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STUDIES ON BIOACTIVE PROPERTIES OF THE CATFISH *Plotosus canius* (Hamilton, 1822*) STING* VENOM AND EPIDERMAL MUCUS

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ABSTRACT

Catfish of the family plotosidae was extensively rampant all the way through the watercourses of India that let liquid flow away into the Indian Ocean. Catfish species are endemic to the most extreme freshwater environment of the Parangipettai and possess a toxic mucous-covered sting, responsible for most of the injuries, as well as a protective epidermal secretion. The envenomation causes immediate, local and intense pain, soft tissue edema, and a variable extent of bleeding.The present study was carried out on studies on Bioactive properties of mucus and spine extract from catfish *Plotosus canius*. The crude was extracted with three different solvents aqueous, acetone, chloroform and it is screened for antimicrobial properties and were tested against 6 pathogenic bacteria and 5 pathogenic fungi. The result of present investigation reported that spine and mucus extract of catfish having significant antimicrobial activity. In particularly mucus extracts showed much activity than spine extracts. The crude extract was partially purified by using DEAE cellulose. The antioxidant property of the extract was assessed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method .The toxic activity of crude extracts were determined by illeal loop in chick respectively. The haemolytic activities in chick, goat, and human blood erythrocytes were recorded. The result reported that mucus extracts showed a very strong haemolytic activity than spine extracts. The estimation of protein gist were intervened by SDS –PAGE and the results showed that all the extracts showed significant number of protein bands in range from 7 KDa to 21 KDa respectively. It could be surmised from the contemporary study that *Plotosus canius* venom boasts a assorted mixture of peptides, protein and pharmacologically active components.

INTRODUCTION

Catfish (sub-order siluroidei) are multifariously located across pelagic zone from sea shore and scattered around worldwide but being largely concentrated in tropical areas .The most important catfish families are the Ariidae, Doradidae and Pimelodidae, many of which are found in rivers of south America. In aquatic environment, large numbers of venomous and poisonous animals exist out of which more than 200 species of marine fish *vis a vis* stingray, scorpion fish, zebra fish, stonefish, weever fish, toadfish and some species of shark, ratfish, catfish, surgeonfish are known or suspected to be venomous (Russrell FE, 1996). The Ictaluridae family found in the rivers and estuary ;the plotosidae family members that are frequently found in brackish, coastal and fresh water environments (Nelson *et al* 1994). In India fresh water catfish are included in *Plotosidae* family, which comprise three valid genera *Plotosus lineatus* (Shiomi *et al.*, 1986), *Plotosus canius* (Auddy *et al.*, 1994) *Plotosus limbatus* (Haddad, 2000). Many catfish have three serrated bony stings on dorsal and pectoral fins, which was used for defence against predators (Haddad, 2000; Halstead, 1953). Venomous catfish have a sharp and stout sting immediately in front of the soft –rayed portion of pectoral and dorsal fins, where stings are derived from fin rays and are covered by thin integumentary sheath (Rodrigues, 1972). In addition to venom, the skin of fish plays a passive role in protective immunity, serving as an anatomical and physiological barrier against the external environment (Cameron and Endean, 1973; Shiomi, 1988).The Mucus, such as that produced by skin of the catfish may include Aminoacids, proteins, peptides, complex carbohydrates, Glycoproteins, Glycotids and other chemicals (Klesius, 2008). In this present study series of Aqueous, acetone, and chloroform extracts of epidermis of mucus and spine from marine catfish species were screened for there in vitro. *Plotosus canius*, one of the most abundant species of catfish in sea shore of India and Srilanka. Enevenomation by catfish is relatively

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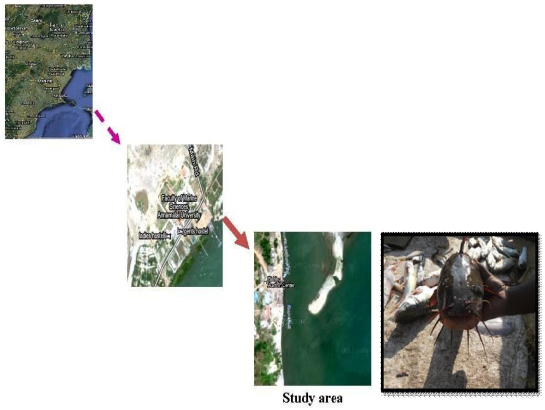
common among fisherman, anglers, bathers, and swimmers in which injuries may be very painful causing complication such as erythema, edema, pain, sudoresis, fever, nausea, vomiting and secondary infection. Taking in this view of frequency of accidents provoked by catfish *Plotosus canius*. The present study was undertaken to evaluate the bioactive properties of catfish *Plotosus canius* by two types of venoms (a)venom found in glandular epithelium which covers the sting (sting venom) and (b) venom found in body mucus(mucus venom).

