Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 13 [7] June 2024: 135-140

Online ISSN 2277-1808
Journal's URL: http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Biomedical Profiling of Fish Venom *Pterois volitans* (Linnaeus, 1758)

Debarati Paul, Bandana Kumari, Kanchana Sankar, Sangeswari Thirukumar, Srichandan Rath, Arumugam Muthuvel *
Centre of Advance Studies in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai – 608502, Tamilnadu
***Corresponding Author:** Arumugam Muthuvel,
Email: arumnplab@gmail.com

ABSTRACT
Pterois volitans, commonly known as lionfish, are fish species native to Indo-Pacific waters that are spreading to other areas, including the Caribbean and Atlantis. Lionfish possess venomous dorsal spines to protect themselves from enemies in underwater environments. Our current study aims to extract the venom from dorsal spines and to estimate the biological activity. This venom protein was quantified as 347 µg/mL. The haemolytic activity showed the highest (59%) venom concentration in the chicken blood group, whereas the moderate haemolytic activity in the Goat (54%) and Human Blood (52%) groups compared to the control condition. This venom protein has shown good efficacy in human plasma coagulation assay compared to the control condition. Antioxidant activity has the highest Inhibition percentage (78.13%) at a lower concentration (100µL). This present study indicates that P. volitans venom is a potent source of bioactivity to use in the biomedical industry.
Keywords: *Lionfish, Venom, Anti-oxidant, Lytic-assays.*

Received 09.04.2024 Revised 21.05.2024 Accepted 27.06.2024

INTRODUCTION

Numerous taxa, including the kingdom Animalia, contain venomous organisms. Throughout their evolutionary history, animals have evolved unique organs that enable them to produce and inject venom [1,2]. Animals secrete venom, which is made up of chemicals that, when injected, cause disruptions to normal physiological processes. This allows the organism to kill prey and protect itself from predator attacks [3,4,5]. More than 200 different species of fish are known to be venomous, meaning they can cause severe injuries that could be fatal. Less than 5% of this group have even the most basic research done on them, and the fundamental chemical makeup of even a single fish venom has not yet been determined. The fundamental and severe instability of fish venoms is the primary cause of this seeming ignorance. The vast biomedical potential of venoms from a wide range of alien species has been extensively studied and highlighted [6,7]. However, compared to terrestrial organisms—of which snakes, scorpions, and spiders are the most well-known and extensively researched—the scientific gap regarding the biomedical potential of marine organisms is relatively large [8,9,10,11,12,13,14,15,16]. As members of the same order Scorpaeniformes as stonefish, lionfish are notoriously venomous due to the presence of venomous glandular tissues in their dorsal, pelvic, and anal spines. Three species of lionfish—*Pterois antennata*, *Pterois lunulata*, and *Pterois volitans*—have been known to produce proteinaceous toxins, some of which have been described along with their primary structures [17,18]. According to Hornbeak and Auerbach, lionfish venom contains neurotoxin and acetylcholine, which can have systemic effects like severe pain, fever, vomiting, dizziness, and even cardiac arrhythmias and pulmonary oedema [19]. But there haven't been any reports of fatalities from lionfish injuries in the books [20,21]. Lionfish meat is safe for ingestion by humans once the poisonous spines have been carefully removed [21]. Since the 1950s, numerous studies have examined the bioactivity of homogenates made from the venomous spines of lionfish [6].

MATERIAL AND METHODS

Sample collection, Venom Preparation and Protein Quantification

25 Specimens of *P. volitans* were collected from Mudusalodai Landing Centre (11°29'43"N 79°46'01" E), Parangipettai coast, Tamilnadu India. Fishes were immediately transferred to the marine natural products

laboratory, washed with distilled water and preserved at -40°C. The venom was extracted from the dorsal spines [22]. Dorsal spines were dissected carefully and the unwanted tissues were scrapped. These dorsal spines were crushed and ground using a sterile mortar pester by using PBS and centrifuged at 7000 rpm for 10 min at the cooling condition. The supernatant was lyophilized and stored at -40°C. Protein quantification was determined by the Lowry method using Bovine Serum Albumin (BSA) as a standard (1mg/mL) [23].

