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(RESEARCH ARTICLE)

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Epidermal mucus as a potential biological and biochemical matrix for fish health analysis

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Abstract

Fish reside in ecosystems teeming with pathogens, so their mucus has developed antimicrobial properties that help inhibit these pathogens. The fish's epidermal mucus serves as the initial line of defense against pathogens. This study aimed to characterize the antibacterial activity and biochemical makeup of fish skin mucus against various bacterial strains. The analysis was conducted on Labeo rohita, a fish species chosen for its mucus sample. The mucus was tested for its antibacterial activity against multiple Gram-positive and Gram-negative bacteria. The results showed that the crude mucus extract had higher activity than the saline mucus extract. The fish mucus's antimicrobial potential was assessed using the well diffusion method. The crude mucus extract displayed slightly better antibacterial activity against Gram-negative bacteria like *Escherichia coli* and *Pasterulla multocida*, as well as Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus subtilis*, compared to the saline mucus extract of *Labeo rohita*. The samples were also tested for their hemolytic and thrombolytic activities. The activity of antioxidants in fish mucus was evaluated using DPPH, reducing power, TPC, and TFC assays. Additionally, biochemical analysis was performed, including measurements of CAT, POD, SOD, and protein content. Advanced techniques such as Fourier infrared spectroscopy (FTIR) and UV spectroscopy were employed for the characterization of fish mucus. FTIR analysis of fish mucus revealed the presence of aliphatic primary amines (N-H) and alkenes as functional groups at various peaks in the spectrum. The results were analyzed using mean and standard deviations.

Keywords: Fish Mucus; Mucus Molecules; Interspecific Communication; Biological Activity; Biochemical Activity**.**

1. Introduction

Labeo rohita belongs to the family Cyprinidae. It is mostly present in indigenous, Pakistan, and it is present generally all over the streams and in naturally occurring water along with commercially originated in fish tanks and fishpond (Hussain *et al*., 2011). Rohu (*L. rohita)* is generally more important in Indian major carp (IMC) and it is commonly used in polyculture system of carp. This fish species is the common indweller of arctic and midway India, and present in the rivers of Bangladesh, Myanmar and Pakistan. Now it has also been originated in frequent other countries like Japan, Nepal, Malaysia, China, Sri Lanka and some other regions of Africa (Khan *et al*., 2011).

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The congruity of rohu is more as compared to other carps such as mrigala *(Cirrhinus mrigala)* and catla (*Catla catla)* and that's why it is excellent applicant for polyculture practice of carp (Jhingran, 1991). All the fishes live in such surroundings that are rich in microorganisms and are susceptible to attack by opportunistic and pathogenic microbes. The water environment for fish is very competitive due to the presence of large number of microorganisms, so mucus of fish provides protection against these microorganisms and pathogens (Subramanian *et al*., 2007). The basic natural protected element in fish contains the mucus sheet on the gills, epidermis and digestive tract and contains the component of blood like phagocytes (Ellis, 2001). Mucus slime makes the fish silky (lubricious). Its slipperiness is due to the presence of immense water constituents, and it is also due to the existence of large molecular weight and gel-forming macromolecules (Martinez *et al*., 2006).

Normally the body of fish is protected by the layer of mucus and this layer is secreted by the many kinds of biological constituents in ectoderm, these are the mucus cells, the sacciform cell, club cells and the epithelial cells (Pearson *et al*., 2005). The composition of mucus is that it is gel like slimy, viscous and having the diverse mixture of ions, water and enzymes (Tkachenko *et al*., 2006; Sumi *et al*., 2004; Nigam *et al*., 2012b).

Mucus of the fish have the constant connection among their aquatic surrounding and it behave as obstacle in opposition of mechanical, substantial and chemical agents and this layer is also a barrier against biological infectious agents (bacteria, virus, fungi etc). The mucus layer of the fish plays various biological tasks for example osmoregulation (Handy *et al*., 1989); mucus provides security from injury during excavate (Mittal *et al*., 2002) and epidemic (Ellis, 1999); increase in damage curing (Al-Hassan *et al*., 1983); help in intra-species transmission; and cocoon production in some type of fish species (Shepherd, 1994).The mechanical boundaries of the fish epidermis disrupt the entrance of microorganisms into the body. Digestive tract, respiratory tract and genitourinary systems are lined with mucus layer. The function of this layer is to capture the foreign microorganisms outside of the body (Arockiaraj *et al*., 2013a).

The epidermis of the fish constitutes as the major protection line. Mucus cells disguise the mucus and in fish it functions as defense against dynamic, chemical, physical, biological, semipermeable and natural barriers (Subramanian *et al*., 2007; Raj *et al*., 2011). Moreover, fish mucus also has natural resistant parameters, like antimicrobial proteins and enzymes (Jung *et al*., 2011). The mucus of fish also has some resistance compounds such as immunoglobulins, lecitin, interferon, agglutinin, calmodulin, C-reactive proteins, lysozymes, proteolytic enzymes and antimicrobial peptides (Nigam *et al*., 2012).

2. Material and method

2.1. Isolation of fish mucus

The sample of fish mucus will be obtained from the fishpond of department of Zoology, Wildlife and Fisheries of Agriculture University. For the collection of fish mucus plastic scraper will be used, mucus will be obtained from the upper side of the fish. After that, this material will be shifted into test tubes and will store at 4^0C in refrigerator.

2.1.1. Preparation of mucus extract

Fish mucus will be obtained from the skin of fish and fused with brine 0.85% NaCl solution. Then unsinkable portion of solution will be used for the evaluation of biochemical compounds (Balasubramanian *et al*., 2012).

2.2. Antimicrobial activity

The fish mucus antibacterial activity will be tested against the Gram-positive, Gram-nagative bacteria. And this antimicrobial activity will be done by agar well diffusion method (Al-Arifa *et al*., 2011).

2.2.1. Antibacterial activity

For the assessment of antibacterial activity of fish mucus agar diffusion process will be used. On the culture plate various strains of bacteria will be spread. Sterilized water will be used to regulate the antibacterial activity (Cavalieri *et al*., 2005).

2.2.2. Antifungal activity

The fish mucus antifungal activity will be determined with the help of disc diffusion method, and microplate dilution techniques will be used. Agar culture will be prepared for the test microorganisms. Sterilized filter paper will be used for the screening of mucus extract (Hellio *et al*., 2002).

2.2.3. Minimum inhibitory concentration (MIC)

Test of micro plate dilution will be performed to calculate the MIC of epidermal mucus across all the selected microorganisms. And these plates will be incubated at 37^0C (Tyor and Kumari, 2016).

2.3. Biochemical analysis

2.3.1. Catalase activity (CAT)

CAT activity will be measured by the process of (Medeiros *et al*.,2016) Catalase enzyme will be used to observe the decomposition of hydrogen peroxide, and it will be used in a place of substrate. For this purpose, UV spectrophotometer will be used.

2.3.2. Superoxide Dismutase (SOD)

SOD will be used to determine the quantity of protein required to minimize the source amount 50 percent with respect of more resistance. SOD will be used to define the capability of enzyme that block the NBT (nitro blue tetrazolium) reduction (Sun *et al*., 1988).

2.4. Antioxidant

2.4.1. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH)

DPPH will be used for the evaluation of the antioxidant activity through the method of (Garcia *et al*., 2014). This DPPH radical will be deliberated at 515nm.

2.4.2. Reducing Power

The epidermal reducing power of the fish mucus sample will be determined by the process of Garcia *et al*. (2014). Potassium ferricyanide and Phosphate buffer will be added to determine reducing power.

2.4.3. Total phenolic content (TPC)

Total phenolic concentration will be measured according to the process of Mamelona *et al*. (2007). Reagent of Folin-ciocalteau phenol will be used for this purpose. Gallic acid will be used as a standard for the measurement of the of the total phenolic contents through calibration curve.

2.4.4. Total flavonoid content (TFC)

The total flavonoid content will be measured through process of Ordonez *et al*. (2006). $AlCl_3$ ethanol solution will be added in the sample solution of mucus. After approximately 1 hour the absorption will be measured at 420nm at room temperature in the form of curve.

