides an ethical, reliable, and balanced approach for assessing venom's characteristics, helping to improve the evaluation of antivenom and ultimately may help in ensuring good health and well-being.

EXPERIMENTAL

Material and Method

The *Daboia russelii* (Russell's viper) venom used in the study was standardised to a unitage of 25 mg (dry weight) and was obtained in a lyophilised (freeze-dried) form from the Central Drugs Laboratory (CDL), Kasauli. MTT dye was purchased from SRL Chemicals (India) and DMEM and FBS from Gibco (USA). Vero cells (ATCC® CCL-81™) were sourced from CDL, Kasauli. Chemicals for RP-HPLC and SDS-PAGE were from Sigma-Aldrich (USA). Fresh RBCs and whole blood for hemolytic and anticoagulant assays were collected from a healthy male donor.

General Procedure

Daboia russelii's venom was characterised biochemically after determining the protein content and protein profiling using SDS-PAGE and RP-HPLC. The biological effects of the venom were determined using functional assays such as the haemolytic assay and anticoagulant activity. MTT assay was used to examine the IC_{50} value in Vero cells.

Protein Estimation

Standards were prepared using Bovine Serum Albumin from 10 - 2000 µg/ml. The lyophilised venom (25 mg) was suspended in 0.9% saline, centrifuged, and diluted to 1000 µg/ml. In the assay, 100 µL of each venom concentration was combined with 100 µL NaOH, heated and cooled to room temperature (25 °C), whereupon the solution reacted with the complexometric reagent.[16] Absorbance was read at 750 nm post addition of 100 µL of Folin's reagen.[16,17]

Purity Assessment of *D. russelii* Venom using SDS-PAGE

To separate the protein components from the *Daboia russelii's* sample, SDS-PAGE (reducing) was carried out. Polyacrylamide gel at the concentration of 12.5% was used to prepare Tris-Glycine-SDS (TGS) buffer solution, onto which venom samples (12 µL each; L2 and L3) and a protein standard (3 µL; L1) were loaded. A constant voltage of 80 V resolved the proteins in the gel based on their molecular weight. The PageRuler™ prestained Protein Ladder was the marker used for the determination of molecular weight (10–180 kDa) and was procured from Thermo Fisher Scientific. Post-electrophoretic run, Coomassie Brilliant Blue G-250 (Merck) was used to stain the gel. To improve the band visibility, the gel was subjected to destaining. The iBright CL1000 Gel Documentation System (Thermo Fisher Scientific, USA) was used to capture the image of the gel.[18,19]

Purity Assessment of *D. russelii* Venom using RP-HPLC

Analysis of HPLC was conducted using a Dionex Ultimate 1100 system (USA) fitted with a reversed-phase C16 column (5µm, 4.6×250 mm) and a guard column. The venom sample load was 200 µg for analysis. Elution was detected at 210 nm. Mobile phase of 95% water, 0.1% trifluoroacetic acid (TFA), and 5% acetonitrile (Solvent A), and a flow rate of 0.6 mL/min was used to balance the column. A non-linear gradient, with a second mobile phase made up of 95% acetonitrile, 5% water and 0.1% TFA (solvent B). The flow rate of 0.6 ml/min resulted in elution under the following conditions: B = 10% at t = 0 min, B = 10% at t = 20 min, B = 25% at t = 30 min, B = 45% at t = 60 min, B = 45% at t = 85 min, B = 65% at t = 110 min, B = 10% at t = 130 min. The column temperature was kept at 40°C, and the samples were kept at 6°C. The mobile phases were sonicated for 10 minutes before their use. Chromeleon 7 software was employed for the collection and analysis of data.[20]

Haemolytic Assay

Lysis of the RBC is referred to as haemolysis, and the potential of the snake venom was assessed using a haemolytic assay. The undiluted suspension of RBCs from whole blood was achieved by washing the whole human blood with Phosphate-Buffered Saline (PBS) (pH 7.4) and centrifuging it at 3000 × g for 10

minutes at 4^0C. The process was carried out five times to completely remove unwanted blood components and remaining cellular material. The prepared 1% RBC suspension was combined in a 10:1 ratio with varying venom concentrations (5, 10, 20, and 40μg), and this mixture was further incubated for 24 hours at 37°C. After the sample was incubated, it was centrifuged at 3000 × g for 10 minutes, and at 540 nm, a microplate spectrophotometer was used to determine the absorbance of the supernatant.[21]

