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Biochemical Components, Immune Characteristics and Antimicrobial Activity of Epidermal Mucus of the Three Indian Major Carp Species

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ABSTRACT

Fish live in an environment that is full of phylogenetically diverse collections of bacterial pathogens. Fish epidermal mucus is regarded as a barrier between fish and infectious microorganisms in their surroundings protecting it against pathogens. The current study aimed to evaluate the biochemical components of skin mucus, mucosal immunity, and the bactericidal effect of epidermal mucus in three carp species, including *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala*. Biochemical composition showed the presence of protein, carbohydrate and lipid in the mucus of all three carp species, and concentration varied with respect to species. Protein and lipid were higher in *C. catla*, followed by *L. rohita* and *C. mrigala*; whereas, carbohydrate was high in the mucus of *C. mrigala*. Mucosal immunity indicating parameters, viz. lysozyme, alkaline phosphatase, myeloperoxidase, and protease activity was observed in the epidermal mucus of these species, showing the role of mucus in immunity. Lysozyme and alkaline phosphatase were high in *C. mrigala*, and myeloperoxidase and protease were high in *C. catla*. To understand the bactericidal role of raw and aqueous mucus, extracts of the epidermis of these species were tested against the common fish pathogen *Aeromonas hydrophila* by well diffusion assay. The results showed that raw as well as aqueous extracts showed the zone of inhibition (ZOI) against *A. hydrophila*; however, ZOI was high for raw mucus compared to aqueous extract. The variation in biochemical composition and mucosal immunity parameters may be due to variation in their habitat, ecological niche, and the presence of pathogens in the surrounding medium.

INTRODUCTION

Fish, like other living organisms, interact with a wide range of pathogenic and non-pathogenic bacteria because they have complex defense mechanisms to help them survive. The innate immune system fight against infections, from the moment they make contact with the organism **(Kimbrell & Beutler, 2001)**. Mucus is the initial physical barrier protecting fish from various infections and microorganisms in the environment (**Bhatnagar & Rathi, 2021**). **Nagashima *et al.* (2003)** reported that, epidermal mucus is continuously released so that any bacteria or other particles that get caught are washed

away, assisting the removal of highly infectious pathogens that have colonized the skin. Disease susceptibility differences between species and strains have long been assumed to be related to the host's ability to get rid of pathogen attachment and entry at the place of mucosal epithelial **(Ourth & Chung, 2004; Griffin & Mitchell, 2007)**.

Proteins and fatty acids are major components of fish skin mucus **(Bergsson *et al.*, 2005)** and the amount of phospholipids present in fish mucus helps to determine its viscosity. Several humoral defense factors, including immunoglobulin, complement, lysozyme, alkaline phosphatase, protease, and myloperoxidase are also present in the skin mucus of fish **(Watts *et al.*, 2001; Dash *et al.*, 2014; Timalata *et al.*, 2015)**.

Lysozyme cleaves the glycosidic linkages of the peptidoglycan layer, and its presence in fish mucus causes lysis of the outermost peptidoglycan layer of bacteria, which is effective against both Gram-negative and Gram-positive bacteria **(Whang *et al.*, 2011)**. In epidermal mucus, alkaline phosphatase (AP) is a significant indicator of stress in Atlantic salmon **(Ross *et al.*, 2000)**. Due to its hydrolytic activity, alkaline phosphatase, a lysosomal enzyme, has been linked to show a defensive role in the early phases of wound mending in fish, and it acts as an antibacterial agent **(Subramanian *et al.*, 2007)**. Proteases, which play a critical role in innate immune systems, are also found in fish mucus **(Palaksha *et al.*, 2008)**. Protease helps to break the bacterial proteins which consequently damage the pathogen. The proteases in skin either directly attacks pathogen or indirectly prevent pathogen invasion by altering the thickness of mucus **(Esteban & Cerezuela, 2015)**. However, there is currently a lack of comprehensive data on the antibacterial activity and innate immune characteristics of epidermal mucus in freshwater fish. The biochemical composition of fish epidermal mucus varies with respect to the habitat ecological niche because some major carp species reside at the surface, while others live in the column and at the bottom. The purpose of this study was to investigate the role of epidermal mucus in the defence system by examining biochemical, immunological, and antibacterial activity in epidermal mucus of three major carps, while taking into account the fact that it is a non-invasive method.

