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Studies on anticancer, haemolytic activity and chemical composition of crude epidermal mucus of fish *Mugil cephalus*

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Abstract
The healthy live fish *Mugil cephalus* were purchased from lower Anaikkat in Kollidam River, Thanjavur District. Tamilnadu, India. Mucus was carefully scraped from the dorsal body using a sterile spatula, the collected fish mucus was stored separately at 4 °C for further use. Anticancer activity was done by laryngeal cancer cell lines. Primarily the hemolytic activity was done in chicken and goat blood erythrocytes were recorded. Further characterization of crude mucus was done through FT - IR.Studies on the anticancer activity of the skin mucus from *Mugil cephalus* has remarkable anticancer activity. The mucus sample showed absence of hemolytic activity in sheep and chick blood erythrocytes, FT-IR analyze revealed the presence of bioactive compounds signals at different ranges. *Mugil cephalus* mucus may also contain some biologically active agents which has anticancer activity and absence of hemolytic activity. In future, purification and structural identification of compounds will be carried out in mucus of fish *Mugil cephalus*.

Keywords: *Mugilcephalus-AMPs* antimicrobial peptides-MTT assay-laryngeal cancer cell lines-Haemolytic activity

1. Introduction
Among the most effective methods of treatments, the natural productsare proved to have much less side effects than the chemical or radioactive treatments. On various experimental models few natural products has been tested for anticancer activity [1, 2]. From the prehistoric period, many traditional medicines were prepared by using essential ingredients from animals and their products [3, 4]. Diverse structural classes such as polyketides, terpens, steroids and peptides have been used as anticancer molecules from marine organisms [5]. However, fish tissues also constitute a potential source of anticancer molecules to be explored.

Majority of fisheries by products are presently utilized to produce fish oil, fish meal, fertilizer, pet food and fish silage [6]. However, most of these recycled products possess low economic value. Most of thestudies have been reported as bioactive compounds were obtained from the fish body such as collagen, gelatin, fish oil, fish boneand internal organs [7]. Tumours have been inhibited by protamine and fish antibacterial peptides [8]. Marine fish such as shark, globefish, devil ray (*Manta birostris*), king crab and hairy clam (*Arca subcrenata*) have been reported to possess few proteins with potent antitumor activity [9].

Few bioactive peptides isolated from various fish protein hydrolysates have been shown to involve numerous activities such as antihypertensive, antithrombotic, immunomodulatory antioxidative activities and anticancer activity [7, 10]. Recently, few studies described the haemolytic activity of epidermal mucus from *Anguilla Anguilla* [14]. Further studies described the haemolytic activity of mucus from two fishes, *Cynoglossus arel* and *Arius caelatus* [15]. Mucus of *Mastacembelus armatus* was shown to have haemolytic activity [16]. Few studies were carried out to analysis the chemical composition of mucus by using FT-IR in fishes *Cynoglossus arel* and *Arius caelatus* [15, 17].

More than six thousands of new marine natural products with low molecular weight (MW) comprising alkaloids and peptides, steroids and lipids, phenolic and quinoid compounds, have been obtained till date [18]. Few compounds have been tested clinically and were found to possess powerful antitumor, antiviral, anti-inflammatory, haemolytic activity and antinociceptive activities [19]. Molecules of fish tissues and mucus also have potential for

Anticancer activity to be explored. In several animal models squalamine, peptide isolated from the liver of the dogfish shark *Squalus acanthias* for potent to inhibit angiogenesis and tumour growth [11, 12, 13]. Hence, the present study has been attempted to screening the potential anticancer properties, haemolytic activity and FT – IR analysis of mucus of fresh water fish *Mugilcephalus.*

2. Materials and Methods

2.1 MTT assay- Cytotoxicity activity

The Cytotoxicity of samples on Laryngeal cancer cell lines was determined by the MTT assay [20]. Cells (1×105/well) were plated in 1ml of medium/well in 24 - well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37 °C. After removal of the sample solution and washing with phosphate - buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3- (4, 5- dimethyl - 2 - thiazolyl) - 2, 5-diphenyl - tetrazolium bromide cells (MTT) solution was added. After 6-7 hrs incubation, 0.04M HCl/isopropanol were added. Viable cells were determined by the absorbance at 595nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 595 nm was measured with a UV -Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of Laryngeal cancer cell lines were expressed as the % cell viability, using the following formula: Cell viability (%) = Mean OD/Control OD x 100.