Haemolytic activity

The microtiter plate method was used to assess the crude venom's haemolytic assay. In EDTA-coated tubes, three blood samples—two from each of the slaughterhouse's chicken and goat—as well as human blood (myself) samples were taken. The blood was centrifuged for five minutes at 6,000 rpm after it was collected. After washing the pellet in regular saline (pH 7.4), the supernatant was thrown away. After three saline washes, the obtained erythrocytes were kept at -20°C for storage. In 96-well plates, the haemolytic assay was carried out.100 µl of 1% RBC was combined with 50, 75, and 100 µL of crude venom. Used as a positive control was 1% RBC. Saline was used as a negative control. The samples in the 96-well plate were kept at room temperature for three hours and the haemolytic activity was recorded as a percentage of inhibition [24].

Plasma Coagulation Assay

The effect of lionfish venom on coagulation was assessed through the recalcification time measured using the SpectraMax Plus 384 microplate reader. This methodology allows to monitoring of clot formation and kinetic parameters for coagulation through a minimalized plate per user. A 50 µl of citrated human plasma was incubated with various concentrations of stingray venom (10,20,30,40 and 50µL) in 90 µl of 20 mMHEPES, pH 7.4. After incubating for 5 min at 37°C, 10 µl of 150 mMCaCl2 was added and clot formation was observed at 37°C for 20 min and measured in the SpectraMax system at 650 nm. To get to calcium-free coagulation action, EDTA was added rather than $CaCl_2$ to a last grouping of 10 mm. The experiments were carried out thrice [25].

Antioxidant Assay 2,2-diphenyl-1-picrylhydrazyl (DPPH)

DPPH solution was made at 125 µM by weighing 2.5 mg DPPH in 50 mL of ethanol in a brown bottle and cover up with aluminium foil. Different sample concentrations were 50,75 and 100 µL of samples and100µL DPPH solution into a microplate. Blank made for 200 µL ethanol. Cover up the microplate with aluminium foil and incubate for 30 min. Measure the absorbance of blank solution and sample using Microplate Elisa Reader at wavelength 517 nm. Plot absorbance into the equation to obtain inhibition value [26].

$$\% \text{ of inhibition} = \frac{\text{Absorbance of control- Absorbance of samples}}{\text{Absorbance of Control}} * 100$$

RESULT AND DISCUSSION

Collected Sample and Protein Quantification from extracted Venom

Pterois volitans ((Linnaeus, 1758) samples were collected from the Mudusalodai Landing Centre in 2022. These fish species were identified morphologically [27, 28, 29]. The figure (1) represents the collected *Pterois volitans* sample from Mudasalodai Landing Centre, Tamil Nadu (2022). Using Bovine Serum Albumin as a standard, the Lowry method was used to estimate the amount of crude protein. It was discovered that the venom protein concentration was 347 µg/mL. A previous study has revealed the protein concentration in crude venom extracted from *Pterois volitans* was reported as 330.81µg/mL 309.83 µg/mL, 64.40 µg/mL 262.38 µg/mL 351.24µg/mL by using different types of solvents [30]. So, our current study also quantified a high concentration of protein.

Haemolytic assay

Extracted venom from *Pterois volitans* venom assay was performed on three different blood (chicken, Goat and human) and followed by serial dilutions. The venom shows activity in chicken blood in a very low concentration- 10 µL (15%) and human erythrocytes (52%) and in a higher concentration (50µL) and moderate lysis effect on goat blood (54%). Figure 2 represents the Haemolytic assay in several animal's blood. This haemolytic effect of *Pterois volitans* venom has relative significance results to the earlier findings. Fig 45µg/mL of crude venom concentration of lionfish can have a maximum haemolytic activity on Rabbit erythrocytes [31]. Moreover, human erythrocyte lysis was moderately induced by the lion's venom extract. This outcome aligns with the findings published by Shiomi and the team who showed that the haemolytic activity in *Pterois volitans* venom was extremely selective against rabbit erythrocytes [32]. A previous study stated that after venom injection into the mice, analysis of blood caused notable changes to the haematological parameters. Reduced blood cell count, which was caused by increased haemolysis, can be linked to reductions in Hb, HCT, MCV, MCH, and PLT [33].