MATERIALS AND METHODS

Three carp species; *Catla catla*, *Cirrhinus mrigala* and *Labeo rohita* with a range weight between 150g – 240g were procurred from fish farm located in Karnal, district of Haryana. Prior to mucus collection, fishes were acclimatized in the laboratory for 7-10 days in the Aquaculture Research Unit of the Department of Zoology, Kurukshetra University, Kurukshetra (29º58’ N, 76º51’ E). Fishes were kept in aquariums (60×30×30 cm) connected with low pressure aerators and motors. About 40 liter of water in the aquariums was replaced on a daily basis with previously equilibrated water to maintain proper hygienic standards. The water quality parameters (**APHA, 2017)** such as dissolved oxygen (6.90±0.09 mg L^{-1}), pH (7.66± 0.03), conductivity (668.0± 1.70 µmhos

cm^{-1}), temperature (26.37± 0.24ºC), alkalinity(190.9 ± 0.90 mg L^{-1}), chloride (38.28±0.35 mg L^{-1}), calcium (18.5±0.26 mg L^{-1}), total ammonia secretion (0.21±0.004 mg L^{-1}) and ortho-phosphate production (0.149± 0.001 mg L^{-1}) were within the ideal range during the experiment **(Bhatnagar & Devi, 2013)**. Fish samples were fed daily at a rate of 4% of their body weight. Isocaloric and isonitrogenous diet with 40.34 % of protein content, 9.16% of crude fat, 7.64% of crude fiber, 7.24% of total ash, 4.32% of moisture and 38.54% of nitrogen free extract was prepared by using cheap source of protein i.e. duckweed **(Bhatnagar & Raparia, 2015)**. Groundnut oil cake, rice bran, duckweed, wheat flour, mineral mixture, and chromic oxide were used to make the basic diet. Feeding frequency was two times a day at 0900 and 1700 hrs.

I) Mucus collection

Mucus was collected from 8 fishes of each species by scrapping epidermis from the body of fish. The fish had no chemical or anesthetic treatment in order to collect skin mucus. Mucus was obtained from fish dorso-laterally by moving aseptic plastic spatula from head to tail in an anterior-posterior direction. Mucus was collected according to the technique of **Chong *et al.* (2005)** in which the lower part of fish was escaped to get rid of alimentary and genitourinary contamination. To avoid bacterial contamination, the scraped mucus was initially discarded. The samples of mucus were collected in vials, labeled and kept preserved at -20°C to get rid of bacterial augmentation and proteolysis.

II) Biochemical components of fish skin mucus

a) Protein estimation

Protein estimation of fish skin mucus was done by the Lowry assay **(Lowry *et al.*, 1951)**. In a glass test tube, 2.5ml of lowry reagent was mixed with 500µL of fish mucus and kept at room temperature for 10 minutes. After that, 250µL of folin reagent was mixed and shaken well. The mixture was kept at ambient temperature (37ºC) for 30 minutes. The optical density (OD) was measured using a UV-1900i UV-VIS spectrophotometer at 660 nanometers (nm). Concentration of protein from the mucus samples was determined by using a Bovine serum albumin (BSA) standard curve.

b) Carbohydrate estimation

Carbohydrate presence was determined using phenol–sulfuric acid method **(Dubois *et al.*, 1956)**. A volume of 0.5ml of fish skin mucus was poured in a sterile test tube, and 0.5ml of phenol solution was added to the sample and mixed. A rapid addition of 2.5ml concentrated sulphuric acid was made. The mixture was kept at room temperature for 20 minutes. The OD was measured using a UV-1900i UV-VIS spectrophotometer at 490 nm. The concentration of test samples was calculated using a glucose standard curve.