2.2 Hemolytic assay

The hemolytic activities of crude extracts of *Mugilcephalus* epidermal skin mucus were assayed on chick and goat erythrocytes the method described by Paniprasad and Venkateshvaran [21].

2.3 Fourier Transform-Infra Red spectrum analysis (FT-IR)

Lyophilized sample of *Mugilcephalus* epidermal skin mucus was analysed by FT-IR spectroscopy (Nicolet Avatar-360) the method described by Akkas *et al.,* [22]. The dry lyophilized mucus sample (10mg) was mixed with 100mg of dried potassium bromide (KBr) and compressed to prepare as a salt disc (10mm diameter) for reading the spectrum further.

3. Results

The concentration dilution and death of cancer cells are presented in Table 1 and Fig 2, Fig 3.The concentration of 1000µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml shows the percentage of cell death as 86.33 ± 1.67, 80.92 ± 1.59, 74.44 ± 0.67,70.19 ± 1.32 and 64.46 ± 1.87 respectively. From the results it can be inferred that as the concentration of mucus decreases, decrease in cell death was observed. In the dilution of 1:16, 1:32, 1:64, 1:128 and 1:256 the observed percentage of dead cells were 58.33±1.09, 54.02±1:45, 45.88±0.99 24.34±1.03 and 12.88±1.78 respectively.

Table 1: Anticancer activity of crude mucus from *Mugilcephalus against* the laryngeal cancer cell lines

S. No	Concentration (µg/ml)	Dilutions	Cell viability
1	1000	Neat	86.33 ± 1.67
2	500	1:1	80.92 ±1.59
3	250	1:2	74.44 ±0.67
4	125	1:4	70.19 ±1.32
5	62.5	1:8	64.46 ±1.87
6	31.25	1:16	58.33 ±1.09
7	15.625	1:32	54.02 ±1.45
8	7.8125	1:64	45.88± 0.99
9	3.906	1:128	24.34±1.03
10	1.953	1:256	12.88 ±1.78
11	Cell control	-	0

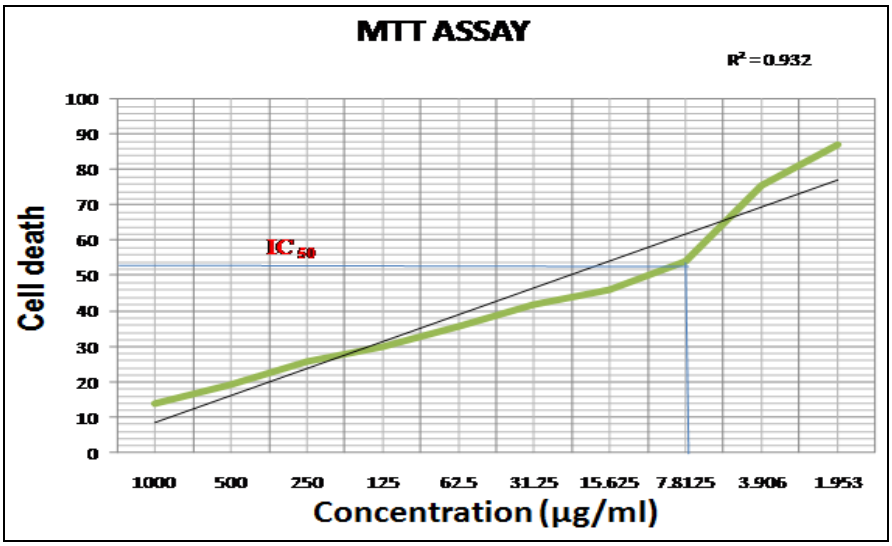

Fig 1: Anticancer activity of crude mucus from *Mugilcephalus against* the laryngeal cancer cell lines

