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Research Article

Fish Epidermal Mucus from *Labeo rohita*: Hemolytic Activity and Antibacterial Investigation Against Human Pathogen

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Abstract

The fish mucus was extracted from *Labeo rohita* by using aqueous solution and the chemical composition showed 27.5% of protein, 5.8% of carbohydrate and 0.21% of lipids. The haemolytic activity of the mucus extract showed 43.47 and 37.73 HT/mg in human blood. Further the antibacterial activity of the mucus extract showed against *S. aureus, K. pneumoniae, S. typhi, V. cholerae, K. oxytoca, E. coli, V. parahaemolyticus* and *S. pyogenes* were 14, 12, 15, 16, 11, 17, 13 and 12mm at the highest concentration of 100µg/ml. 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 above antibacterial activity of the mucus suggested as a source antimicrobial agent in future pharmacological for the development of new antimicrobial drugs.

Keywords: Mucus; Biochemical; Antibacterial Activity; MIC; MBC

Introduction

Fishes are a miscellaneous group of animals in the animal kingdom and comprise nearly half of the vertebrate species in reality nowadays. Nearly 20 million metric tons of fish by-products are discarded yearly from the world fisheries [1]. These fish by-products are rich in proteins, minerals, enzymes, and pigments. The mucus gel matrix is primarily comprised of O-glycosylated proteins (GPs) called mucins, but it also contains a diverse array of other molecules such as proteins, lipids and smaller molecules such as crinotoxins [2]. Among the, epidermal mucus includes diverse bioactive metabolites which play an immense role in defense mechanisms and other significant cellular activities [3].

The fish mucus contains a variety of biologically active compounds such as lysozyme, lectins, proteolytic enzymes, flavoenzymes, immunoglobulins, C-reactive protein, apolipoprotein A-I [4]. Generally, several solvent methods are used to extract fish mucus viz aqueous, ethanol, dichloromethane and acidic acid [5]. Several antimicrobial molecules have been found in fish external mucus including pore-forming glycoproteins, enzymes, proteins and crinotoxins [6,7]. Antibacterial peptides (AMPs), which are one of the main molecules to fight pathogens, have also been observed in fish mucus [8].

Nowadays, fish mucus extract has been reported many biological functions such as antibacterial, antiviral, antifungal, antipara-

sitic, and their potential use in human medicine and in fish farming [9,10]. Further, the fish mucus is considered further valuable and has been reported that contains antimicrobial proteins. In recent years, many investigators have examined the antibacterial properties of skin mucus from many a fish species against several human and fish pathogenic microbes [11,12]. 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. Hence, the present study to evaluate the hemolytic activity and antibacterial activity against human pathogens from the fish epidermal mucus of *Labeo rohita*.

Materials and Methods

Collection of mucus from fish

The fish *L. rohita* was collected from the nearby fish culture pond. 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°C for 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°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].

Results and Discussion

Chemical composition of mucus extract

The chemical composition of the fish mucus recorded 27.5% of protein, 5.8% of carbohydrate and 0.21% of lipids (Figure 1). The chemical composition of the crude mucus extract from *H. nobilis* showed the protein as a major component followed by carbohydrate and lipids [13]. Similarly, 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 [18]. Wei., *et al*. [11] also reported protein content in both crude and aqueous mucus extract of *Channa straitus*. Dhotre., *et al*. [19] also reported the similar biochemical composition of freshwater fishes viz. *Channa punctatus*, *C. gachua*, *C. carpio* and *A. dussmieri*.

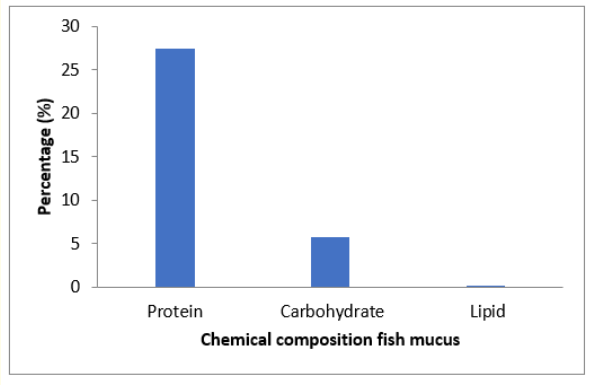

Figure 1: Chemical composition of fish mucus extract from *L. rohita.*

Hemolytic activity of mucus extract

The mucus extract showed the haemolytic activity of 43.47 and 37.73 HT/mg in human blood (Table 1 and 2). The hemolytic activity was differed considerably depending on the type of blood. The crude mucus extracts of *Cynoglossus arel* and *Arius caelatus* showed the haemolytic activity on chicken blood maximum of 64 HU/mg and 8 HU/mg; whereas the goat blood showed maximum of 32 64 HU/mg for crude mucus of *C. arel* and *A. caelatus* [20].