c) Acid value of fat test

Acid value of fat was tested by method of **Zaharah *et al.* (2005)**. In a 100ml conical flask, 1ml of fish mucus was poured. The materials were suspended in a 25-ml organic

solvent (1:1 ether: alcohol) (95 percent). After adding a few drops of phenolphthalein solution, everything was well mixed together. The solution was titrated against 0.05 M KOH for 10 to 15 seconds until the faint pink color remained.

d) Lipid estimation

Lipid was determined by the procedure of **Folch *et al.* (1957)**. To extract the lipid, 1ml of mucus sample was mixed with 20ml chloroform – methanol (2:1) solvent, followed by continuous shaking for 7-8 hours. After shaking, the extract was filtered and the filtrate was taken in a separating funnel and was washed with solution of saline (0.89%), and the funnel was left at room temperature overnight. The difference in initial and final weights of the sample gave the amount of total lipid content.

III) Immune parameter of fish skin

a) Lysozyme activity

Lysozyme activity was measured by turbidimetric assay **(Sankaran & Gurnani, 1972)** with slight modification. Mucus sample (100µl) was mixed with 2.5ml solution of lyophilized *Micrococcus lysodeikticus* cells (0.1 mg/ml) made in phosphate buffer (0.067 M) at pH 6.25. Optical density was measured at ambient temperature (37ºC) using a UV-1900i UV-VIS spectrophotometer at 450nm for 1-3 min. Change in absorbance of 0.001 per minute was defined as one unit of lysozyme activity. The concentration of test samples was calculated using egg white lysozyme standard curve.

b) Alkaline phosphatase activity

The activity of alkaline phosphatase in mucus was determined using the method of **Rosalki *et al.* (1993)** with a few changes. About 3ml of the chemical agents (magnesium chloride, ammonium bicarbonate, and p-nitrophenyl phosphate) were first poured into an aseptic glass test tube, and 50µl of mucus was then incubated with 3ml of the chemical reagent. Test sample and chemical reagent were completely mixed and the initial optical density was measured, using a UV-1900i UV-VIS spectrophotometer at 405nm, followed by three consecutive absorbance recorded at minute intervals.

c) Myeloperoxidase assay

With few modifications, the content of myeloperoxidase in epidermal mucus was obtained following the method of **Quade and Roth (1997)**. Mucus (100µl) was dissolved in Hank's balanced salt solution (900µl) without Ca^{2+} or Mg^{2+}. Then, 20mM 3,30,5,50-tetramethylbenzidine hydrochloride (350 µl) and 5mM H_2O_2 were combined together. To stop the process, 350µl of 4M sulphuric acid (H_2SO_4) was added after 2 minutes. The absorbance was measured at 450nm using UV-VIS spectrophotometer.

d) Protease activity

The casein hydrolysis test was used to assess protease activity **(Dash *et al.*, 2014)**. For around 10 minutes, a mucus sample (100 µl) was incubated in casein substrate (5ml) comprising potassium phosphate buffer with pH 7.4 at 37°C. Afterward, this reaction was restricted with 110mM trichloroacetic acid (5 ml), followed by placement in a water bath for 30-minutes at 37°C. Later on, samples were sieved using Whatman paper, and an aliquot (2ml) of it was dissolved in 5ml of sodium carbonate (500mM) with Folin's reagent (1 ml). Finally, these samples were incubated for 30 minutes at 36-37°C in a universal incubator, and their optical density was measured at 660nm using a UV-VIS spectrophotometer. The concentration of test samples was calculated by using a tyrosine standard curve.

IV) Antimicrobial activity

a) Mucus extracts preparation

(i) Crude mucus extract was produced under sterile condition. Aseptic mucus was centrifuged at 10000 r.p.m. for 10 minutes, and then the supernatant was stored at -20°C for further procedures.

(ii) The aqueous extract of mucus was made. To dilute the raw mucus, an equal amount of physiological saline (0.89% NaCl) was added and centrifuged for 10 minutes at 10000 r.p.m. Afterward, the supernatant was preserved in -20°C for future processing. Sterile physiological saline was used as negative control at the time of aqueous mucus extract preparation.