2.5. Cytotoxic activity

2.5.1. Haemolytic activity

The fish mucus haemolytic activity will be measured through the plates of blood agar base. Crude extract dilutions will be made in (PBS) phosphate buffered saline. Then the plates will be incubated at room temperature (Bragadeeswaran *et al*., 2011).

2.5.2. Thrombolytic activity

By pursing the United States Pharmacopeia (USP) process, sample anticoagulant activity will be determined. The small amount of heparin sodium will be determined as a standard by adding saline solution that will sustain the movement of plasma. Before and after incubation, weight the clot disruption (Kumar *et al*., 2011).

2.6. Characterization

Different advanced techniques will be used for characterization of the fish mucus.

- Scanning Electron Microscopy (SEM) will be used to examine dorsal and ventral cells of the fish mucus with the help of electron microscope. Sample will be stained with Giemsa for 1 hour and then it will be washed through the water, and sample will mount with the DPX (Mcdowell and Trump, 1976).

- X-Ray diffraction (XRD) will be used to determine the structure of mucus. The examined field of tissues will be analyzed with the help Energy-dispersive spectrometer (Humbert *et al*., 1986).
- Fourier transformed infrared spectroscopy (FTIR) will be used for the spectroscopic analysis of solid portion of mucus (Abu *et al*., 1991).
- Fish mucus UV absorbance will be determined by the fibril optical spectrometer. Then the mucus will be fixed on a UV-translucent microscope for the evaluation of UV absorbance. The compactness of every sample of mucus will be standardized by using glass slips (Zamzow *et al*., 2004).

2.7. Statistical analysis

Statistical analysis such as one sample and two samples t-test will be used to examine the study hypothesis for the characteristics of interest.

3. Results and Discussion

3.1. Antioxidant activity of fish mucus

Antioxidant activity of fish mucus was determined through various assays.

3.1.1. Reducing power activity

The reducing power assay is mostly used to determine the capability of an antioxidant to give an electron due to the reducing capacity of a compound. It is the major indicator of antioxidant activity (Duan *et al*., 2007).

For the determination of the samples extract, ability to reduce iron (III) reducing power assay is used. This reducing power assay depend upon concentration for all samples. Increased the reducing power of sample or mixture means that increased in absorbance of reaction mixture. The sample which has high reducing power means higher ability to donate electrons and Fe3+/ ferricyanide complex reduced into the ferrous form formation of blue colour. The colour of test sample changes from yellow to green or blue depending upon reducing power of sample. Absorbance was measured at 700 nm.

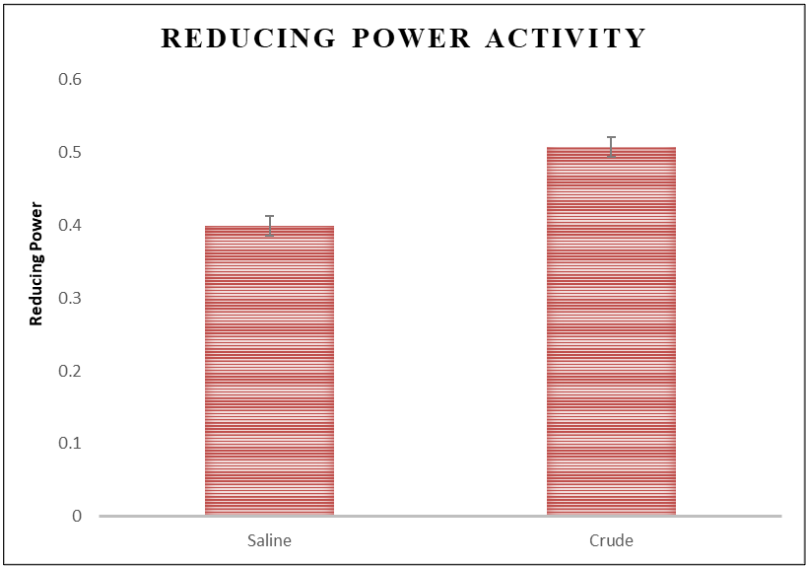


Figure 1 Compression of reducing power assay of saline and crude mucus of *Labeo rohita.* Given data is an average of three replicates ± S.E.

The result observed that the reducing power of saline mucus was less than the reducing power of crude mucus because saline mucus was diluted form of mucus whether crude mucus was without dilution, so it has higher reduced power than saline.

3.1.2. Total phenolic content (TPC)

Total phenolic content (TPC) of plant extract samples is higher than that of fish mucus extract. Fish mucus extract has low phenolic contents, and its antioxidant activity is also low. Total phenolic content of the mucus extract was evaluated by using Folin-Ciocalteu colorimetric procedure, and regression equation of gallic acid calibration curve was used for this purpose. The amount of phenolic per each extract was expressed as gallic acid equivalent. The results obtained from the assay were expressed as means ± standard deviation of triplicate analyses and are presented (Turkoglu *et al*., 2010a).

In my research work the total phenolic contents were also determined by Folin- Ciocalteu reagent, because it is the fast and simple way for quick determination of samples phenolic contents. Many earlier reports were found related to the use of this Folin- Ciocalteu reagent (Yadav *et al*., 2014).

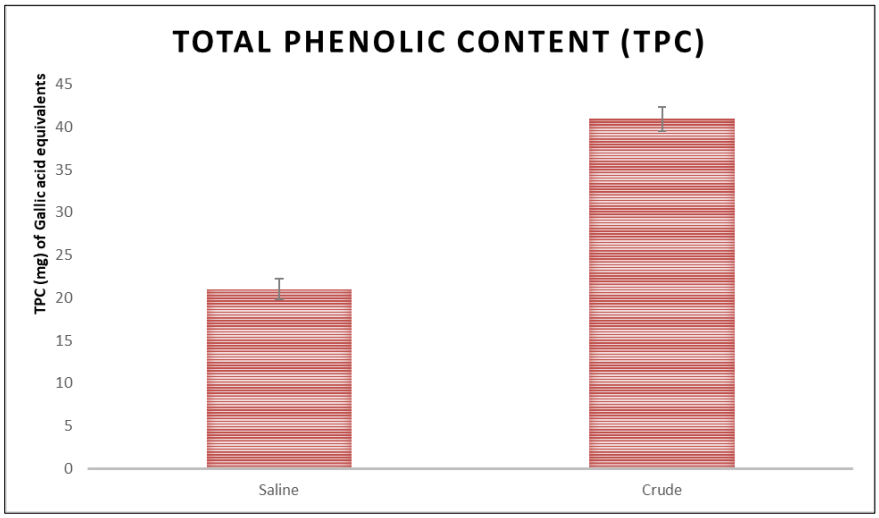


Figure 2 Comparison of total phenolics contents in saline and crude extract of mucus of *Labeo rohita*. Given data is an average of three replicates ± S.E.

The above graph shows that the total phenolic content of saline mucus extract of rohu is less in range of almost 20-22 mg GAE/g, and TPC of crude mucus extract is in range of 40-42 mg GAE/g. Therefore, total phenolic content was observed high in crude mucus extract and less in saline mucus extract.

3.1.3. Total Flavonoid content (TFC)

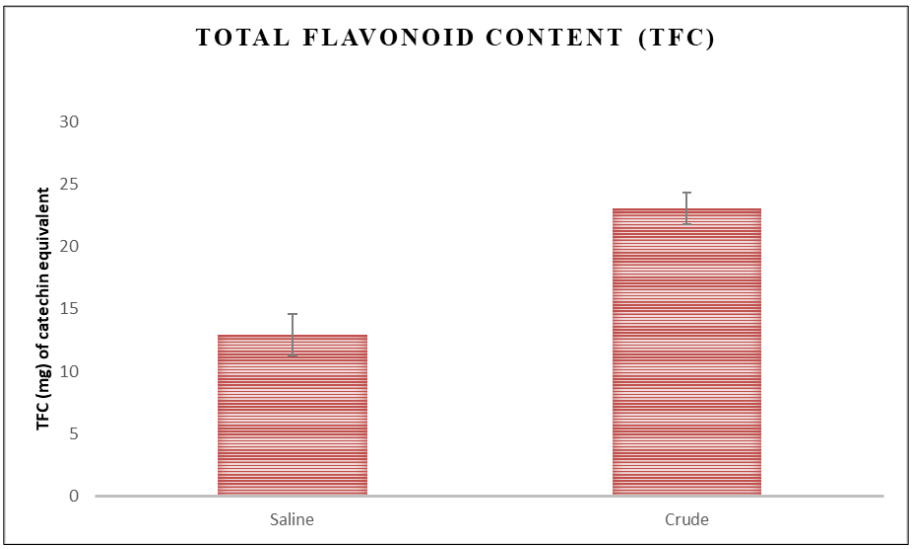


Figure 3 Comparison of total flavonoid contents in saline and crude extract of mucus of *Labeo rohita*. Given data is an average of three replicates ± S.E.