Antibacterial activity of mucus extract

The fish mucus was screened for the antibacterial activity against ten bacterial strains. The fish mucus showed antibacterial activity against *S. aureus, K. pneumoniae, S. typhi, V. cholerae, K. oxytoca, E. coli, V. parahaemolyticus* and *S. pyogenes* were 14, 12, 15, 16, 11, 17, 13 and 12mm at the highest concentration of 100µg/ml. Whereas, the *S. paratyphi* and *P. mirabilis* strains not inhibited at the above concentration (Table 1 and Figure 2). The fish mucus showed maximum inhibition zone of 17mm was recorded against *E. coli* and minimum of 11mm inhibition zone was recorded against *K. oxytoca* at 100µg/ml concentration. 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* [13].

The ethanol extracts of hypobranchial gland of *C. virgineus* showed 10mm of inhibiton zone against *S. typhi*, 7mm (excluding-disc) against *Shigella flexineri*, 6mm against *V. cholerae*, 5mm (excluding disc) against *K. pnemoniae* and 4mm against *B. subtilis* and *E. coli*, but methanol extract exhibited inhibition against *S. pyogenes* only [21]. 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* [22]. In the present study mucus of *L. rohita* demonstrated rich in protein content may be responsible for antibacterial activity.

S. No	Name of the strains	Zone of inhibition (mm)					
		25µg/ml	50µg/ml	75µg/ml	100µg/ml	+ve	-ve
1	*S. aureus*	10	12	13	14	24	Nil
2	*K. pneumoniae*	8	10	11	12	25	Nil
3	*S. typhi*	10	11	13	15	26	Nil
4	*V. cholerae*	12	13	14	16	26	Nil
5	*K. oxytoca*	8	8	9	11	27	Nil
6	*S. paratyphi*	-	-	-	-	27	Nil
7	*E. coli*	12	14	15	17	25	Nil
8	*P. mirabilis*	-	-	-	-	25	Nil
9	*V. parahaemolyticus*	9	10	12	13	23	Nil
10	*S. pyogenes*	8	9	11	12	24	Nil

Table 1: Antibacterial activity of the mucus extract from *L. rohita* against human pathogen.

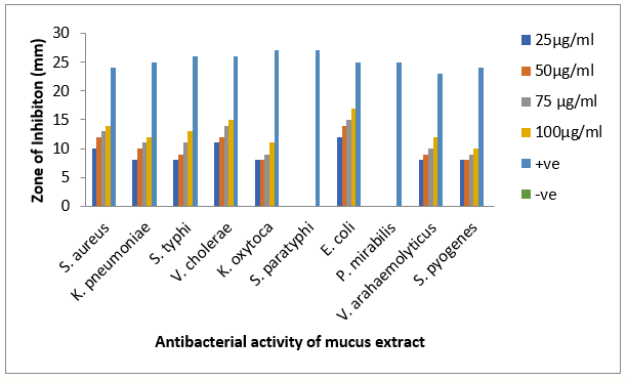

Figure 2: Antibacterial activity of mucus extract from *L. rohita* against human pathogen.

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*	+++	+++	+++	+++	++	-	+++

Table 2: MIC of the mucus extract from *L. rohita* against human pathogen.

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

Minimum inhibitory concentration of mucus extract

The fish mucus observed MIC values of 100µg/ml against *E. coli* and the *V. cholerae* slightly arrested at the above concentration. Whereas, the recorded 180 and 200µg/ml respectively. Whereas, the *S. aureus, K. pneumoniae, S. typhi, K. oxytoca, S. paratyphi, P. mirabilis, V. parahaemolyticus* and *S. pyogenes* not arrest at any concentration (Table 2). In the present study, the result of MIC was higher when compared to that of the methanol, ethanol mixture (1:2) extract from *Phallusia arabica* in which the MIC was 0.80mg/ml against *S. aureus* [23]. Ramasamy., *et al*. [24] described the MIC of acetone extracts of tissue and egg mass extract from *Chicoreus ramosus* was 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. Rao., *et al*. [25] reported 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.

Minimum bactericidal concentration of mucus extract

The fish mucus showed the MBC values of 100µg/ml against *E. coli* and the *V. cholerae* slightly arrested at the above concentration. Whereas, the recorded 180 and 200µg/ml respectively. Whereas, the *S. aureus, K. pneumoniae, S. typhi, K. oxytoca, S. paratyphi, P. mirabilis, V. parahaemolyticus* and *S. pyogenes* not arrest at any concentration (Table 3). In antibacterial activity, MBC are excellent and comparatively reasonable tools to concurrently assess many antimicrobial agents for effectiveness. Many studies have demonstrated similar results about the antimicrobial property of epidermal mucus in variety of fishes Channa punctatus [26], catfish *Arius maculates* [19], hagfish *Myxine glutinosa* [27], and eel fish *Anguilla Anguilla* [12]. Another hand, the brook trout mucus extract recorded MBC value as 10 and 273µg/ml against *S. Typhimurium* and *P. aeruginosa* [28]. In addition, the aqueous mucus extract of rainbow trout did no show any activity against any of the bacterial strains tested [29].

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*	+++	+++	+++	+++	++

Table 3: MBC of the mucus extract from *L. rohita* against human pathogen.

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

Conclusion

In conclusion, the present study, the fish mucus from *L. rohita* had good antibacterial activity against clinical pathogens at notable concentration. Therefore, the antibacterial activity of the mucus suggested as a source antimicrobial agent in future pharmacological for the development of new antimicrobial drugs.

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