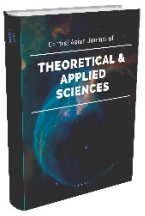


**CENTRAL ASIAN JOURNAL OF THEORETICAL AND APPLIED SCIENCES**

**Volume: 03 Issue: 07 | Jul 2022 ISSN: 2660-5317**
**https://cajotas.centralasianstudies.org**

# In Vitro Evaluation of Binding of Fish Mucus by Nanoparticles Induce Oxidative Stress on Nile Tilapia

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*Received 24th May 2022, Accepted 13th Jun 2022, Online 22th Jul 2022*

***Abstract:*** *The emerging species of fish, Nile tilapia have extremely practical importance in the field of aquaculture in Pakistan and other country of Asian subcontinents. The natural fresh water bodies are quickly contaminated with the various harmful chemicals the improvement in industrialization. The main objective of this research was to designate the fish mucus in respect to its biochemical investigation and representation. This involves various procedures such as agar diffusion for assessment of antimicrobial activity. And total phenolic content was also be inspected and as well as the total flavonoid content (TFC) of mucus obtained from fish. The cytotoxicity of mucus of fish was checked by the haemolytic and thrombolytic tests. Moreover, various other techniques such as UV-visible spectra, FTIR was used for the assessment of biochemical processes. The greatest inhibition zone was observed in S.areus and lowest zone of inhibition for saline mucus in P. multocida. Protein contents in fish mucus. Similarly, 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 spectrum .The results obtain were analyzed through mean and standard deviations.*

 

## REVIEW OF LITERATURE

Khan *et al*. (2016) In Thailand to Pakistan, Muhammad Naeem explored the purpose of common Tilapia aquaculture. Due to powerful tolerance to turbidity and temperature breeding practices and inexhaustible lakes, the canal settles natural inland water in most natural habitats such as river streams. Food fish cyprinid species in waters in Pakistan, although one of the most significant was common carp. Recruiting more than 400,000 individuals in the beginning of the project will gradually increase the country's economy. Freshwater carps are grown exclusively in the provinces of Punjab and Sindh throughout Pakistan.

From some earlier research information found that, for humans, fish mucus can be useful. It might be probable that protein fibers in the slime of hagfish formulate new materials and fabrics. Recent findings proposed that mucus formed by some coral reef types fishes may be used to produce a new sunscreen. Fish mucus consists of other substances except water and mucin, including antibodies, salts and enzymes. Fish which live in the area of coral reefs are been present to contains chemicals in the mucus that is a called as mycosporine-like amino acids. These chemicals obstruct the ultraviolet (UV) light.

Cordero *et al*. (2015) stated that, to check mucus, *Anguilla anguilla, Anguillidae, Pagellus bogaraveo, Sparidae and Dicentrarchus labrax, Moronidae* were studied, tissues samples and blood sera for antibacterial and agglutinating activity against diversity of Gram positive and negative bacteria. These samples were also tested against RBCs of sheep to analysis their haemolytic activity. Out of these the highest antibacterial activity was determined in blackspot seabream and European eel (against Vibrioalginolyticus). Haemolytic activity against RBCs of sheep sea bass, and black spot mucus, along with the sera of eel and sea bass. The sea bass and eel sera showed agglutination activity against Staphylococcus aureusand Pseudomonas aeruginosa, the sea bass mucus was capable to agglutinate isolates of *V. Alginolyticus, A. hydrophilaand Vibrio fluvialis.* Nevertheless, on haemolytic and other biological activities of fish, little possibility of relative studies on different species in relevant research that make this subject a growing interest. Furthermore, in past few years search for control from infectious disease and for also natural antibacterial agents are gaining importance in relative to occurrence of widespread antibiotic resistant phenomenon.

Food fish cyprinid species in Pakistan's waters, although common carp was one of the most important. At the start of the project, recruiting more than 400,000 people will gradually boost the economy of the country. Freshwater carp is cultivated solely throughout Pakistan in the provinces of Punjab and Sindh. Banaee *et al*. (2009) is particularly vulnerable to the environmental contamination of water fish. Physiology and biochemical processes affected by various types of insecticides can cause severe impairment of health status and impairment of fish physiology by pollutants such as insecticides.

Ritvo *et al*. (2004) complete the C combination stoking ratio. Mrigla is generally adopted as a bottom feeding species because of its common carp that is normally kept away from the polyculture of the pond. The present set-up of culture is a controversial introduction of common carp. Exposing debris to sunlight releasing nutrients for microscopic plants believed that this species of fish could bear various climatic conditions.

Magnado *et al*. (2005) investigated as wild species were initially settled for aquaculture purposes in the freshwater ecosystem common carp. The introduction of this alien species has also contributed to a substantial decline in local commercial fish species catches. Lysozymes, lectin, peptides and proteases active against bacteria and viruses found in the secretions of fish skin and gill mucus. These have played an important role in innate immunity.