Plasma Coagulation Assay

The plasma coagulation assay was used to evaluate the blood coagulation activity of venom samples. Before the induction of coagulation by calcium, venom samples were incubated with citrated chicken plasma to examine the impact of the samples on clotting. Figure 3 clearly illustrates the clotting time of human plasma from the *Pterois volitans* venom sample. Concurrently, it takes over 17 seconds for the control plasma and venom samples to start coagulating. To initiate coagulation, plasma was incubated with venom samples (10 µL,20 µL,30µL,40µL,50µL) obtained approximately 2 seconds after the addition of calcium. Recalcification Time (RT) increased by nearly three times in the venom protein sample when compared to the control. Both in the presence of EDTA and the absence of calcium chloride, normal plasma was unable to clot. Coagulation activity has been carried out in earlier times where it has shown that the extracted venom from *Pterois volitans* has a procoagulant activity (respective 6 secs and 8.5 secs respectively). Previously Nomega-Nitro-L-arginine was reported from the venom of *P. volitans* which has been proved a coagulant factor in human plasma [34]. The coagulant activity of *Pterois russeli* venom was reported in another finding where it has shown good coagulant activity in a minimum quantity of venom (7.5 µg) [35]. This current study has supported the early findings.

DPPH Antioxidant Activity

The DPPH antioxidant activity was performed to estimate DPPH elimination by different venom concentrations. 3 different crude venom concentrations were performed. 100 µL concentration has shown a higher percentage of antioxidant activity (78.13%) compared to the other two different concentrations (50 and 75 µL). Compared to the commercialised Antioxidant compound (Ascorbic acid) it has a lesser percentage inhibition activity. Previous data has shown Ammonium Sulphate (80%) precipitated crude venom had the highest (57.08 %) inhibition percentage at a higher concentration of 2000µg/mL [30]. Fera Ibrahim and his colleague have reported this *P. volitans* venom possesses a higher percentage inhibition (76.13 %) by the ammonium Sulphate precipitation (40-60%) in the year 2021 [36]. A comparative study has reported a higher value inhibition percentage (78.13%) by using the Phosphate Buffer Saline. So, extraction of venom by using Buffer has a good efficacy in reducing Cell damage efficacy.

Fig. 1 The figure represents the collected *Pterois volitans* sample from Mudasalodai Landing Centre, Tamil Nadu (2022).

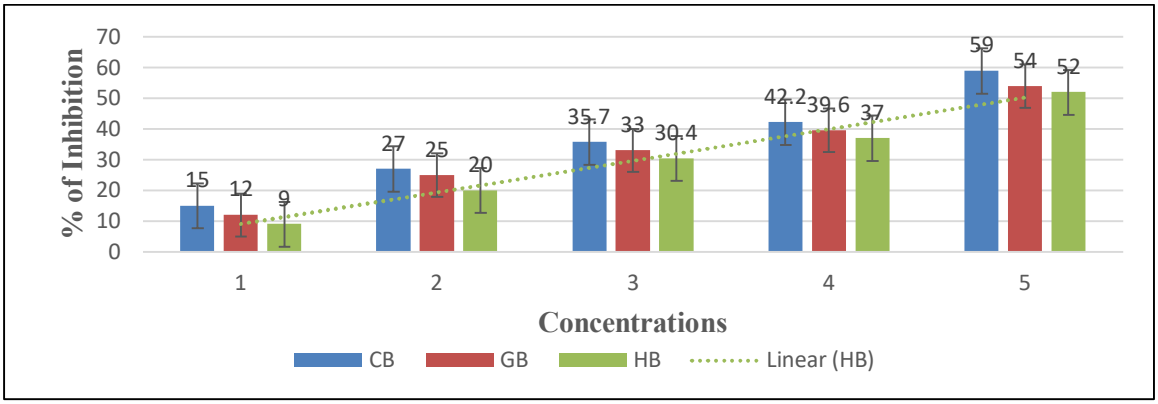

Fig. 2 CB- Chicken Blood, GB- Goat Blood, and HB- Human Blood are different blood samples – Haemolytic Assay