MATERIALS AND METHODS

Collection and processing of sample

The live sample of *Plotosus canius were* collected from Annankoil landing centre (Lat 11° 29 n: Long 79° 46 E), Parangipettai, southeast coast of India. The collected animals were kept at – 2° C for 1 hour. Mucus was collected from surface of fish body by scrapping with dull blade (Al Hassen, 1982).Sting venom was collected by cutting spins of fish approximately 3- 5 mm from base of dorsal and pectoral spine (Gisha, 2007).Further, the spine venom and mucus 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, 000g x15 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 and mucus respectively.

DESCRIPTION OF THE STUDY AREA



Study area

EXTRACTION OF VENOM

Aqueous extraction

The aqueous extract of *Plotosus canius* 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 ^{o} C in a refrigerator for the further use as aqueous extract.

Acetone Extraction

For Acetone extraction, *Plotosus canius* was put into 200 ml of Acetone, covered and kept standing for 5 hours. The solvent was evaporated at low pressure by using a Buchi Rotavapor R-200 at 45 ^{o} C in refrigerator for further use as crude Acetone extracts.

Chloroform Extraction

Crude toxin was extracted following the method of Bakus *et al.,* (1981).For Chloroform extraction, *Plotosus canius* 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 ^{o} C in refrigerator for further use as crude Chloroform extracts.

Partial purification of crude protein

Partial purification of the crude extract *Plotosus canius* was carried out using DEAE Cellulose Anion Exchange chromatography according to the procedure of Stempion *et al.,* (1970).

Protein estimation

Protein content from crude extracts was estimated by Lowry and Lopaz method. (1946)

Microbial Strains Used

Antibacterial effect of *Plotosus canius* was determined against 6 different bacterial strains viz. *Pseudomonas* sp, *Streptococcus aureus, Vibrio cholerae, Bacillus sp, E.coli* and *Lactobacillus brevis* similarly Antifungal effect was determined against 5 different fungal strains viz. *A.flavus, A.niger, Candida albicans A.oryzae and A.sojae* These pathogenic strains were obtained from the department of Medical Microbiology (Raja Muthiah Medical College hospital) Annamalai University, Annamalai Nagar.

Antimicrobial activity

Petri dishes with nutrient agar and Potato Dextrose Agar (PDA) were inoculated with five different species of bacteria and fungus. *Plotosus canius* spine and mucus 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 and fungal colonies were allowed to grow overnight at 37ºC and 20ºC respectively, and then the inhibition zone around the disc was measured.

Hemolytic assay

The hemolytic activity of crude extracts of *Plotosus canius* were assayed on chick, goat and human erythrocytes followed by the method of Pani Prasad and Venkateshwaran (1997).

Ileal loop Assay

The effect of drug at lower and higher doses in the intestine of the chick was found by injecting 25µl/ml and 100µl/ml to its intestinal wall. This was then stored in phosphate buffer saline. The extract would attach to the acetyl choline receptor and the sodium potassium pump will be blocked as a result it brings out the fluid secretion. It proves that presence of toxin and the intensity of low dose can be used as therapeutic protein

DPPH Radical Scavenging Assay

The scavenging effects of samples for DPPH radical were monitored according to the method described by (Yen and Chen 1995).

SDS-PAGE

Crude extracts were analyzed by SDS –PAGE 12%, topped by 7% stacking gel according to (Laemmli 1970).

Statistical Analysis

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

RESULTS

Preparation of Crude Extracts

Aqueous extracts yield a total amount of 1.06g of crude extract from 50g of spine venom. Similarly, chloroform extracts given a total amount of 0.98g of crude extract and for acetone a total amount of 1.00 crude extracts were obtained. In case of mucus, aqueous extracts yield a total amount of 1.02g of crude extract from 50g, chloroform extract yield a total amount of 2.25 g of crude extract and for Acetone a total amount of 1.25 g crude extracts obtained.