The antimicrobial activity of both the mucus extracts was tested against the pathogenic bacteria *Aeromonas hydrophila* (was procured from CSIR-Institute of Microbial Technology, Chandigarh, India with MTCC No. 1739).

b) Screening of mucus extracts for antimicrobial activity

• Agar-well diffusion method

The agar well diffusion method was used to test the antibacterial activity of mucus extracts on a pathogenic bacterial strain for all the experimental fishes **(Perez *et al.*, 1990)**. Freshly cultivated bacteria were sown in petri-plates with about 20ml of nutrient agar media. Wells were cut down with sterile borer, and 100µl of fish skin mucus extract (crude and aqueous mucus) was poured. After that, plates were incubated at 37°C for 24-36 hours. The diameter of the inhibition zone created around the well was used to calculate antibacterial activity (NCCLS, 1993). In millimeters (mm), the diameter of the zone of inhibition (ZOI) was measured.

V) Statistical analysis

Data was subjected to analysis of variance using SPSS (version 16.0). The difference among data was determined by Tuckey's test. Data with *P* value more than 0.05 are

considered as non-significant, while those lesser than 0.05 are considered as of significant value. Data are expressed as Mean ± SEM.

RESULTS

The present study evaluated different epidermal mucus parameters and compared between the studied carp species, including *L. rohita, C. catla* and *C. mrigala*. Among these freshwater fish species, there was a significant difference in the levels of biochemical components. Mucus was secreted in varying volumes in the carp species studied, and the appearance of the mucus differed from species to species. *C. catla* secretes large amount of mucus with higher viscosity; whereas, the mucus of *L. rohita* and *C. mrigala* was quite clear though found in low quantity. The epidermal mucus showed the presence of different biochemical parameters, such as protein, carbohydrate and lipid.

Protein recorded the highest concentration among all biochemical parameters with a range from 0.69 mg ml^{-1} to 1.01 mg ml^{-1}. The analysis of skin mucus protein level (Table 1) depicted that the high protein was found in *C. catla* (1.01 ±0.00091 mg ml^{-1}), followed by *L. rohita* and *C. mrigala* (0.97 ±0.00061 mg ml^{-1} & 0.69±0.00167 mg ml^{-1}, respectively). While, the carbohydrate level was high in *C. mrigala*, with a value of 0.1331 ± 0.00024 mg ml^{-1}, compared to *L. rohita* and *C. catla* (0.0971 ± 0.00184 mg ml^{-1} and 0.0776 ± 0.00281mg ml^{-1}, respectively).

Compared to the other carps under study, *C. catla* secreted the highest amount of mucus, and the amount of lipid was also high, with a value of 0.4607 ± 0.00318 mg ml^{-1}, compared to *L. rohita* (0.2140 ± 0.00289 mg ml^{-1}) and *C. mrigala* (0.1587 ± 0.00203 mg ml^{-1}). The acid value of fat of *C. mrigala* (0.367 ± 0.003 mg ml^{-1}) was higher than that recorded in *L. rohita* and *C. catla*, and values were statistically significant ($P< 0.05$).

Table 1. Biochemical components of fish skin mucus

	Protein (mg ml^{-1})	**Carbohydrate (mg ml^{-1})**	**Lipid(mg ml^{-1})**	**Acid value of Fat(mg ml^{-1})**
C. catla	1.01 ± 0.00091^{A}	0.0776 ± 0.00281^{C}	0.4607 ± 0.00318^{A}	0.267 ± 0.03^{A}
L. rohita	0.97 ± 0.00061^{B}	0.0971 ± 0.00184^{B}	0.2140 ± 0.00289^{B}	0.267 ± 0.03^{A}
C. mrigala	0.69 ± 0.00167^{C}	0.1331 ± 0.00024^{A}	0.1587 ± 0.00203^{c}	0.367 ± 0.03^{A}

All values are Mean± S.E of mean.
Means with different letters in the same column are significantly ($P< 0.05$) different.
(Tuckey's test)

Mucus lysozyme is a significant factor of mucosal immunity. The range of lysozyme varied from 2.26 µg ml^{-1} to 3.56 µg ml^{-1} in the epidermal mucus samples. *C. mrigala* showed the highest range (3.56 ± 0.098µg ml^{-1}), compared to *C. catla* (3.16µg ml^{-1}) and *L. rohita (*2.26µg ml^{-1}) as shown in Fig. (1a). Myloperoxidase, an agent of antibacterial activity, was significantly ($P< 0.05$) higher in *C. catla* (OD_{450}=1.38 ± 0.013) than *L. rohita* (1.02 ± 0.045) and *C. mrigala* (0.462 ± 0.038) (Fig. 1b).

