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Chemical composition and biological activity of fish epidermal mucus from *Catla catla*

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Abstract

The fish mucus was extracted from *C. catla* by using aqueous solution and the chemical composition showed 27.93% of protein, 5.9% of carbohydrate and 0.35% of lipids. The antibacterial activity of the mucus extract showed 17, 11, 14, 16, 11, 18, 12 and 13mm zone of inhibition at the highest concentration of 100µg/ml against *S. aureus, K. pneumoniae, S. typhi, V. cholerae, E. coli, V. parahaemolyticus* and *S. pyogenes*. The fish mucus observed MIC values of 100µg/ml against *E. coli* and the *V. cholerae* slightly arrested at the above concentration. The fish mucus showed the MBC values of 100µg/ml against *E. coli* and the *V. cholerae* slightly arrested at the same concentration. The anticancer activity of the fish mucus recorded 14.37 – 51.09% at 50 – 250µg/ml concentration against A549 cell line. Hence, above antibacterial and anticancer activity of the mucus recommended as good source of biological agent in future pharmacological industry.

Keywords: Mucus; Biochemical; Antibacterial activity; MIC; MBC; Anticancer.

Introduction

Fishes are a highly diverse group of animals and comprise half of the vertebrate species in existence today [1]. Approximately, 20 million metric tons of fish wastes are discarded annually from the world fisheries [2]. The fish by-products are rich in proteins, minerals, enzymes, pigments or flavors [3]. Among the fish by-products in external skin produced mucus and the composition and rate of mucus secretion vary from species to species. The mucus gel matrix is mostly comprised of O-glycosylated proteins (GPs) called mucins, but it also contains other molecules such as proteins (structural proteins, immune-related proteins, and antimicrobial peptides and proteins) and lipids [4, 5].

Further, the fish mucus is considered as a valuable biological protein and has been reported that contains antimicrobial proteins. These antimicrobial proteins contained fish mucus was extracted used for different solvent methods such as aqueous, ethanol, dichloromethane and acidic acid [6]. Besides, the external fish mucus provides nonlethal alternatives for the early detection of infections [7]. Nowadays fish mucus research has been increased mainly due to the discovery of various bioactive molecules (antibacterial, antiviral and antifungal) and their potential application in human medicine and in aquaculture [8, 10].

In recent years, many investigators have been isolated the antimicrobial peptide (AMPs) in fish mucus, many of the fish species active against several human and fish pathogenic microbes [11]. The antibacterial activity in fish mucus has been demonstrated in several fish species; therefore the activity seems to differ from species to species and can be specific to the chemical variation. Furthermore, some studies have also shown cytotoxic activities of external fish mucus against specific cancer cell lines, indicating the potential of fish mucus in the development of new pharmacological antitumoral strategies [12]. So, the field of fish mucus research is growing rapidly to development of new biological activities of

fish mucus in mind, in the present study to investigate the chemical composition and biological activities such as antibacterial and anticancer fish epidermal mucus of *C. catla*.

Materials and Methods

Collection of mucus from fish

The fish *C. catla* was collected from Mettur dam, Tamil Nadu, India. Immediately, the collected fish was starved for 24 hours prior to mucus collection and then kept out of water in specimen tray for 1 hour. After one hour mucus was secreted on the epidermal surface of the body of fish was collected as sample. Mucus was carefully scraped from the dorsal body surface using a sterile spatula. Mucus was not collected in the ventral side to avoid intestinal contamination. The collected fish mucus was stored at 4 ˚C for further use to avoid bacterial growth and protein degradation

Preparation of mucus extract

The aqueous extraction was prepared from the previously preserved mucus as described [13]. To prepare aqueous mucus extract, collected mucus was thoroughly mixed with equal quantity of sterilized physiological saline (0.85% NaCl) and centrifuged at 5000 rpm for 5 minutes. The supernatant was collected and stored at 4 ˚C for further use.

