

ORIGINAL RESEARCH ARTICLE

Bioactive Properties of the Stone Fish *Synanceia horrida* (Thomas, 1984) Spine Venom

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ABSTRACT

Stonustoxin (SNTX) is a two-subunit protein toxin purified from the venom of the stonefish (*Synanceia horrida*), which induces potent hemolytic activity. The fish *Synanceia horrida* were collected from Mandapam coast by local fisherman. The present study was focused on the bioactive properties of the stonefish *Synanceia horrida* spine venom. The crude extract was partially purified by using DEAE cellulose. The crude was extracted with two different solvents aqueous and methanol and it is screened for antimicrobial properties and was tested against 5 pathogenic bacteria. The results showed that very low antibacterial activity has been recorded for the both extract. The aqueous extract inhibits the growth of *Vibrio cholerae* whereas in the methanol extract a clear inhibition zone was observed only against *Pseudomonas* sp. The hemolytic activity in chick blood erythrocytes was recorded. The result reported that both the crude extracts exhibited hemolytic activity which was estimated as 8.9 HT/ml for aqueous extract and 12.49 HT/ml for methanol extract.

Key words: *Synanceia horrida*, Hemolytic activity, Antimicrobial properties and Protein.

1. INTRODUCTION

A Marine biodiversity has been the source of unique biochemical compounds with the potential for many industrial applications. In recent years, a significant number of novel metabolites with potent pharmacological properties have been discovered from the marine organisms. Many bioactive compounds extracted from various marine animals like sponges, tunicates, soft corals, sea hares, nudibranchs, bryozoans, sea slugs, Conus and other marine organisms. More than 200 species of marine fishes, including stingrays, weever - fish, stonefish and other, are known to be venomous [1]. The venom (stonefish *Synanceia horrida*) was stored in the dorsal fine spines. The stings produced by the spines induce pain, respiratory arrest, and damage to the cardiovascular system and skeletal muscle paralysis, sometimes leading to death [2, 3, 4]. Verrucotoxin (VTX), a tetrameric glycoprotein with a molecular weight of 322 KDa [5] and a dimeric 166-KDa protein [6] isolated from the venom. However, the toxicity of most venomous fish is still unknown probably due to the marked

instability of venoms as well as the difficulty in collecting sufficient samples.

The stone fish *Synanceia horrida*, one of most dangerous venomous fishes its shows potent hemolytic activity. In addition to hemolytic activity, the toxin possesses various other biological activities, such as edema induction, vascular permeability, platelet aggregation and endothelium-dependent vasorelaxation and hypotension. Stone fish venom contains enzymatic proteins. Several enzymatic activities detected in the venom of *S.horrida* include hyaluronidase, proteinase (thrombin-like), phosphodiesterase, alkaline phosphomonoesterase, arginine esterase, arginine amidinase, 5-nucleotidase and acetylcholinesterase [7]. Therefore the potent hemolytic activity of SNTX is most probably mediated through a non-enzymatic mechanism.

Many cytolytic toxins isolated from venoms and bacteria show various enzymatic activities, such as phospholipase, phospholipase, C and phingomyelinase. They lyses cell directly or make cells more susceptible to damage by hydrolyzing

membrane lipids through enzymatic action. On the other hand, a wide variety of non -enzymatic proteins and peptides that possess cytolytic activity have been isolated from reptilian, amphibian, insect, microbial and mammalian origins.

Because of the recent and rapid proliferation of reach in the area of marine biotoxinology, there have increasing interest not only from the point of view obtaining biomedically active substance from marine resources, and to have a better knowledge of the pharmacologically active substance, especially on marine biotoxin. Bioprospecting for potential new drugs continues to be the leading force behind the efforts of marine natural product researchers to tap the fascinating chemical diversity encountered in the sea. The success of this approach in highlighted by several compounds that are in the late stages of clinical development and are expected to enter the drug market shortly in the areas of anticancer chemotherapy or as an analgesic. Therefore, the present investigation was undertaken to elucidate the bioactive properties of the venom of stone fish *Synanceia horrida* were collected from Mandapam coast, Tamil Nadu, South east coast of India and partial purification of protein for their suitability against antimicrobial activity to pathogenic bacteria.

2. MATERIALS AND METHODS

2.1. Collection and processing of sample

The live samples of *Synanceia horrida* were collected from Mandapam coast, Tamil Nadu, South east coast of India. The collected animals were kept at -2°C for 1 hour. Spine venom was collected by cutting spines of fish approximately 3-5 mm from base of dorsal spine^[8]. Further, the spine venom and were placed in tubes and stored at -40°C until use. Briefly, homogenization and all subsequent procedures were carried out at 4°C . The homogenate was centrifuged at $8,000\text{g} \times 15\text{ min}$. The pellets were collected, re-extracted with extraction buffer (0.005m sodium phosphate buffer pH 7.5 containing 0.14 NaCl) recentrifuged as before and the supernatant was subsequently called spine venom respectively.

