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ORIGINAL RESEARCH ARTICLE

Biologically Active Principles of the Seer Fish *Scomberomorus commerson* (Lacepede, 1800) Epidermal Mucus

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ABSTRACT

The present investigation, the *Scomberomorus commerson* mucus were collected from Parangipettai south east coast of India. The purified ethanol extract of *Scomberomorus commerson* mucus yielded a total amount of 4.9g from 2 kg of fish and chloroform extract yielded the total amount 5.9g.The protein content in crude mucus extract was found to be 2.4 mg/ml in ethanol extract and 2.8 mg/ml in chloroform extract. The ethanol and chloroform extract of mucus showed hemolytic activity on chicken blood were 12 HT/mg and 9HT/mg of protein respectively. The antibacterial assay were studied on ethanol and chloroform extract over 5 bacterial species among these the crude ethanol extract inhibit the growth of *Vibrio cholera* whereas in the chloroform extract shows a clear inhibition zone against *Pseudomoynas sp.*The results of antifungal indicate that the crude extract has inhibited the fungal colonies. The DPPH reaction on the both extracts changed the colour which indicated the presence of antioxidant molecule supporting the thin layer chromatography result.The SDS – PAGE on 12% gel, the crude protein toxins yielded distinct 5 bands in ethanol extracts and 8 bands in chloroform extract ranging from 7.8 to 116 KDa with three well defined bands 109.9, 28.2 and 12.4KDa in the both extracts.

Key words: Seerfish**,** *Scomberomorus commerson*, Hemolytic activity, antimicrobial properties, SDS-PAGE.

INTRODUCTION

Marine environment has been source of unique biochemical compounds with potential for many bioactive natural products exhibiting tremendous structural and chemical features. Marine organisms produce chemical substance for reproduction, communication, protection against predation and competition. These compounds exhibit antitumor, anti-inflammatory and antiviral activities in the medical field. In recent years, significant number of novel metabolites with potent pharmacological properties has been discovered from the marine organisms. Many bioactive compounds extracted from various marine animals like sponges, tunicates, soft corals, seahorse, nudibranchs, bryozoans, sea slugs, Conus and other marine organisms .More than 200 species of marine fishes, including stingrays, weever –fish, stonefish and others known to be venomous [1].

In the less derived vertebrates such as fish, the specific immunity including antibody and specific cell-mediated responses are significantly less diverse than those of higher vertebrates [2] .The specific immune mechanisms in fish are slow and limited by temperature constrains on their metabolism. Therefore, fish are likely to rely highly on their innate immune mechanisms for protection against invading pathogens. In fish the epidermal mucus is considered a key component of innate immunity. The epidermal mucus is produced primarily by epidermal goblet or mucus cells and is composed mainly water and gel-forming macromolecules including mucins and other glycoprotein [2, 3]. The mucus layer on the fish surface performs a number of function including diseases resistance, respiration, ionic and osmotic regulation, locomotion, reproduction, communication, feeding and nest building [3, 4]. The antimicrobial property of crude epidermal mucus against infectious pathogens was initially demonstrated in rainbow trout *Oncorhynchus mykiss* [5]. The removal mucus in *Plecoglossus altivelis* and *Scophthalmus maximus* after

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challenging them with *Listonella anguillarum* resulted in increased mortiality [6].

