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Biological Activity and Antiproliferative Assay of Fish Mucuspeptides

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Abstract

Antimicrobial proteins (AMPs) are small cationic or amphipathic proteins, they have capability to interrupt microbial cell membranes by associating negatively charged phospholipids.The mixed cationic and hydrophobic composition of AMPs makes them well suited for interacting with and penetrating microbial phospholipid bilayer.In the current research,the fish mucus AMPs of bacterial infected fishes displayed significantantibacterial,antifungal and anti-cancer properties. This broad antimicrobial and anticancer properties of fish mucus peptides makes them potent therapeutic agents.
Keywords: Fish mucus, Antimicrobial activity,anticancer activity

1. Introduction

Antimicrobial peptides (AMPs), ubiquitous in nature, are a versatile class of naturally occurring biologically active proteins. AMPs are the peptides of short amino acid sequences, and generated by variety of living beings such as bacteria, fungi, plants, animals including mammalian species [1]. The primary role of AMPs is to provide first line of defence by exerting cytotoxicity on a wide range of foreign attacks such as viruses, bacteria, fungi, yeasts, and even cancer cells therefore AMPs are also known as host defence peptides (HDPs) [2]. AMPs are considered as a promising and potential drug candidate with added advantages to conventional chemotherapeutic agents such as their lesser toxicity, produced from natural sources, wide spectrum of activity, and decreased resistance development by the target cells. Different environmental sources such as plants, insects, marine invertebrates, and mammals have been exploited for the isolation as well as biological evaluation of AMPs [3]. Although Marine fishes are also a rich source of antimicrobial peptides [4], fish peptides are under explored for evaluation of their biological activities. The exploitation of fish skin or mucus for research purposes to examine their biological activities could be a delightful utilization. In fishes, mucus secreted from epidermal and epithelial cells of external skin considered as one of the main sources of AMPs, because skin involves in intra and interspecific chemical communication [5]. Mucus serves as a dynamic biochemical and physical barrier, expressing numbers of biological functions such as chemical communication [6], protection against environmental toxins and heavy metal toxicity [7], protection against pathogens [8], and parental feeding [9]. Literature reports have demonstrated that mucus offers a role in the inhibition of colonisation by parasites, fungi, and bacteria. The outcomes from the extensive researches on AMPs from fish showed that the antibacterial and anti-fungal actions were evaluated well [10]. Although several reports on anti-

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cancer activities of AMPs from fish are available, the more exploration of AMPs from fish mucus against cancer, a worldwide threat, is highly desirable and need of the current scenario of pharmaceutical sciences.

2. Methodology

2.1 Fishes were collected from different fresh water bodies of kashmir collection of fish mucus including khag ,Mansbal and Kokernag fish farms.The fish were killed with a sharp blow to the head and mucus was collected by using a wooden spatula .To avoid contamination,the mucus was kept in frozen conditions.The mucus was subjected to centrifugation at 1000xg for 10 min and supernatant was precipitated by ammonium sulphate precipitation to obtain the final saturation upto 55%.

2.2Agar well diffusion method

Agar well diffusion protocol as followed by Perez et al. [11] was employed to determine the anti-microbial properties of ammonium sulphate precipitated fish mucus extract against selected microbial strains. Prior to antimicrobial susceptibility, microbial strains (bacterial and fungal strains) were sub-cultured on their respective agar media. From the recently produced microbial suspension, 100µl of 0.5 Mac-Farland standard inoculum from bacterial and fungal strain were mixed with molten Mueller Hinton agar media and potato dextrose agar media respectively in culture tubes and added into 90 mm sterile petri plates. The laminar air flow hood was allowed to solidify plates and after that wells were made taking 5 mm standard cork borer. 50 µl of each extract dissolved in DMSO was poured in respective wells. Streptomycin for bacterial strain and Nystatin for fungal strain were explored as positive controls and DMSO was used as negative control. The parafilms were used to seal the plates and then incubated at 37°C for 24 hours in incubator.

