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ORIGINAL RESEARCH ARTICLE

A Study on Biochemical and Molecular Characterization of a Marine Fish *Lutjanus campechanus* from Nagapattinam, Tamilnadu

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ABSTRACT

Antimicrobial Peptides (AMPs) are regarded as an important component of the first-line defence in more important in fish when compared with mammals as fish rely more on their innate immune system. The physic-chemical properties of the water samples carried out by soil analysis kit. We analyse the antibacterial activity of protein, and biochemical estimation in *Lutjanus campechanus* fish. From the present work, extraction of protein from *Lutjanus campechanus* muscle and purification of protein using SDS-PAGE method and purified protein was tested in five human's pathogen. Hence the protein was estimated by TLC method. The biochemical studied revealed that the order of concentration was carbohydrate, protein and lipid. The higher protein content was present in the muscles of *Lutjanus campechanus*. Marine *Lutjanus campechanus* always having rich source of biochemical constitutes enzyme and in with dietary consumption as supplementary food for fishes, prawn species and human. It is confirmed by the present study that *Lutjanus campechanus* shows maximum concentration of protein, carbohydrate and lipid.

Keywords: Marine *Lutjanus campechanus*, Antibacterial activity, Biochemical constitute, Marine fish, Nagapattinam.

INTRODUCTION

Antimicrobial Peptides (AMPs) are regarded as an important component of the first-line defence in more important in fish when compared with mammals as fish rely more on their innate immune system. In addition to highly specific cell-mediated immune system, vertebrates and other organisms of distinct groups of broad-spectrum antibacterial peptides that marine crabs Charybdis lucifera possessan antimicrobial peptide in their hemolymph. The peptides as factors of innate immunity is that they can function without either high specificity or marine is isolated from

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Thalamita crenate shows immense activity towards human bacterial pathogens peptides are promptly synthesized at low metabolic cost, easily stored in large amounts and readly. The haemolymph proteins of marine invertebrates are unique in composition, as they do not contain proteins and the protein.[1]

Nuclear basic proteins from fish spermatozoan known as protamines are known to possess antibacterial action particularly against Gram-negative organisms and Bacillus spores. But until now, the mechanisms of action of protamine have not been completely elucidated. In our previous studies, we described that fish protamine bind to the bacterial cell wall, change the cellular morphology and release the cytoplasmic materials from the intact cells. It was also reported that, protamine interact with the phospholipid of the bacterial cell membrane, increase the membrane-ATPase activity and induce changes in the membrane structure.[2]

Natural product has been an important resource for the maintenance of life for ages. Several life-saving drugs have been developed from the plants. The plant kingdom has provided an endless sources of medicinal plants first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders. Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs. Marine species are known to produce a large number of structurally diverse secondary metabolites. Sea grass meadows the biodiversity and habitat diversity of coastal water.[3]

The epithelical surface of fish, such as the skin, gill and alimentary tract, first provide with potential pathogens.[4] The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural/chemical features not found in terrestrial natural products. Antimicrobial peptides are important the first line of host defence system of many animal species. Their value in innate immunity lines in their ability to function without either high specificity or memory. More over their small size makes then easy to synthesize without dedicated cells or tissues and they rapidly diffuse to the point of infection. They have been defined molecules of less than 10kDa which show stoichiometric, as opposed to enzymatic, antimicrobial properties. Penaeus vannamei and P.stylirostris. On shrimp, P. Vannamei, allowed full characterization of three members of a new family of antimicrobial peptides which were named as penaeidins. Cloning of a crustin-like, single whey-acidic-domain, antibacterial peptides from the haemocytes of the European lobster, Homarcus gammarus and its response to infection with bacteria has been investigated. Partially characterized a cysteine-rich 11.5kDa gram-negative. Specific antimicrobial peptide from C.maenas. Callinectin is a cationic

antimicrobial peptide of 3.7kDa that represents the major antibiotic activity from the blue crab - sapidus.[5]

Fish consumption is associated to health benefits because of rich content in proteins of high nutritional value, minerals, vitamins and distinctive lipids. Estimates for 2003, based on reports from major fishing countries, indicate that proved more than 2.6 billion people with at least 20% of their average animal protein intake. However, fish stocks are decreasing and the annual world fish catch is stabilized. Moreover, a rational valorization of by-products is extensively investigated, as the yearly average discards are estimated to 7.3 million tonnes. Fish protein hydrolysates (FPH), obtained by controlled enzymatic hydrolysis, are among the best protein hydrolysate in term of nutritional properties balanced amino acid composition, high digestibility, but are mainly used for animal nutrition because of their bitter flavour and fishy odour.[6]

Systematic

Kingdom : Animalia

Phylum : Chordata

Class : Actinopterygii

Order : Perciformes

Family : Lutjanidae

Genus : Lutjanus

Species : ***L. campechanus***

The present study is designed to study the following aspects of in the marine fish *Lutjanus campechanus*.