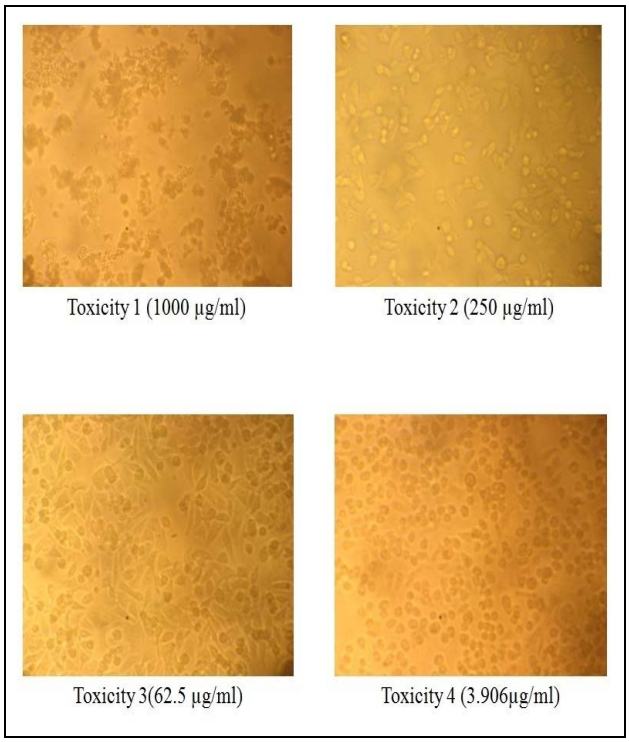

Fig 2: Cytotoxicity effect of samples on laryngeal cancer cell lines against *Mugilcephalus skin* mucus

Results of the present study showed that the concentration of 1000µg/ml causes more damage to cancer cells which is indicated that the anticancer activity is concentration dependent. More dead cells are found in the higher concentration of mucus than in the less concentration. Moreover the cell shrinkage was higher in the wells with higher concentration of mucus. The microscopic observation of wells revealed that there is a change in the morphology of cells. 1000µg/ml shows significant lytic activity of cell membrane. On the contrary the cells of the control well show 0% death of cancer cells.

The haemolytic activity of crude epidermal mucus of *Mugilcephaus* on goat RBC (gRBC) and chicken RBC (cRBC) have been carried out. The hemolysis is due to RBC membrane destruction which resulted from lysis of membrane lipid bilayer. The first two wells containing distilled water with gRBCs are showing hemolysis. The wells containing different concentrations of mucus and gRBCs are forming the button which indicates the absence of hemolysis. The negative control wells having PBS with RBCs are also showing buttons implies the absences of haemolysis (Fig 3).

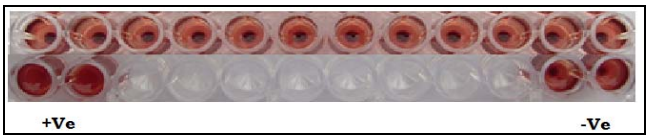

Fig 3: Haemolytic activity of crude mucus from *Mugilcephalus*on goat RBC (gRBC)

First two wells containing distilled water with cRBC showed hemolysis. Whereas the wells containing various concentrations of fish mucus with cRBCs are showing button in their wells which indicate the absence of hemolysis. On the contrary the wells containing PBS with cRBCs are also showing buttons confirming that they are also not causing hemolysis which act as negative control. The haemolytic activity in the wells with cRBCs is presented in Fig4.

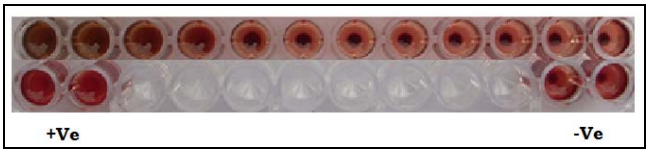

Fig 4: Haemolytic activity of crude mucus from *Mugilcephalus*on Chicken RBC (cRBC)

+Ve - Positive
-Ve - Negative

The FT-IR spectra of epidermal mucus are shown in Fig. 5. The intense bands which are strong occurring at 2379 cm^{-1}, 2346 cm^{-1}, 2092 cm^{-1}, 762 cm^{-1}, 737 cm^{-1}, 710 cm^{-1}, 566 cm^{-1}, 539 cm^{-1} and509 cm^{-1}. Their corresponding functional groups are given in the Table 3.