In natural compounds, flavonoids are the vital group, containing vegetables, fruits and cereals. Due to their wide spectrum of biological and chemical activities, including free radical scavenging properties flavonoid are the most likely important phenolics. Flavonoids are also therapeutic agent against large number of diseases (Gulcin, 2005). TFC of mucus extracts were calculated as catechin equivalents.

Figure 4.3 explains how quantity of flavonoids varies in different extracts of fish mucus. High flavonoid contents were found in crude mucus of fish as compared to the saline extract of mucus.

3.1.4. Free radical Scavenging Activity (DPPH)

DPPH is a well-known free radical which gave strong absorption band at 517 nm. The colour of DPPH solution is deep violet and it's colour disappears and changes to yellow when neutralized by antioxidant compound. Free radical scavenging activity of mucus extract was determined by DPPH scavenging assay. The DPPH scavenging activity of different mucus extracts has been shown in the Table and Fig 4.

The scavenging activities of all samples were concentration dependent. Lower absorbance of the reaction mixture indicated higher DPPH radical-scavenging activity (Gulcin *et al.,* 2006a).

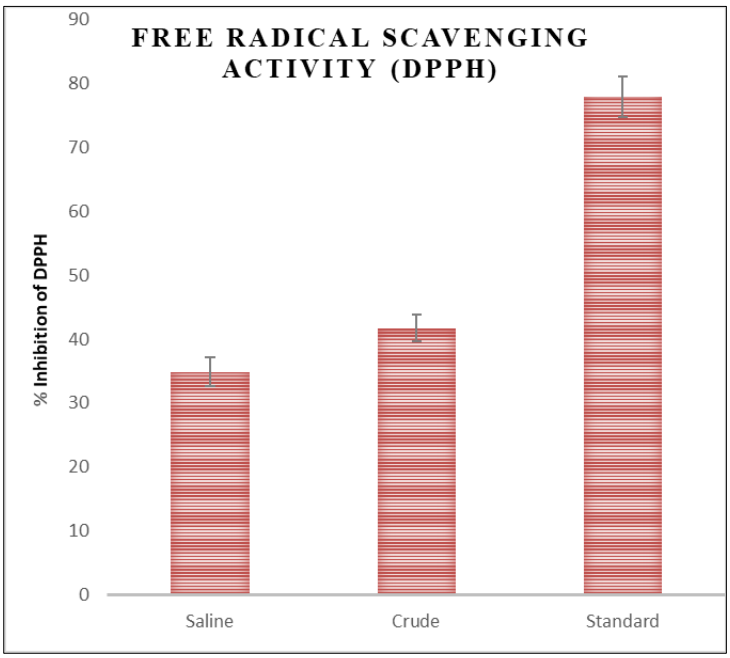


Figure 4 Inhibition of DPPH radical of crude and saline mucus of rohu fish. Given data is an average of three replicates ± S.E.

The DPPH scavenging activity of different mucus extracts has been shown in Table 4.1 and Fig 4.4. Higher absorbance of saline extract indicated that lower DPPH radical-scavenging activity of saline mucus. Whether high DPPH radical-scavenging activity of crude mucus extract due to the lower absorbance.

Table 1 Antioxidant activities of fish mucus by different assays

	Sample	**DPPH**	**Total phenolic contents (TPC)**	**Total flavonoid contents (TFC)**	**Reducing Power**
1	Saline	34.8958± 2.1933	23.993 ± 10.210	12.938 ± 1.6359	0.4993± 0.0135
2	Crude	41.744 ± 2.066	200.933± 12.1182	23.0263 ± 1.2631	0.707 ± 0.0141
	Standard	77.916 ± 3.143	_____	_____	_____

Biofilm inhibition

Biofilm is a thin layer of mucilage adhering to a solid surface. It comprises a group of microorganisms in which cells attached to the surface. These cells become surrounded within a slimy extracellular matrix that is composed of extracellular polymeric substances such as DNA, proteins and polysaccharides (Flemming and Wingender, 2010).

The resistence of biofilm is due to the occurrence of some polysaccharides and enzymes that cause the molecules inhibition or receptors inhibition in the pathway of quorum (necessary for formation of biofilm). Lecitin that are important for colonization and bacterial infection and also play significant role in formation of biofilm have been inhibited by the polysaccharides (Valle *et al*., 2006; Rendueles *et al*., 2013).

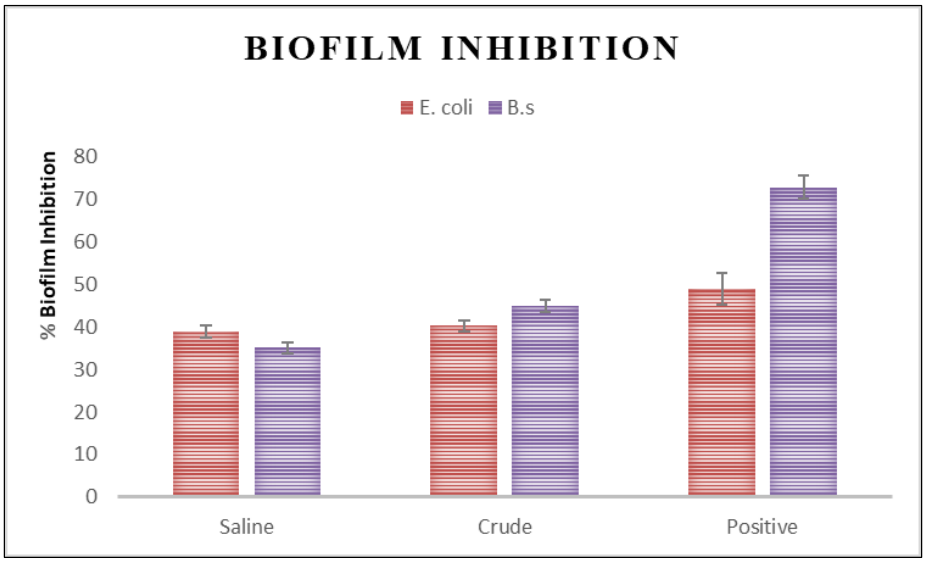
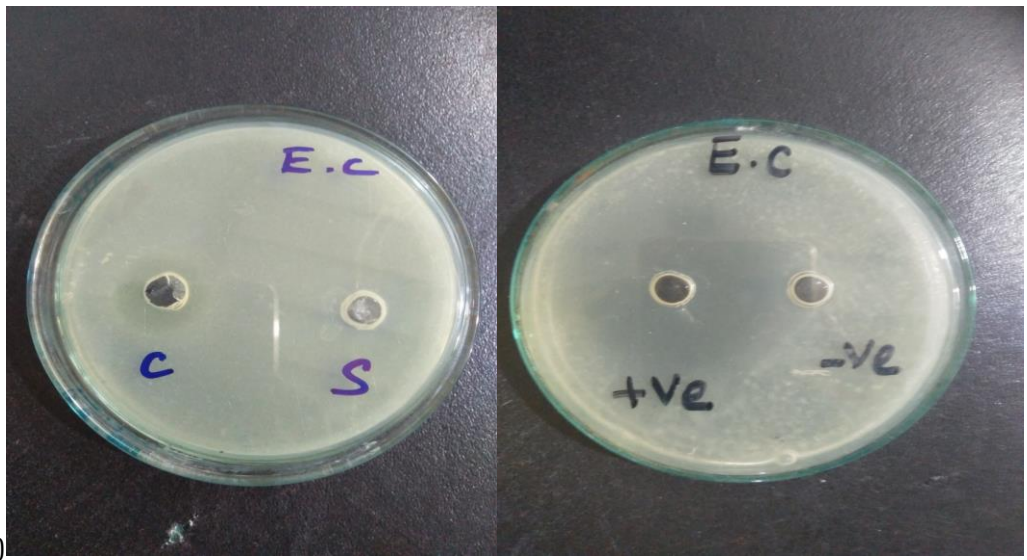


Figure 5 Inhibition of *Bacillus subtilis* and *E.coli* biofilm by the mucus extracts of rohu fish. The given data is average of three replicates ± S.D.