- To identify the fish *Lutjanus campechanus*.
- To estimate the biochemical parameters.
- To determine the molecular weight of protein.
- To determination of antibacterial activity against various bacterial pathogen.

The study support the pharmaceutical industry to effectively use these species for therapeutic purposes, dietary consumption supplementary.

MATERIAL AND METHODS

SAMPLE COLLECTION

Fish sample were collected from the fish market, Nagapattinum East coast of Tamilnadu. Collection of fish *Lutjanus campechanus*.

Lutjanus campechanus collected by cutting muscle of the animal with a fine sterile scissor and knife. The homogenate was collected in the presence of TCA buffer (4.6). Homogenate was centrifuged at 5000rpm for 15 minutes at 40C storeage.

PHYSICO –CHEMICAL ANLAYSIS OF WATER

The physic-chemical properties of the water samples carried out by soil analysis kit (Model 191E). The water texture, soil pH, electrical conductivity (EC), turbidity, salinity, Total Dissolve solids (TDS) and dissolve oxygen (DO) was determined by using deluxe water analysis kit.

pH

The pH meter electrode was dipped into the buffer solution (pH-7.0) and the knob was adjusted and calibrated to 7.0. Then the electrode was dipped into the water suspension. The pH value of the sample was displayed and noted down.

TOTAL DISSOLVED WATER

The TDS cell was dried and the TDS cell was dipped into the water solution and the reading was noted.

TURBIDITY

For measuring turbidity distilled water used as a blank solution and adjusted to zero. Then the standard solution was placed in turbidity sampler (200 NTU) buffer. The turbidity was measured as 200 NTU by calibration. Then the turbidity of the water sample was measured.

SALINITY

The salinity cell was cleaned with distilled water, dried and the salinity cellwas dipped in the water solution and the reading was note.

ELECTRICAL CONDUCTIVITY

The conductivity cell was cleaned with distilled water, dried and the conductivity cell was dipped into the water solution and determined the value in ms/cm.

DISSLOVED OXYGEN (DO)

The estimation of dissolved oxygen was made to study about the oxygen content in relationship to the number and type of organisms.

CALCULATION

$$\text{D.O. (In mg /liter)} = \frac{8 * X\ 1000\ XN}{V} \times V$$

Where,

- V- Volume of the sample taken (ml)
- V- Volume of titrant used p
- N- Normally of the titrant eumonia
- 8 is the constant since the 1ml of 0.025 sodium thiosuphate is equivalent to 0.2mg oxygen.V-
- Volume of the sample taken (ml)

MICROBIAL STRAINS USED

Antibacterial activity of proteinwas determinate against five different bacterial strains is Staphylococcus aureus, Salmonella typhi, Enterobacter ashuria, Klebsiella pneumonia,

Bacillus subtilis. These clinical strains were obtained from the department of Sri Gowri Biotech Research Academy, Naagi Road, Thanjavur.

ANTIBACTERIAL ACTIVITY OF PROTEIN

Collection of animal tissue (*Lutjanus campechanus*). The fish sample were collected from fish market Nagapattinam and used for present investigation. The fish sample were collected and muscles are cutted small pieces homogenate in the presence of sodium citrate buffer, pH 4.6 and Equal volume of physiological saline (0.85% , NaCl, w/v) was added to Homogenate was centrifuge at 10,000 rpm for 15minutes at 4oC and until use further study.

MICROBIAL CULTURE TUBE USED

Bacterial culture were obtained from Sri Gowri Biotech Research Academy, and separate inoculums is maintained for further studies.