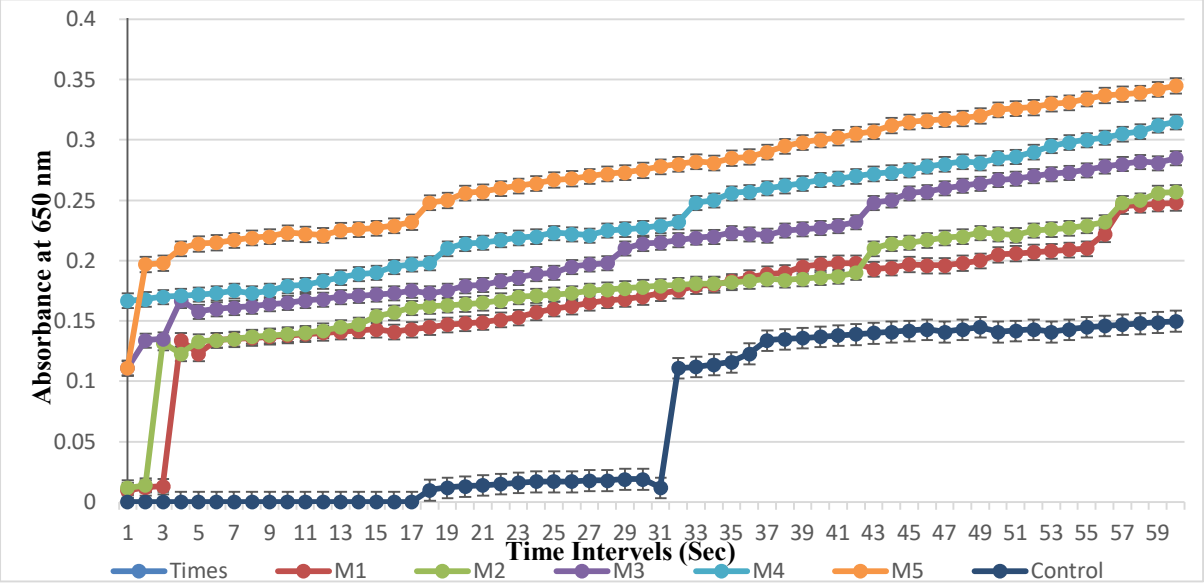

Fig. 3 M1- 10 µL, M2- 20 µL, M3- 30 µL, M4- 40 µL, M5- 50 µL different concentrations – Plasma Coagulation Assay

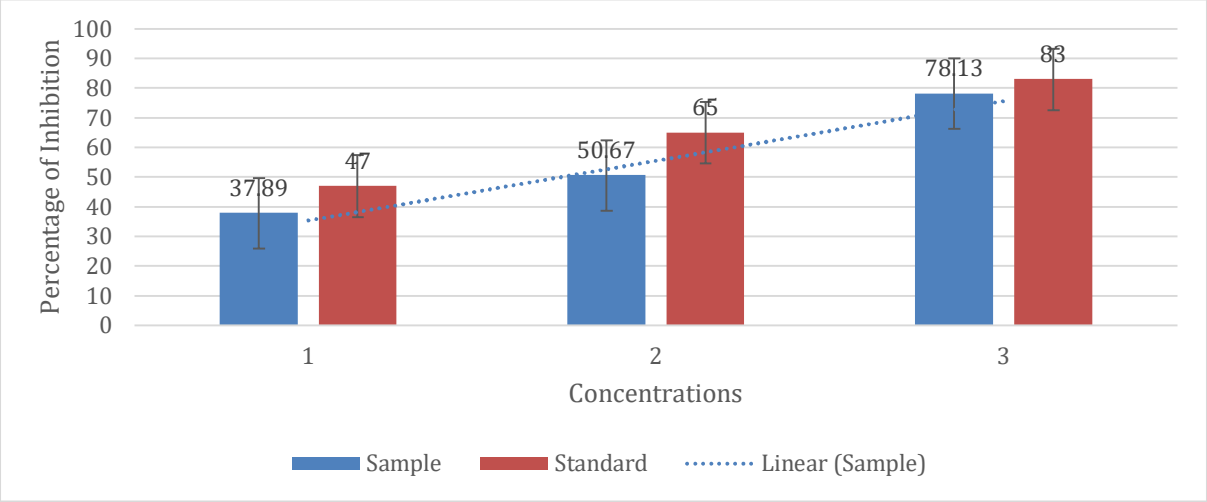

Fig. 4 Different Sample Concentrations – 50 µL, 75 µL, 100 µL compared to Standard Concentrations- Antioxidant Assay

CONCLUSION

The present results show that Fish venom has a satisfactory bioactivity. This venom can act as an antioxidant drug to reduce the free radicals. Further characterisation of targeted molecules can be a good drug shortly.

ACKNOWLEDGEMENTS

All Authors want to Acknowledge the Marine Natural Product Laboratory for providing all necessary chemicals and equipment.

REFERENCES

1. Mebs, D. (1994). Anemonefish symbiosis: vulnerability and resistance of fish to the toxin of the sea anemone. Toxicon, 32(9), 1059-1068.
2. Utkin, Y. N. (2015). Animal venom studies: Current benefits and future developments. World journal of biological chemistry, 6(2), 28.
3. Arbuckle, K. (2017). Evolutionary context of venom in animals. Evolution of venomous animals and their toxins, 24, 3-31.
4. Gokulalakshmi, E., Vijayaraj, R., & Sri Kumaran, N. (2018). A mini review on bioprospecting of fish venom. Int. J. Creative Res. Thoughts, 6(2), 1177-1181.