In saline mucus extract, the biofilm inhibition against *E. coli* is more than that of inhibition against *Bacillus subtilis*. Whether in case of crude mucus extract the biofilm inhibition against *Bacillus subti*lis is more than against the *E. coli.*

Antibacterial activity

Fish mucus extract was tested for their antimicrobial activities. For this activity four bacterial cultures were selected (two gram negative and two-gram positive bacteria). *E. coli, B. subtilis, S. aureus* and *P. multocida* these four bacterial cultures or strains were used in antibacterial activity.

(A) 

(B)

(C)

Figure 6 (A) Zone of inhibition of crude and saline fish mucus against *E. coli*. (B) Zone of inhibition of positive and negative control of *E. coli*. (C) Zone of bacterial inhibition of *B. Subtilis*. (D) Zone of inhibition of positive and negative control of *B. Subtilis.* (E) Zone of inhibition of *Staphylococcus aureus*. (F) Zone of bacterial inhibition of *P. multocida.*

Above figures demonstrated that fish mucus of *Labeo rohita* (rohu) showed highest antibacterial activity against *E. coli* bacteria with inhibition zone of 12 mm in saline case and 16 mm in crude mucus case. The lowest antibacterial activity was found against the *P. multocida* with inhibition zone of 7 mm in saline case and 11 mm in crude mucus case. The crude mucus extract showed more antibacterial activities than the saline mucus against in all bacterial culture (in all cases). Ampicilin was used as positive control to compare the bacterial zones of fish mucus.

Table 2 Antimicrobial activity against different bacterial of fish (*Labeo rohita)* mucus

Sample	***E. coli* (mm)**	***B. subtilis* (mm)**	***S. aureus* (mm)**	***P. multocida* (mm)**
Saline	12	11	10	7
Crude	16	15	13	11
(Ampicilin)	40	30	41	38

The native fish species like *Labeo rohita* and *Catla catla* showed high antimicrobial activity rather than that of foreign fish species like *Ctenopharyngodon Idella* and *Hypophthalmicthys molitrix* (Balasubramanian *et al*., 2012). Antibacterial proteins are secreted by the fish that make the fish able to permeablize the target cell membrane and in this way perform

as a protection obstacle. Antibacterial activity is due to the antibacterial glycoproteins that are found in the fish mucus capable to destroy bacteria by formation of huge pores in the membrane of target cells (Kuppulakshmi *et al*., 2008).

Cytotoxic activity

- Hemolytic activity

This assay is used to check the hemolysis of different samples. EDTA was used to safe the blood from clotting. The percentage hemolysis of saline and crude mucus extract was shown in the graph below and in table number 4.3. The more hemolytic activity was measured in crude mucus extract of rohu that is 25% and less hemolytic activity 18% was measured in saline mucus extract of rohu. As a positive control Triton-X was used, and its percentage hemolysis was 89%.

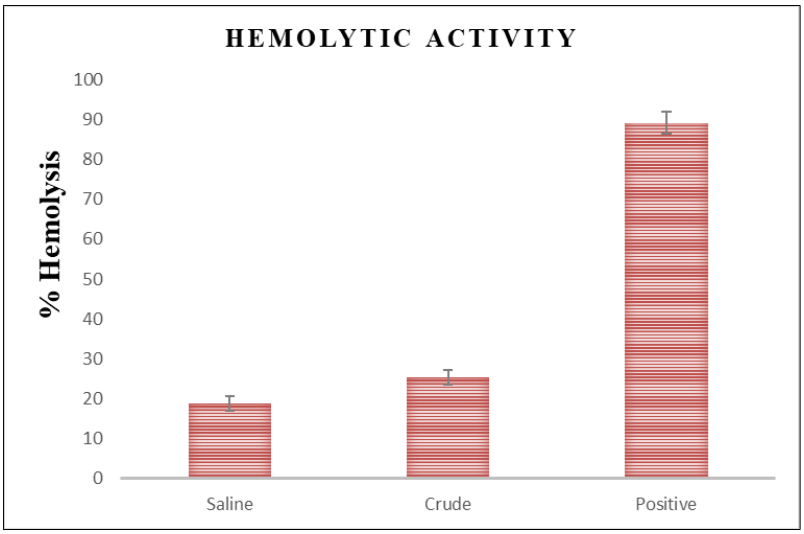


Figure 7 % Hemolysis of fish mucus extract against human RBCs

- Thrombolytic activity

For myocardial treatment many thrombolytic drugs are used. Among all these drugs Streptokinase is mostly used. This assay was used to determine the clot lysis ability of different samples (Wu *et al*., 2001).

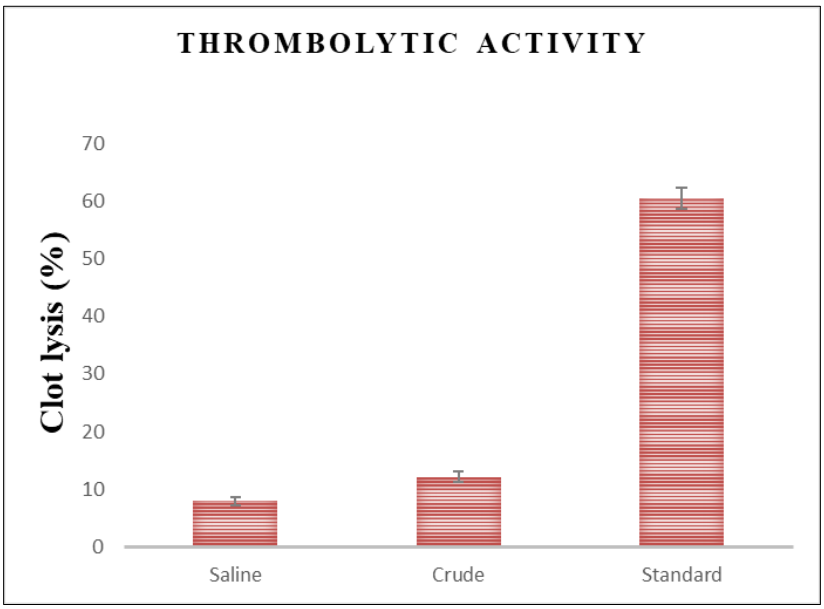


Figure 8 Thrombolytic activity of fish mucus extracts. Given data is average of three replicates ± S.E.

The above graph showed that crude mucus thrombolytic activity 12% is more than that of saline mucus extract that is 8%. The streptokinase was used as standard or positive control and its thrombolytic activity measured as 61%.

Table 3 Cytotoxicity and thrombolytic activities of fish mucus by different assays

Sample	Haemolytic activity	Thrombolytic activity
Saline	18.825 ± 1.8671	7.942 ± 0.751
Crude	25.346 ± 1.7677	12.128 ± 0.952
Standard	89.189 ± 2.685	61.4415 ± 1.8573

3.1.5. Biochemical analysis

- Catalase (CAT)

In this case, H_2O_2 was used as a substrate and the decomposition of H_2O_2 by the catalase enzyme was observed using UV-vis spectrophotometer. The absorbance measured at 240 nm.

