

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: <u>www.ijirset.com</u> Vol. 6, Issue 7, July 2017

Studies on a Natural Biopolymer Prepared From Catla Fish for Phamacological Utility

Dr. R. Kalaivani *1, Bakiyalakshmi S. V²

Assistant Professor, Department of Botany & Biotechnology, Bon Secours College for women, Vilar Bypass,

Thanjavur, India

Assistant Professor, Department of Botany & Biotechnology, Bon Secours College for women, Vilar Bypass,

Thanjavur, India

ABSTRACT: Fish processing wastes are utilized as a potential source of proteins, amino acids and oils. The omega-3 fatty acids have beneficial bioactivities including prevention of atherosclerosis, protection against maniac–depressive illness and various other medicinal properties. To develop ecofriendly and economically practicable techniques for extracting omega-3 fatty acids is the major challenge. This study is indented on isolation and extraction of omega-3 fatty acids from *Catla*. The extracted α linolenic acid showed high antibacterial activity against *Proteus mirabilis*, antioxidant activity (39.3%) and anti inflammatory activity (84.7%). Hence, the study attributed the use of biopolymer as a natural therapeutic agent with high efficiency.

KEYWORDS: biopolymer, omega-3 fatty acids, *Catla*, α linolenic acid, therapeutic agent.

I. INTRODUCTION

Fish is considered to be one of the most perishable human foods throughout the world. It has an excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals including phosphorus, magnesium, selenium, as well as iodine in marine fish [1]. Fish processing generates wastes of up to 50% of the body weight of the processed fish [2]. Fish processing involves scaling, cutting, filleting, cooking, salting and canning. Large amounts of processing waste are discarded every year, including the fins, frames, trimmings, heads, skin and viscera. The waste produced from fish processing can be utilized for the production of fish meal and fish oil [2].

Fish lipids have gained great attention due to the presence of health beneficial omega-3 fatty acids (polyunsaturated fatty acids) which include Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) [3]. The importance of polyunsaturated fatty acids in the prevention of disease and nutritional worth was scientifically recognized. Enhanced intake of marine lipid will in turn increase omega-3 fatty acids. The health benefits of the omega-3 fatty acids include a major role in the prevention of atherosclerosis, heart attack, depression, stroke, diabetes, obesity, premature aging, hyper tension and cancer as well as improving vision and memory [4, 5]. Concentration of omega-3 polyunsaturated FA from fish oil has been carried out by urea complication [6, 7], supercritical fluid extraction [8, 9] and enzymatic hydrolysis [10, 11]. Hence methodology for extraction and preparation of natural potential biopolymer from fish should be simplified. This study was designed to investigate the potential of developing ecofriendly and cost effective techniques for the extraction of omega-3 fatty acid from *catla* fish and its pharmacological efficacy.

Description of the fish

Scientific classification Kingdom: Animalia Phylum: Chordata Class: Actinopterygii



International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: <u>www.ijirset.com</u> Vol. 6, Issue 7, July 2017

Order: Cypriniformes Family: Cyprinidae Genus: *Catla* Species: *C. catla*

II. EXPERIMENTAL SECTION

2.1 Sample Collection

The Sample *Catla* fish was collected from Fish market at Thanjavur District in Tamil Nadu. The fish was dissected to remove the skin used to isolate the Omega 3 fatty acid which is used for preparation of biopolymer.

2.2 Isolation of Lipid

5g fish sample was grinded well with 10 ml of phosphate buffer. 2ml of 80% phenol and 2 ml of 80% chloroform were added and centrifuged at 3000 rpm for 15 minutes. The supernatant was removed and Protinase k was added to the pellet. After it was mixed with 2 ml of 80 % ethanol then centrifuged at 3000 rpm for 15 minutes. The pellet was stored in 4° C for further use.

2.3 Isolation of Fatty acids by TLC

TLC is one of the valuable and versatile methods for analysis of wide range of biomolecules. The stationary phase was prepared with water or buffer at 1: 2 and applied to a glass plate as thin uniform layer by a TLC applicator. Silica gel; (Gypsum) (10 - 15%) is incorporated to adsorbent as a binder, After application of the adsorbent, the plates were air dried for 10 - 15 minutes and then oven dried for 10 - 15 minutes at 100° C $- 110^{\circ}$ C. The plates can be used immediately. The omega-3-fatty acid spots were separated using the solvent mixture chloroform, glacial acetic acid, methanol and water in the ratio of 16: 8:3:2. The color and r_f value of the separated omega- 3 - fatty acid were recorded both under ultra violet and visible light after spraying with anisaldehyde and sulphuric acid.

2.4 Isolation of a Linolenic acid from Omega- 3 - Fatty Acid [12]

The α linolenic acid spots were separated using the mobile solvent mixture methanol and Sulphuric acid in the ratio of 20:20. The color and R_f value of the separated α linolenic acid were recorded both under ultra violet and visible light after spraying with anisaldehyde and sulphuric acid.