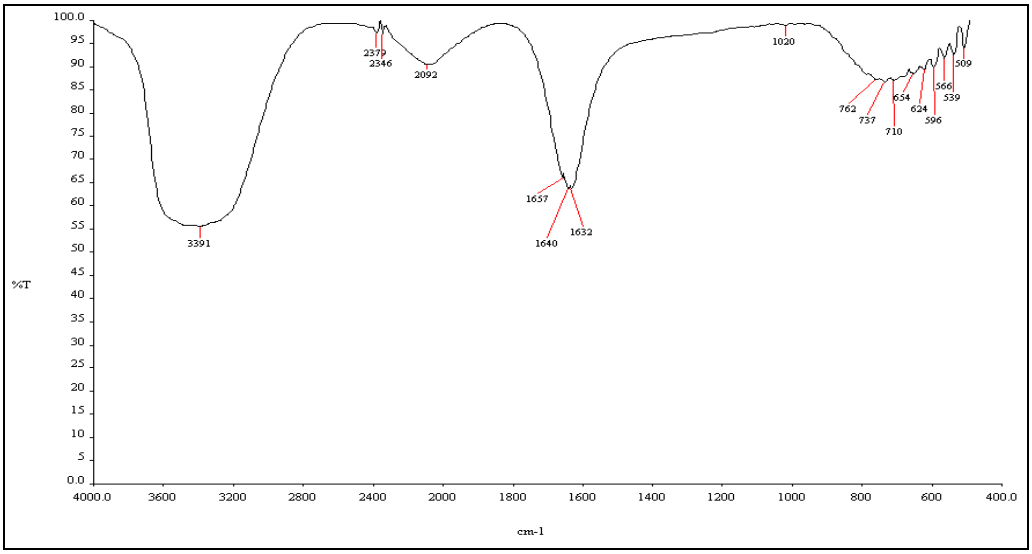

Fig 5: FT-IR spectra of skin mucus from *Mugilcephalus*

Table 3: Analysis of chemical composition of crude mucus from *Mugilcephalus*

S. No.	Frequency ranges	Intensity	Functional groups
1	3400-3300	(m)	N-H stretching- amines
2	2700-2250	(s)	$N\text{-}H^{+}{}_{2}$ stretching- secondary amine salts
3	2090-2100	(s broad)	$CO\text{-}CHN_2$ stretchingdiazoketone
4	1660-1600	(w)	$NH_3{}^{+}$ asymetric bending amino acids
5	1040-980	(s)	Three ring stretching bands prizoles, triazoles
6	900-650	(m, s)	C-H out of plane bending Thiazoles
7	690-620	(w)	CH bending vibration RC=CH
8	600-500	(s)	I-stretching Iodo compounds

The peak at 3391 cm^{-1} which falls in the range of 3400-3300 is correspond to N-H stretching of amines. The spectrum show peak at 2379 cm^{-1} and 2346 cm^{-1} indicates the presence $N\text{-}H^{+}{}_{2}$ stretching of secondary amine salts. The bands between 2092 cm^{-1} represent $CO\text{-}CHN_2$ stretching of diazoketone compounds. The peak areas 1657 cm^{-1}, 1640 cm^{-1}, 1632cm^{-1} are correspond to $NH_3{}^{+}$ asymmetric stretching indicates the presence of mainlyamino acids. The band at 1020 cm^{-1} is showing the presence of three ring stretching bands prizoles and triazoles. The various peaks presents at 762 cm^{-1}, 737 cm^{-1} and 710 cm^{-1} indicatesthat CH out of plane bending thiazoles. The weak frequencies of 654 cm^{-1}, 624 cm^{-1}, 596 cm^{-1} are showing CH bending vibration RC=CH. The peak area at 566 cm^{-1}, 539 cm^{-1} and 509 cm^{-1} is having I-stretching Iodo compounds.