5. Schendel, V., Rash, L. D., Jenner, R. A., & Undheim, E. A. (2019). The diversity of venom: the importance of behavior and venom system morphology in understanding its ecology and evolution. Toxins, 11(11), 666.
6. Church, J. E., & Hodgson, W. C. (2002). The pharmacological activity of fish venoms. Toxicon, 40(8), 1083-1093.
7. Ziegman, R., & Alewood, P. (2015). Bioactive components in fish venoms. Toxins, 7(5), 1497-1531.
8. Escoubas, P., De Weille, J. R., Lecoq, A., Diochot, S., Waldmann, R., Champigny, G., ... & Lazdunski, M. (2000). Isolation of a tarantula toxin specific for a class of proton-gated Na+ channels. Journal of Biological Chemistry, 275(33), 25116-25121.
9. Terlau, H., & Olivera, B. M. (2004). Conus venoms: a rich source of novel ion channel-targeted peptides. Physiological reviews.
10. Koh, D. C. I., Armugam, A., & Jeyaseelan, K. (2006). Snake venom components and their applications in biomedicine. Cellular and Molecular Life Sciences CMLS, 63, 3030-3041.
11. King, S. F. (2015). Models of neutrino mass, mixing and CP violation. Journal of Physics G: Nuclear and Particle Physics, 42(12), 123001.
12. Lopes-Ferreira, M., Grund, L. Z., & Lima, C. (2014). *Thalassophryne nattereri* fish venom: from the envenoming to the understanding of the immune system. Journal of venomous animals and toxins including tropical diseases, 20, 1-12.
13. Ortiz, E., Gurrola, G. B., Schwartz, E. F., & Possani, L. D. (2015). Scorpion venom components as potential candidates for drug development. Toxicon, 93, 125-135.
14. Casewell, N. R., Visser, J. C., Baumann, K., Dobson, J., Han, H., Kuruppu, S., ... & Fry, B. G. (2017). The evolution of fangs, venom, and mimicry systems in blenny fishes. Current Biology, 27(8), 1184-1191.
15. Jawad, L. A., & Jawad, L. A. (2018). Poisonous Fishes. Dangerous Fishes of the Eastern and Southern Arabian Peninsula, 155-229.
16. Ashwood, L. M., Undheim, E. A., Madio, B., Hamilton, B. R., Daly, M., Hurwood, D. A., ... & Prentis, P. J. (2022). Venoms for all occasions: the functional toxin profiles of different anatomical regions in sea anemones are related to their ecological function. *Molecular Ecology*, *31*(3), 866-883.
17. Madokoro, M., Ueda, A., Kiriake, A., & Shiomi, K. (2011). Properties and cDNA cloning of a hyaluronidase from the stonefish *Synanceia verrucosa* venom. Toxicon, 58(4), 285-292.
18. Kiriake, A., Suzuki, Y., Nagashima, Y., & Shiomi, K. (2013). Proteinaceous toxins from three species of scorpaeniform fish (*lionfish Pterois lunulata*, devil stinger *Inimicus japonicus* and waspfish *Hypodytes rubripinnis*): close similarity in properties and primary structures to stonefish toxins. Toxicon, 70, 184-193.
19. Hornbeak, K. B., & Auerbach, P. S. (2017). Marine envenomation. Emergency Medicine Clinics, 35(2), 321-337.
20. Haddad, V., Lupi, O., Lonza, J. P., & Tyring, S. K. (2015a). Tropical dermatology: marine and aquatic dermatology. Journal of the American Academy of Dermatology, 61, 733-750. https://doi.org/10.1016/j.jaad.2009.01.046.
21. Haddad, V., Stolf, Jr., H. O., Risk, J. Y., França, F. O., & Cardoso, J. L. (2015b). Report of 15 injuries caused by lionfish (Pterois volitans)in aquarists in Brazil: a critical assessment of the severity of envenomation's. Journal of VenomousAnimals and Toxins Including Tropical. Diseases,21, 1-7. https://doi.org/10.1186/s40409-015-0007-x
22. Haddad Jr, V., Neto, D. G., de Paula Neto, J. B., de Luna Marques, F. P., & Barbaro, K. C. (2004). Freshwater stingrays: study of epidemiologic, clinic and therapeutic aspects based on 84 envenomings in humans and some enzymatic activities of the venom. Toxicon, 43(3), 287-294.
23. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. J biol Chem, 193(1), 265-275.
24. Wang, Y., Zhang, Y., Zhu, Z., Zhu, S., Li, Y., Li, M., & Yu, B. (2007). Exploration of the correlation between the structure, hemolytic activity, and cytotoxicity of steroid saponins. Bioorganic & Medicinal Chemistry, 15(7), 2528-2532.
25. Rajasekhar, A., Gowing, R., Zarychanski, R., Arnold, D. M., Lim, W., Crowther, M. A., & Lottenberg, R. (2011). Survival of trauma patients after massive red blood cell transfusion using a high or low red blood cell to plasma transfusion ratio. Critical care medicine, 39(6), 1507-1513.
26. Chang, S. T., Wu, J. H., Wang, S. Y., Kang, P. L., Yang, N. S., & Shyur, L. F. (2001). Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. Journal of Agricultural and Food chemistry, 49(7), 3420-3424.
27. Myers, R.F., 1999. Micronesian reef fishes: a comprehensive guide to the coral reef fishes of Micronesia, 3rd revised and expanded edition. Coral Graphics, Barrigada, Guam. 330 p DOI / ISBN0-9621564-5-0
28. Linnaeus, C. (1758). Systema Naturae per regna tria naturae, secundum classes, orders, genera, species, cum characters, differences, synonymis, loci. Tenth edition, reformata. [10th revised edition], vol. 1: 824 pp. Laurentius Salvius: Holmiae. , available online at https://biodiversitylibrary.org/page/726886
29. Chaves, L. C. T., Hall, J., Feitosa, J. L. L., & Côté, I. M. (2016). Photo-identification as a simple tool for studying invasive lionfish Pterois volitans populations. Journal of Fish Biology, 88(2), 800-804
30. Wenningmann, N., Knapp, M., Ande, A., Vaidya, T. R., & Ait-Oudhia, S. (2019). Insights into doxorubicin-induced cardiotoxicity: molecular mechanisms, preventive strategies, and early monitoring. Molecular pharmacology, 96(2), 219-232.
31. Manso, L., Ros, U., Valdés, G., del Rivero, M. A., Lanio, M. E., & Álvarez, C. (2015). Proteolytic and hemolytic activity in the venom of the lionfish *Pterois volitans*, an invasive species of Cuban sea coasts. Cuban Journal of Biological Sciences/Revista Cubana de Ciencias Biológicas, 4(2).
32. Balasubashini, M. S., Karthigayan, S., Somasundaram, S. T., Balasubramanian, T., Viswanathan, V., Raveendran, P., & Menon, V. P. (2006). Fish venom (*Pterios volitans*) peptide reduces tumor burden and ameliorates oxidative stress in Ehrlich's ascites carcinoma xenografted mice. Bioorganic & medicinal chemistry letters, 16(24), 6219-6225.