Chemical composition analysis

Protein, Carbohydrate and Lipid estimation

The total protein was estimated using Bradford method [14]. The total carbohydrate was estimated by following the phenol - sulfuric acid method [15]. The extraction of lipid was done by the chloroforms- methanol mixture [16].

Microbial cultures

Ten strains of bacteria were used as test organisms. The bacterial strains included Gram positive strains (*Staphylococcus aureus* and *Streptococcus pyrogus*) and Gram –

negative strains (*Salmonella typhi, Klebsiella pneumoniae, Vibrio cholerae, Klebsiella oxytoca, Escherichia coli, Salmonella paratyphi, Vibrio parahaemolyticus* and *Proteus mirabilis*). All the bacterial strains were clinical isolates, obtained from Raja Muthyiah Medical College Hospital, Annamalai University, Tamil Nadu, India.

Inoculums Preparation

Nutrient broth was prepared in test tubes and autoclaved at 15 lbs pressure for 15 mins. All the bacterial and fungal strains were individually inoculated in the sterilized nutrient broth and incubated at 37^{o} Cfor 24 h.

Antibacterial activity

The antibacterial activity was evaluated using agar well diffusion according to the method Seedevi *et al.* [17]. The 24 h old cultures were swabbed in nutrient agar plates by using a sterile cotton swab aseptically. The wells were punched on swabbed plates using a sterile 5mm well cutter. The stock solution was prepared at 10mg/ml concentration in 10% DMSO. The mucus extract used four different concentrations such as 25, 50, 75 and 100µg/ml. The standards tetracycline (1mg/ml dissolved in 10% DMSO) and control (10% DMSO) were loaded into the respectively labeled wells. The plates were incubated at 37^{o}C for 24 h, the results were obtained by measuring the diameter of inhibition zone for each well and expressed in millimeter.

Minimum inhibitory concentration (MIC)

The mucus extract used for the determination of MIC following the method of Seedevi *et al.* [17]. A stock solution of 1mg/ml was prepared and was serially diluted to obtain various ranges of concentrations between 20 - 100µg/ml. 0.5 ml of each of the dilutions containing 2.0 ml of nutrient broth were taken in test tube and to each of which 0.5 ml of old bacterial culture was inoculated. The test tubes containing broth alone was used as

control. All test tubes and control were incubated at 37°C for 24h. After the period of incubation, the tube containing the least concentration of extract showing no visible sign of growth was taken as the minimum inhibitory concentration.

Minimum bactericidal concentration (MBC)

MBC was characterized following the above MIC assay by spreading 5µl of sample on nutrient agar plates and then incubated at 37 ◦C for 18–24 h and the MBC values were noted [17].

Anticancer activity

The anticancer activity of the fish mucus was examine against lung carcinoma (A549) cell line by using MTT assay as described by Seedevi *et al.* [17]. Vero cells were seeded (3×10^4/well) in 96-well plates in 100µl of growth medium (MEM) containing 10% FCS mixture in each well incubated at 37°C in a 5% CO_2 incubator. After 24 h of monolayer cell cultivation, the medium was removed and replaced by a 100µl of varying concentrations (25 - 250µg/ml) of the sample in MEM medium containing 2% FCS in respective wells. Control cells were maintained in MEM medium containing 2% FCS incubated at 37°C in a 5% CO_2. After 72 h of incubation, 20µl of MTT (5mg/ml) in PBS solution/well were added and incubated at the above said condition for 4 h, and then were observed for the crystal formation. The medium was replaced by 100µl of DMSO solution in each well and the Optical Density (OD) of each well was measured by using an Elisa reader at 620 nm.

Results and Discussion

Yield and Chemical composition of mucus extract

The yield of the fish mucus from *C. catla* was 30.2% and the chemical composition was recorded 27.93% of protein, 5.9% of carbohydrate and 0.35% of lipids respectively (Fig.1).