Table 1 Crude extract

S. No	Samples	Extract	Amount of crude extract (g)
1		Aqueous	1.06
2	P.C Spine	Acetone	1.00
3		Chloroform	0.98
4		Aqueous	1.02
5	P.C Mucus	Acetone	1.25
6		Chloroform	2.25

Protein Estimation

The protein content in crude extracts of aqueous spine venom sample was found to be 0.95 mg/ml, in case of Acetone extract 1.40 and 0.97 mg/ml in chloroform extract. In case of mucus venom sample, aqueous exacts showed 1.05 mg/ml, in case of acetone extract it was 1.20 mg/ml in case of chloroform extract 1.05 mg/ml was found.

Table 2 Protein Estimation

S. No	Sample	Extract	Protein estimation
1	P.C	Aqueous	0.95
2	Spine	Acetone	1.40
3		Chloroform	0.97
4	P.C	Aqueous	1.05
5	Mucus	Acetone	1.20
6		Chloroform	1.05

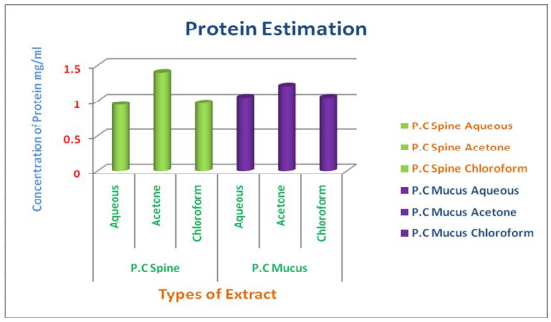


Fig. 1 Protein Estimation

ANTIMICROBIAL ACTIVITY

Antibacterial Activity

The crude of Aqueous, Acetone and Chloroform extracts were tested against 6 species of bacteria viz. *Pseudomonas* sp, *Streptococcus aureus, Vibrio cholerae, Bacillus sp, E.coli* and *Lactobacillus brevis.*

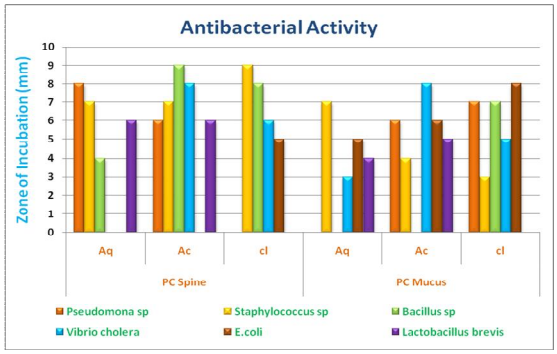


Fig. 2 Antibacterial activity

Antifungal Activit

The crude of Aqueous, Acetone and Chloroform extracts were tested against 5 species of fungai viz. *A.flavus, A.niger, Candida albicans, A.oryzae* and *A.sojae*

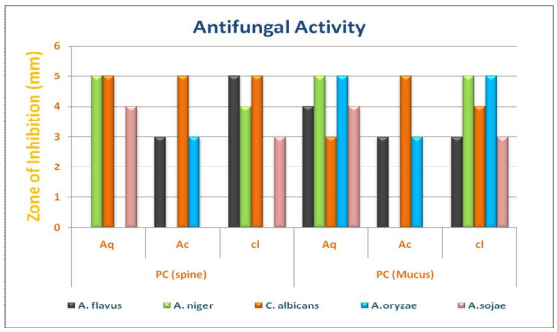


Fig. 3 Antifungal activity

Hemolytic Assay

The results of the hemolytic assay on chick, goat and human blood sample erythrocyte were done using crude aqueous, acetone and chloroform solvents. The results were shown in figure. The crude extracts in spine venom induced hemolysis in chick blood sample**.** The hemolytic titre in case of aqueous extract was found to be 2 and its specific hemolytic activity was estimated to be 4.8 HT/mg of protein. For acetone extract, it was found to be 7 and its specific hemolytic activity was 1.5 HT/mg of protein. for chloroform extract, the titer was found to be 3 and its specific hemolytic activity was 3.2HT/mg of protein, In case of mucus venom sample, aqueous extract it was found to be 4 and its specific hemolytic activity was estimated to be 2.6 HT/mg of protein. For Acetone extract it was found to be 7 and its specific hemolytic activity was 1.7 HT/mg of protein, for chloroform extract it was found to be 6 and its specific hemolytic activity was 1.8 HT/mg of protein.