Anticoagulation Activity

Russell's viper venom dilutions ranging from 2ug-10ug were prepared in Buffered Saline with Phosphate (PBS) and were added to the 2ml of the fresh human blood in a sterile and dry glass tube separately. To ensure even distribution of the venom and blood, each of the dilutions was mixed thoroughly. The precise addition of venom allowed for the evaluation of its dose-dependent effects on the blood, particularly its influence on the coagulation process. The test tube containing the mixture of blood and venom was maintained in a stationary and undisturbed position at ambient room temperature (typically around 25^0C) for a duration of 20 minutes.[21,22,23]

Detection Methods (MTT Assay)

The Vero cells (African green monkey kidney epithelial cells, ATCC CCL-81) used for the toxicity assessment of Russell's viper venom were cultivated in DMEM in 25 cm² flasks in a CO_2 incubator (5% CO_2 atmosphere) enriched along with 10% Fetal Bovine Serum (FBS) and 2% antibiotic solution (combining 100 IU/mL penicillin and 100 mg/mL streptomycin). To assess the toxicity induced by venom, the Vero cells were subjected to the MTT assay, which was originally a colourimetric method developed by Mosmann in 1983. In order to measure the viability of the cells, the cells were cultured under the same above-mentioned culture conditions. The cells were seeded into 96-well culture plates at a density of 5×10^4 cells per well to enable the development of a confluent monolayer. After the monolayer formed, the culture medium was removed and replaced with fresh medium with varying quantities of venom (125.44 ug/mL to 1.96μg/mL). To ensure reproducibility, the experiment was carried out three times. Following a 4-hour incubation period, 20 μL of sterile 0.5% MTT solution was filtered through a 0.22 μm filter to replace the medium. MTT solution was used to incubate the cells for four hours at 37^0C, after which the activity of mitochondrial succinate dehydrogenase during this period had reduced the MTT to crystals of formazan. Phosphate-buffered saline (PBS) was used to wash the plate after the MTT solution was withdrawn. To dissolve the blue formazan crystals in each well, 150 μL of acidified isopropanol (made up of 100 μL of strong hydrochloric acid diluted with 100 mL of isopropanol) was applied. The number of live cells in each well is directly correlated with the absorbance of the solubilised formazan, which was measured at 570 nm.[24,25]

Morphological Changes

After the four-hour exposure to the venom, the cellular morphological changes and evidence of damage were assessed qualitatively through an inverted microscope. Photographs were taken to record the changes observed.

Statistical Analysis

Experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). IC_{50} value was calculated using a dose-response curve (sigmoidal model).

RESULTS AND DISCUSSION

Protein Content of *D. russelii* Venom

The venom's protein content was estimated to be 22.6 mg per 25 mg of venom, estimated using the Lowry method; the BSA standard curve ($y = 0.0018x + 0.0611$; $R^2 = 0.9995$) revealed the linear relationship between the dilutions and the protein concentrations of the samples. Therefore, this finding authenticates protein dominance of the venom, which can be attributed to the presence of a mixture of enzymes, toxins and other protein bioactive agents.[26]

Electrophoretic and Chromatographic Assessment of *D. russelii* Venom Purity

SDS-PAGE results indicated six clear protein bands, reflecting the biochemical complexity of the *Daboia russelii* venom (Fig.-1A). The low and high molecular weight proteins observed in venom are

approximately 70 kDa to 23 kDa and indicate a structural and functional multiplicity of the venom. This range suggests a combination of non-enzymatic and enzymatic agents that can elicit a wide range of toxicity. The lower molecular weight bands (23–40kDa) are likely to be attributable to enzymatic toxins like phospholipases A_2, serine proteases, and specific metalloproteinases that may disrupt coagulation, promote bleeding, and cause cellular destruction, which are consistent with the medically relevant consequences of envenomation. Conversely, the protein bands between 55 and 70 kDa could represent more complex enzymes or high-molecular-weight toxins like L-amino acid oxidases, Phosphodiesterase, venom serine proteases and venom metalloproteinases. The protein diversity detected in this study illustrates the complexity of the venom and highlights its potential to produce miscellaneous pathologies, mostly mediated by the enzymatic components of the venom, including phospholipases A_2, metalloproteinases, serine proteases, and L-amino acid oxidases, which can invoke toxicity, haemorrhage, coagulopathy, and breakdown of tissues. The results of this study are an important basis for further studies to investigate larger numbers of animals with a more direct form of protein analysis and individual variations.[27,28]