Chong *et al*. (2006) conducted an experiment to check the concentration of various oral mucosal substances in tilapia in concentrations, including mucins and glycoproteins, related to mouth breeding. We have already described the presence of biochemical changes in the epidermal mucus through critical to larval development and parental fish protection during the parental care phase.

**Mucus as Osmoregulation in Fish:**

Sarmasik (2002) Mucus partly blocks the water movement or flow into and out of body of fish through its connection with the fish scales that's why mucus is useful for the fish. They assist to remain constant conditions in the fish. Other portions of fish body also effect the concentrations of water and salt in the fish. The urine contains less or more salts and waters, as required. In addition, gill absorbs or excretes salts, depending upon the fish's requirements. Movement of water and ions in freshwater fish, the indicator inside and outside of skin are small because of the occurrence of scales and mucus.

**Mucus Cocoons:**

Like the other skin of fishes, mucus are also produced by the parrotfish's skin. In addition, in parrotfish gill chambers, mucus glands are present. For protection, they make cocoon of mucus at night and enfold themselves in cocoon for their protection. Gill glands secreted mucus for the cocoon and then released from fish mouth. But the mucus cocoon function is not completely known. A general assumption is that it masks the fragrance of parrotfish that avoid the aggression by predators when it is sleeping. Another assumption is that these mucus cocoons saved the fish by the attack of small blood-sucking parasites known as gnathiid isopods.

**Fish Mucus as a Natural Sunscreen:**

Noga (2000).The experimenters have revealed that if they combine the chemical found in crustaceans shells with the chemical present in fish mucus, then as a result of such combining the substance produced, block the both rays of ultraviolet B and A from the sun. These ultraviolet rays are that rays which a cause of skin cancer and sunburn. These jointed chemicals might be helpful as the natural, environment friendly sunscreen for human beings. Some amino acids known as mycosporine-like amino acids (MAAs) which is chemicals of light-blocking in the fish mucus. These chemicals are also been present in some algae, cyanobacteria and fungi like in reef-dwelling fish.

Scientists added that chitosan made MAAs to lattice. From the crustacean's shells, a chemical known as chitosan is obtained. Chitosan is an appealing chemical in its specific right due to its good ability of wounds healing. Chitosan occurs as a large substances called as polymers. MAAs disperse through the transporter, sunscreen part in its place of staying where MAAs are applied. Attaching them with chitosan lattice avoids this problem. +6+

Shephard (1993) reported that fish produces mucus in both types of skin surfaces such as skin on non-gill surface and skin on gill-surface that might form a distinct layer between water and tissues. Mucus in bulk is produced by the goblet cells, but other types of cells are implicated. The rank of mucus covering in unstressed healthy fish, especially on its gills is unsure. In mucus layer, neither the concentration nor the thickness of macromolecules has been resolute with precision. However, it is clear that disease and stress frequently show the way to the mucus release and increase the mucus thick layers.

Fish Mucus is considered to have various roles, but some are fine financed by experimental testing. With few superficially applied chemicals, mucus of fish has definite relations, although for other chemicals it performs only as an unmoved layer. In some conditions, it can help to impermeability of epithelial. In techniques, there is some significance to change the mucus characters or eliminate it, mainly to increase the diffusion of beneficial agents.

**Antimicrobial activity:**

Manivasagan *et al*. (2009) show higher activity in Rita's acidic solvents (0.1% trifolin acid or 3% acetic acid) mucus extract in the aqueous media channel punctuates. Rita described a wide range of pathogens in this study also reported antimicrobial activity of brook trout, haddock and hag-fish epidermal mucus extracts that the acid mucus extracts showed the highest level of bactericidal activity against Salmonella enterica serovar Typhimurium C one-sixty. More recently, in 0.01 percentage acetic acid from catfish Arius maculatus, antibacterial activity was extracted in skin mucus.

Wei *et al*. (2010) The extract from the H was obtained. Nobitis had a very strong antibacterial potential for all the microbes treated. Several other studies also observed the same observations intended with strong antibacterial potential for the extract obtained from the crude fish. From the overall results, it was concluded that both the aqueous mucus extract and the crude mucus extract for C. straitus had an inhibitory effect on fish pathogenic bacteria but no inhibitory effect towards K. pneumonia, E. coli (human pathogenic bacteria). On the other hand, extracts were obtained from the H-shaped aqueous mucus and crude mucus. Nobitis showed strong antibacterial activity towards the pathogenic human and fish bacteria described above.