Alkaline phosphatase, a significant lysosomal enzyme, is associated with the innate immune system in fishes; it exsists in the epidermal mucus of fish. Activity of alkaline phosphatase in skin mucus varied from 34.09 U L^{-1} to 58.04 U L^{-1} and was significantly different among the three fresh water carp species. The highest was recorded in *C. mrigala* (58.04 ± 1.60 U L^{-1}). Whereas, the lower levels of alkaline phosphate activity was detected in *L. rohita* (39.62 ± 3.322 U L^{-1}), followed by *C. catla* (34.09 ± 0.461 U L^{-1}) (Fig.1c).

Protease activity was significantly high in *C. catla* (1.2680±0.03142U ml^{-1}), followed by *C. mrigala* (0.9812 ±0.01743) and *L. rohita* (0.9007 ± 0.0219) (Fig. 1d).

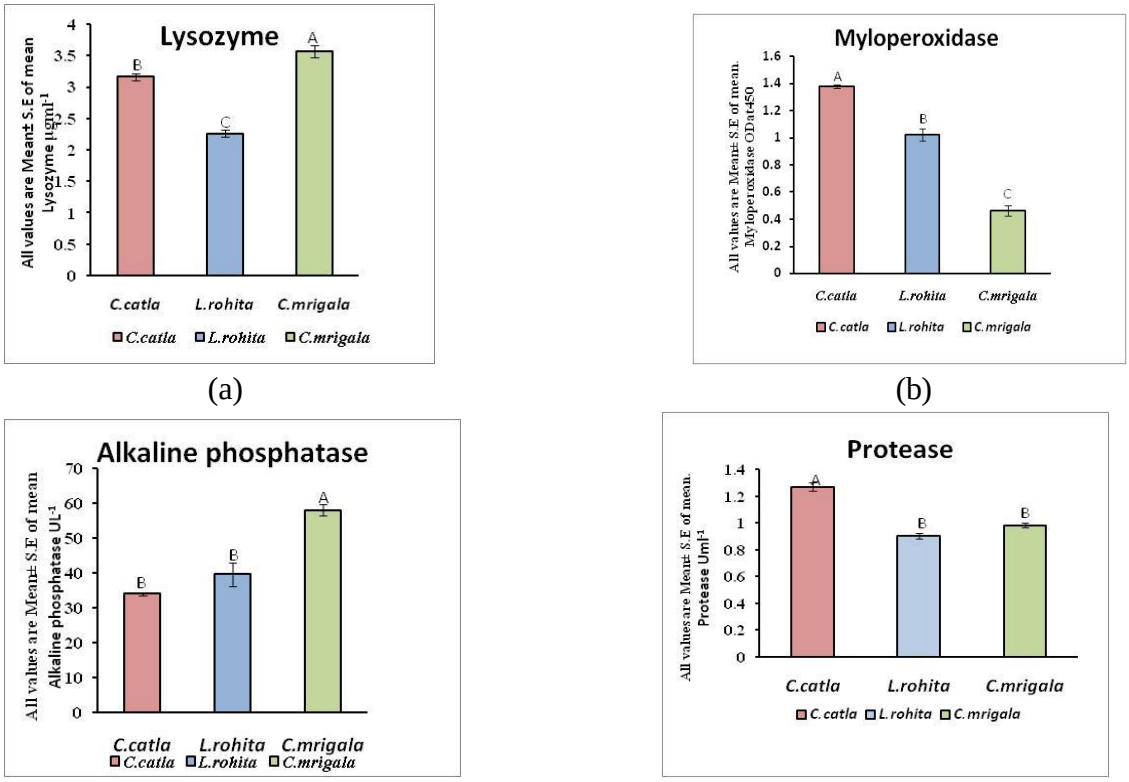

Fig. 1. Comparison of different immune parameters for the experimental fishes (a) Lysozyme (b) Myloperoxidase (c) Alkaline phosphatase (d) Protease.