BACTERIAL CULTURE

- Staphylococcus aureus
- Salmonella typhi
- Enterobacter ashuria
- Klebsitella pneumonia
- Bacillus subtilis

ANTIBACTERIAL ACTIVITY OF PROTEN

The antibacterial activity of protein extract (*Lutjanus campechanus*) was tested against the selected bacterial culture. The sterilized nutrient agar medium was poured into each sterile pertriplate and allowed to solidification. Bacterial cultures were swapped on the medium by using sterile cotton swab. Fresh bacterial culture was spread over the plates by following spread plate technique. As well as protein was isolated and sterile disc was added in protein 24 hours stored in 4oC at plate disc was center plates on the bacterial culture plate were incubated for 24 hours at 37oC for bacteria. After the incubation period the results were observed and the diameter of the inhibition zone was observed around the plates.

ANTI BIOTIC SENSTIVITY TEST ON BACTERIA

The antibiotic sensitivity test using standard antibiotics (streptomycine for bacteria) were analysed by following the method of Bauer et al., 1996.

The sterile nutrient agar was poured into each sterile petriplates and allowed to solidify by using a sterile cotton swabs, a fresh bacterial culture with known population count was spread over the plate by following spread plate technique. Then the selected standard antibiotic discs namely streptomycine were placed on the bacterial culture

plates, then the plate were incubated for 24 hours at 37oC for bacteria. After the incubation period the result were observed and the diameter of the inhibition zone was measured around the isolates.

ANTIBACTERIAL EFFECT OF SOLVENT

The antibacterial activity of TCA buffer were tested against the selected bacterial strains. The sterilized nutrient agar medium to solidify. By using a sterile cotton swabs, a fresh bacterial culture with known population count was spread over the plates by following spread plate technique. The selected TCA buffer discs was center the plate. Then, the plates were incubated for 24 hours for bacteria. After the incubation period the result were observed and diameter of the inhibition zone was measured around the isolate.

THIN LAYER CHROMATOGRAPHY[7]

PRINCIPLE

The general principle in TLC is similar to that of column chromatography. In the absorption process, the solute complete with the solvent for the surfaces sites of the adsorbent. Depending on the distribution co-efficient the compounds are distributed on the surface of the adsorbent of course in TLC the partition effect in the separation is also not rule out. The abing by adsorbent normally used contains a binging agent such as calcium sulphate which facilitates the holding of the absorbent to the glass plate.

PROCEDURE (TLC)

As the soluble fractions showed ninhydrin positive spots they were subjected to purification chromatography on using method as eluent and monitoring by TLC. Diagnostiv thin layer chromatography was performed on Methanol and Chloroform extracts. They were also spotted and plates development proportions. Detection was done with the specific colour reagent ninhydrin, for detecting the compounds.

EXTRACTION OF SAMPLE

The crude extract was mixed with the Methanol and Chloroform (1:1). Shake the contents at 55oc for 10 minutes. Centrifuge the contents at 10,000 rpm for 10 minutes. Collect the supernatant. Repeat the extraction of the pellet at 55oC at least twice. Dissolved the residue in known volume of absolute ethanol or water for analysis.

PROTEIN DETECTION

The active fraction was resolved by TLC using silica gel with mobile phase's Chloroform and Methanol (9:1). The spots were indentified by spray with 0.1% ninhydrin in acetone and heat the plates for 5 minutes at 100-110oC.

BIOCHEMICAL ESTIMATION

ESTIMATION OF PROTEIN[8]

Principle

The blue colour developed by the reduction of the phosphotongstic compounds in the folin phenol reagent by the amino acid tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of protein with the alkaline capuire are measured in the Lowr's method.

Calculation

Test OD / STD OD **X** Con. of STD **X** 1 / Vol. of Sample

Express the amount of protein mg/g or 100g of sample. The amount of protein present in the sample was read from the standard graph calibrated with the help of standard solution (Albumin).

ESTIMATION OF CARBOHYDRATE

Total carbohydrate content of isolated *Lutjanus campechanus* was estimated by anthrone reagent method.

Calculation

Amount of carbohydrate present in 100mg of the sample / mg of glucose X 100 = Volume the test sample

The amount of carbohydrate present in the sample was read from the graph calibrated with the help standard solution (Glucose)

ESTIMATION OF LIPID

The total amount of lipid in the sample was estimated by chloroform and Methanol.

Calculation:

Percentage of lipid = Mg of lipid in the same / Weight of the sample X 100

The amount of lipid present in the sample was read from the standard graph calibrated with the help of stamdard solution (Cholesterol).