(Esteban, 2012) The mucus obtained from the fish skin is considered the main source of defense against microbes, according to a study. In addition, it was also reported from the above study that fish including scale less fish are likely to produce epidermal mucus in higher proportions than those produced by fish with scales above them. While the fish named as bighead produces the higher amount of mucus among the fish with scales. The cause of the increase in water turbidity is due to the intensive secretion of skin mucus by the fish infected with the attached organism, which caused an increase in epidermal mucus Secretions. However, it has also been suggested that the secretion of mucus from the infected catfish from the gastrointestinal tract and skin in greater proportions may also be another cause of the increase in water turbidity.

(Bergsson *et al*., 2005) Fish epidermis and its skin-secreted mucus consists of several biocidal and biostatic elements including agglutinin, antimicrobial peptides, proteolytic enzymes, proteases, antibodies, immunoglobulins, hemolysins, complements, lysozymes, lectins, and C-reactive substances. Despite the availability of large amounts of work on the mucus protective agent derived from the fish epidermis, great interest in skin function as isolation for potent antimicrobial components has been developed

A brief overview of the immune components of the mucus of fish is now available. There are several types of antimicrobial elements with the mucus secreting cells from the fish's epidermis. The cause of the inhibition effect is pore from properties to different bacterial strains and it is further suggested that fish have secreted several antibacterial properties in the response to the target cells and thus developed resistance and developed defense. The presence of antibacterial property is due to the presence of glycoproteins in the mucus that hold the ability to destroy bacteria by forming a slightly large pores in the target cell membrane. (Tirupathi *et al*., 2011).

Balakumar *et al*., (2011) The overall results of the following study show that fish mucus possesses the great potential as an antimicrobial agent used in drugs to cure certain infectious diseases due to certain pathogens and also favors fish folk practice. Lectin is well known in the fish body for having several antibacterial and antifungal properties. Mucus is well known to have various types of trypsin and proteases including (metalloproteases, cysteine proteases and serine proteases) in it together with the property of acting as an antibacterial agent.The mucus secreted from epithelial cells and epidermal cells play meaningful roles as the shield from environmental pathogens as well as biological fish barriers.

In addition, the mucus secreted by the epidermis provides significant defense against several pathogens and thus acts as a strong source of unique antimicrobial elements (Ellis, 2001; Nagashima *et al*., 2001 ; Sarmasik, 2002). The mucus secreted from the epithelial and epidermal cells varies from species to species and therefore affects their composition. Furthermore, according to both physiological and ecological conditions, the biological and chemical elements of the mucus differ. The mucus produced by the various fishes was highly resistant to several microbes in the current study.

The collected mucus from a total of four fishes presents different property the tested bacteria. It is generally acknowledged that physical and chemical stresses, including (rough handling, injuries, ectoparasite infestations) affecting the skin's mucus, cause a significant increase in the susceptibility of the fish to multiple pathogens (Rob). In the current study, we evaluated the innate immune barrier presented by the mucus secreted towards CyHV-3 entrance by the epidermal cells. The results show that skin-screened mucus neutralizes the property of CyHV-3 and hinders CyHV-3 binding to the epidermal cells. Mucus present on the fish's upper surface provides a defense against pathogens entering. There are limited studies of the innate mechanism of immune protection to multiple bacterial infections for the mucus derived from the epidermal cells. The mucus obtained from the fish named as carp impedes the binding of the CyHV-3 to the epidermal cells. From the introduction, this is already clear that the mucus from the epidermis created an innate response as a shield for several pathogens to enter. Such protection depends on the mechanical decline of pathogens for pathogens to enter the epidermal cells.

And ultimately involves pathogens-related neutralization through active molecules. The mucus obtained from the fish's epidermal cells consist of large quantities of elements that participate in the neutralization of multiple viruses, such as C-reactive proteins, defensins, immunoglobulins, lectins, immunoglobulins, and complement factors. It is suggested, however, to find out the mechanisms by which the epidermal cells mucus neutralizes the CyHV-3. The presence of heterogeneity (thickness) in the fish may be able to represent physiological variances or results may occur through physical contact removal of the mucus.

Palakash *et al*., (2008) Further constant studies show the facts that the main sites associated with the primary infection are on the fins boundary. Thioredoxin peroxidase belongs to the family of peroxiredoxins that includes thioredoxin and thioredoxin reductase and is essentially responsible to mediate cellular oxidative stress. Studies have also shown localization of peroxiredoxins which are stress-inducible in mammalian olfactory epitheliums and mucus secretion (Gon *et al*., 2001).

Alexander *et al*., (1992) Lectin, a group of carbohydrate binding proteins, has been reported in fish skin and mucus with role of pro- viding external defense mechanism via agglutination process to stop pathogenic penetration or colonization. Here we identified the unique expression of a C-type lectin in discus parental mucus. The presence of this lectin in the mucus could therefore be a form of defensive mechanism to protect fish during the constant biting and nipping activities of fry. This present study showed the up regulation of three key metabolic enzymes: fructose-biphosphate aldolase C, lactate dehydrogenase, and malate dehydrogenase in parental fish mucus. Fructose-bisphosphate aldolase is an omnipresent glycolytic enzyme frequently found in mammalian skeletal muscle. Fish studies have shown that there is a greater allocation of metabolic energy during stress for tissue repair and restoration reasons and hence an increase in the pace of glycolysis.