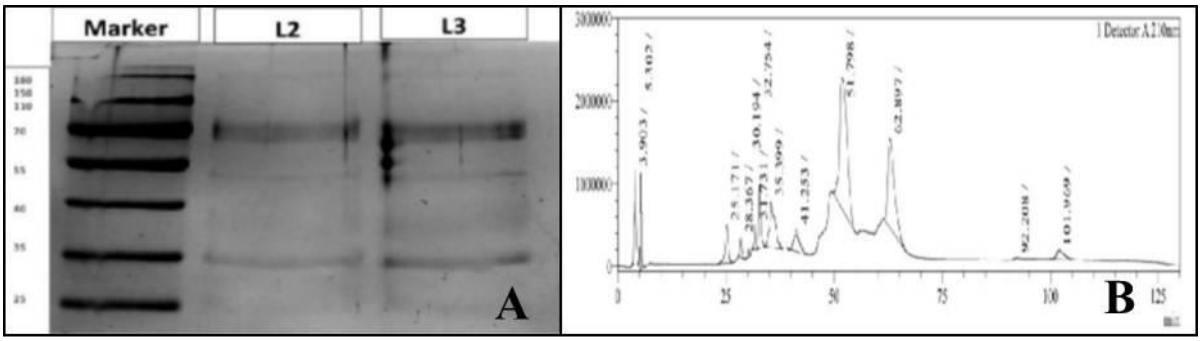

Fig.-1: Electrophoretic and Chromatographic Assessment of *D. russelii* Venom Purity

Here A: SDS-PAGE showing the protein pattern of *D. russelli venom*; B: Represents the reverse-phase HPLC separation of *D. russelii* venom molecules*;* L1: Marker; L2, and L3: Venom samples. The RP-HPLC chromatogram of *D. russelii* venom displayed thirteen distinct and well-defined peaks, highlighting the intricate composition of the venom (Fig.-1B). The distinct separation characteristics show a clear indication for a diverse range of proteins and peptides, along with the well-known complexity of the venom's toxicological characteristics. The high resolvability (Minimum = 3.90) and reasonable asymmetry (Range = 3.90-5.30) indicate the utility of the method for fractioning the venom into individual components, which can be used for future structural and functional studies. These chromatographic peaks likely correspond to specific venom molecules, encompassing both enzymatic elements such as phospholipases A_2 , metalloproteinases, and serine proteases[29,30] and non- enzymatic components of the venom include snake C-type lectins (Snaclecs), Kunitz-type serine protease inhibitors (KSPIs), a small peptide with pro-angiogenic properties, a bifunctional inhibitor targeting both thrombin and factor Xa, as well as a peptide exhibiting cytotoxin-like activity.[31]

Haemolytic Activity

The venom displayed low to no direct haemolytic activity, even with the highest concentration of venom (50 µg). This implies that the hemotoxicity of the venom may be through indirect action, such as enzymatic destruction of blood vessel structure (integrity) and/or coagulation pathways (rather than through direct lysis of erythrocytes). This suggests that other elements of the venom, such as endothelial damage and/or platelet impairment, may be more significant contributors to the venom's overall toxicity.[32,33]

Anticoagulation Activity

The venom demonstrated strong anticoagulant activity, as evidenced by its inhibition of clot formation at lower concentrations (2ug, 4ug, 6ug, 8ug and 10µg). The literature indicates that enzymatic activity (PLA_2) is necessary for snake venom anticoagulant activity, and this anticoagulant reactivity varies based

upon mechanisms dependent upon, or independent of, their enzyme activity.[32] However, some investigations show that the venom may have anticoagulant activity and do so *via* a variety of nonenzymatic mechanisms.[33] Their precise mechanism remains complicated, as there are conflicting findings from previous investigations.