33. azuo Shiomi, K., Kazama, A., Shimakura, K., & Nagashima, Y. (1998). Purification and properties of phospholipases A2 from the crown-of-thorns starfish (*Acanthaster planci*) venom. Toxicon, 36(4), 589-599.
34. Sommeng, A. N., Eka, A. K., Ramadhan, M. Y. A., Ginting, M. J., Sahlan, M., Hermansyah, H., & Wijanarko, A. (2020, March). Protein isolation and identification of *Pterois volitans* spine venom coagulant activity. In IOP Conference Series: Earth and Environmental Science (Vol. 462, No. 1, p. 012039). IOP Publishing 333-2338.
35. Memar, B., Jamili, S., Shahbazzadeh, D., & Bagheri, K. P. (2016). The first report on coagulation and phospholipase A2 activities of Persian Gulf lionfish, *Pterois russelli*, an Iranian venomous fish. Toxicon, 113, 25-31.
36. Ibrahim, F. E. R. A., Sahlan, M., Ginting, M. J., Pratami, D. K., Hermansyah, H. E. R. I., & Wijanarko, A. (2021). Isolation of protein from the spine venom of *Pterois volitans* found in the Indonesian ocean, using a heating process, for anticancer, antiretroviral, antibacterial, and antioxidant assays. Int J App Pharm, 53-8.

CITATION OF THIS ARTICLE

Debarati Paul, Bandana Kumari, Kanchana Sankar, Sangeswari Thirukumar, Srichandan Rath, Arumugam Muthuvel *. Biomedical Profiling of Fish Venom *Pterois volitans* (Linnaeus, 1758) . Bull. Env.Pharmacol. Life Sci., Vol 13 [7] June 2024:135-140