(Larsen & Olsen 2001) Greater chemo-attractive gesture toward mucus from a distinct place is not to be expected since intestinal mucus is as appealing as skin mucus and more chemo-attractive as gill mucus.Epidermal mucus extract of C's antimicrobial activity. The inclusion of gariepinus juveniles in diet was explored and contrasted with the activity of C epidermal mucus extract. Juvenile gariepinus (control) without dietary ginger. This research shows the antimicrobial function of ginger in enhancing fish protection against bacterial infection as shown by the greater inhibition areas found in the diet for

epidermal mucus extract of fish-fed ginger relative to control. For Bacillus, Escherichia, Staphylococcus and Streptococcus species, inhibition regions for epidermal mucus of therapy fish were 30.7 mm, 29.8 mm, 26.3 mm and 19.3 mm respectively.

Gariepinus, (2011) Although these values were not considerably greater ($P > 0.05$) than those acquired for Bacillus, Escherichia, Staphylococcus and Streptococcus species for control fish with inhibition areas of 25 mm, 11.2 mm, 9.0 mm and 7.3 mm respectively, the greater levels reported for the fish therapy indicate that the incorporation of ginger in the fish diet had an antibiotic impact on bacterial isolates in fish samples from cultivated lakes. The C. ginger add-on. Gariepinus diet is promoted because its action indicates the potential of ginger to prevent the development of resistant bacteria and to improve the antimicrobial function of fish mucus and hence the quality of C. gariepinus,.Nearly all fishes in the setting are enhanced with microbes and micro-organisms and are discovered to be prone to pathogens attack. For the broad society of pathogenic and non-pathogenic microbes, the aquatic environment of fish postures a huge challenge to fish.

Nwabueze, (2012) The bad cultural conditions and pollution in the natural resources of water are the primary causes of degradation of the environment and contain big quantities of pathogens. Fish skin is a complex structure that provides chemical, mechanical and immune protection against pathogens and wounds induced by their interaction with these pathogens (Fotenot and Neiffer 2004).The mucus layer offers the entry of pathogens with an inherent immune shield. There are primarily two distinct types of mechanisms associated to the description of mucus protection, firstly, the mucus acquired by means of a skilled mechanical barrier that also moves downstream along the trailing edges and fish. For instance, the mechanism of the respiratory tract muco-ciliar escalator discovered in the pulomnate animals.

(Wei *et al*., 2010) The mucus extracted from the fish's epidermis is taken as the main protective agent. Its rate and structure have been altered overtime in reaction to changes in the environment (Ellis, 2001), analyzing that the removal of skin mucus along with epidermal lesions in *C.caprio* through in-vitro methods has enhanced the introduction of CyHV-3 vrius while its existence offers protection for the entry of pathogens. According to various findings on innate immunity, the mucus in the fish body shows that the mucus acquired from the epidermis blocks the development of few bacteria and is therefore regarded to be the primary cause of many antimicrobials.

Kasai *et al*., (2009) observed the inhibition induced by the growth of Staphylococcus and S.aureus epidermis and discovered that, in the event of S. epidermis proliferation, it was effectively suppressed in some respects and its impact was regarded to be the most effective among of all the bacterial strains that died. Epithelium mucosal lining is ensured against the pathogens in the body fluid layer and the mucins it contains ,However, microscopic organisms ' ability to connect to body fluid components can actually simplify the colonization scheme through the mucosal epithelium surfaces by immobilizing the microorganisms within the body fluid gel. For fruitful colonization, which is essential as soon as they are bound to body fluid, the microscopic organisms establish themselves in the body fluid gel. Ordinary body fluid gel disintegration is likely to wash away microscopic organisms from followers. Along these lines, the rate of replication of microscopic organisms linked to body fluid needed to be equal to or greater than the rate of disintegration of the body layer of fluid itself is to achieve fruitful colonies development. Subsequently, microscopic organisms ' ability to use body fluid as a supplement hotspot for their growth will be worthwhile. Body fluid from the rainbow trout's skin and digestive tract has been recognized as an attractive to V. anguillarum since late.

Nagashima *et al*., (2003) The chemotactic response of V. anguillarum to body fluid from the skin, digestive tract, and rainbow trout gills was quantized to clarify the portion of chemotactic motility in the fundamental interaction between the pathogen and the host. For instance, rockfish (*Sebastes schegeli*),

which displays specific antibacterial action against gram-negative microscopic organisms, are of importance to high-atomic mass antibacterial proteins in fish body fluid. We discovered an antibacterial genius tein in the flounder *Platichthys stellatus* ' epidermal body liquid in the current inquiry. This species, which on its body surface has a wealthy covering of body fluid, has rough water at the mouths of rivers. Antibacterial motion against Staphylococcus epidermis, *Staphylococcus aureus* and methicillin secure *S.aureus* (MRSA) has emerged to apply this body fluid protein.