In vitro Toxicity of Russell's Viper Venom on the Vero Cell Line

D. russelii venom cytotoxicity was systematically tested in a Vero cell line to establish a reliable *in vitro* assay model. The study demonstrated that cell death was directly proportional to the increasing venom concentration. The highest venom concentration evaluated was 125µg/ml; it was shown to be cytotoxic and decrease the viability of cells to 81.93±2.52% (Fig.-2X and 2Y). The IC_{50} value was determined from a linear regression equation (y=0.5048x+28.598; R^2= 0.7597), which was 42.39µg/ml, demonstrating moderate cytotoxicity of venom in a normal epithelial kidney cell, signifying that the venom is not a potent cytotoxic agent in this non-cancerous model.[34] This *in vitro* model provides an ethical option that is reproducible and can take the place of animal testing for toxicity assays. The current study utilised normal cell lines (Kidney epithelial cells), but previous work utilising *D. russelii* venom in cancerous cell lines showed varying sensitivities, suggesting a possible role of cell type in venom cytotoxicity.[35] This presumed cytotoxicity may be linked to key enzymatic components of viperid venoms; specifically, phospholipases A_2, metalloproteinases, proteases, and L-amino acid oxidases (LAAOs). PLA_2 enzymes hydrolyse phospholipids, resulting in cellular membrane disturbances.[36]

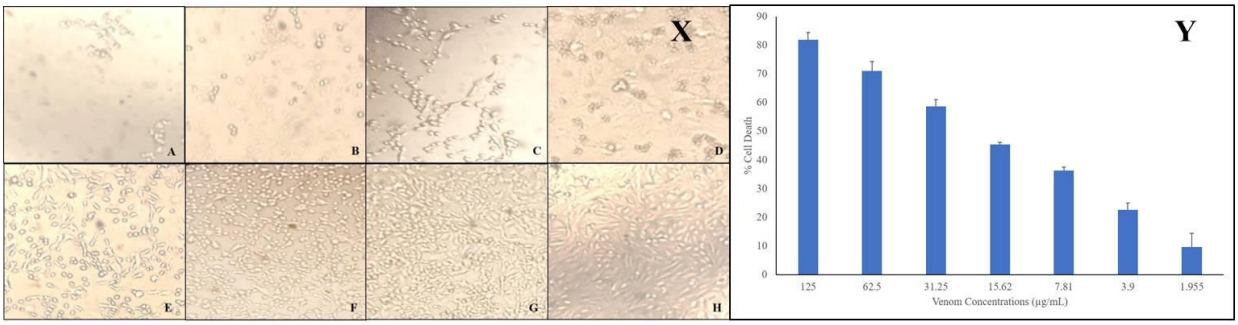

Fig.-2: In Vitro Toxicity of Russell's Viper Venom on Vero Cell Lines

Here X: Morphological characterisation of Vero cells after 4 hours of exposure to different concentrations of venom at 125µg/ml (A), 62.5µg/ml (B), 31.25µg/ml (C), 15.62µg/ml (D), 7.81 µg/ml (E), 3.9µg/ml (F), 1.95µg/ml (G) Control cells (H) are depicted; Y: In vitro cytotoxic activity of Russel viper venom on Vero cell lines in a dose dependent manner.

CONCLUSION

The study provides key biochemical and cytotoxic traits of *D. russelii* venom. The *in vitro* IC_{50} value of 42.39µg/mL in Vero cells, which suggested moderate cytotoxicity caused by enzymatic and oxidative actions. The Vero cell-based assay offers a justifiable and morally acceptable alternative to animal testing, corresponding with the 3Rs (Replacement, Reduction, Refinement) principle. The results of the MTT assay provided ample evidence that *in vitro* methods could potentially replace animal-based methods without losing any reliability. To understand the complex modalities of action of the venom, a myriad of biochemical analyses was performed, such as protein quantification, haemolytic and anticoagulant activity, and protein profiling (SDS-PAGE and RP-HPLC). In summary, this study provides support for the value of *in vitro* systems for ethical and sustainable toxicology, as well as a more progressive viewpoint against the use of antivenom in treatment and non-animal models in venom studies/research to ensure good health and well-being.

ACKNOWLEDGMENTS

The authors would like to acknowledge the management of Lovely Professional University, Central Drugs Laboratory (CDL), and Amity University Rajasthan for providing support to carry out this work.

CONFLICT OF INTERESTS

This study received no funding. This study doesn't have any conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

K. Sharma https://orcid.org/0009-0004-2436-6231
M. Sharma https://orcid.org/0009-0002-4446-9508
G. Kumar https://orcid.org/0000-0002-9464-7811

Cite this article as: K. Sharma *et al.*, *Rasayan J. Chem.*, 19(2), 851-857(2026), https://doi.org/10.31788/RJC.2026.1929623.