Bars with different letters on the same graph are significantly different (Tuckey's Post hoc test)

The inhibition effect of crude and aqueous mucus extracts of all experimental fish species was measured in mm against selected microorganism strain (*Aeromonas hydrophila*). The zone of inhibition (ZOI) values of mucus extracts (crude and aqueous) against this pathogen *A. hydrophilla* varied between 14.16± 0.12019mm and 31.40± 0.40415mm.

For *A. hydrophila*, the crude mucus extract of *C. catla* exhibited high ZOI (31.40± 0.40415mm), followed by *C. mrigala* (31.26±0.18559 mm) and *L.rohita* (30.90±0.12019 mm) (Fig. 2). Like crude mucus, aqueous mucus extract showed the zone of inhibition with *C. catla* (14.53± 0.08819mm), followed by *C. mrigala* (14.43± 0.08819mm) and *L. rohita* (14.16± 0.12019mm) (Fig. 3).

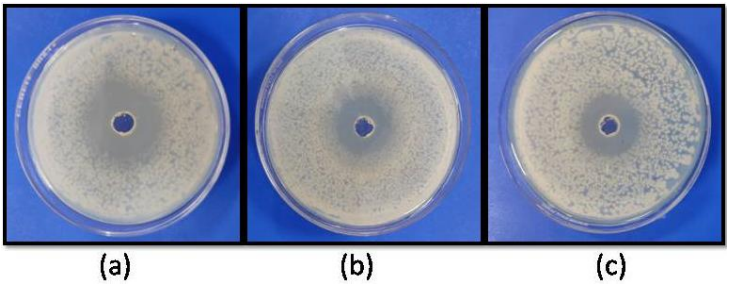

Fig. 2. Crude mucus extracts from experimental fishes showing a zone of inhibition against the common pathogenic bacterial strain *A. hydrophila*; (a) *C. catla* (b) *L. rohita* (c) *C. mrigala*

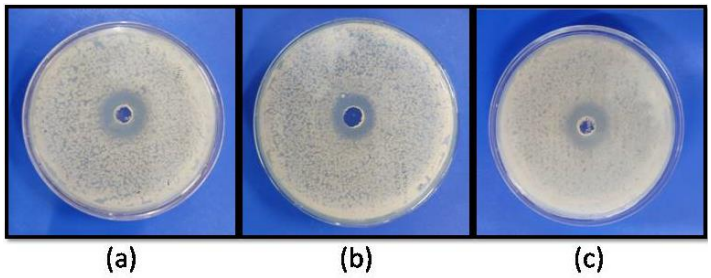

Fig. 3. Aqueous mucus extracts from experimental fishes showing a zone of inhibition against the common pathogenic bacterial strain *A. hydrophila*; (a) *C. catla* (b) *L. rohita* (c) *C. mrigala*

The amount of mucus in aqueous extracts of the experimental test sample was almost 50% of crude mucus extracts (Table 2). Furthermore, the findings reveal that the aqueous mucus extract has a decreased antibacterial impact, compared to crude mucus extract against the pathogens examined; in addition, it showed a significantly different antibacterial effect against bacteria.