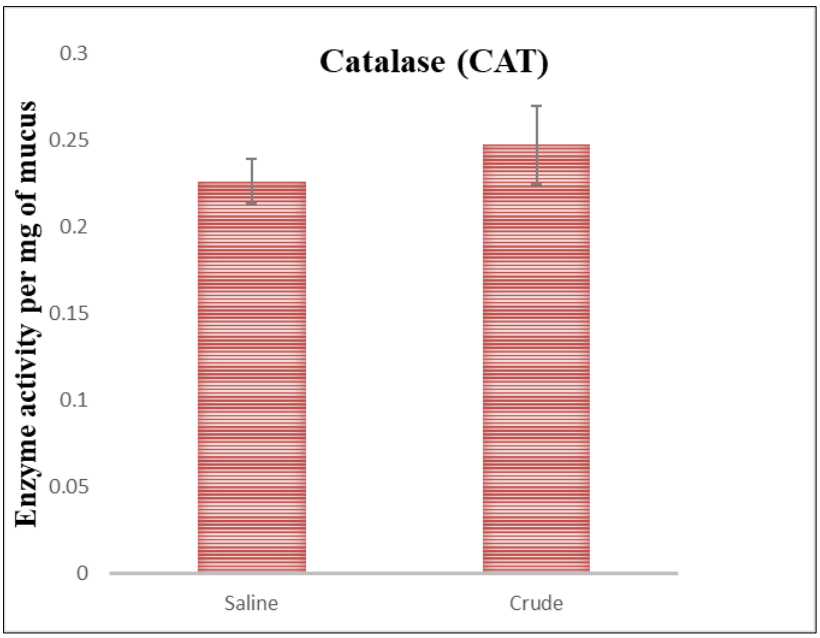


Figure 9 Graphical representation of comparative analysis of catalase activity of crude and saline extract of fish mucus.

The above graph showed that the crude mucus extract has the more Catalase activity than that of saline mucus extract of *Labeo rohita.*

- Peroxidase (POD)

The POD activity was assayed using guaiacol as a hydrogen donor by measuring the change at 470 nm

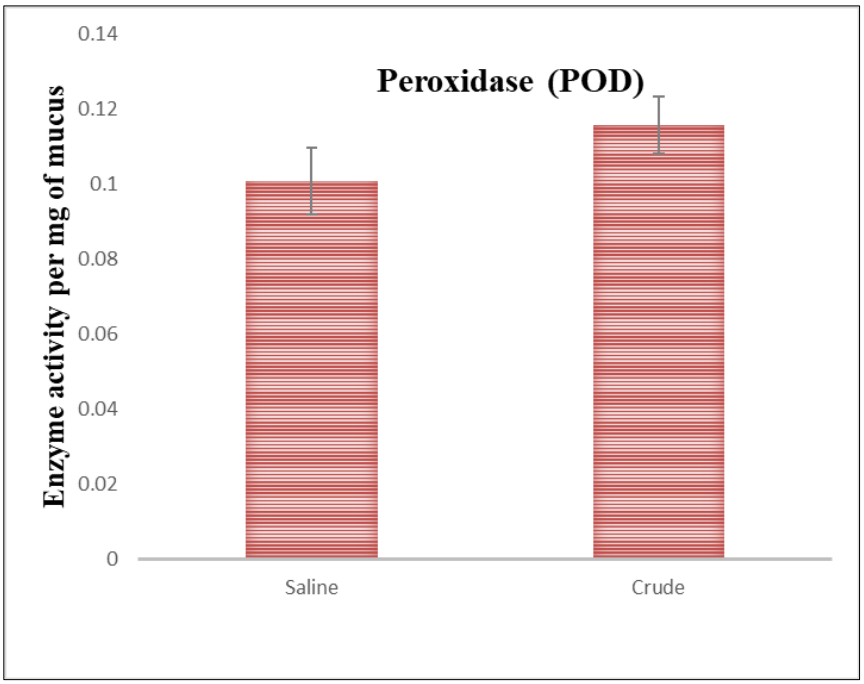


Figure 10 Graphical representation of Comparative analysis of Peroxidase activity of crude and saline extract of fish mucus.

The peroxidase activity of saline mucus extract was less than that of crude mucus extract of fish, because saline mucus extract was dilution mixture or sample means not as such pure like crude mucus. There was no dilution of crude mucus, so it exhibited more activity.

- Superoxide dismutase (SOD)

The reagents used in SOD assay included phosphate buffer (pH 7.5), riboflavin, nitro blue tetrazolium, Triton-X and methionine. After exposure of 15 min in UV light added riboflavin at the end. The absorbance was measured at 560 nm.

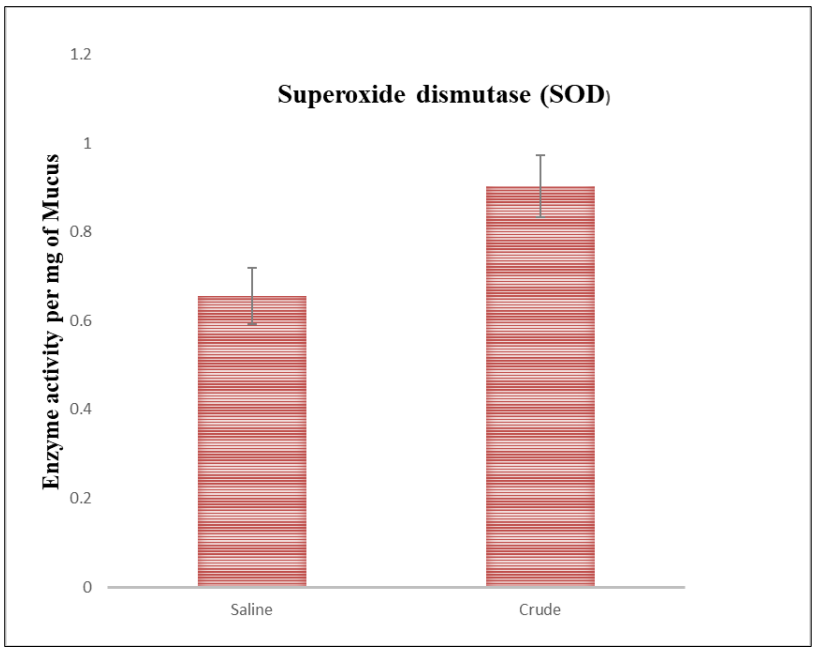


Figure 11 Graphical representation of Comparative analysis of Superoxide dismutase activity of crude and saline extract of fish mucus.

The graph represented that the saline mucus extract exhibited less SOD activity than the crude mucus extract of fish *Labeo rohita*.

- Protein estimation

In protein estimation assay samples were diluted to obtain protein. Bovine serum albumin used as a standard. The standard was prepared containing a range of 200 to 2000 micrograms protein (Bovine Serum albumin 2 mg/ml in 1000 µl volumes for setting up the standards). The absorbance (OD) was measured at 595 nm with the help of spectrophotometer. The results showed that the crude mucus extract had higher protein content of approximately 2 mg/mL, while least protein concentration was exhibited by saline mucus extract near to 1.7 mg/mL.

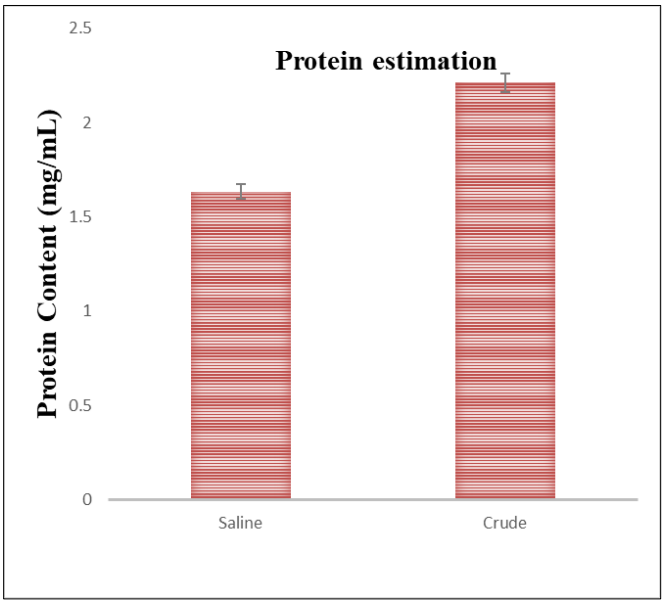


Figure 12 Graphical representation of Comparative analysis of Protein contents of saline and crude mucus extract by using Bovine serum albumin (BSA).