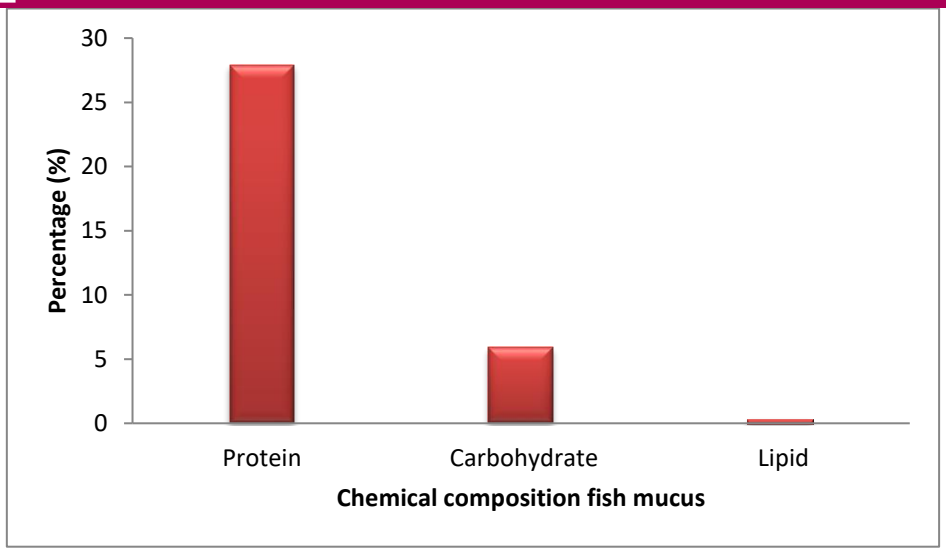

Fig. 1. Chemical composition of fish mucus extract from *C. catla*

Antibacterial activity of mucus extract

The fish mucus was screened for the antibacterial activity against ten clinically isolated human bacterial strains. The fish mucus presented antibacterial activity against 8 bacterial strains out of 10 bacterial strains. The fish mucus showed the antibacterial activity of 17, 11, 14, 16, 11, 18, 12 and 13mm zone of inhibition at the highest concentration of 100µg/ml at the highest concentration of 100µg/ml against *S. aureus, K. pneumoniae, S. typhi, V. cholerae, E. coli, V. parahaemolyticus* and *S. pyogenes* respectively. Whereas, the *K. oxytoca* and *P. mirabilis* strains not inhibited at the above concentration (Table 1 and Fig. 2). The fish mucus showed maximum inhibition zone of 18mm was recorded against *E. coli* and minimum of 11mm inhibition zone was recorded against *K. oxytoca* and *P. mirabilis* at 100µg/ml concentration.

Table 1. Antibacterial activity of the mucus extract from *C. catla* against human pathogen

S. No	Name of the strains	Zone of inhibition (mm)

		25µg/ml	50µg/ml	75µg/ml	100µg/ml	+ve	-ve
1	*S. aureus*	12	13	15	17	25	Nil
2	*K. pneumoniae*	8	9	10	11	23	Nil
3	*S. typhi*	11	12	13	14	24	Nil
4	*V. cholerae*	13	14	15	16	25	Nil
5	*K. oxytoca*	-	-	-	-	21	Nil
6	*S. paratyphi*	-	8	10	11	26	Nil
7	*E. coli*	13	14	16	18	24	Nil
8	*P. mirabilis*	-	-	-	-	23	Nil
9	*V. parahaemolyticus*	8	9	11	12	20	Nil
10	*S. pyogenes*	8	10	12	13	21	Nil