Table 2. Zone of inhibition shown by crude and aqueous mucus extract

	Crude mucus(mm)	Aqueous mucus(mm)
C. catla	31.40± 0.40415^{A}	14.53± 0.08819^{A}
L. rohita	30.90±0.12019^{A}	14.16± 0.12019^{B}
C. mrigala	31.26±0.18559^{A}	14.43± 0.08819 ^{AB}

Zone of inhibition including well diameter
All values are mean± S.E of mean
Means with different letters in the same column are significantly (P< 0.05) different.
(Tuckey's test)

DISCUSSION

Fish are the most common ectothermic aquatic vertebrates, and they play a vital role in food webs containing a variety of pathogenic species in aquatic habitats **(Ellis, 2001)**. Fish mucus serves a variety of functions, and the antibacterial capacity of epidermal mucus against contagious pathogens has been demonstrated in a variety of carp species, implying that epidermal mucus protects fish from infectious diseases. According to **Subramanian *et al.* (2008)**, the mucus-secreting cells of skin have a variable mucus composition in different fish species. The findings showed that all three major carp species (*C.catla*, *L.rohita*, and *C.mrigala*) emitted a high amount of mucus, with a significant difference in the components of epidermal mucus.

The present study examined the protein content of the mucus of three different fish species. In comparison to *L.rohita* and *C.mrigala*, the *C.catla* had the highest protein content. Some studies have found that differences in fish mucus protein content is primarily caused by physiological conditions, such as growth, development, smoltification, and food shortages. Moreover, **Fagan *et al.* (2003)** and **Elavarasi *et al.* (2013)** considered the presence of protein as main constituent among the skin mucus of *Clarius batrachus* and *Tilapia mossambicus*. The protein content of *Channa straitus* crude mucus (0.589 mg/ml), aqueous (0.291 mg/ml) and acidic (0.267 mg/ml) was determined in the study of **Wei *et al.* (2010)** and revealed that, protein constitutes a high potential of antimicrobial source. The protein composition of the cutaneous mucus of young and fully grown discussed fish, *Symphysodon* spp., is different **(Chong *et al.*, 2005)**. **Sumi *et al.* (2004)** used gas liquid chromatography to confirm the presence of carbohydrates in rainbow trout skin mucus. According to **Al-Hassan *et al.* (1987)**, mucus is high in proteins and lipids but low in carbohydrates, which supports our findings. **Venkaiah and Lakshmipathi (2000)** findings revealed the low range of carbohydrates than lipids in the epidermal mucus secretions of *Aiolopus thalassinus*, *Ariid* cat fish, *C.*

batrachus and *Heteropneustes fossilis.* The current findings coincide with the previous ones. Additionally, lipids present in mucus secretions include covalently attached fatty acids that enhance the viscoelasticity of the gel. **Ebran *et al.* (1999)** observed that, presence of lipids and protein molecules in skin mucus are associated with antibacterial properties. The present result is in line with that of **Hussin *et al.* (2017)** who investigated the epidermal mucus of *Clarias gariepinus* and *Clarias* sp.1 and detected a high range of protein, followed by lipids and carbohydrates, respectively.

The value of lysozyme activity in the cutaneous mucus of the three different carp species were found significantly different in this investigation. As compared to *C. catla* and *L. rohita*, *C. mrigala* had a high level of lysozyme activity. Seawater fish species have larger levels of lysozyme in their epidermal mucus than species present in freshwater **(Ross *et al.*, 2000; Subramanian *et al.*, 2007)**. Several factors such as diet, sex, type of species, handling stress and genetic variation could be the reason for the variation in the activity of lysozyme **(Balfry & Iwama, 2004)**.

The present study found that the levels of myloperoxidase varied among the three major carp species, with *C. catla* recording the greatest level of myeloperoxidase, compared to *L. rohita* and *C. mrigala*. **Stosik *et al.* (2001)** reported that, the enhanced level of myloperoxidase activity is due to the stimulatory action of microbial and parasite substances on neutrophilic granulocytes revealed in ill carps. This indicates that myloperoxidase plays an important part in the removal of pathogenic factors in fish. Moreover, **Dash *et al.* (2014)** observed that, the highest myloperoxidase activity was present in the mucus of *C. catla*, indicating that it has a well-developed immune system capable of dealing with infections. Myloperoxidase is an important enzyme with an antimicrobial activity.