2.5 Preparation of Biopolymer (Omega – 3 Fatty Acid) [13]

A bio polymer was prepared by adding aliquots of the acid to water to produce a range of concentrations. NaOH (1.0 M) was then added drop wise to the solution, vortexing between additions, until the acid was completely dissolved as micelles, typically near pH 11. HCl (1.0 M) was then added drop wise, vortexing and checking the pH after each addition. At this point the solution appeared opalescent. This process was monitored by plotting absorbance of the solution at 480 nm as the titration progressed. If the pH was lowered further, droplets formed as the protonated acid became insoluble. Bio Polymer formed immediately upon vortexing.

2.6 In vitro Antibacterial Activity of Biopolymer (Kirby-Bauer Disc Diffusion Method) [14]

In vitro antibacterial activity of Biopolymer was examined by disk diffusion method. The zone of inhibition was determined by comparing standard antibiotic. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C for bacteria.

2.7 In-Vitro screening for anti-inflammatory activity of biopolymer (HRBC Method) [15]

The 2ml blood was centrifuged and the packed cells were washed with isosaline and 10% suspension was made with Isosaline. The drug samples were prepared by suspending the residues in hot water. The assay mixture contained the drug, 1 ml phosphate buffer, 2 ml hypo saline, 0.5 ml HRBC suspension, hydrocortisone sodium was used as the



International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

reference drug. 2 ml of distilled water was used in the control. All the assay mixture were incubated at 370 °C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated at 560 nm. The percentage of haemolysis was calculated. The percentage of HRBC membrane stabilization was calculated using the formula,

100 – Optical density of sample

x 100

Optical density of control

2.8 In-Vitro anti-Oxidant activity of biopolymer by Lipid Peroxidation Method (LPO) [16]

0.1ml sample (different concentration) was mixed in a test tube and volume was made up to 1ml by adding distilled water. Finally 0.5ml FeSO4 (0.07 M) was added to the above mixture and incubated for 30 min to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5ml of 0.8% TBA (prepared in 1.1% sodium dodecyl sulphate), 0.05ml 20% TCA were added, vortexed and then heated in a boiling water bath for 60 min. After cooling, 5.0 ml of n-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The Absorbance of the organic upper layer was measured at 532nm.

III. RESULTS AND DISCUSSION

3.1 Isolation of Omega 3 Fatty acid by TLC

The R_f value observed was 0.89. Fatty acids are the simplest form of the biopolymers in that they consist of a carboxylic acid functional group with a hydrocarbon chain. The selected sample contains high amount of Omega 3 fatty acid which is widely used for various health problems.

3.2 FTIR Analysis of α Linolenic acid

Percentage protection =

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectrum profile was illustrated in the **Fig.1**. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in sample.IR spectral analysis of eluted bands of α linolenic acid was presented in Fig.1



Fig .1: FTIR Spectrum for α Linolenic acid



International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: <u>www.ijirset.com</u>

Vol. 6, Issue 7, July 2017

Infrared spectra of Linolenic revealed a broad OH group absorption at 3468.24 cm-', absorptions at 1891.12 cm-l attributed to vibrations of methyl and methylene groups in the sterol molecule and the fatty acid chain; and broad absorptions in the region of 1101.21 characteristic of sugar.

Then the anti-inflammatory, antioxidant and antibacterial activity of isolated polymer was studied. The minimum concentration of Omega 3 fatty acid necessary to form biopolymer in aqueous solution (starch) was found to be a function of chain length, with longer hydrocarbon chains permitting vesicle formation at lower concentrations. The results are described as follows.

3.3 In vitro anti inflammatory activity

The synthesized biopolymer possesses the good anti-inflammatory activity and the inhibition rate was found to be 84.7% at 0.5ml of concentration where as the other concentrations such as 0.1, 0.2, 0.3 and 0.4 possess 65.5%, 70.4%, 74.9% and 78.5% of anti inflammatory activity respectively (Graph 1). Hence, the biopolymer might be widely used in medicinal preparation.





3.4 Study on In vitro anti bacterial activity

Antibacterial activity test was conducted for eight bacterial species. The high inhibition was showed in selected bacterial species which is similar to the antibiotic disc. The results are showed in Graph 2.







International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: <u>www.ijirset.com</u>

Vol. 6, Issue 7, July 2017

The use of biocompounds for pharmaceutical purposes has gradually increased in India. About 80% of individuals from developed countries use traditional medicine, which involves compounds derived from biological sources. The prepared biopolymer showed maximal antibacterial activity against *Proteus mirabilis* (12mm), *Bacillus subtilis and Clostridium perfringens* (11mm), *Bacillus megaterium* (10mm), *Klebisella pneumoniae* (10mm) and *Proteus vulgaris* (10mm) whereas the minimum zone of inhibition was observed against *Listeria Monocytogen* and *Salmonella typhi* possess 9mm of inhibition respectively. Therefore, this kind of natural sources should be better analyzed to understand their properties, safety and efficiency. The antimicrobial property of afore said biopolymer has their own significance in therapeutic treatments. Hence, the studies involving the use of biopolymer as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes.