4. Discussion

Potentially valuable proteins, minerals, enzymes, pigments or flavours have been obtained from fish by products [23]. Use of fish skin or mucus for research on biologically active compounds could be an interesting one. The biological interface between fish and their aqueous environment consists of a mucus layer composed of biochemically diverse secretions from epidermal and epithelial cells [24]. Many Anti-microbial peptides kill both Gram-positive and Gram-negative bacteria, few bactericidal peptides have been shown to posses anticancer and antiviral properties [25]. Some of these peptides reported to have lipopolysaccharide with the capacity to recruit the adaptive immune response [26].

Few molecules isolated from marine organisms are having anticancer properties belong to classes including polyketides, terpenes, steroids and peptides [27]. These molecules are found in animals such as corals, sponges and ascidians, which protect themselves from predation by producing substances. However, fish tissues also constitute a potential source of anticancer molecules to be explored. Many AMPs are small, containing 5 to 40 amino acid residues, while a few of them have more than 40 residues. Positively charged residues such as Lys and Arg and substantial hydrophobic residues are commonly seen in these peptides. An important set of AMPs, such as tritrpticin, lactoferricin and indolicidin, are rich in Trp and Arg residues [28].

In the present study showed laryngeal cancer celllysis and it is shown in Fig. 3. Because, the mucus of *Mugilcephalus*has highest quantity of lysine which causes the cationiccity and which in turn causes the lysis of cancer cells [29, 30]. The AMPs are having ~30% or more lysine and arginine. Generally the AMP categorised into two types, namely few AMPs which are able to kill bacteria and cancer cells but not normal mammalian cells and others highly potent against bacteria, cancer cells and normal mammalian cells [36]. In the present investigation the property of the mucus of the *Mugilcephalus* is similar to the first category which is potent against cancer cells whereas it is not showing any lytic activity against cRBC and gRBC.

Several studies have been made to know the composition of plant extracts which are having antimicrobial activities [31]. The absorption bands as per the table 3 are having amines, secondary amine salts, diazoketone, amino acids, prizoles, trizoles, thiazoles and iodo compounds. The presence of NH stretching amines, secondary amine salts, diazoketone, amino acids, prizoles, trizoles, thiazoles and iodo compounds are responsible for the antibacterial activity of mucus. It is well known that the compounds with halogens are having antiviral, anticancer, antifungal and anti-inflammatory activity [32]. Antibacterial activity of triozoles was studied by Chanda *et al.*,[28] and they concluded that triozole compounds have pharmacological activities.1,2,4-triazole compounds are known to exhibit a wide range of biological activities such as antibacterial and antifungal activity[33], anticancer and antiviral [27].

From the foregoing studies it can be concluded that the mucus of *Mugilcephalus* is having broad spectrum of biological activities such as antibacterial, antifungal and anticancer activities by the presence of triazoles. The above conclusion may be supported by our anticancer activity. The present study demonstrated that the mucus of *Mugilcephalus* had a strong and potent anticancer activity, thus supporting folkoric usage of the mucus and suggests that the mucus of fish can be used as an antimicrobial agent in the new drugs and for treatment of infectious diseases. These properties of mucus suggest that it may be beneficial in aquaculture and human health related applications. Further investigation of present study also demonstrates that the mucus had broad spectrum of antimicrobial activity [33] but comparatively no haemolytic activity. Therefore the study stresses that further in depth haemolytic activity of epidermal mucus sample need to be carried out before final conclusion about its role on haemolytic activity.

Absence of haemolytic activity is an early indication for potentially low toxicity [34, 35]. Magainins are antifungal peptides produced by the African frog are not having haemolytic property. Our investigation was supported by the study made by Graham [35] who stated that parasin I showed a strong anticancer activity without haemolytic activity.