2.3MTT ASSAY

Living cells cleave the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)], a pale yellow substrate, into a dark blue formazan product. The MTT assay protocol needs active mitochondria, and it is observed that even freshly dead cells are not able to cleave MTT in substantial extent. Hence the extent of MTT cleavage is directly proportional to the number of viable cells present that is further quantified by colorimetric analysis. The MTT assay was conducted at Deshpande Laboratories, Bhopal following the standard operating protocols. In short, DMSO was used to dissolve the compounds and then serial dilutions were performed with complete medium to obtain were performed with complete medium to obtain the concentrations to a range of test concentrations. The concentration of DMSO was maintained < 0.1% in all the samples and A549 kept in suitable parameters were cultured in 96 well plates. Then it was treated with various concentrations of the test samples followed by the incubation at 37 °C, 5% CO2 for 72 hours. Addition of MTT reagent was done to the wells and system was incubated for the period of 4 hours. The cells produced the dark blue formazan product by the cleavage of MTT. Next, dark blue formazan product was dissolved in DMSO under a safety cabinet and measure at 550 nm. Percentage inhibitions were determined and formation of plot was performed with the concentrations used for the assessments of IC50 values.

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3. Results

3.1Antimicrobial assay

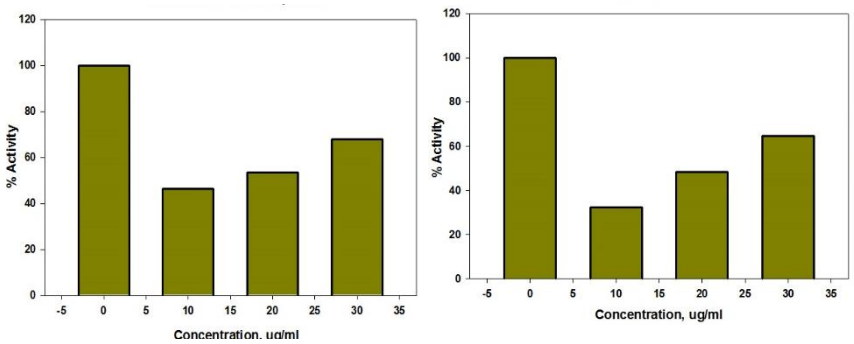
The mucus extract was evaluated against *Lactobacillus acidophilus* and *Aspergillius niger* for antimicrobial activity. The results showed that fish mucus protein was active against both fungal and bacterial strains (Table 1and 2, fig. 1). Antimicrobial spectrum was attained by disc diffusion process of fish mucus on the agar plates. The samples of purified mucus protein limited the growth of different bacteria and fungi on petri plates. Notably, the mucus protein was sensitive towards *Lactobacillus acidophillus* with the zone of inhibition of 19 mm with activity of 67.9% at 30 µg concentration to that of streptomycin used as positive control and *Aspergillus niger* having activity of 60.6% with zone of inhibition of 20mm to that of nystatin used as positive control .So, It seemed that the antibacterial and antifungal activity of the skin mucus protein was high.

Table No.1: Antibacterial activity of Fish mucus proteins against bacterial strain *Lactobacillus Acidophilus*

Fungal strain	**Zone of inhibition**			**Streptomycin 100 µg/disc**
LactobacillusAcidophilus	10µl/disc	20µl/disc	30µl/disc	28mm
	13mm	15mm	19mm	

Table No.2:Antibacterial activity of Fish mucus proteins against Fungal strain*Asperigillus niger*

Fungal strain	**Zone of inhibition**			**Nystatin 100 µg/disc**
Asperigillus niger	10µl/disc	20µl/disc	30µl/disc	33mm
	10mm	15mm	20mm	



(A) Lactobacillus acidophilus **(B) Aspergillus niger**

Fig 1: Bar diagrams representing the antimicrobial activity of purified fish mucus protein against bacterial pathogens (A) *Lactobacillus acidophilus* and (B) *Aspergillus niger.* The streptomycin and nystatin has been used as control and consigned as 100 % activity

3.2Anticancer Activity

The fish mucus protein in earlier studies represented the potent anti-microbial and anti-fungal activities with the range of fungal and bacterial strains. Furthermore, we intentionally performed MTT assay and targeted against lung a549 cell line using purified protein fraction to observe the