Mozumder, (2005) Fish live in insinuated contact with their thickly populated microorganism's fluid setting. The defensive portion of the fish's epidermal body fluid has long been known to show a hotspot for antimicrobial segment detachment. The purpose of this inquiry was to identify antimicrobial components of sound Atlantic cod (*Gadus morhua*) from the skin body fluid. The way in which body fluid components are observed to be dynamic against both Gram-positive and Gramnegative bacteria in a situation that is likely to mirror the standard cod habitat further supports the body fluid component as a safeguard block. Since we watched pepsin therapy canceling the concentrate exercises, we thought that the exercises were starting point for protein / peptide. The antimicrobial motion for the expansive range of irresistible pathogenic organisms was provided with the epidermal fluid of the fish body. We late portrayed catfish (*Cathorops spixii*) body liquid antimicrobial motion (Ramos *et al*.,2001).Furthermore, two ribosomal proteins and histone H2B proteins are instances of antimicrobial-moving proteins disengaged from Atlantic cod's epidermal body liquid.

In angle, skin is one of the major pathogen section locations as it is a mucosal surface with all-round living cells. The skin body fluid has a crucial role to play in maintaining angle well-being, especially in intensified cultivation where stress and contamination levels may be high. Skin body fluid of fish includes an assortment of insusceptible important factors against microbial peptides and proteins including lectins, lysozymes, calmodulin, immunoglobulins, complement, core proteins, proteolytic catalysts (Esteban, 2012).

Jurado *et al*., (2015) Biochemical barrier acts as the primary line of protection created by these variables against a wide spectrum of microbes. From a variety of perspectives, ski mucus characteristics were determined focusing on protein group and protein particular interest.Current studies have used high techmics to characterize fish mucus skin. They comprises of variance Atlantic cold skin mucus proteome upon native infection with V. anguillarum occurs, mucus of gilthead seabream (*Sparus aurata*) proteomic profile, Venom and mucus of skin of marine catfish (*Cathorops spixii*) see-through functional toxin modification, skin mucus proteomic map of Atlantic cod (*Gadus morhua)*, discus fish (*Symphysodon aequifasciata*) proteomic profile mucus of skin displayed parental care, European sea bass (*Dicentrarchus labrax*) proteomics profile, In Atlantic salmon (*Salmo salar*) protein configuration variations mucus followed by infection of sealice.

Whyte (2007) According to their immune system, fish are said to be highly dependent. Mucus was the first line of defense against microbes and acts as a physical barrier between the atmosphere and fish mucus. Fish mucus also determined antimicrobial activity through lysozyme, proteolytic enzyme, c-reactive proterins, immunoglobulins and lectins .Further studies have shown that fish mucus surfaces have AMPs. To determine the antimicrobial peptides that interfere with the immunity of fish, many fish mucus of fish species were evaluated to check the antimicrobial activity. The skin mucus has the greatest antimicrobial activity in yellow cat fish (Pelteobagrus fulvidraco, Richardson). Characterization and purification of antimicrobial peptide from yellow cat fish mucus, pelteobagrin was also researched and the epidermal mucus in the defensive role of the immune system in fish was also determined. Earlier studies on fish skin AMPs describe the association of antimicrobial activity with their habitats in fish species AMPs separated from fish seawater species such as cod, mucus histone-H2B-derived peptide, myxinidin and pleurocidin are salt-tolerant, whereas oncorhyncin III separated from rainbow trout skin mucous

showed little activity at high levels of salt. Yellow catfish is restricted to freshwater habitats, a salt-sensitive technique and a small concentration of NaCl, pelteobagrin that conducts it, its role.

Birkemo *et al*., (2003) From pathogen protection, Hagfish believed it had a strong defense mechanism. Several cathepsin B have been evaluated as distinct immune parameters in hagfish epidermal mucus, proteases and lysozyme. Earlier trials have also shown that acidic hagfish mucus epidermis has proteins, powerful activity among various human fish and pathogens, as well as enzymes and peptides. This research concentrated on the structural characterization and antimicrobial activity of these peptides using bioassay-guided fractionation from hagfish acid mucus of the epidermis. The existence of these peptides in hagfish enables the function of immune protection. Fish mucus plays a key role in the host defense mechanism as it has many antibacterial compounds, consisting of AMPs. Consists of pleurocidin, monorhinic III, SAMP-H1, pardaxin, monorhinic II and pardaxin, myxinid in and $H_2B$ histone peptide current in Atlantic cod have been acknowledged in prior years