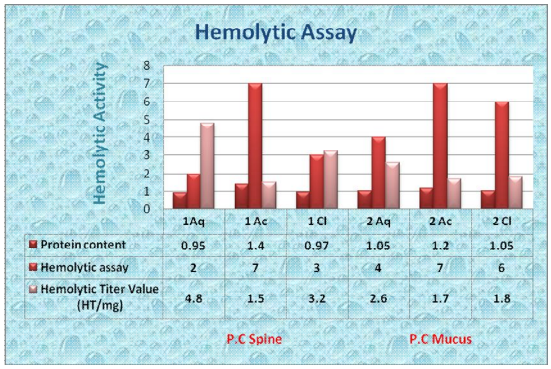


Fig. 4 P.C. Spine & Mucus of Chicken

In goat blood sample aqueous extract found to be 7 and its specific hemolytic activity was estimated to be 1.4 HT/mg of protein. For Acetone extract found to be 5 and its specific hemolytic activity was 2.1 HT/mg of protein, For chloroform extract found to be 7 and its specific hemolytic activity was 1.4 HT/mg of protein, similarly In case of mucus venom sample, aqueous extract it was found to be 4 and its specific hemolytic activity was estimated to be 2.6 HT/mg of protein. For Acetone extract found to be 5 and its specific hemolytic activity was 2.4 HT/mg of protein, for chloroform extract found to be 3 and its specific hemolytic activity was 3.5 HT/mg of protein.

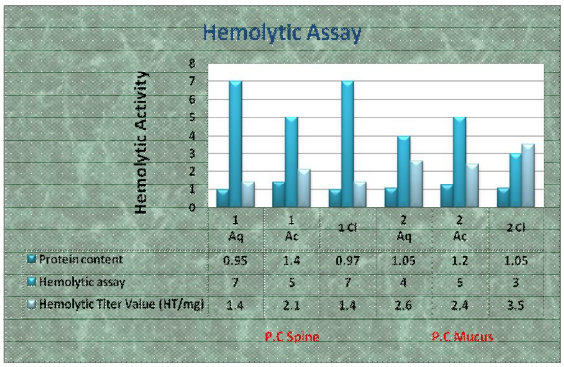


Fig. 5 P.C. Spine & Mucus of goat

In human blood sample The aqueous extract found to be 3 and its specific hemolytic activity was estimated to be 3.1 HT/mg of protein. For Acetone extract found to be 3 and its specific hemolytic activity was 4.6 HT/mg of protein, For chloroform extract found to be 3 and its specific hemolytic activity was 9.7 HT/mg of protein, Similarly In mucus venom sample, the aqueous extract found to be 4 and its specific hemolytic activity was estimated to be 2.6 HT/mg of protein. For Acetone extract found to be 6 and its specific hemolytic activity was 2.0 HT/mg of protein, for chloroform extract found to be 2 and its specific hemolytic activity was 5.2 HT/mg of protein. All the extractions were significantly showed hemolytic activity against chick, goat and human blood samples. In the present study it was found that P.C mucus venom showed a very strong hemolytic activity when compared to PC spine venom.

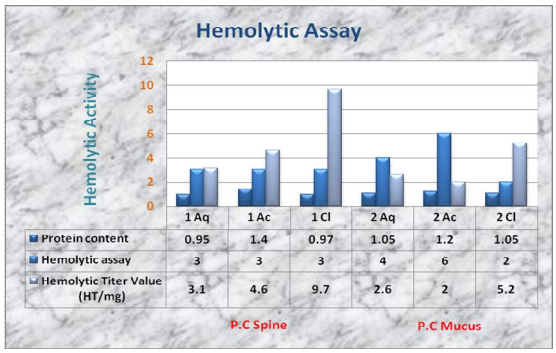


Fig. 6 P.C. Spine & Mucus of human

lleal Loop Assay

The minimal and maximal dose of both extracts developed inflammation and accumulation of fluid. But the intensity of inflammation was less in 25µg/ml of both the extracts and high fluid accumulation was observed in higher dose of 100µg/ml. The intestinal loop assay found the toxicity levels in mammalian tissues. It was identified by the accumulation of fluid in the intestinal loop. The more fluid in the loop leads to more toxicity to the tissues. The fluid secretion was observed which may be due to the effect of toxic protein present in the extracts. These results indicated that presence of toxic substances in the extracts and the intensity of low dose can be used in therapeutic purpose

Table 3 P.C. Spine & Mucus of chick

S. No	Sample	Types of Extract	Results
1	P.C Spine	Aqueous	-
2		Acetone	+
3		Chloroform	++
4	P.C Mucus	Aqueous	-
5		Acetone	-
6		Chloroform	+

++ = More accumulation of fluid
+ = Less Accumulation of Fluid
- = No Accumulation of Fluid

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

The DPPH reaction with both venom samples showed a significant colour change pattern. This result confirmed that the presence of antioxidant molecule in the sample.