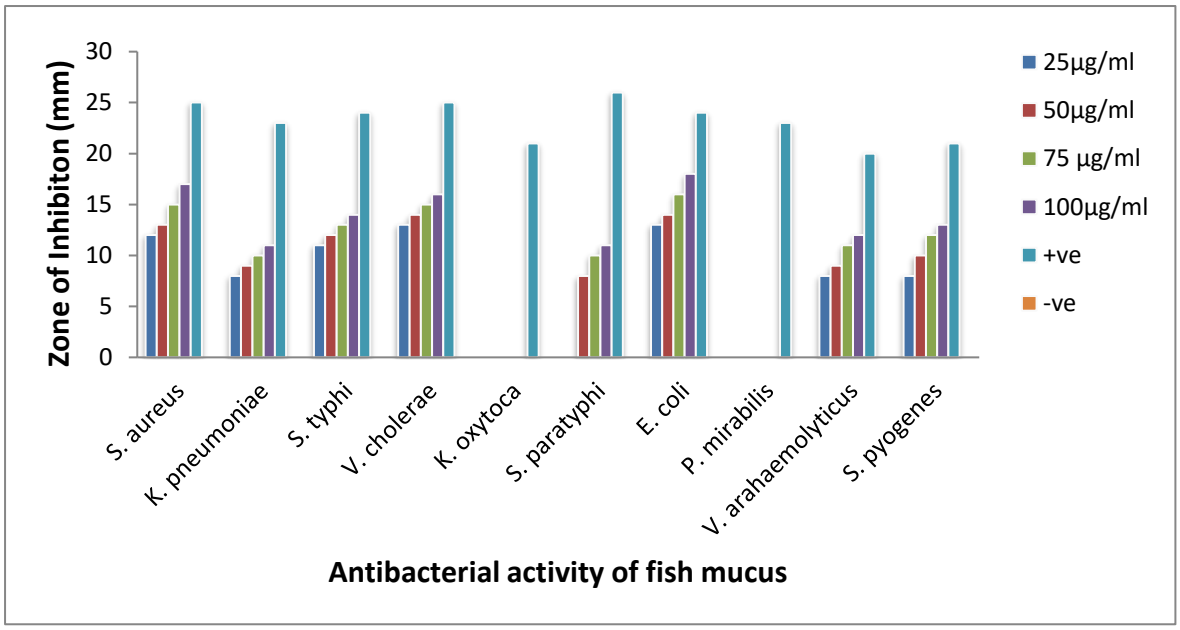

Fig. 2. Antibacterial activity of mucus extract from *C. catla* against human pathogen

Minimum inhibitory concentration of mucus extract

The fish mucus exposed the MIC values of 80µg/ml against *S. aureus* and *E. coli* followed the *V. cholera* was arrested at 100µg/ml concentration. Whereas, the, *K. pneumoniae*, *S. typhi*, *K. oxytoca*, *S. paratyphi*, *P. mirabilis*, *V. parahaemolyticus* and *S. pyogenes* not arrest at any concentration (Table 2).

Table 2. MIC of the mucus extract from *C. catla* against human pathogen

S. No	Name of the strains	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	+ve	-ve

1	*S. aureus*	+++	++	++	*	-	-	+++
2	*K. pneumoniae*	+++	+++	+++	+++	++	-	+++
3	*S. typhi*	+++	+++	+++	++	+	-	+++
4	*V. cholerae*	+++	+++	++	+	*	-	+++
5	*K. oxytoca*	+++	+++	+++	+++	+++	-	+++
6	*S. paratyphi*	+++	+++	+++	+++	++	-	+++
7	*E. coli*	+++	++	+	*	-	-	+++
8	*P. mirabilis*	+++	+++	+++	+++	+++	-	+++
9	*V. parahaemolyticus*	+++	+++	+++	+++	++	-	+++
10	*S. pyogenes*	+++	+++	+++	++	+	-	+++

- MIC concentration; - No growth; * - considerably arrest; + - Cloudy solution; ++ - Turbid solution; +++ - Highly turbid solution.

Minimum bactericidal concentration of mucus extract

The fish mucus revealed the MBC values of 80µg/ml against *S. aureus* and *E. coli* followed the *V. cholera* was arrested at 100µg/ml concentration. Whereas, the, *K. pneumoniae*, *S. typhi*, *K. oxytoca*, *S. paratyphi*, *P. mirabilis*, *V. parahaemolyticus* and *S. pyogenes* not arrest at any concentration (Table 3).