2.2. Systematic position

Phylum	:	Chordata
Sub-phylum	:	Vertebrata
Class	:	Actinopterygii
Sub-class	:	Neopterygii
Order	:	Scorpaeniformes
Family	:	Scorpaenidae
Genus	:	<i>Synanceia</i>

Species : *horrida*
Description of the Study Area



Stone fish (*Synanceia horrida*) attains the growth of 50 cm, those seen about 15-20 cm. The eyes are close together with a bony ridge between them. It has a deep depression below each eye. The huge mouth faces upwards and is curved into a perpetual frown, it is also found in muddy places and estuaries. During the day, it lies motionless on the sandy bottom, in a shallow depression that it creates by scooping sand out and piling sand around its sides with its pectoral fins. At the night, it is more active and often moves on top of reefs.

2.3. Extraction of venom

2.3.1. Aqueous extraction

The aqueous extract of *Synanceia horrida* was prepared by squeezing the sand – free specimens in triple distilled water. The resultant solution was filtered and dialyzed by using Sigma dialysis membrane – 500 (Av Flat width -24.26 mm, Av. Diameter -14.3 mm and capacity approx – 1.61ml/cm) against D-glucose to remove the excess water. The supernatant so obtained was lyophilized (Labcono Freeze Dry System) and stored at 4°C in a refrigerator for the further use as aqueous extract.

2.3.2. Methanol Extraction:

Crude toxin was extracted following the method for Chloroform extraction *Synanceia horrida*, was put into 200 ml of chloroform, covered and kept standing for 5 hours. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 45°C in refrigerator for further use as crude Chloroform extracts.

2.3.3. Partial purification of crude protein:

Partial purification of the crude extract *Synanceia horrida* was carried out using DEAE Cellulose Anion Exchange chromatography according to the procedure of Stempion *et al.*^[9].

2.3.4. Protein estimation

Protein content from crude extracts was estimated by Lowry *et al.* [10].

2.3.5. Microbial Strains Used

Antibacterial effect of *Synanceia horrida*, was determined against 5 different bacterial strains viz., *Pseudomonas* sp., *Streptococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Escherichia coli*. These pathogenic strains were obtained from the department of Medical Microbiology (Raja Muthiah Medical College hospital), Annamalai University, Annamalai Nagar.

2.3.6. Antimicrobial activity

Petri dishes with nutrient agar were inoculated with five different species of bacteria. *Synanceia horrida* extracts were sterilized by passing each through a 0.22 m Millipore GV filter (Millipore, U.S.A). Round paper discs with a radius of 0.8 cm were dipped into each extract of different concentration of 5mg/ml and 10mg/ml and placed

in the center on inoculated petridishes. The bacterial colonies were allowed to grow overnight at 37°C and 20°C respectively, and then the inhibition zone around the disc was measured.

2.3.7. Hemolytic assay

The hemolytic activity of crude extracts of *Synanceia horrida* were assayed on chick blood erythrocytes followed by the method.

2.3.8. Statistical Analysis

Tests were carried out in triplicates. The mean values were calculated from the triplicate values. Values are expressed as the mean \pm SD and differences between groups were considered to be significant if $p < 0.05$

3. RESULTS

3.1. Preparation of Crude Extracts

Aqueous extracts yield a total amount of 5.09g of crude extract from 500g of fish. Similarly, Methanol extract yield a total amount of 5.4 g of crude extract.

Table 1: Crude extract

S. No	Samples	Extract	Amount of crude extract (g)
1	<i>Synanceia horrida</i>	Aqueous	5.09
2		Methanol	5.4

3.2. Protein estimation

The protein content in crude extracts of aqueous sample was found to be 1.43 mg/ml and 1.63mg/ml in methanol extract.

Table 2: Protein Estimation

S. No	Samples	Extract	Protein estimation (mg/ml)
1	<i>Synanceia horrida</i>	Aqueous	1.43
2		Methanol	1.63

3.3. Antibacterial Activity

The crude of aqueous and ethanol extracts were tested against 5 species of bacteria viz., *Pseudomonas* sp., *Streptococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Escherichia coli*. The results showed that the aqueous extract inhibits the growth of *Vibrio cholerae* where as in the methanol extract clear inhibition zones were observed only against *Pseudomonas* sp. (Table 3).