**Antioxidant activity:**

Schaeck I*et al*., (2013) Fish mucus acts as a barrier to their surroundings and acts as a significant element of the fish's immune system. Alkaline pH destroys the formation of biologically active mucus compounds, leading to the pathogens ' decreased immune response. In addition, fish mucus protein may be useful in studying the overall functions of these variables. In flavors, enzymes, proteins, minerals and pigments, fish by-products are useful. In bony fish mucus, these fishes find few antimicrobial agents that bind and deteriorate with the microorganisms. They indicated that bacteriostatic characteristics and unique in the distribution between living organisms are lysozyme separated from fish mucus. Sloughing in mucus microbes comprises of lectins, antibacterial peptide, proteases, and lysozyme. Approximately 20 million tons of fish by-products are published from world fisheries per year. Fishes are different groups of animal’s current today, consisting of half of vertebrates.

Fast *et al*., (2002) It has been acknowledged that the quality and amount of fish mucus differs in seasons and environmental circumstances such as managing pressure, fish age and pH, which supports the outcomes that the amount of mucus secretion was large in summer compared to winters. These variables play a main role against infection in the weakness of the fish. Epithelium has defended the body surfaces of multicellular organisms, providing physical barriers between the outside world and the inner environment. The body is coated by the skin that saves it from the entry of pathogens and also created the leakage of water, nutrients and solutes. The internal and external obstacles depend on the epidermis cellular stratified sheet. The epidermal barrier in reptiles, mammals, birds and amphibians is found in cornified cellular sheets and amphibian tadpoles in fish epidermis.

Ogawa *et al*., (2004) Mucus formation varies across the epithelium surface and is a multi-faceted fluid. Environmentally coated fish mucus and skin mucus proteins are required to keep harsh circumstances such as hydraulic pressure and greater stress. The mucus surface is sticky, except for a tiny region not sticky. Many organisms used the mucus's sticky action from bacteria to barnacles on which they reside. Small fish collect nutrients to water suspended from the Mucus secretions in lipids that have covalently attached fatty acids that participate in fibrine activities that increased the viscoelasticity of the gel and researched vertebrate gastric mucus. The thickness of the mucus blanket is assessed by the equilibrium between shedding frequency, degradation rate and secretion frequency. Irritating and toxic substances can greatly increase mucus secretions and also increase the thickness of the mucus cover. The features and structure of skin mucus are very important for the maintenance of fish immune functions. Tear and saliva liquids are less viscoelastic in mobile bacteria and are absorbable. Known viscoelasticity that is determined by hydration is needed for the transport of mucus.

Nigam *et al*., (2012) Mucus epithelia may therefore control the viscoelasticity of the secreted mucus gel and it is achieved in the portion by regulating the ionic atmosphere to control viscoelasticity and hydration of the mucus. Trefoil factor, pH, lipid, non-mucin glycoprotein, and calcium were secreted in many variables.The main enzymes studied in fish mucus are lysozymes. Lysozyme (muramidase) is a distinctive bactericidal enzyme, which is also found in fish in a wide spectrum of livestock. Lysozyme is found in most species of lymphoid tissue, serum and mucus, but is not found in others such as wolfish and wolfish. Lysozyme bacteriolytic activity in fish mucus contributes to the mechanism of host defense among bacterial infections. Two lysozyme isoforms have been identified in different fish species skin mucus. Many kinds of biologically active compounds are found in fish mucus. The hydro lysates of antioxidant activity against radicals of peroxyl, hydroxyl and DPPH were determined by using spectrometer. But the hydro lysate of pepsin exhibited the minimum IC52 value for scavenging activities of hydroxyl radical, the second lowest value IC52 are also indicated by hydro lysate of a-chymotrypsin. Consequently, hydrolysate a-chymotrypsin was choosen. Hydrolysis of different proteins causes the formation of antioxidant peptides imitative from organisms of marine. They stated that the sole protein of yellow fin fish is hydrolyzed by using of a-chymotrypsin to form the antioxidant peptides and these peptides have fine antioxidant activity.

Najafian and Babji. (2012) identified that the fish might be act as a basis for functional material, like polysaccharides, vitamins, enzymes, polyunsaturated fatty acids, bioactive peptides, minerals and antioxidants. Currently, much awareness has been focused on structure characterization, sequence of bioactive peptides, composition and identification .These active biological peptides which perform an essential role in metabolic modulation and regulation. These peptides might be performed as functional food components and in pharmaceuticals and nutraceuticals to prevent diseases and for improvement of humans' health. Thus the fish with new bioactive compounds growing fastly. Three methods are used for the formation of bioactive peptides, these methods are: enzymatic hydrolysis, food proteins microbial fermentation and solvent extraction. These bioactive peptides that are fish-derived are depending on their composition of amino acids, its sequences, nutrient utilization and amino acids and structural properties. These peptides might be take part in different biological functions that are the restriction of ACE (angiotensin-I-converting enzyme), immunomodulatory, anticoagulant, antimicrobial and antioxidant activities.