Table 3. MBC of the mucus extract from *C. catla* against human pathogen

S. No	Name of the strains	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
1	*S. aureus*	+++	+++	+++	++	+
2	*K. pneumoniae*	+++	+++	+++	+++	++
3	*S. typhi*	+++	+++	+++	+++	++
4	*V. cholerae*	+++	++	++	+	*
5	*K. oxytoca*	+++	+++	+++	+++	+++
6	*S. paratyphi*	+++	+++	+++	+++	+++
7	*E. coli*	+++	++	+	*	-
8	*P. mirabilis*	+++	+++	+++	+++	+++
9	*V. parahaemolyticus*	+++	+++	+++	+++	++
10	*S. pyogenes*	+++	+++	+++	+++	++

- MBC concentration; - No growth; * - considerably arrest; + - Cloudy solution; ++ - Turbid solution; +++ - Highly turbid solution.

Anticancer activity

In the present study, the anticancer activity of fish mucus from *C. catla* recorded 14.37 – 51.09% at 50 – 250µg/ml concentration against A549 cell line (Fig. 3). The highest cancer inhibition was recoreded 51.09% at 250µg/ml concentration. The anticancer activity of the fish mucus exposed dose depended activity; whereas in increasing concentration of fish mucus, the activity level also increased.

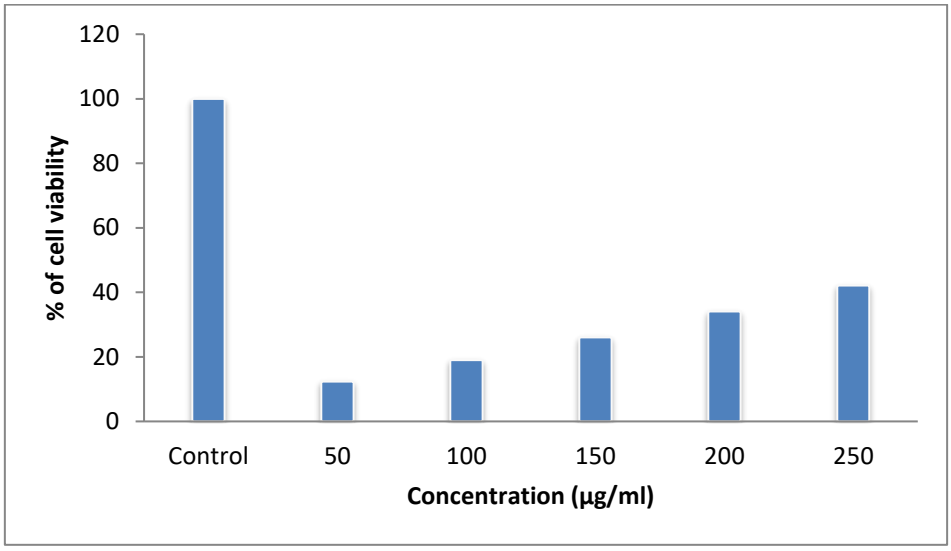

Fig. 3. Anticancer activity of mucus extract from *C. catla*

Discussion

Aquatic organisms are wealthy sources of natural products and many compounds are derived from different aquatic organisms that have produced interest both as challenging problems for structure elucidation and synthesis as well as for their biological activities [18]. Many substances with biomedical potential, has risen towards the aquatic organisms as a source of new drugs [19]. Especially, fish products have various chemical composition and rich in protein, and controlled many infective disease in which the human bacteria. In the present study, the chemical composition of the mucus from *C. catla* recorded 27.93% of protein, 5.9% of carbohydrate and 0.35% of lipids. Similarly, the fish mucus from *Labeo rohita* recorded 27.5% of protein, 5.8% of carbohydrate and 0.21% of lipids [20]. Tyor and

Kumari [13] reported protein as a major component followed by carbohydrate and lipids were the crude mucus extract from *H. nobilis.* The soluble gel of *A. maculates* recorded 12.64µg/g of protein content, 0.08µg/g of carbohydrate content and 0.005µg/g of lipid content [21]. Similar results were observed in freshwater fishes such as *Channa punctatus*, *C. gachua*, *C. carpio* and *A. dussmieri* [22].