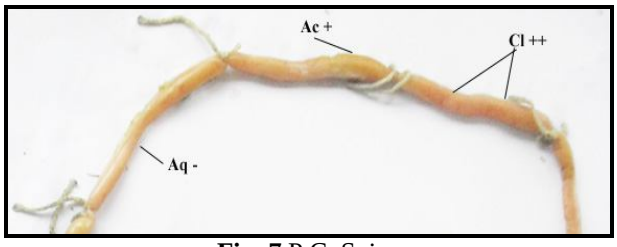


Fig. 7 P.C. Spine

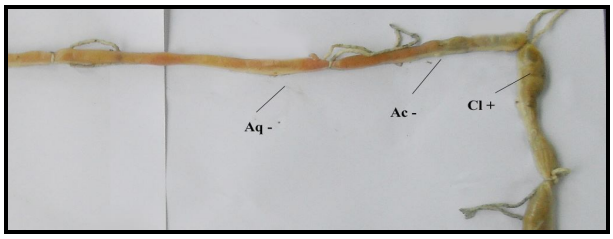


Fig. 8 P.C. Mucus

DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical DPPH to the yellow coloured 1-1diphenyl 2picrylhydrazine. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction.

SDS – PAGE

Electrophorectic separations of proteins were showed significant banding patterns in 12% SDS – PAGE. Fig shows the banding pattern of both spine and venom samples. It demonstrated the presence of some toxic protein in the sample. The range of protein was predicted about 7 – 21 kDa. All the extraction of both venoms yielded very clear distinct bands in the gel. Numbers of bands were high in mucus extraction when compared to spine samples. All the extraction of mucus sample showed a similar banding pattern. The band around 14 to 21 KDa were intensely observed in both mucus and spine samples.

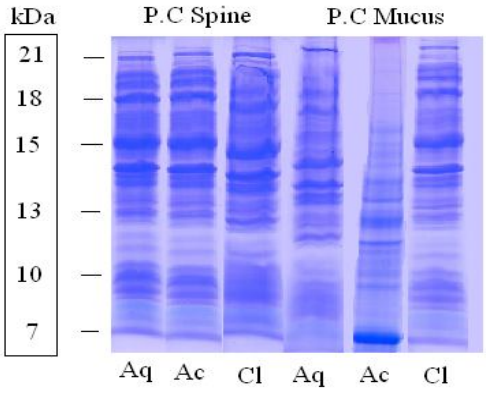


Fig. 9 SDS-PAGE of P.C. Spine & Mucus

DISCUSSION

Discoveries of toxins from venoms, for the most part from marine resources that are racing ahead because of their extremely complex and notable action on various mammalian physiological systems (G. Sivan *et al.,* 2007). Toxic proteins serve in a number of adaptive roles such as immobilizing paralyzing, killing, liquefying competitions. Other venom proteins may act synergistically by enhancing the activity or spreading of toxins (Garnier *et al.,* 1965).

In the usual course of events, epidermal toxins, spine venom and poisonous fishes are produced by protein elaborating cells (Al –hassan *et al.,* 1982). Thus the epidermal secretions contain a mixture of highly active biochemical and pharmacological components that are different from typical fish mucus and spine, which is composed of catfish proteinaceous secretions (Al-hassan *et al.,* 1985). Thus knowing importance of toxins and venom in this present study was carried out to evaluate the bioactive properties of catfish *Plotosus canius* spine venom and epidermal mucus. The present investigation of spine venom and mucus of *Plotosus canius* were collected from Annankoil, South east coast of India while this species was identified by Hamilton, 1822. The three extracts of spine and mucus venom were purified by DEAE - anion exchange chromatography. In purified spine venom of catfish (*Plotosus canius*), aqueous extract, acetone extract and chloroform extract yielded 1.06 g, 1.00g and 0.98 g of mucus extract whereas the total amount yielded mucus from 2 kg fish.

In case of epidermal mucus, aqueous extract yielded 1.02 g of mucus extract, and acetone extract yielded 1.25g in mucus extract. Corresspondingly, chloroform extract yielded the total amount of 2.25g of mucus extract from 2kg of fish. Poh *et al.,* 1991 had also done the same extraction in stone fish *Synanceja horrida.* SNTX was purified from crude venom by atleast two step procedure on Sephacryl S-200 high resolution gel – permeation and DEAE bio gel an anion - exchange chromatography.