3.1.6. Characterization

- Fourier infrared spectroscopy (FTIR)

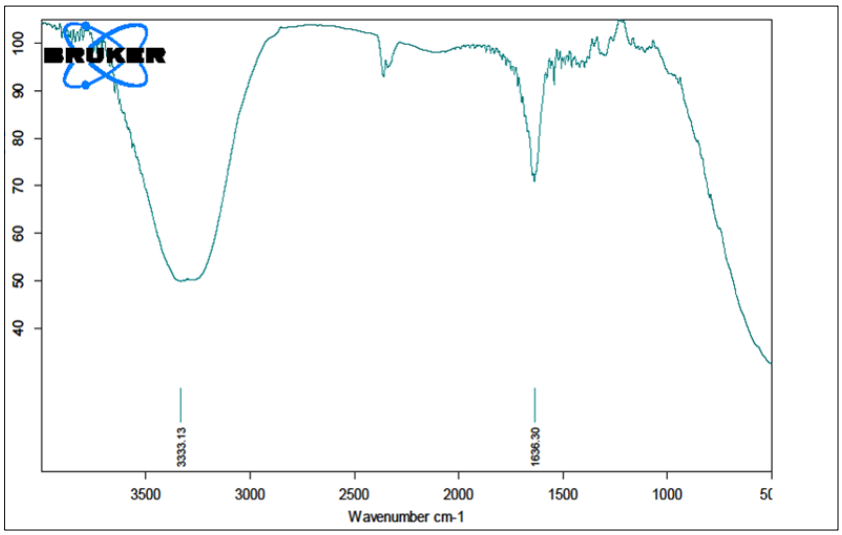


Figure 13 FTIR curve of saline mucus extract of *Labeo rohita.*

Table 4 FTIR spectra of *Labeo rohita* saline mucus extract

Sr.No	Range	Detected Functional group
1	3333.13	Aliphatic primary amines (N-H)
2	1636.30	Alkene (C=C)

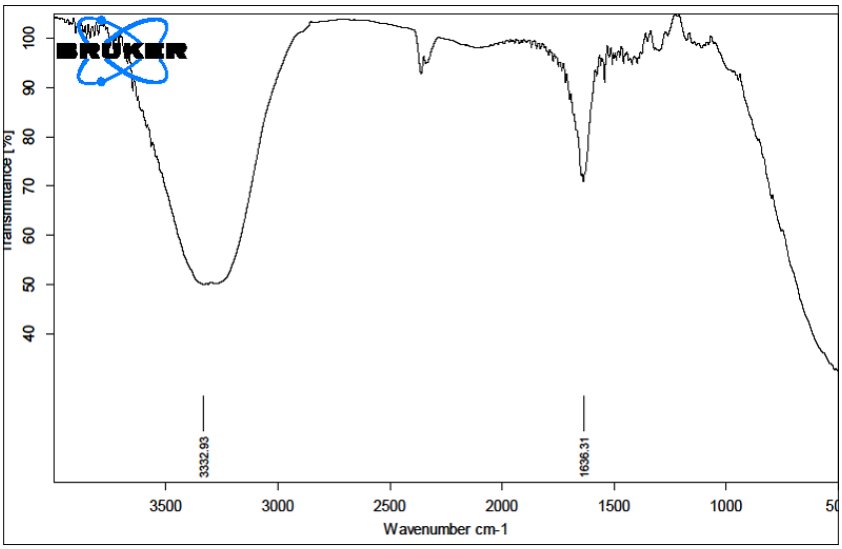


Figure 14 FTIR curve of crude mucus extract of *Labeo rohita.*

In the FT-IR spectrum different peaks observed of saline mucus. Total two peaks of the saline mucus of *Labeo rohita* observed in this FT-IR spectrum. The first peak observed at 3333.13nm it detected the aliphatic primary amines (N-H) functional group. The alkene functional group (C=C) detected at the second peak of 1636.30nm.

Table 5 FTIR spectra of *Labeo rohita* crude mucus extract

Sr.No	Range	Detected Functional group
1	3332.93	Aliphatic primary amines (N-H)
2	1636.31	Alkene (C=C)

In the FT-IR spectrum different peaks observed of crude mucus. Total two peaks of the crude mucus of *Labeo rohita* observed in this FT-IR spectrum. The first peak observed at 3332.93nm it detected the aliphatic primary amines (N-H) functional group. The alkene functional group (C=C) detected at the second peak of 1636.31nm.

- UV spectra

Normal range of UV-vis spectra used is ranged from 200 nm to 1100 nm through which peaks of different functional groups are find. In these spectra the maximum peak observed at 250nm, and the lowest peak observed at 1100 nm (0.5 nm). The observed spectrum peak is highest at between 200-300 nm but after 300nm the peak begins decline. The maximum absorbance is at 250 nm.

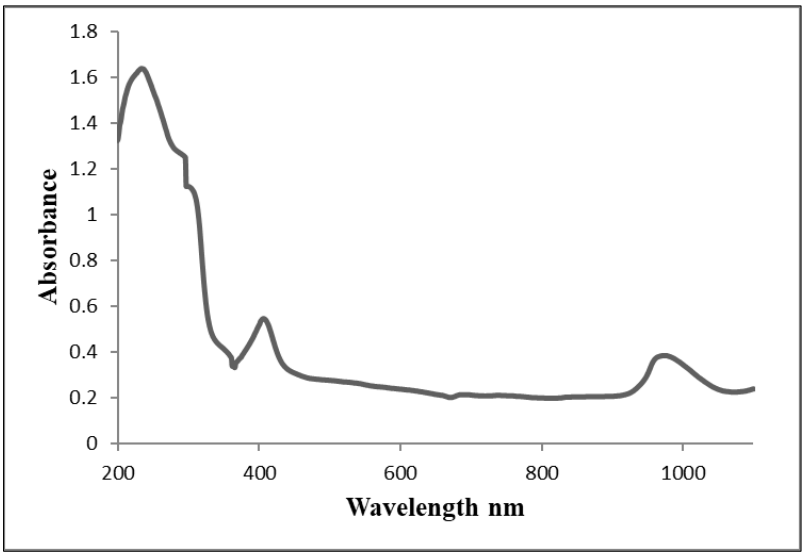


Figure 15 Graphical representation of UV spectra, saline mucus of fish

4. Discussion

The water environment for fish is very competitive due to the presence of large number of microorganisms, so mucus of fish provides protection against these microorganisms and pathogens (Subramanian *et al*., 2007). In fish immune defense, the native protective system is primarily important. This system is separated into 3 major components, the cellular machinery, the humoral components and the mucosal/epithelial obstruction. In fish the important ailment barrier is mucosal and epithelial barrier of digestive, skin and gill region, that is being persistently absorbed in media containing injurious agents. The fish mucus has numerous resistant defense parameters which are immunoglobulins, antimicrobial peptides and complement factors that provides both mechanical and physical protection to the fish (Suzuki *et al*., 2003; Magnadottir, 2006; Whyte, 2007).

The present study was conducted to test the biochemical analysis and characterization of fish epidermal mucus in *Labeo rohita*. For this purpose, two samples were collected as saline and crude mucus. Their protein contents, zone of inhibition, antioxidant and cytotoxicity of crude and saline mucus were measured. Characterization was also done.

Fish mucus of *Labeo rohita* (rohu) showed highest antibacterial activity against *E. coli* bacteria with inhibition zone of 12 mm in saline case and 16 mm in crude mucus case. Lowest antibacterial activity was found against the *P. multocida* with inhibition zone of 7 mm in saline case and 11 mm in crude mucus case.