The present study, the fish mucus of *C. catla* exposed the maximum antibacterial activity of 17mm and 18mm zone of inhibition at the highest concentration of 100µg/ml against *S. aureus,* and *E. coli*. The present study antibacterial activity of fish mucus showed higher when compared to the previous study of fish mucus from *L. rohita* was 17mm against *E. coli* at 100µg/ml concentration [20]. Similarly, the aqueous fish skin mucus extract showed maximum inhibition of 16.71±1.04 mm, 16.55±1.10mm and 16.03±0.16mm against *S. epidermidis*, *E. coli* and *A. hydrophilla* [23]. Likewise the methanol extract from the whole body of *H. pugilinus* exhibited 0.5mm inhibition zone against *E. coli*, 1mm against *B. subtilis* and 0.5mm against *K. pnemoniae*. Whereas the ethanol extract from the hypobranchial gland of *C. virgineus* exhibited 10mm against *S. typhii*, 6mm against *V. cholerae*, 4mm against *B. subtilis* and minimum activity was recorded against *S. aureus* and in *E. coli* [24]. In the present study mucus of *C. catla* demonstrated the protein content and other derivatives may be responsible for antibacterial activity [20].

The MIC values for the fish mucus recorded 80µg/ml against *S. aureus* and *E. coli* followed the *V. cholera* was arrested at 100µg/ml concentration. In the present study, the result of MIC was higher when compared to that of the fish mucus from *Labeo rohita* observed the MIC values of 100µg/ml against *E. coli* [20]. In addition, the MIC value of acetone extracts from the tissue and egg mass extract of *Chicoreus ramosus* showed 12, 12, 8, 8 and 4 mg/ml and 8, 8, 12, 4 and 4 mg/ml against *A. hydrophilla, S. typhi, S. paratyphi, V. cholerae* and *E. coli* respectively [25]. The methanol, ethanol mixture (1:2) extract from

Phallusia arabica in which the MIC was 0.80mg/ml against *S. aureus* [26]. Likewise, the MIC value of Gaint snakehead, striped snakehead, tilapia and bagrid catfish (*C. nigrodigitatus*) were 11.96µg/ml to 31.91µg/ml against different pathogen [27].

MBC are excellent and comparatively reasonable tools to concurrently assess many antimicrobial agents for effectiveness. The present study, the MBC values for the fish mucus from *C. catla* exposed 80µg/ml against *S. aureus* and *E. coli* followed the *V. cholera* was arrested at 100µg/ml concentration. Similarly, Abreethan et al. reported the MBC value of the fish mucus from *L. rohita* was 100µg/ml against *E. coli*. Many studies have demonstrated similar results about the antimicrobial property of epidermal mucus in variety of fishes Channa punctatus [28], catfish *Arius maculates* [21], hagfish *Myxine glutinosa* [29], and eel fish *Anguilla Anguilla* [12]. Some brook trout mucus extract recorded MBC value as 10 and 273µg/ml against *S. Typhimurium* and *P. aeruginosa* [30]. In addition, the aqueous mucus extract of rainbow trout did no show any activity against any of the bacterial strains tested [31].

The fish mucus from *C. catla* showed the anticancer activity of 14.37 – 51.09% against A549 cell line at 50 – 250µg/ml concentration. Gavamukulya *et al*. [32] reported the anticancer activity of ethanolic extract from the leaves of *Annona muricata* against two human breast cancer cell lines MDA and SKBR3 were 32.9% and 26.7% at 250µg/ml concentration. Jiang *et al.* [33] reported that if compounds can enhance the level of anti-oxidation and remove the reactive oxygen species in cancer cells, they may inhibit the cell growth.

Conclusion

In conclusion, the present study, the fish mucus from *C. catla* had admirable antibacterial activity against clinical pathogens at notable concentration and also the

anticancer activity. Therefore, the antibacterial and anticancer activity of the mucus recommended as good source of biological agent in future pharmacological industry.

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