The catfish has proteinaceous secretory cells in its epidermis (Al-Hassan *et al.,* 1982). In the present study, the protein content of spine venom of *Plotosus canius* was found to be 0.95mg/ml in aqueous extract, 1.40mg/ml in acetone extract and similarly 0.97mg/ml in chloroform extract. In case of epidermal mucus, the protein content was found to be 1.05mg/ml in aqueous extract, 1.20mg/ml in acetone extract and similarly 1.05 mg/ml in chloroform extract. Similarly, the protein concentration of *Synanceja horrida* were estimated in the native and modified SNTX with a concentration of 1 mg/ml which showed an absorbance at 280 nm by (Chen *et al.,* 1997).

The results of hemolytic assay showed good activity in blood samples. The crude extracts of spine venom *Plotosus canius* showed maximum hemolytic titre values (3.3HU) recorded in chick blood and minimum value (1.1HU) was recorded in human blood. In case of epidermal mucus of *Plotosus canius,* crude extracts showed maximum hemolytic unit (3.3 HU) recorded in human blood and minimum titre values (2.0 HU) recorded

in chick blood. Hemolysis of human and sheep red blood cells was studied by Al- Hassan *et al.,* 1982. In earlier study reported by (Al –ladhan *et al.*. 1987), they studied the specific activity of catfish epidermal factor of 20.6 mg^{-1} protein. The Illeal loop assay showed positive results with inflammation and fluid accumulation in both spine and mucus extracts which may due to the effect of toxic protein present in above mentioned extracts. These results indicated that presence of toxic substances in the extracts as observed from previous study (Bose, 2010).

The DPPH reaction of the spine and mucus extracts sample changed the colour from light green to pale yellow in 96-titre wells which indicated the presence of antioxidant molecule in the extracts. DPPH is a useful reagent for investigating the free radical – scavenging activities of compounds. In the DPPH test, spine and mucus extracts were able to reduce the stable radical DPPH to the yellow coloured 1-1diphenyl 2-picrylyhydrazine.This method is based on reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to formation of non – radical from DPPH reaction (Shon *et al.,* 2003). Both the spine and mucus sample exhibited antioxidant property which was indicated by appearance of yellow colour. Similiary, Babd *et a., l* (2008) observed the antioxidant property in fish mucus.

While the SDS –PAGE on 12% gel, the spine venom and mucus of *Plotosus canius* both yielded distinct bands in gel. All the extraction of both venoms yielded clear distinct bands. During SDS –PAGE analysis, the present study revealed medium sized proteins in both crude venom extracts protein sample. Number of bands appeared on gel were high in mucus extraction compared to spine venom samples. In comparison of crude venom from various extracts, acetone extract showed a higher number of bands in range of 14 KDa when compared to other solvents. Prominent bands indicated proteins of 109.9, 52.3, 48.5, 28.2 and 12.4KDa to be common in the protein toxin which have been reported from a number of marine species. These results assume significance considering the presence of bands at 14 KDa. The experiment again proved from the previous study on the stone fish venom containing SNTX having proteins molecular weight (57KDa) as characterized by Chen *et al.,* (1997).

CONCLUSION

Hitherto from by gone few decades, marine products research ceded a alluring insight into the structural intricacy of secondary constituents produced by marine toxic fishes which are mostly for defensive incentive. Bioprospecting for potential new drugs has been and continues to be the leading force behind the efforts of marine natural products researchers to tap the fascinating chemical diversity encountered in the sea. With the advance of molecular biology, target oriented screens have become available that will accelerate the hit rate in the quest for drugs from the sea. The present study gives us information about biochemical and toxicological characters of catfish *Plotosus canius* spine venom and mucus. In general, both spine venom and mucus are proteineous in nature with different enzymic as well as toxicity properties The bioactivity of the sample differs because of extreme liability of fish venom toxins even their biological activity is lost during storage. There results also suggest that the cause serve envenomation upon introduced into an open skin not only responsible by spine venom but also accompanied by mucus secretion that enters wound at the time sting increase virulence effect, thus these biochemical properties of catfish *Plotosus canius* venom may pave a way for the isolation of specific protein or novel compounds and or development of new therapeutic strategies complementary to conventional therapy. Further detailed studies could be made on purification and characterization of the venom into several active components which may lead to the discovery of new potent antimicrobial drugs in future.

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