Jin *et al*., (2010) with enhancement of knowledge about functional characters of hydrolysate fish protein, there are numerous studies that are also conducted about nutraceuticals and the fish-derived food applications.Currently, a numerous studies are established that from various fish species, peptides derived and protein hydrolysates work as probable antioxidants. From the conger eel (Conger myriaster), bioactive peptides of antioxidants are separated such as Leu-Gly-Asp-Val-Gly-Asn-Asp-Asn, that showed the higher antioxidant activity level. Fish peptides that are enzymatically hydrolyzed showed diverse biological and physiochemical activities depending upon composition of the amino acids and their molecular weight. Therefore the bioactive peptide molecular weight is the mainly essential factor in formation of these bioactive peptides along required biological actions. The ultrafiltration membrane process could isolate the peptides that are required for the functional properties and molecular weight from hydrolysate fish protein. Such type of system could control the distribution of molecular weight of appropriate peptides.Ion exchange membranes, column chromatography and Nano filtration can also be used for this purpose.

Whyte, (2007) Common procedures are utilized to determine the antioxidant enzyme action of Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase. The SOD change the radical of superoxide into the molecular oxygen and hydrogen peroxide, while in the case of peroxidases (POD) and Catalase (CAT), changed the hydrogen peroxide into the water. So by this processes, two poisonous

species hydrogen peroxide and radical of superoxide's are altered into the non-toxic or safe water product. Some other procedures contains immunogold and immunohistochemistry that can more determine the different antioxidant enzymes levels in cells and tissues. The difference in activity of lysozyme from the mucus of fish can be linked to many factors like response to maturity, sex, genetic variation, stress handling, species variation and diet (Balfry and Iwama, 2004). In species of seawater, greater lysozyme activity determined, which can be associated to species specific developmental variations to different conditions of environment and genetic changes of all these environmental factors.

Marel *et al.,* (2010) In the fish mucus, lysozymes are present. Lysozyme of fish is present in two modes, out of these one is that it develope like greatly bactericidal than the lysozyme present in higher vertebrates. Lysozyme is mainly humoral and cellular factors linked with first line of defense and native immunity in all vertebrates. Previously, circulating lysozymes have been determined in teleosts through evaluating enzymatic action against *Mycococcus lysodieticus* and whole plasma or serum at the low level pH. In addition to lactoferrin, immunity can be increased and allergic reactions decreased. As a consequence, plasma cortisol levels have been recognized as an indicator of positive stress.

Chong, *et al*., (2005) Such as microscopic observations showed the presence of peroxidase in mucus at the moment of exposure to various stressful stimuli that caused plasma cortisol levels to rise (Iger and Wendelaar Bonga 1994). The relative assessment of lipids, carbohydrates and proteins in the skin mucus secretions of two freshwater fish species Heterop- neustes fossilis and catfish-Clarias batrachus-was reported. Present trials have shown important similarities between the biochemical composition of *Catla catla, Cirrhinus mrigala, Labeo rohita, Channa punctata,* and *Rita rita* living in different ecological niches. In R.rita (50.21%) and L.rohita (54.77%), the protein content in their mucus was higher than in C.mrigala (19.99%), C.catla (39.33%) and C.punctata (20.64%). Proteins were reduced than 35% in these fish mucus, lipids (2.51-8.60%) and carbohydrates (1.28-4.53%) are also present in tiny amounts in fish mucus. Protein bands in these fish species range from 50 kDa to 205 kDa in L. rohita, C. mrigala and C. catla. These bands have been small in C. punctata. Bands in R. rita Protein ranged from 17 kDa to 50 kDa. Studies have also shown that the protein content of skin mucus in all fish species is higher than (19.9 to 54.7 percent) with regard to the protein content of detergent (0.3 percent Triton X-100) which has less protein content (less than 3 percent).Like mucus, fluid helps to move fish in mud and prevents skin mucus from drying under harsh circumstances.

Manivasagan *et al*., (2009) The current research showed that fewer carbohydrates of 1.2-4.5% (w / w) are present in fish species relative to protein content A.thalassinus skin mucus secretions also found low carbohydrate levels (Venkaiah and Lakshmipathi, 2000).The presence of lipids combines with protein substances linked to antifungal and antibacterial characteristics to assess the viscosity of the fish's skin mucus. It is a multifunctional substance that plays a major role in disease resistance, communication, breathing, osmotic and ionic regulation, reproduction, feeding and nest building. The two marine fishes ' crude mucus showed antimicrobial activity against various pathogens. Also evaluated were the antioxidant activity of fish mucus Clarias sp.1 and Clarias gariepinus. C.sp1 aqueous extracts with a high protein level [602.04 mg g-1 new weight] compared to other extracts, whether C.sp1 organic extract provides a higher protein level (43.50 mg g-1 fresh weight) than both species in the dichloromethane phase.