SDS –PAGE[9]

SDS –PAGE is used for method quantitatively analysing any protein mixture, maintain protein purity and to determine their molecular weight. It is based on the separation of protein according to their size and coating them binding of bye. Sodium dodecyl sulphate (SDS) is an anion detergent the binds strongly to protein, causing their denaturation. In the Presence of excess SDS about 1.4gm of the detergent binds to each gram of protein giving the protein a constant negative.

As a result proteins SDS complex make towards the anode during electrophoresis is during to molecular saving properties of the polyacrylamide gel get separated phase based on the molecular weight same. The principle of this technique is separation of protein based on the molecular weight same. The principle of this technique is separation of protein based on size difference by running standard protein of unknown protein can be determined mobility of

protein. In SDS electrophoresis is expressed as relative mobility (Rf) with respect to the tracking blue the technique consist of 3 basis steps.

STEP I

PREPARATION OF POLYACRLAMIDE GEL

Cross linked polyacrylamide gel are formed by co-polymerization acryl amide monomer and a cross linked agent. N, N Methylene bis acrylamide. This reaction is catalysed by N. N, N1 N1titrated methylene diamine (TEMED) and initiated by ammonium per sulphate porosity of the gel is determined by the amount of acrylamide and bis-acrylamide mix used. Lower percentage gel have large pore size, there by offering loss resistance to passage of larger molecular higher percentage gel favour separation carried out using gel raining from 12% acrylamide.

STEP I I

ELECTROPHORESIS

The polyacrylamide gels slab is prepared and fixed to a vertical electrophoresis apparatus. Protein sample are usually denatured by boiling in the sample loading buffer containing Tris-buffer of pH – SDS, β - mercaptoethanol (reduces disulphide bonds, sucrose or glycol to increase density).

Resolution of the protein bonds are greatly increased by applying the sample into a stacking gel on top the separating gel differences in pH and composition between these to gel cause sample tube concentrated into narrow bonds by isoelectrophoresis. As the sample migrate as through the separating gel proteins get resolved depending as their molecular weight electrophoresis is stopped. As the dye front reaches the bottom of the gel.

STEP III

DECOLOURIZATION OF PROTEIN

Generally proteins are colourless, and hence can't be visualized directly suitable dyes are used to stain the sample, Example comasie brilliant blue.

RESULT

PHYSICO-CHEMICAL ANALYSIS OF WATER

The physic-chemical parameters such as pH (7.5), Electrical conductivity (7.3ms/cm), Salinity (16.7ppt), Turbidity (3.30NTU, Total Dissolve Water (15.14ppt) and Dissolved Oxygen (1.4mg/l), were recorded from the water samplers of Nagapattinam, Tamilnadu.

EXTRACTION OF PROTEIN

The protein was extracted from 10gm muscle by using Rameshkumar et al method with TCA buffer.

ANTIBACTERIAL ACTIVITY OF PROTEIN

The protein extracted was tested for their antibacterial activity against 5 human pathogenic bacteria Staphylococcus aureus, Salmonella typhi, Enterobacter ashuria, Klebsiella pneumonia, and Bacillus subtilis .

Protein extract exhibited maximum zone of inhibition against Bacillus subtilis (20mm), Klebsiella pneumonia (20mm) and moderate zone of inhibition against Staphylococcus aureus (12mm), Salmonella typhi (10mm), Enterobacter ashuria (0.5mm)

ANTIBIOTIC SENSITIVITY TEST (POSITIVE CONTROL)

The antibiotic sensitivity test using standard antibiotics such streptomycin. Were tested against human pathogenic bacteria. The result of antibiotic sensitivity test .

All the antibiotics used were confirmed that the protein extract exhibited a lesser antibacterial activity against Staphylococcus aureus, Salmonella typhi, , Enterobacter ashuria, Klebsiella pneumonia, and Bacillus subtilis.

ANTIBACTERIAL EFFECT OF SOLVENT (NEGATIVE)

The result of antibacterial effect of Chloroform and Ethanol realed no activity against 5 pathogenic bacteria studied

PROTEIN DETECTION BY USING TLC TECHNIQUES

The extract of protein were alone used for compound analysis using Thin Layer Chromatography the TLC of extract of protein related the presence of protein (figure 5).

SEPARATION OF PROTEIN (SDS – PAGE)

The molecular weight of the protein was determined by SDS-PAGE using standard protein ranging markers from 29 kDa – 43 kDa. The molecular weights of protein were found to be 66 KDa.

ESTIMATION OF BIO CHEMICAL TEST

Higher activity in lipid – 0.75g

Moderate activity in Protein and carbohydrate - 0.45g and 0.33g.