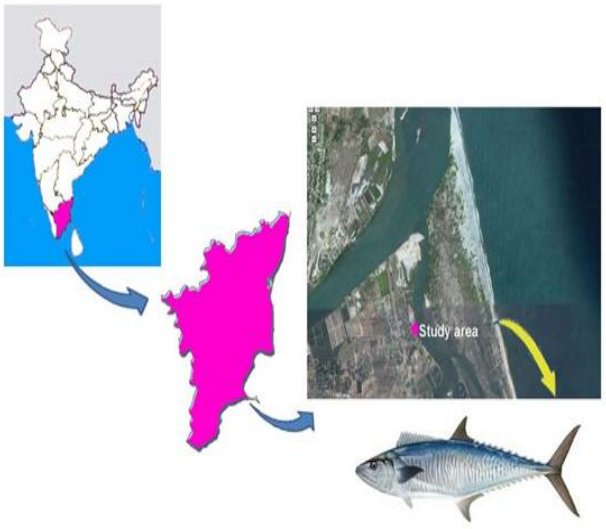
Many cytolytic toxins isolated from fish mucus show various enzymatic activities, such as phospholipase, phospholipase C and phingomyclinase.They lyses cells directly or make cells more susceptible to damage by hydrolyzing membrane lipids through enzymatic action. Bioprospecting for potential new drugs continues to be the leading force behind the efforts of marine natural products 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 biologically active principles of the seer fish mucus *Scomberomorus commerson* were collected from Parangipettai coast, south east coast of India.

MATERIALS AND METHODS

Collection and processing of sample:

The live sample of *Scomberomorus commerson* was collected from Parangipettai (Lat 11° 29 n: Long 79° 46 E), 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 [7]. Further, mucus was 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 g x15 minute pellets were collected, re-extracted with extraction buffer (0.005m sodium phosphate buffer pH 7.5 containing 0.14 NaCl) centrifuged as before and the supernatant was subsequently called mucus respectively.

DESCRIPTION OF THE STUDY AREA



Systematic position

Phylum	:	Chordata
Sub-phylum	:	Vertebrata
Class	:	Actinopterygii
Sub-class	:	Neopterygii
Order	:	Perciformes
Family	:	Scombridae
Genus	:	*Scomberomorus*
Species	:	*commerson*

Extraction of venom

Ethanol extraction:

The Ethanol extract of *Scomberomorus commerson* mucus sample *was* prepared by dissolving the mucus in ethanol. 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.

Chloroform Extraction:

Crude toxin was extracted following the method. For Chloroform extraction *Scomberomorus commerson*, 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 *Scomberomorus commerson* was carried out using DEAE Cellulose Anion Exchange chromatography according to the procedure of Stempion *et al.*[8] (1970).

Protein estimation:

Protein content from crude extracts was estimated by Lowry *et al*. [9].

Microbial Strains Used: Antibacterial effect of *Scomberomorus commerson*, mucus was determined against 5 different bacterial strains viz. *Pseudomonas* sp, *Streptococcus aureus, Vibrio cholerae, Vibro parahaemlyticus, E.coli* and similarly Antifungal effect was determined against 3 different fungal strains viz. *A.flavus, A.niger, Candida albicans.* 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.

Scomberomorus commerson, 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 *Scomberomorus commerson*, were assayed on chick blood followed by the method of Pani Prasad and Venkateshwaran. [10].

DPPH Radical Scavenging Assay:

The scavenging effects of samples for DPPH radical were monitored according to the method.[11] .

SDS-PAGE: crude extracts were analyzed by SDS –PAGE 12%, topped by 7% stacking gel according to [12] .

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:

Ethanol extracts yield a total amount of 4.9g of crude extract from 2kg of fish. Similarly, chloroform extract yield a total amount of 5.9 g of crude extract.

Table 1: Crude extract

S. No	Samples	Extract	Amount of crude extract(g)
1	*Scomberomorus commerson*	Ethanol	4.9
2		Chloroform	5.9

Protein estimation

The protein content in crude extracts of ethanol mucus sample was found to be 2.4 mg/ml and 2.8mg/ml in chloroform extract.

Table 2: Protein Estimation

S. No	Samples	Extract	Protein estimation (mg/ml)
1	*Scomberomorus commerson*	Ethanol	4.9
2		Chloroform	5.9

Antibacterial Activity:

The crude of Ethanol, and Chloroform extracts were tested against 5 species of bacteria viz. *Pseudomonas* sp, *Streptococcus aureus, Vibrio cholerae, Vibrio parahaemolyticus, E.coli.*