REFERENCES

1. J.M. Gutiérrez, D. Williams, H.W. Fan and D.A. Warrell, *Toxicon*, **56**, 1223(2010), https://doi.org/10.1016/j.toxicon.2009.11.020.
2. H.S. Bawaskar, *Journal of the Association of Physicians of India*, **52**, 11(2004).
3. R.K. Kamal, N. Sahu, J. Rahul and S.P. Singh, *International Journal of Pharmaceutical Sciences Review and Research*, **30**, 227(2015).
4. S. Kumar, S. Kanwar, V. Bansal and R. Sehgal, *Biologicals*, **37**, 297(2009), https://doi.org/10.1016/j.biologicals.2009.05.002.
5. J.M. Gutiérrez, M. Vargas, Á. Segura, M. Herrera, M. Villalta, G. Solano, A. Sánchez, C. Herrera and G. León, *Frontiers in Immunology*, **11**, 617429(2021), https://doi.org/10.3389/fimmu.2020.617429.
6. The Principles of Humane Experimental Technique, Methuen & Co.,pp.45-88 (1959).
7. C. Venkatesan, M. Sarathi, G. Balasubramanaiyan, S. Vimal, N. Madan, N.S. Raj, S.M. Bilal, A.N. Basha, M.A. Farook, A.S. Hameed and G. Sridevi, *Biologicals*, **42**, 8(2014), https://doi.org/10.1016/j.biologicals.2013.09.002.
8. J.C.R. Oliveira, H.M. de Oca, M.M. Duarte, C.R. Diniz e C.L. Fortes-Dias, *Toxicon*, **40**, 321(2002), https://doi.org/10.1016/S0041-0101(01)00229-X.
9. A.V. Feofanov, G.V. Sharonov, M.V. Astapova, D.I. Rodionov, Y.N. Utkin and A.S. Arseniev, *Biochemical Journal*, **390**, 11 (2005), https://doi.org/10.1042/BJ20041892.
10. R. Thangam, P. Gunasekaran, K. Kaveri, G. Sridevi, S. Sundarraj, M. Paulpandi and S. Kannan, *Process Biochemistry*, **47**, 1243 (2012), https://doi.org/10.1016/j.procbio.2012.04.020.
11. K. Ebrahim, H. Vatanpour, A. Zare, F.H. Shirazi and M. Nakhjavani, *Iranian Journal of Pharmaceutical Research*, **15**, 101 (2016).
12. L. Lopes-de-Souza, F. Costal-Oliveira, S. Stransky, C.F. de Freitas, C. Guerra-Duarte, V.M. Braga and C. Chávez-Olórtegui, *Toxicon*, **170**, 68(2019), https://doi.org/10.1016/j.toxicon.2019.09.010.
13. R.N. Nundes, A.E. Almeida, W.C. Moura, M.S. Gonzalez and H.P. Araújo, *Alternatives to Laboratory Animals*, **52**, 82 (2024), https://doi.org/10.1177/02611929241237
14. J. Pathan, S. Mondal, A. Sarkar and D. Chakrabarty, *Toxicon*, **127**, 11(2017), https://doi.org/10.1016/j.toxicon.2016.12.013
15. A. Nalbantsoy, N.Ü. Karabay-Yavaşoğlu, F. Sayim, I. Deliloğlu-Gürhan, B. Göçmen, H. Arıkan and M.Z. Yıldız, *Journal of Venomous Animals and Toxins Including Tropical Diseases*, **18**, 208(2012), https://doi.org/10.1590/S1678-91992012000200011.
16. *Indian Pharmacopoeia*, Indian Pharmacopoeia Commission, Ghaziabad, Vol. 1, (2022).
17. O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *Journal of Biological Chemistry*, **193**, 265(1951).
18. R.R. Senji Laxme, S. Khochare, S. Attarde, V. Suranse, A. Iyer, N.R. Casewell, R. Whitaker, G. Martin and K. Sunagar, *PLoS Neglected Tropical Diseases*, **15**, e0009247(2021), https://doi.org/10.1371/journal.pntd.0009247