Table 1: Physico-chemical analysis of marine water samples from Nagapattinam.

S.No.	Physical-Chemical Parameters	Marine water sample
1	Water	Water
2	pH	7.5
3	Electrical Conductivity (ms/cm)	7.3
4	Salinity (ppt)	16.7
5	Turbidity (NTU)	3.30
6	Total Dissolved Solides (ppt)	15.14
7	Dissolved Oxygen (mg/litre)	1.4

Figure 1: *Lutjanus campechanus*

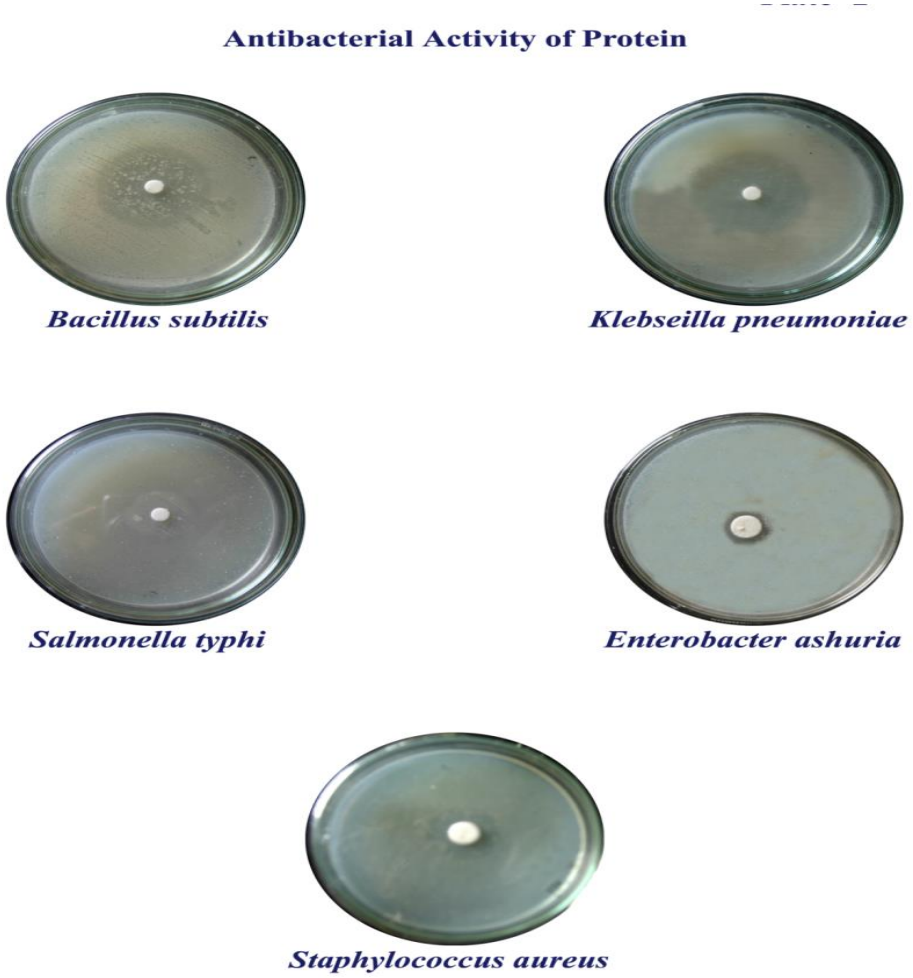

Figure 2: Antibacterial activity of Protein

Estimation of Protein

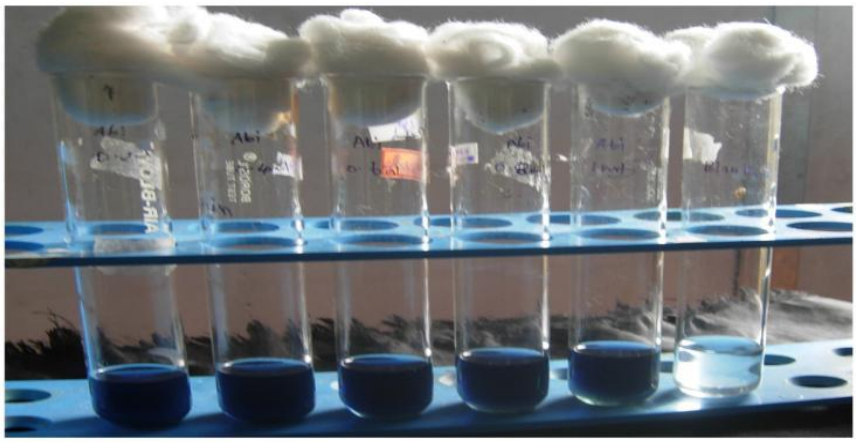

Figure 3: Estimation of Protein

Estimation of Carbohydrate

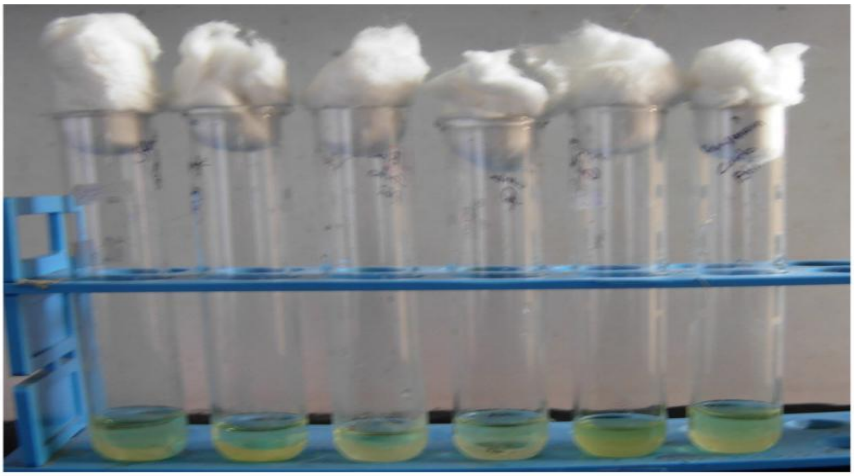

Figure 4: Estimation of Carbohydrate

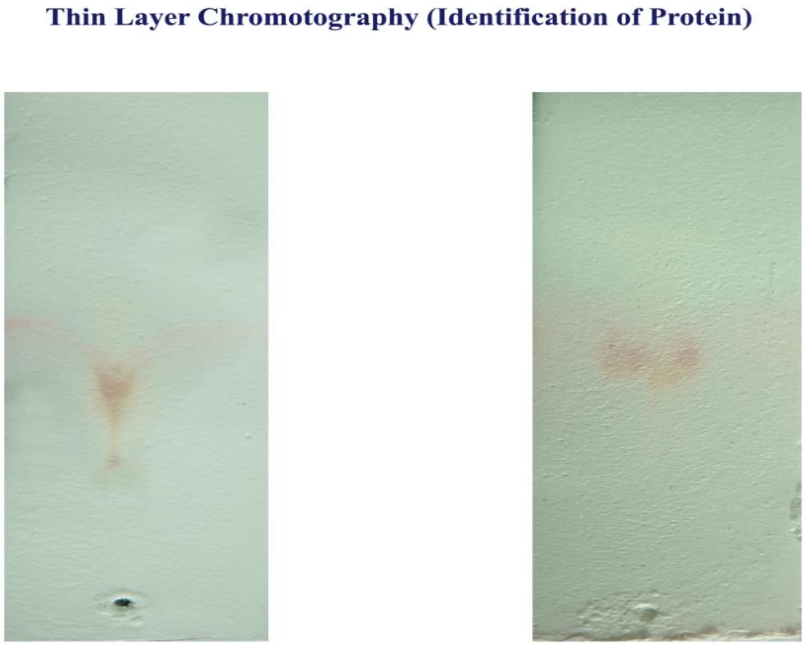

Figure 5: Thin Layer Chromatography for identification of Protein

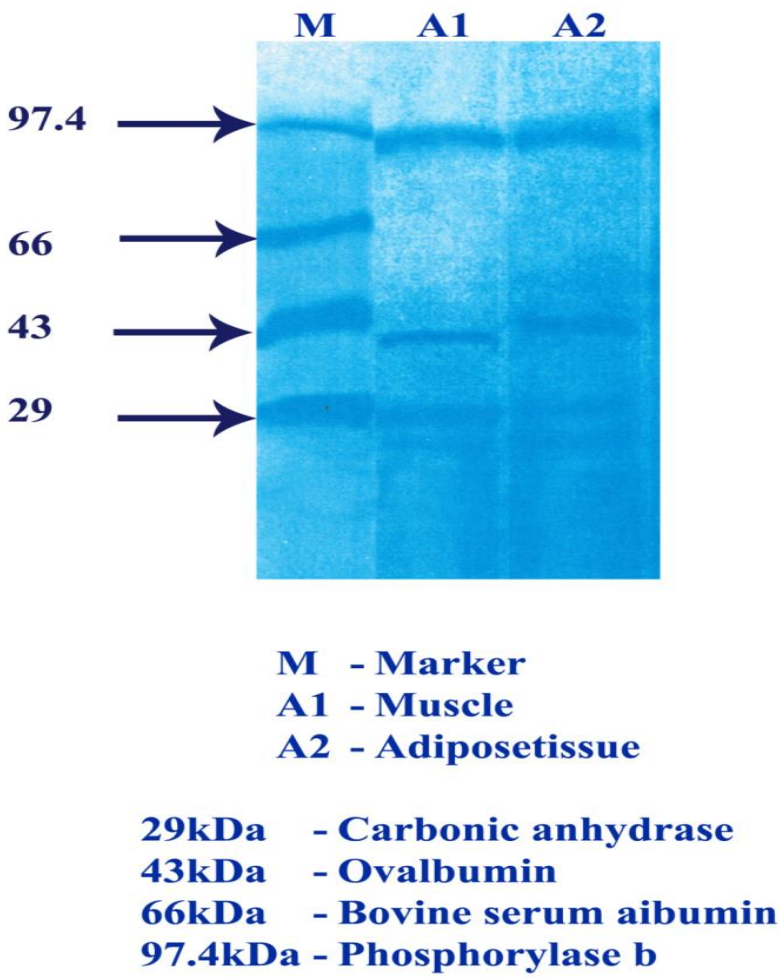

Figure 6: SDS - PAGE

DISCUSSION and CONCLUSION

In recent years, great attention has been paid to study the bioactivity of natural products due to their potential pharmacological utilization. The rationale of searching for drug from marine environment stems from the fact that marine plants and animals have adapted to all sorts of marine environments and these creatures are constantly under tremendous selection pressure including space competition, predation, surface fouling and reproduction. Many of these of organisms have been antibacterial properties, although most of the