Table 3: Antibacterial activities

S. No	Organism	*Scomberomorus commerson* (Mucus) Zone of Incubation (mm)	
		Ethanol	chloroform
1	*Pseudomona sp*	5mm	6mm
2	*Staphylococcus sp*	4.5mm	5mm
3	*Vibrio cholera*	4mm	5mm
4	*Vibrio parahaemlyticus*	4mm	6mm
5	*E.coli*	6mm	7mm

Antifungal Activity:

The crude of Ethanol, and Chloroform extracts were tested against 3 species of fungai viz. *A.flavus, A.niger, Candida albicans,*

Table 4: Antifungal activities

S. No	Organism	*Scomberomorus commerson*(Mucus) Zone of Incubation (mm)	
		Ethanol	Chloroform
1	*A.niger*	7mm	7mm
2	*A.flavus*	4mm	5mm
3	*Candida albicans*	6mm	7mm

Hemolytic Assay

The results of the hemolytic assay on chick, blood sample erythrocyte were done using crude ethanol and chloroform solvents. The results were shown in table 5 The crude extracts in mucus venom induced hemolytic activity in chick blood sample**.** The hemolytic titer in case of ethanol extract was found to be 14 and its specific hemolytic activity was estimated to be 12 HT/mg of protein. Similarly In case of chloroform extract found to be 10 and its specific hemolytic activity was 9 HT/mg of protein. In the present study it was found that mucus *Scomberomorus commerson* venom showed a very strong hemolytic activity on both extracts.

Table 5: Hemolytic assay of *Scomberomorus commerson* in Chick Blood

S. No	Sample	Extract	Protein content	Hemolytic assay	Hemolytic Titer Value (HT/mg)	Hemolytic Value (%)
1	*Scomberomorus commerson*	Ethanol	2.4	12	14	2.80
2		Chloroform	2.8	9	10	3.11

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

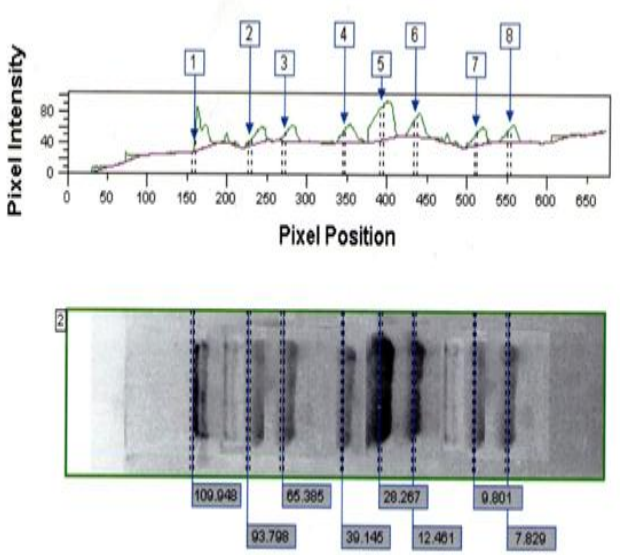
The DPPH reaction with both venom samples showed a significant colour change pattern. This result confirmed that the presence of antioxidant molecule in the sample. DPPH is a useful reagent for investigating the free radical-scavenging activities of compounds. In the DPPH test, the extracts were able to reduce the stable radical DPPH to the yellow colored 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. Fig1 shows the banding pattern of mucus samples. It demonstrated the presence of some toxic protein in the sample, the crude protein toxins yielded 5 bands in ethanol extract and 8 bands in chloroform extract *Scomberomorus commerson* of ranging from 7.8 to 116 KDa with three well defined bands 109.9, 28.2 and 12.4KDa in the both extracts.

Fig 1: SDS-PAGE showing various bands in ethanol and chloroform extracts



DISCUSSION

The present investigation of the mucus *Scomberomorus commerson* were collected from Parangipettai, south east coast of India and this species was identified based on the morphological characters given by Lacepede, 1800.The two extracts were purified by DEAE Anion exchange chromatography. The purified ethanol extract of *Scomberomorus commerson* yielded a total amount of 4.9g mucus extract from 2 kg of fish. Similarly extract yielded the total amount 5.9g of mucus extract. [13] Also done in the stone fish *Synanceja 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 anion – exchange chromatography.