In vitro anti oxidant activity

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. The prepared biopolymer shows the maximum anti oxidant activity (39.3%) in 1 ml of sample where as the other concentrations such as 0.2, 0.4, 0.6 and 0.8 possess 9.5%, 11.4%, 16.8% and 25.5% of anti oxidant activity respectively.

As a result the biopolymer produced from the catla fish will be the efficient natural bioactive compound surely facilitates good medicinal practices in future.

INVITRO ANTI OXIDANT ACTIVITY OF BIOPOLYMER (Omega 3 Fatty acid) BY LIPID PEROXIDASE METHOD 40 35 30 25 20 % of Activity 15 10 5 n 0.2 0.4 0.6 0.8 1

Graph: 3 In vitro Anti Oxidant Activity Of Biopolymer (Omega 3 Fatty Acid) By Lipid Peroxidase Method

IV. CONCLUSION

This study is proposed to provide a brief outline to under way in the area of biodegradable polymer research and development. The biopolymer products may come in the forms of tablet, capsule, and powder to fulfill the demands of pharma and nutraceutical industries. Further refinement and research in the same biopolymer will be the chief resource by improving quality and expand healthy life in future.

V. ACKNOWLEDGEMENT

I thank the Management and Principal of Bon Secours College for women, for their technical support to complete the research.

Copyright to IJIRSET



International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

REFERENCES

- 1. FAO. 2005. Post-harvest changes in fish. In: FAO fisheries and aquaculture department, Food and Agriculture Organization, Rome, Italy, (Retrieved on 2nd March, 2010) http://www.fao.org/fishery/topic/12320/en.Holub, D.J. and B.J.
- 2. Babbitt, J.K. (1990). Intrinsic quality and species of North Pacific fish. In the proceedings of International by-products conference, April 25-27, 1990, Alaska Sea Grant College Program, University of Alaska Fairbanks, Alaska, USA.
- 3. Holub. 2004. Omega-3 fatty acids from fish oils and cardiovascular disease. Molecular and cellular biochemistry, Kluwer academic publishers, Netherlands. 263, 217-225.
- Chin, J.P.F. and A.M. Dart. 1995. How does fish oil affect vascular function? Clinical and Experimental Pharmacology and Physiology, 22, 71-81.
- 5. Connor, W.E. 2000. Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition, 71(1), 171S-175S.
- 6. Gamez-Meza, N., J.A. Noriega-Rodri, L.A. Medina-Juareza, J. Ortega-Garcaa, J. Monroy-Riverab, F.J. Toro-Vazquezc, H.S. Garcia and O. Angulo-Guerrerob.
- 7. Tor, C. and H. Yi. 2001. Polyunsaturated fatty acid concentrates from borage and linseed oil fatty acid. Journal of America Oil Chemists' Society, 78(5), 485-488.
- 8. Letisse, M., M. Rozieres, A. Hiol, M. Sergent and L. Comeau. 2006. Enrichment of EPA and DHA from sardine by supercritical fluid extraction without organic modifier. I optimization of extraction conditions. Journal of Supercritical Fluids, 38, 27-36.
- 9. Mishra, V.K., F. Temelli and B. Ooraikul. 1993. Extraction and purification of omega-3 fatty acids with an emphasis on supercritical fluid extraction, a review. In food research international, 26, 217-226.
- 10. Zuta, C.P. 2003. Synthesis of novel triglycerides from mackerel by-products and vegetable oils. PhD thesis submitted to McGill University, Montreal, Canada, 20- 200.
- Hoshino, T., T. Yamane and S. Shimuzu. 1990. Selective hydrolysis of fish oils by lipase to concentrate ω3 polyunsaturated fatty acids. Agricultural Biological Chemistry, 54, 1459-1467.
- 12. Stahl E. Thin layer chromatography. 2nd ed. Springer-Verlay Berlin Publisher; 2005. p.21.
- Charles L. Apel, David W. Deamer, and Michael N. Mautner 2002. Self-assembled vesicles of monocarboxylic acids and alcohols: conditions for stability and for the encapsulation of biopolymers. Biochimica et Biophysica Acta (BBA) - Biomembranes, Volume 1559, Issue 1, 10, 1–9.
- 14. Bauer, A. W., D. M. Perry, and W. M. M. Kirby. 1959. Single disc antibiotic sensitivity testing of Staphylococci. A.M.A. Arch. Intern. Med. 104:208-216.
- 15. Kumar V, Bhat ZA, Kumar D, Khan NA, Chashoo IA, 2012. Evaluation of anti-inflammatory potential of leaf extracts of Skimmia anquetilia. Asian Pac J Trop Biomed. ;2(8):627-30.
- 16. Ruch, R. T. S. J. Cheng, J. E. Klaunig, Methods Enzymol., 1984, 5, 198-209.