antibacterial agents that have been isolated from marine sources have not been active enough to complete with classical antimicrobial obtained from micro organisms.[10]

The phyico-chemical parameters such as pH (7.5), Electrical Conductivity (7.3ms/cm), Salinity (16.7ppt), Turbidity (3.30NTU), Total Dissolved water (15.14 ppt), Dissolved Oxygen (1.4mg/l) were recorded from the water samples of Nagapattinam (Table 1) .[11]

Antibacterial activity has been reported earlier in the blue crab Callinectes Sapidus. It was highly inhibitory to gram-negative bacteria. Although there were several antibacterial activity in seminal plasma few antibacterial peptides have reported in Scylla serrate.[12]

The present study indicated that antibacterial activity of the highest zone of inhibition was observed in the muscle of *Lutjanus campechanus* against Klebsiella pneumonia and Bacillus subtills and lowest zone of inhibition was observed in the muscle of *Lutjanus campechanus* against Enterobacter ashuria. On the test pathogenic strains.

In this present study the amino acids separated from protein by using Thin Layer Chromatography.

The extract nature of the antraquinone is yet to be determined, rechrfomatography and elution of the dye from section were done in order to obtain a pure compound as monitored with TLC. Optimum staining was achieved in 15 minutes in conclusion the antraquinone obtained from Morinda lucida is a promising natural histological stain for collagen fibers, muscle and red blood cells.

In the present investigation the molecular weight of protein were determined by SDS-PAGE (figure) in with standard marker protein.

Lane M indicates the marker protein lane A1 and A2 was protein from fish respectively 29kDa and 43kDa (Carbonic anhydrase and ovalbumin).

Electrophoresis separation of soy flour protein fractions confirmed the finding. The insoluble gluten fraction (pH 6.1) from dough composed of NSE plus wheat lacked bands of molecular weight characteristics of soy protein (37-6KDA).

Denaturation of the texturized soy flour proteins may expose formely hidden hydrophobic groups, which may afford soy proteins the ability to bridge lipid-aqueous interfaces in much the same way as glycolipids. Successful performance of textured soy flours in meat products is evidence of its emulsification ability.

Its conformed in the present study that *Lutjanus campechanus* showing maximum concentration of protein, carbohydrate and lipid.

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