Antibacterial proteins are secreted by the fish that made the fish able to permeablize the target cell membrane and in this way perform as a protection obstacle. Antibacterial activity is due to the antibacterial glycoproteins that are found in the fish mucus capable to destroy bacteria by formation of huge pores in the membrane of target cells (Kuppulakshmi *et al*., 2008). Antibacterial activity may be due to the variation in comparative levels of alkaline, cathepsin, proteases, lysozyme and phosphatase of all fish's epidermal mucus.

Flavonoids are the group of secondary metabolites with significant antioxidant and chelating potential. Antioxidant activities of fish mucus was less and minute. Total phenolic content of saline mucus extract of rohu is less in range of almost 20-22 mg GAE/g, and TPC of crude mucus extract is high in range of 40-42 mg GAE/g and same the case for total flavonoid content of fish mucus that may not be determined as a rich and good source of antioxidant properties, while the plants are known best for their high antioxidant properties such as vegetables, fruits and medicinal plants. The phenolics activity is due to their redox properties, trench which act as quench singlet oxygen, hydrogen donators and reducing agents.

Biofilm activity was used to find out the fish mucus extract potential to inhibit biofilm formation. But fish mucus extracts were rarely tested for their antibiofilm activity. In saline mucus extract, the biofilm inhibition against *E. coli* is more than that of inhibition against *Bacillus subtilis*. Whether in case of crude mucus extract the biofilm inhibition against *Bacillus subtil*is is more than against the *E. coli*. This inhibition may be due to occurrence of polysaccharides and certain enzymes in fish mucus extracts that are concerned in molecules inhibition or receptors important for formation of biofilm. These

polysaccharides have the capability to resist lectin that are important for bacterial colonization, infection and play significant role in biofilm formation (Valle *et al*., 2006; Rendueles, 2013).

No more work done on the cytotoxicity of fish mucus so less research data available on it. The high cytotoxicity was measured in crude mucus extract of rohu and less in saline mucus extract. Formation of clot is severe blood circulation problem. By the action of thrombin fibrinogen produced blood clot that is break through plasmin. Many thrombolytic drugs are now a days used for the treatment of myocardial infarction.

In characterization, specific wavelength absorbed by sample that detect the different compounds. One peak of fish mucus is observed at the 250-300 nm that determine the presence of phenolics and flavonoids. In the FT-IR spectrum different peaks observed of saline and crude mucus. Total two peaks of mucus of *Labeo rohita* observed in this FT-IR spectrum. The first peak observed at 3333.13nm it detected the aliphatic primary amines (N-H) functional group. The alkene functional group (C=C) detected at the second peak of 1636.30 nm.

The present study findings determined that epidermal mucus of *L. robita* contains some biochemical substances in fish resistant system to protect it from pathogenic microbes. The mucus of fish released from the skin acts as a defensive wall between external and internal environment.

5. Conclusion

The conclusion of this study was that slight or very little biological activities were exhibited in fish mucus of *Labeo rohita*. As antibacterial activity, the greatest inhibiton zone was observed 18 mm and lowest zone of inhibition for *P. multocida* was 14 mm in crude mucus case. Protein contents in fish mucus, that was exhibited higher in crude mucus extract of *L. rohita* as compared to saline mucus which was (2.212±0.048) and (1.634±0.038) respectively. Similirarly, from characterization point of view, FTIR results in fish mucus showed the presence of aliphatic primary amines (N-H) and alkenes as a functional group (C=C) at different peaks of spectra and UV spectrum showed maximum peak at 250 nm. The results obtain were analyzed through mean and standard deviations. Fish mucus having many antimicrobial agents that might be used to prepare the new drugs for the treatment of infectious diseases which are caused by opportunistic and pathogenic microorganisms. Such mucus properties proposed that it might be useful in aquaculture.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Reference

[1] Abu-Ruwaida, A. S., Banat, I. M., Haditirto, S., Salem, A., & Kadri, M. (1991). Isolation of biosurfactant-producing bacteria, product characterization, and evaluation. *Acta biotechnologica*, *11*(4), 315-324.

[2] Al-Arifa, N., M.S. Mughal, A. Hanif and A. Batool. 2011. Effect of alkaline pH on bioactivemolecules of epidermal mucus from *Labeo rohita*. Turk. J. Biochem., 36: 29-34.

[3] Al-Hassan, J.M., M. Thomson and R.S. Criddle. 1983. Accelerated wound healing by apreparation from skin of the arabian gulf catfish. Lancet, 1: 1043-1044.

[4] Arockiaraj, J., A.J. Gnanam, D. Muthukrishnan, R. Gudimella, J. Milton, A. Singh, S.Muthupandian, M. Kasi and S. Bhassu. 2013a. Crustin, a WAP domain containing antimicrobial peptide from freshwater prawn *M. rosenbergii*: immune characterization. Fish. Shellfish Immunol., 34: 109-118.

[5] Balasubramanian, S., R.P. Baby, A.A. Prakash and M. Prakash. 2012. Antimicrobial properties of skin mucus from four freshwater cultivable Fishes (*Catla catla, Hypophthalmichthys molitrix, Labeo rohita and Ctenopharyngodon idella)*. Afr. J. Microbiol. Res., 24: 5110-5120.

[6] Balasubramanian, S., R.P. Baby, A.A. Prakash and M. Prakash. 2012. Antimicrobial properties of skin mucus from four freshwater cultivable Fishes (*Catla catla, Hypophthalmichthys molitrix, Labeo rohita and Ctenopharyngodon idella)*. Afr. J. Microbiol. Res., 24: 5110-5120.

[7] Bragadeeswaran, S. and S. Thangaraj. 2011. Hemolytic and antibacterial studies on skin mucus of eel fish, *Anguilla anguilla* Linnaeus. Asian. J. Biol. Sci., 4: 272-276.

[8] Cavalieri, S.J., R.J. Harbeck, Y.S. McCarter, J.H. Ortez, I.D. Rankin, R.L. Sautter and C.A. Spiegel. (2005). Manual of Antimicrobial Susceptibility Testing. Coordinating Editor, M.B. Coyle. Amr. Soci. Microbiol (ASM).

[9] Duan, X., Y. Jiang, X. Su, Z. Zhang and J. Shi. 2007. Antioxidant properties of anthocyanins extracted from litchi (Litchi chinensis sonn) fruit pericarp tissue in relation to their role in the pericarp blowing. Food Chem., 101:1365-1371.

[10] Ellis, A.E. 1999. Immunity to bacteria in fish. Fish Shellfish Immunol., 9: 291-308.

[11] Ellis, A.E. 2001. Innate host defense mechanisms of fish against viruses and bacteria. Dev. Comp. Immunol., 25: 827-839.

[12] Flemming, H. and J. Wingender. 2010. The biofilm matrix. Nat. Rev. Microbiol., 8: 623-633.

[13] Garcia-Moreno, P.J., *et al.*, 2014. Antioxidant activity of protein hydrolysates obtained from discarded Mediterranean fish species, Food Res. Int., 65: 469-476

[14] Garcia-Moreno, P.J., *et al.*, 2014. Antioxidant activity of protein hydrolysates obtained from discarded Mediterranean fish species, Food Res. Int., 65: 469-476

[15] Gulcin, I. 2005. The antioxidant and radical scavenging activities of black pepper (Piper nigrum) seeds. J. Inter. Food Sci. Nut., 56: 491-499.

[16] Gulcin, I., R. Elias, A. Gepdiremen and L. Boyer. 2006a. Antioxidant activity of lignans from fringe tree (Chionanthus virginicus). Eur. Food Res. Technol., 223: 759-767.

[17] Handy, R.D., F.B. Eddy and G. Romain. 1989. Invitro Evidence for the Ionoregulatory Role of Rainbow trout Mucus in Acid, Acid Aluminum and Zinc Toxicity. J. Fish Biol., 35: 737-747.

[18] Hellio, C., A.M. Pons, C. Beaupoil, N. Bourgougnon and Y. Le Gal. 2002. Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. Int. J. Antimicrob. Agents. 20: 214-219.