In the present study protein content in crude mucus extract of *Scomberomorus commerson* was found to be 2.4 mg/ml in ethanol extract and 2.8 mg/ml in chloroform extract. Similarly the protein concentration of *Synanceja horrida* were estimated the native and modified SNTX with a concentration of 1 mg/ml showed an absorbance at 280 nm [11].

The crude chloroform extract induced pronounced hemolytic activity on chicken blood. The hemolytic titre in case of chloroform extract found to be 10 and its specific hemolytic activity was estimated to be 9 HT/mg of the protein. The hemolytic titre of ethanol extract of fish mucus was found to be 12 HT/mg of protein. Thus result of the present study indicate a very hemolytic activity of both chloroform and ethanol extracts of *Scomberomous commerson* it is well known by Chen *et al.* [11] tested *Synanceja horrida* hemolytic activity by pore formation in the cell membrane and to examine the role of colloid – osmotic shock in SNTX-induced hemolysis, and studied the effect of various osmotic protectants on SNTX-induced hemolsis. This approach is based on the concept that colloid – osmotic lysis can be suppressed by an osmatic protectant of appropriate size which, being too large to penetrate the induced membrane pores, is capable of balancing the osmotic drag of intercellar impermeant solutes such as hamologin and organic phosphates .The results presented here with clearly demonstrate the presence of hemolytic factors in *P.berghei*, which can lysis normal rat and sheep erythrocytes. The lytic factor could be extracted with aqueous medium and hemolytic action was a temperature dependent process. The observations substantiate the early reports of Fife *et al.* [14] on the presence of a similar factor in *P. knowlesi*.

The first attempt to locate antimicrobial activity marine organisms was initiated around the 1950 Brukholder and Burkholder. [15]. Antimicrobial activity (inhibition of bacterial growth by chemical reaction in mucus) was tested using *Scomberomorus commerson* mucus samples by testing different bacterial strains, dilute samples to the same protein content and monitoring growth of bacterial strains, Growth curves of different bacteria were measured against *Scomberomorus commerson* mucus samples for the crude ethanol and chloroform extract were tested against 5species of bacteria viz. *Pseudomonas sp., Streptococcus aureus, Vibro parahaemolyticus, Vibro cholera and E.coli.* Growth curves of different fungus were measured against *Scomberomorus commerson* mucus samples for the crude of ethanol and chloroform extract were tested against 3 species of fungal strains *viz. A.flavus, A.niger and Candida albicans.* The results shown that the crude ethanol extract inhibit the growth of *Vibrio cholera* whereas in the chloroform extract shows a clear inhibition zone against *Pseudomonas sp.* Burkholder. [15] Isolated two bromo compounds from *Verongi fistularies* and *V.vauliformis* that inhibited the growth of gram positive and gram negative bacteria. There is no obvious correlation between incidences of antibacterial activity and

latitudinal occurrence, systemic group or growth from the fish mucus. However, the present results indicate that the crude extract has inhibited the bacterial and fungal colonies. The DPPH reaction of the both extracts sample changed the color 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, mucus extracts were able to reduce the stable radical DPPH to the yellow colored 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. Both the extracts sample exhibited antioxidant property which was indicated by appearance of yellow color.

While the SDS –PAGE on 12% gel, the crude protein toxins yielded distinct 5 bands in ethanol extracts and 8 bands in chloroform extract *Scomberomous commerson* of ranging from 7.8 to 116 KDa with three well defined bands 109.9, 28.2 and 12.4KDa in the both extracts gel. During SDS –PAGE analysis, the present study revealed medium sized proteins in both crude venom extracts protein sample. Prominent bands indicated proteins of 109.9, 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 28.2 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.* [11] . Though,the present study made on hemolytic ,antibacterial, antifungal activity of *Scomberomorous commerson* mucus, provided baseline information on their pharmacological potential. It could be inferred from present investigation that the *Scomberomorus commerson* mucus 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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