5. Conclusion

The global emergence of many new infectious diseases, as well as concern about the antibiotic resistance of increasing number of microbial pathogens, necessitates that new approaches be sought in combating these serious infection. Approximately 600 peptide antibiotics have been isolated from various animals relatively few have been identified from fish, the fish represent a vast, untrapped chemical library. The present study were concludes for the isolation of bio active component which are useful for therapeutic applications.

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7. References

1. Ramakrishna Y, Manohor AI, Mamata P, Shreekant KG. Plants and novel anti-tumour agents: *A review Indian Drug.,* 1984; 21:173-185.
2. Ramnath V, Kuttan G, Kuttan R. Anti-tumour effect of a brin on transplanted tumours in mice. *Indian J Physiol Pharmacol.,* 2002; 46:69-77.
3. Almeida AV. Zooterapia indígena brasileira do século XVII nas obras de Guilherme Piso, Georg Marcgrave e Joannes de Laet. Sitientibus, 2007; 7:261-272.
4. Ferreira FS, Brito SV, Ribeiro SC, Almeida WO, Alves RRN. Zootherapeutics utilized by residents of the community Poço Dantas, Crato-CE, Brazil. *J Ethnobiol Ethnomed.* 2009; 5:21-31.
5. Mayer AMS, Gustafson KR. Marine pharmacology in antitumour and cytotoxic compounds. *Eur J Cancer.,* 2004; 40:2676-2704.
6. Chaudhary J, Munshi M. Scanning electron microscopic analysis of breast aspirates. *Cytopatholo,* 1995; 6:162-167.
7. Kim SK, Choi YR, Park PJ, Choi JH, Moon SH. Screening of biofunctional peptides from cod processing wastes. *J Korean Soci Agricul Chem and Biotech.,* 2000; 43:225-227.
8. Li J, Zhao GZ, Chen HH, Wang HB, Qin S, Zhu WY *et al.,* Antitumour and antimicrobial activities of endophyticstreptomycetes from pharmaceutical plants in rainfrest. *Lett Appl Microbiol.,* 2008; 47(6):574-580.
9. He LH, Huang JH, Sheng SD, Sun SY. Anti-tumor substances of shark and their action mechanisms. *Mar Sci.,* 2005; 29:63-67.
10. Choi JS, Lee CK, Jeon YJ, Byun HG, Kim SK. Properties of the ceramic composites and glass ceramics prepared by using the natural hydroxyapatite derived from tuna bone. *J Korean Industrial and Engineering Chem.,* 2000; 10:394-399.
11. Moore KS, Wehrli S, Roder H, Rogers M, Forrest JN, McCrimmon D. Squalamine: an aminosterol antibiotic from the shark. *Proc Natl Acad Sci USA.,* 1993; 90 (4):1354-1358.
12. Cho J, Kim Y. Sharks: a potential source of antiangiogenic factors and tumor treatments. *Mar Biotechnol (NY).,* 2002; 4(6):521-525.
13. Pedrono F, Martin B, Leduc C, Le Lan J, Saiag B, Legrand P. Natural alkylglycerols restrain growth and metastasis of grafted tumors in mice. *Nutr Cancer.,* 2004; 48(1):64-69.
14. Bragadeeswaran S, Thangaraj S. Hemolytic activity and antibacterial studies on skin mucus of eel fish *Anguilla Anguilla Linanaus 1758. Asian J biological sci.,* 2011; 4(3):272-276.
15. Bragadeeswaran S, Priyadharshini S, Prabhu K, Sophia Rani S. Antimicrobial and hemolytic activity of fish epidermal mucus *Cynoglossusarel* and *Arius caelatus*. *Asian Pacific J Tropi Medici.,* 2011, 305-309.
16. Uthaya kumar V, Ramasubramanian V, Senthil kumar D, Brindha Priyadarisini V, Harikrishnan R. Biochemical characterization, antimicrobial and hemolytic studies on skin mucus of fresh water spiny eel *Mastacembelusarmatus. Asian Pacific J Tropic Biomedici,* 2012, 863-869.