[19] Humbert, W., R. Kirsch-Simonneaux. 1986. Is mucus involved in biocrystallization? Study of the intestinal mucus of the sea water eel *Anguilla anguilla* L. Cell Tissue Res., 245: 599-604.

[20] Hussain, S.M., S.A. Rana, M. Afzal and M. Shahid. 2011. Efficacy of phytase supplementation of mineral digestibility in *Labeo rohita* fingerlings fed on corn glutenmeal (30%) based diets. Pakistan J. Agric. Sci., 48: 237-241.

[21] Jhingran, V.G. 1991. Fish and Fisheries of India. Edn 3, Hindustan Publishing Corporation, New Delhi, India.

[22] Jung, T. S., C.S. Del Castillo, P.K. Javaregowda, R.S. Dalvi, S.W. Nho, S. B. Park, I. S. Cha,H. W. Sung, J. Hikima and T. Aoki, 2011. Seasonal variation and comparative analysis of non-specific humoral immune substances in the skin mucus of olive flounder (*Paralichthys olivaceus*). Develop. Compar. Immunol., 38: 295-301.

[23] Khan, N., N.A. Qureshi, M. Nasir, F. Rasool and K.J. Iqbal. 2011. Effect of artificial diet andculture systems on sensory quality of fried fish flesh of Indian major carps. Pakistan J. Zool., 43: 1177-1182

[24] Kumari, U., A.K. Nigam, S. Mittal and A.K. Mittal. 2011. Antibacterial properties of the skin mucus of the freshwater fishes, *Rita rita* and *Channa punctatus*. Eur. Rev. Med. Pharmacol. Sci., 15: 781-786.

[25] Kuppulakshmi, C., M. Prakash, G. Gunasekaran, G. Manimegalai and S. Sarojini. 2008. Antibacterial properties of fish mucus from *Channa punctatus* and *Cirrhinus mrigala*. Eur.Rev. Med. Pharmacol. Sci., 12: 149-153

[26] Magnadottir, B. 2006. Innate immunity of fish (overview). Fish Shellfish Immunol., 20: 137- 151.

[27] Mamelona, J., E. Pelletier, K. Girard-Lalancette, J. Legault, S. Karboune and S. Kermasha. 2007. Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, *Cucumaria frondosa . Food Chem*., 104: 1040-1047.

[28] Martinez-Antün, A., C. de Bolüs, M. Garrido, J. Roca- Ferrer, C. Barranco, A. Xaubet, C. Picado and J. Mullol. 2006. Mucin genes have different expression patterns in healthy and diseased upper airway mucosa. Clin. Exp. Allergy., 36: 448-457.

[29] Medeiros, P. M., Seidel, M., Niggemann, J., Spencer, R. G., Hernes, P. J., Yager, P. L., ... & Hansell, D. A. (2016). A novel molecular approach for tracing terrigenous dissolved organic matter into the deep ocean. *Global Biogeochemical Cycles*, *30*(5), 689-699.

[30] Mittal, S., Pinky and A.K. Mittal. 2002. Characterisation of glycoproteins in the secretory cells in the operculum of an Indian hill stream fish Garra lamta (Hamilton) (Cyprinidae, Cypriniformes). Fish. Physiol. Biochem., 26: 275-288.

[31] Nigam, A.K., N. Srivastava, A.K. Rai, U. Kumari, A.K. Mittal and S. Mittal. 2012b. The first evidence of cholinesterases in skin mucus of carps and its applicability as biomarker of organophosphate exposure.

[32] Nigam, A.K., N. Srivastava, A.K. Rai, U. Kumari, A.K. Mittal and S. Mittal. 2012b. The first evidence of cholinesterases in skin mucus of carps and its applicability as biomarker of organophosphate exposure. Environ. Toxicol.

[33] Ordonez, A. A. L., Gomez, J. D., & Vattuone, M. A. (2006). Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. *Food chemistry*, *97*(3), 452-458.

[34] Pearson, J. and I.A. Brownlee. 2005. A surface and function of mucosal surface. In: Colonization of the mucosal surface, *Nataro JP* (Ed.). ASM Press: Washington DC, USA.

[35] Rajan, B., J.M.O. Fernandes, C.M.A. Caipang, V. Kiron, J.H.W.M. Rombout, M.F. Brinchmann. Proteome reference map of the skin mucus of Atlantic cod (*Gadus morhua*) revealing immune competent molecules. 2011. Fish Shellfish Immunol.,31:224-231.

[36] Rendueles, O., J. Kaplan and J. Ghigo. 2013. Antibiofilm polysaccharides. Environ. Microbiol. 15: 334-346

[37] Rendueles, O., J. Kaplan and J. Ghigo. 2013. Antibiofilm polysaccharides. Environ. Microbiol. 15: 334-346.

[38] Shephard, K.L. 1994. Functions for fish mucus. Review of Fish Biology. 4: 401-429.

[39] Subramanian, S., S.L. MacKinnon and N.W. Ross. 2007b. A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comp. Biochem. Physiol., 148: 256-263.

[40] Subramanian, S., S.L. MacKinnon and N.W. Ross. 2007b. A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comp. Biochem. Physiol., 148: 256-263

[41] Subramanian, S., S.L. MacKinnon and N.W. Ross. 2007b. A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comp. Biochem. Physiol., 148: 256-263.

[42] Sumi, T., Y. Hama, H. Nakagawa, D. Maruyama and M. Asakawa. 2004. Purification and further characterization of a glycoprotein from the skin mucus of Japanese eels. J. Fish Biol., 64: 100-115.

[43] Sun, D., Qin, Y., Chluba, J., Epplen, J. T., & Wekerle, H. (1988). Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T–T cell interactions. *Nature*, *332*(6167), 843-845.

[44] Suzuki, Y., S. Tasumi and S. Tsutsui. 2003. Molecular diversity of skin mucus lectins in fish. Comp. Biochem. Physiol. Biochem. Mol. Biol., 136: 723-730.

[45] Tkachenko, A., Y. Waguespack, J. Okoh and E. May. 2006. Isolation of intact high molecular weight glycoconjugates from the skin mucus of Morone saxatilis (Walbaum). J. Fish Dis., 29: 433-436.

[46] Turkoglu, I., S. Turkoglu, S. Celik and M. Kahyaoglu. 2010a. Antioxidant and Antimicrobial Activities of Turkish Endemic Achillea Species. Afr. J. Microbiol. Res., 4: 2034- 2042.

[47] Tyor, K. and S. Kumari. 2016. Biochemical characterization and antibacterial properties of fish skin mucus of freshwater fish, *Hypophthalmichthys nobilis.* Int. J. Pharm.Pharmaceut. Sci., 8.

[48] Valle, J., S. Da Re, N. Henry, T. Fontaine, D. Balestrino, P. Latour-Lambert and J.M. Ghigo. 2006.

[49] Valle, J., S. Da Re, N. Henry, T. Fontaine, D. Balestrino, P. Latour-Lambert and J.M. Ghigo. 2006.

[50] Whyte, S.K. 2007. The innate immune response of finfish, A review of current knowledge. Fish Shellfish Immunol., 23: 1127-1151.

[51] Wu, D.H., G.Y. Shi, W.J. Chuang, J.M. Hsu, K.C. Young and C.W. Chang. 2001. Coiled coilregion of streptokinase gamma-domain is essential for plasminogen activation. J. Biol. Chem., 276: 15025-15033

[52] Yadav, M., A. Yadav and J.P. Yadav. 2014. In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from Eugenia jambolana Lam. Asian Pacific J.Trop. Med., 7: 256-261.

[53] Zalme, R. C., McDowell, E. M., Nagle, R. B., McNeil, J. S., Flamenbaum, W., & Trump, B. F. (1976). Studies on the pathophysiology of acute renal failure: II. A histochemical study of the proximal tubule of the rat following administration of mercuric chloride. *Virchows Archiv B*, *22*, 197-216.

[54] Zamzow, J.P. 2004. Effects of diet, ultraviolet exposure and gender on the ultraviolet absorbance of fish mucus and ocular structures. Mar. Biol. 144: 1057-1064.