Shephard *et al*., (2011)Catfish are the good cause of mucus in the skin and also bend and nutritious fish complete of proteins, minerals, vitamins and less carbohydrate that have beneficial impacts on human health.They live in the freshwater environment of North America and Tropical South, Asia and Africa, comprising Malaysia.There are few examples of catfish species in Blue catfish (Ictalurus furcatus), Walking catfish (Family: Clariidae), Shark catfish (Family: Pangasiidae), Channel catfish (Ictalurus punctatus) and African catfish (Clarias gariepinus). Catfish is usually consumed fresh, inexpensive,

significant source in some growing nations such as Turkey and has elevated protein characteristics (Itami 1993). More moisture from 88.15 to 90.01 percent is discovered in the epidermal mucus of both species.

Ersoy and Ozeron (2009) Due to elevated molecular weight, gel forming and elevated molecular weight macromolecules, fish ' slipperiness ' occurs. Most constituents of mammalian mucus are also present in fish mucus where glycoproteins are the primary macromolecules. Many other molecules, such as immunoglobulins, glycosaminoglycans, carbonic anhydrase, complements and lysozymes with calmodulin range and lectins current in fish mucus, have been acknowledged Mucus from many species of fish that have up to 20 times more lipids per unit region than human sebum is disclosed to be free fatty acids that could provide antioxidant agents and secure between fungal and bacterial attacks. A full extraction method was used due to screen skin mucus for difference in antioxidant activity. Organic, water and acid extracts from C.sp1 and C have been arranged. Epidermal mucus gariepinus. The acid solvent, which is acetic acid, has been extracted to obtain protein or peptide enhanced mucus extracts.

Subramanian *et al*., (2007) For extract preparing all in water soluble substances in the mucus such as proteases, glycoprotein and lysozyme, the water extraction technique was used. One of the processes to determine the antioxidant characteristics of the epidermal mucus of catfish that measured their free radical scavenging capacity. Free radicals are engaged in the propagation of lipid oxidation and initiation, hence food oxidant could play a key role in radical scavenging. Also researched was the capacity of catfish epidermal mucus to DPPH scavenging radicals. Free radical DPPH and commercially available organic radicals of nitrogen that could accept the electron and become a permanent molecule. DPPH absorbance measured at 517 nm, which sets off the antiradical compound reduction.

Garcia Moreno *et al*., (2014) less absorbance of the response blend indicates the increased activity of DPPH scavenging. The epithelial surface of fish such as gills, food tract and skin provides first contact with pathogens. Environmental and fish biochemical barrier includes mucus layers with distinct epithelial and epidermis cells secretions. It is believed that such a layer performs mechanical function as a lubricant, performs its role in the immune system and also in regulation. Body fluid and fish tissues have naturally occurring semi-immunoglobulin-like glycoproteins or proteins that mix with environmental antigens and may confer natural fish immunity. Numerous parts are available in bodily fluid, out of these are significant I development of gel. These were glycoproteins and water conjugate with incredible sub-atomic weight oligosaccharides called as mucins.

Bansil *et al*.,(1995) More bodily fluid creation in fish on gills and skin decided after presentation to various noses, similar to poisons, pathogenic microorganism and pH conditions. Change in bodily fluid discharge resolved to trade adjust strategy during alkali discharge, osmotic and ionic guideline and in breath. They may have fundamental effect on physiology of fish. After fish introduction to stretch, a few catalysts can be showed up in bodily fluid. Tilapia presented to water (acidic), that water polluted with lead or cadmium, fertilizers and after skin bodily fluid injuring upgraded the amount of electron thick vesicles which was made by asphalt cells and the skin epithelium upper layer of fish bodily fluid.

**Characterization**:

Jill *et al*. (2002) Investigation of UV ghastly M. armadas shows pinnacle esteems extend from 200-400 nm in concentrates of bodily fluid. demonstrated that in ghastly investigation, one pinnacle appeared the majority of fish bodily fluid. Bodily fluid absorbance of numerous fishes ranges from 290-300nm, in light of bodily fluid auxiliary part, similar to protein and nucleic corrosive, gill discharges. Mucus concentrate of M. armadas demonstrated more elevated amount of zone of restraint against contagious and bacterial pathogens. Recently verified that antimicrobial property in fish epidermal bodily fluid like in Cat fish-Arius maculatus, Eel fish - Anguilla, Hag fish, Nile tilapia *Oreochromis niloticus*, They revealed that

epithelial cells that secretes bodily fluid having biochemical anti-toxin substances. Essential antimicrobial substance was serum protein transferrin in Gold fish.

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