17. Wang LL, Johnson EA. Inhibition of *Listeria monocytogenes*by fatty acids and monoglycerides. *Appl Environ Microbiol.,* 1992; 58:624-629.
18. Ireland CM, Molinski TF, Roll DM, Zabriskie TM, McKee TC, Swersey JC *et al*. Natural Product Peptides from Marine Organisms. In P. J Scheuer (ed). *Bioorganic Marine Chem.,* 1989; 3:1-46.
19. Faulker DJ. Marine Natural Products.*Natural Product Report.,* 2000; 17:7-55.
20. Mossman BT. *In vitro* studies on the biologic effects of fibers: correlation with *in vivo* bioassays. *Envt Heal Perspect.,* 1982; 88:319-322.
21. Paniprasad K, Venkateshvaran K, Microhaemolytic Assay: In: training mannual on advance techniques marine Biotoxinology, Venkateshvaran K, Prasad KP (Eds). *Cent Ins Fish Edu, India.,* 1997, 41.
22. Akkas SB, Severcan M, Yilmaz O, Severcan F. Effects of lipoic acid supplementation on rat brain tissue: an FT-IR spectroscopic and neural network study, *Food Chem.,* 2007; 105:1281-1288.
23. Cancre I, Ravallec R, Van Wormhoudt A, Stenberg E, Gilberg A, Le Gal Y, Secretagogues and growth factors in fish and crustacean protein hydrolysates. *Mar Biotech.,* 1999; 1(5):489-494.
24. Ellis A. Immunity to bacteria in fish. *Fish Shellfish Immunol.,* 1999; 9:291-308
25. Zasloff M, Kristensen HH. Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. *Nature.,* 2005; 437:975-980.
26. Rosenfeld Y, Shai Y. Lipopolysaccharide (Endotoxin)-host defense antibacterial peptides interactions: Role in bacterial resistance and prevention of sepsis. *BBA-Biomemb.,* 2006; 1758:1513-1522.
27. Mayer AMS, Gustafson KR. Marine Pharmacology in antitumor and Cytotoxic Compounds. *Eur J Cancer.,* 2008; 44:2357-2387.
28. Chanda S, Baravaliaa Y, Balujab S. Synthesis and antibacterial activity of some new triazole derivatives. *Archi Appl Sci Resear.,* 2010; 2(3):117-126.
29. Balasubramanian S, Gunasekaran G. Fatty acids and Amino acids composition in skin epidermal mucus of selected fresh water fish *Mugilcephalus. Worl. J Pharmacy and Pramaceutl Sci,* 2015; 4(10):1275-1287.
30. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. *Bioch Biophys Acta.,* 2008; 1778:357-375.
31. Ragavendran P, Sophia D, Arul Raj C, Gopalakrishnan VK. Functional group analysis of various extracts of *aervalanata* (l.,) by FT-IR spectrum. *Pharmacol online.,* 2011; 1:358-364
32. Hayakawa I, Shioya R, Agatsuma T, Furukawa H, Naruto S, Sugano Y. 4-Hydroxy-3-methyl-6-phenylbenzofuran-2-carboxylic acid ethyl ester derivatives as potent anti-tumor agents. *Bioorg Med Chem Lett.,* 2004; 14:455-458.
33. Balasubramanian S, Gunasekaran G, Baby Rani P, Arul Prakash A, Prakash M, Senthilraja PA. Study on the antifungal properties of skin mucus from selected fresh water fishes. *Gol. resea. Thoug*, 2013; 2(9):1-6.
34. Zasloff M, Magainins A. A class of antimicrobial peptides from *Xenopus*skin: isolation, characterization of

two active forms and partial cDNA sequence of a precursor. *Proc Natl Acad Sci USA,* 1987; 84:5449-5453.
35. Graham LD. Biological adhesives from nature. In Wnek G and Bowlin G. (eds) Encyclopedia of biomaterials and biomedical engineering, 2nd edition. New York and London: Informa Healthcare., 2008, 236-253.
36. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. *Bioch Biophys Acta.,* 2008; 1778:357-375.