19. E.P. Rybicki, *Manual of SDS-PAGE and Immunoblotting Techniques*, University of Cape Town, Cape Town, (2015).
20. A.K. Mukherjee, *Biochimie*, **99**, 153(2014), https://doi.org/10.1016/j.biochi.2013.11.026.
21. F.L. Tianyi, M. Hamza, S.B. Abubakar, J. Al Solaiss, A. Trelfa, H.L. Abdullahi, G. Iliyasu, N. Mohammed, S.A. Mohammed, N.R. Casewell, R.A. Harrison, D.G. Lalloo, Y. Stienstra and A.G. Habib, *PLoS Neglected. Tropical. Diseases*, **17**, e0011442(2023), https://doi.org/10.1371/journal.pntd.0011442.
22. T. Lamb, M. Abouyannis, S.S. de Oliveira, K.R. Shenoy, T. Geevar, A. Zachariah, S.K. Sharma, N. Bhatt, M. Mukaka, E. Harriss, D.G. Lalloo, E.A. Ashley, W.M. Monteiro, F. Smithuis and M. Eddleston, *PLoS Neglected. Tropical. Diseases*, **15**, e0009657(2021), https://doi.org/10.1371/journal.pntd.0009657.
23. I. Ratnayake, F. Shihana, D.M. Dissanayake, N.A. Buckley, K. Maduwage and G.K. Isbister, *Thrombosis. Haemostasis*, **117**, 500 (2017), https://doi.org/10.1160/TH16-10-0769 .
24. T. Mosmann, *Journal of Immunological Methods*, **65**, 55(1983),https://doi.org/10.1016/0022-1759(83)90303-4.
25. S. Kamiloglu, G. Sari, T. Ozdal and E. Capanoglu, *Food Frontiers*, **1**, 332(2020), https://doi.org/10.1002/fft2.44.
26. A.B. Ghanghro, N.U. Qureshi, I.H. Ghanghro, T.M. Jahangir, M.B. Bhurgri, Z.A. Khoso, S. Ahmed *International Journal of Biology*, Pharmacy and Allied Sciences, **4**, 6808 (2015).
27. B. Kalita, A. Patra and A.K. Mukherjee, *Journal of Proteome Research*, **16**, 583(2017), https://doi.org/10.1021/acs.jproteome.6b00693
28. T. Faisal, K.Y. Tan, N.H. Tan, S.M. Sim, C.A. Gnanathasan and C.H. Tan, *Journal of Venomous Animals and Toxins Including Tropical Diseases*, **27**, e20200177(2021), https://doi.org/10.1590/1678-9199-JVATITD-2020-0177
29. A.K. Mukherjee, *Toxicon*, **51**, 923(2008), https://doi.org/10.1016/j.toxicon.2007.12.008
30. Ş.A. Gâz Florea, D. Ciurca, A. Mare, A. Man, B. Cordoş, A.-L. Grama and D.-L. Muntean, *Acta Medica Marisiensis*, **62**, 90 (2016), https://doi.org/10.1515/amma-2015-0113
31. A. K. Mukherjee and S. P. Mackessy, *Toxicon*, **89**, 55(2014), https://doi.org/10.1016/j.toxicon.2014.06.016
32. E. Condrea, A. De Vries and J. Mager, *Biochimica et Biophysica Acta-Specialised Section on Lipids and Related Subjects,* **84**, 60(1964), https://doi.org/10.1016/0926-6542(64)90101-5
33. D. Saikia, N.K. Bordoloi, P. Chattopadhyay, S. Choklingam, S.S. Ghosh and A.K. Mukherjee, *Biochimica et Biophysica Acta-Biomembranes*, **1818**, 3149(2012), https://doi.org/10.1016/j.bbamem.2012.08.005
34. S. Ahluwalia, M. Sawant, N. Shah, A. Choudhary, *International Journal of Pharmacy and Pharmaceutical Research (Human)*, **3 (3)**, 254 (2015)
35. J. J. Hiu and M. K. Yap, *Biochemical Society Transactions*, **48**, 719(2020), https://doi.org/10.1042/BST20200110
36. G.B. Naumann, L.F. Silva, L. Silva, G. Faria, M. Richardson, K. Evangelista, M. Kohlhoff, C.M.F. Gontijo, A. Navdaev, F.F. de Rezende, J.A. Eble and E.F. Sánchez, *Biochimica et Biophysica Acta-General Subjects*, **1810**, 683 (2011), https://doi.org/10.1016/j.bbagen.2011.04.003

[RJC-9623/2026]