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consequences of this protein on viability of these cancer cells. The result thus obtained has been shown in Table no.3and Fig 3. From this result, it was observed that fish mucus protein lead to the effective decrease in the viability of lung cancer cells. There was concentration dependent effect on the percent inhibition of cancer cells in treatment with increasing concentrations of fish mucus protein. The highest concentration of fish mucus used (10 µM) almost reduced the cell count by around 82.65 % which is highly remarkable. The other serial concentrations used also caused the effective percentage inhibition in viability of cancer cells respectively (58 % in 1 µM, 56 % in 0.1 µM, 12.45 % in 0.01 µM and 3.19 % in 0.001 µM). Altogether, it is revealed by our MTT cell viability assay that fish mucus protein represented overall significant activity against cancer cells with the IC 50 value of 0.06 µM of concentration. The lower value of IC 50 makes the purified mucus protein more potent at even lesser concentrations and thus may prove highly effective for therapeutic approach.

Table No. 3: Cell viability assay by MTT method against a549 lung cancer cells using fish mucus antimicrobial peptides.

Concentration (µM)	**Percentage inhibition (%)**
10	82.65
1	58.46
0.1	56.21
0.01	12.45
0.001	3.19
IC50 Value	0.06

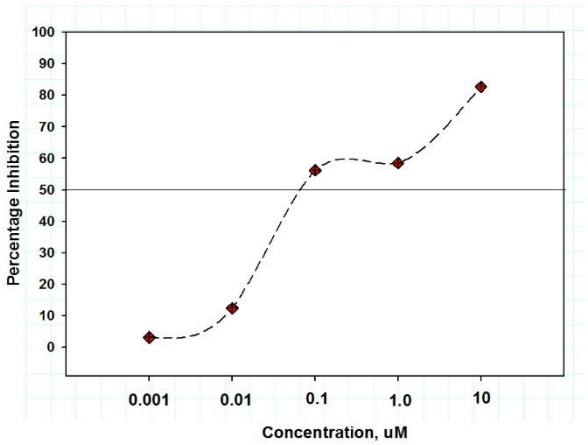

Figure (3): cell viability assay against lung a549 lung cancer cells

References:

[1] R. E. W. Hancock and A. Patrzykat, "Clinical development of cationic antimicrobial peptides: from natural to novel antibiotics," Current Drug Targets, vol. 2, no. 1, pp. 79-83, 2002.

[2] Xia, X., Cheng, L., Zhang, S., Wang, L., & Hu, J. (2018). The role of natural antimicrobial peptides during infection and chronic inflammation. *Antonie Van Leeuwenhoek*, *111*(1), 5-26.

[3] Datta, S., & Roy, A. (2021). Antimicrobial peptides as potential therapeutic agents: A review. *International Journal of Peptide Research and Therapeutics*, *27*(1), 555-577.
[4] Ravichandran, S., Kumaravel, K., Rameshkumar, G., & Ajithkumar, T. T. (2010). Antimicrobial peptides from the marine fishes. *Research Journal of Immunology*, *3*(2), 146-156.
[5] Ángeles Esteban, M. (2012). An overview of the immunological defenses in fish skin. *International Scholarly Research Notices*, *2012*.
[6]Reverter, M., Tapissier-Bontemps, N., Lecchini, D., Banaigs, B., & Sasal, P. (2018). Biological and ecological roles of external fish mucus: a review. *Fishes*, *3*(4), 41.
[7] W. F. Coello, M. Khan, Protection against heavy metal toxicity by mucus and scales in fish. Arch. Env. Contam. Toxicol. 1996, 30, 319-326.
[8] D. Gómez, J. O. Sunyer, I. Salinas, The mucosal immune system of fish: The evolution of tolerating commensals while fighting pathogens. Fish. Shellfish Immunol., 2013, 35, 1729-1739.
[9] K. Chong, S. Joshi, L.T. Jin, A. C. Shu-Chien, Proteomics profiling of epidermal mucus secretion of a cichlid (Symphysodon aequifasciata) demonstrating parental care behavior. Proteomics 2006, 6, 2251–2258.
[10] C. Hellio, A. M. Pons, C. Beaupoil, N. Bourgougnon, Yves Le Gal, "Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus", International Journal of Antimicrobial Agents, 2002, 20, 214-219.
[11] Perez, R., Ineichen, P., Seals, R., Michalsky, J., & Stewart, R. (1990). Modeling daylight availability and irradiance components from direct and global irradiance. *Solar energy*, *44*(5), 271-289.