On the other hand, the alkaline phosphatase activity of the fish species under study ranged from 34.09 to 58.04 U L^{-1}. Similarly, **Ross *et al.* (2000)** found an enhancement in activity of alkaline phosphatase in the Atlantic salmon mucus, contaminated with a parasite *Lepeophtheirus salmonis*, and **Fast *et al.* (2002)** found a high level of mucus alkaline phosphatase activity in fish species present in seawater; namely, *Oncorhynchus kisutch*, *Oncorhynchus mykiss*, and *Salmo salar*. Casein hydrolysis test was employed to identify the proteases activity in this investigation, and the greatest levels were discovered in the epidermal mucus of *C. catla*. **Easy and Ross (2010)** revealed a shift of protease activity in *S. salar* after exposure to a long-term stress. The low level of protease in this study could be attributed to the diverse age groups, behaviours, and habitats of fishes. On the other hand, **Nigam *et al.* (2012)** reported comparatively strong protease activity in *C. mrigala*, compared to *C. catla* and *L. rohita.*

The current findings on the antibacterial activity of major carp epidermal mucus confirmed that it is a source of antimicrobial compounds. In the present study, *C. catla* showed the highest zone of inhibition, compared to *C. mrigala* and *L. rohita.* **Ellis (2001)**, **Nagashima *et al.* (2003), Balasubramanian *et al.* (2012)** and **Nurtamin *et al.***

(2016) recorded a high antibacterial activity in the skin mucus of numerous fishes. These conclusions are supported by the findings of the present analysis. However, **Subramanian *et al.* (2007)** investigated antibacterial compounds in aqueous mucus extracts sampled from various fish species. Similarly, **Dhanraj *et al.* (2009)** investigated the antibacterial activity of cutaneous mucus and intestinal mucus from five distinct freshwater fish *Channa* species and found that each fish species had a different inhibitory impact against pathogenic strains. The fish pathogenic bacteria *A. hydrophila* is inhibited by the mucus of both aqueous and crude extracts of fish *C. straitus* **(Wei *et al.*, 2010)**. **Ebran *et al.* (2000)** revealed, the raw skin mucus contains hydrophobic constituents which give a strong pore-forming property (related to antibacterial activity). **Kumari *et al.* (2019)** tested the antibacterial effectiveness of epidermal mucus from three carp species: *Hypophthalmichthys nobilis*, *Ctenopharyngodon idella*, and *Cyprinus carpio*, against a number of fish and human harmful bacterial strains. They found that mucus from the skin of all the carp species had significant antibacterial activity, suggesting that it may play a role in fish pathogen protection. The antibacterial efficacy of soluble and insoluble fractions of skin mucus obtained from *Arius maculatus* against pathogenic bacteria *Escherichia coli* and *Pseudomonas aueruginosa* was also demonstrated by **Manivasagan *et al.* (2009).** Mucus from *H. fossilis* and *C. batrachus* had antimicrobial property against a wide spectrum of fish and human pathogenic bacteria, according to **Bhatnagar *et al.* (2021)**; they concluded that epidermal mucus is an important component of the natural defensive mechanism. This research also confirmed that fish epidermal mucus is a resource of antibacterial compounds. It was also anticipated in present study that the antibacterial impact would be weaker in aqueous extracts as compared to crude mucus. The richness of immunological components of epidermal mucus demonstrates their importance in the innate immune response, which supports to fight against pathogens and their living in a pathogen-infested environment. Furthermore, in order to prevent infectious disease in fish, a better understanding of mucosal immunity is essential.

CONCLUSION

The findings of this study imply that fish epidermal mucus contains a range of biochemical components such as protein, carbohydrate, lipid and fat and their values vary according to different species and genetic variation. The crude and aqueous mucus extracts of studied fishes demonstrate a significant antibacterial activity against pathogenic bacteria. The presence of lysozome, myloperoxidase, alkaline phosphatase and protease confirms the innate immune parameters that protect fish against pathogens. This study provides an insight for the non invasive method to detect the health status of fishes that means if the fish is under stressed condition, in order to study the stress effect there is no need to dissect the fish we can determine it only by analyzing the epidermal

mucus. Further studies on fish epidermal mucus is needed to increase the understanding of mucosal innate immune components may be beneficial to fish farming, aquaculture, and possibly human health.

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