Table 3: Antibacterial activity of crude of aqueous and methanol extracts

S. No	Bacteria	Aqueous	Methanol
1	<i>Pseudomonas</i> sp.	Resistant	Sensitive
2	<i>Streptococcus aureus</i>	Resistant	Resistant
3	<i>Vibrio cholerae</i>	Sensitive	Resistant
4	<i>Vibrio parahaemolyticus</i>	Resistant	Resistant
5	<i>Escherichia coli</i>	Resistant	Resistant

3.4. Hemolytic Assay

The results of the hemolytic assay on chick, blood sample erythrocyte were done using crude Aqueous and Methanol extract solvents. The results were shown in table. The crude extracts in spine venom induced hemolysis in chick blood sample. The hemolytic titre in case of aqueous

extract was found to be 10 and its specific hemolytic activity was estimated to be 8.9HT/mg of protein. Similarly In case of Methanol extract found to be 14 and its specific hemolytic activity was 12.49HT/mg of protein. In the present study it was found that Spine venom *Synanceia horrida* showed a very strong hemolytic activity on both extracts.

Table 4: Hemolytic Activity of *Synanceia horrida* in Chick Blood

S. No	Sample	Extract	Protein content	Hemolytic assay	Hemolytic Titer Value (HT/mg)
1	<i>Synanceia horrida</i>	Aqueous	1.43	10	8.9
2		Methanol	1.63	14	12.49

4. DISCUSSION

In the present investigation, the stone fish *Synanceia harrida* collected from Mandabam coast, Tamil Nadu and it was identified based on the morphological characters given by Thomas [11]. The two extract were purified stone fish, *Synanceia horrida* yielded a 5.4g of methanolic extract and 5.09g of aqueous extract. Poh *et al.* [7]1991, also done the same in the stone fish *Synanceia horrida* SNTX was purified from the crude venom by a two-step procedure on sephacryl S-200 high-resolution gel-permeation and DEAE Bio-Gel an anion-exchange chromatography.

In this study, protein content in crude extract of stone fish *Synanceia horrida* were found to be 1.62mg/ml in the case of Methanol extract and

1.43mg/ml in the case of aqueous extract. Similarly, the protein concentrations of *Synanceia horrida* were estimated the native and modified SNTX with a concentration of 1mg/ml showed an absorbance at 280nm described by Chen *et al.*^[12]. The result of the hemolytic assay on chicken erythrocyte using Methanol and aqueous extract of stone fish *Synanceia horrida* and the methanol extract induced pronounced hemolysis on chicken blood. The hemolytic titer in the case of methanol extract found to be 14 and its specific hemolytic activity was estimated to be 10 and its hemolytic activity was found to be 8.925 HU of protein. It is well by know by Chen *et al.*^[12] tested *Synanceia horrida* containing hemolytic activity by pore formation in the cell membrane and to role of colloid-osmotic shock in SNTX-induced haemolysis, and studied the effect of various osmotic protestants on SNTX-induced haemolysis. This approach is based on the concept that colloid-osmotic lysis can be suppressed by an osmotic protectant of appropriate size which, being too large to penetrate the induced membrane pores, is capable of balancing the osmotic drag of intracellular impermeant solutes such as hemoglobin and organic phosphates. The results presented herewith clearly demonstrate the presence of a hemolytic factor (s) in *P.berghei*, with aqueous medium and hemolytic action was a temperature dependent process. These observations substantiate the early report of Fife *et al.*^[13] on the presence of a similar factor in *P.knowlesi*.

The first attempt to locate antimicrobial activity in marine organisms was initiated around the 1950s Burkholder and Burkholder,^[14] The aqueous and methonal extract at the concentration of 15mg/ml were tested against 5 species of bacteria *viz.*, *Pseudomonas* sp., *Staphylococcus aureus*, *Vibrio cholerae*, *E. coli* and *Vibrio Parahaemolyticus*. In the present results, very low antibacterial activity has been recorded for the both extract. The aqueous extract inhibits the growth of *Vibrio cholerae* whereas in the methanol extract a clear inhibition zone was observed only against *Pseudomonas* sp. The most obvious reason for the observation of low antibacterial activity for cephalopods in the present may be attributed to the method of extraction. But, the present observation is substantiated by the report of Kawabata *et al.*^[15] who obtained negative of results on bacteriological tests with poisonous cephalopods against human pathogens, and also Rajaganapathi *et al.*^[16] observed negative activity

for the adult *Sepiella inermis* and *Loligo duvancelli* ink at 1600 µg/ml concentration in the anti-retrovial assay but they have observed maximum inhibition at same concentration of ink extract of juveniles. Though, the present study made on hemolytic, antibacterial activity of *Synanceja horrida* spine, provided baseline information on their pharmacological potential which could be inferred from present investigation that the *Synanceja horrida* spine possesses a diverse mixture of bioactive principles. Further detailed studies could be made on purification and characterization of the mucus into several components which may lead to the discovery of new potent antimicrobial drugs in future.

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