Antioxidant and antitumour activity of acid soluble collagen extracted from freshwater snakehead fish *Channa striatus*

A. Palanivel [1]*, G. [1]Gunasekaran, and [2]M. Prakash

*[1]Department of Zoology, Annamalai University, Annamalainagar-608002. Tamilnadu, India,

[2]P.G. and Research Department of Zoology, Periyar Government Arts College – Cuddalore.

ABSTRACT

Fish have often been called the poor man's source of animal protein. The systematic use of fish concentrate has long proven advantageous in animal husbandry and there is growing recognition of its potential for improving human nutrition and health. *Channa striatus*, or snakehead murrel, is an obligate air-breathing freshwater fish which inhabits all types of water bodies. *C. striatus* is commonly consumed as a food fish. *C. striatus* is recorded as high medicinal properties containing fish. The popularity of *C. striatus* as a therapeutic agent is known to folk medicine in its efficacy in treating wounds, relieving pain and boosting energy in the sick and elderly. Hence in the present study effort has been made to study the antitumour and antioxidant activity of the acid soluble collagen isolated from the freshwater snakehead fish *Channa striatus*. The acid soluble collagen extracted freshwater snakehead fish was tested against cancer cells (human colon cancer (HT-29) and human breast adenocarcinoma) at different concentrations. Similarly the free radical scavenging capability was also tested for the acid soluble collagen. The result revealed that the acid soluble collagen extracted from freshwater snakehead fish *C. striatus* exerts both antioxidant and anticancer activity. Whereas at lower concentration the acid soluble collagen has not much effect on the normal Vero cell line having 95 % cell viability which shows a new way for using as medicine against cancer.

Key words: Channa striatus, antioxidant, antitumour, fish collagen.

Introduction

Aquaculture practice plays a major role in world providing more than 142 million tons of fish as human food. It gives direct employment for the farmers and provides more than 180 million jobs as whole in the global fish industry. Aquatic environment is a challenging source of variety of novel biological compounds. Aquatic animals are rich in biologically active secondary metabolites. The biosynthesis of secondary metabolites by these aquatic animals has been speculated as a result of their physical and biochemical adaptation to their environment. In the last two decades, many new compounds have been isolated from these organisms and promoted as candidates for the development of new drugs, especially as anticancer drugs [1].

In spite of modern improvements in chemotherapeutic techniques, infectious diseases are still an increasingly important public health issue. During the past three to four decades research has been made to isolate numerous biologically active novel compounds from the aquatic resources. Those naturally occurring aquatic sources contain compounds are of immense interest for potential drug development as well as ingredients of new leads and commercially successful products for various industrial applications, pharmaceuticals, agrochemicals and nutraceuticals [2].

Aquatic resources produce a great variety of bioactive molecules including natural organic compounds, fatty acids, polysaccharides, polyether peptides, proteins and enzymes. Because of the presence of more amounts of biochemical ingredients, fish has a holistic approach to link medicine and diet. In addition to their nutritive values, fish have a positive impact on individual's health. Fish contains very low fat molecules and more amounts of good sources of protein, essential for the growth of the individual concern. Periodically a number of bioactive compounds have been identified from the fish including peptides, collagens, gelatin, fish oil etc., [3]. Fish contains different types of antimicrobial peptides which are positively charged short amino acid chain molecules involved in host defense mechanism. Fish antimicrobial peptides can be used as antibacterial, antiviral, antifungal, immunomodulatory and antitumor agents [4].

Collagen is a well characterized main structural protein present in the extracellular space of connective tissue which belongs to a group of fibrous protein with very high tensile strength that form the main component in animals. Collagen is a group of naturally occurring protein present in almost all the animals. It is the main protein of the connective tissue and represents about one-fourth of the total protein content in many animals [5]. Collagen is extracted from the different animals and used for various industrial, cosmetic and biomedical purposes. Due to its weak antigenicity, cell attachment ability, biodegradability and biocompatibility, collagen is used in pharmaceutical industries as microparticles, injectable dispersions, shields in ophthalmology sponges, drug delivery system [6]. Collagen based scaffolds play a major role in modern medicines such as cartilage and bone reconstruction, collagen films for wound healing, dental purpose, surgical suture, corneal defects, bone grafting, arthritis and obesity [7].

The fish is the better alternative source for the extraction of collagen rather than Bovine and Porcine. There is no religious barrier, abundant in population and easily culturable one. The bone, skin, fins, scales of fishes are mainly used for this purpose. This in turn help to reduce the environmental pollution by using the wastage of fish during processing. *Channa striatus,* or snakehead murrel, is an obligate air-breathing freshwater fish which inhabits all types of water bodies from small ditches to rice fields, rivers and lakes across tropical and subtropical countries. *C. striatus* is commonly consumed as a food fish. In India, freshwater fish consumption provides an important source of protein constituting upto 70% of total protein requirements [8] and is also recognized as a source of omega-3 fatty acids [9]. *C. striatus* is known for its medicinal value [10-13] and is commonly used as a remedy for wound healing and post-operative recovery [14]. Hence the present study was aimed to extract collagen from the epidermal layer of freshwater snakehead fish *Channa striatus* and to investigate the antioxidant and antitumour activity.

2. Materials and Methods

2.1. Collection of fish

The healthy *C. striatus* were collected from Sirkali fish market, Nagapatinam District, Tamilnadu, India of an average weight 300 $\pm$ 5.67g. The fish were kept in large aerated concrete tank containing potable tap water (pH 7.5 $\pm$ 0.5). The tank were treated with disinfectant sodium hypochloride, with the concentration of 200 ppm for 1 hrs and washed three times with fresh tap water prior to the introduction of the fish in the water.

2.2. Preparation and extraction of Acid-Solubilized Collagen

The Acid-Solubilized collagen was extracted by following the method of Hema *et al* [15]. All the extraction procedures were carried out at 4 ºC. The fish skin was minced and mixed with 30 volumes of 0.1N sodium hydroxide and kept stirred for 24 hours over a magnetic stirrer to remove non collagenous protein. The treated mass was strained through a coarse sieve. The process was repeated twice and the residue was washed twice with 30 volumes of chilled distilled water.

The residue was homogenized in a Polytron homogenizer with 30 volumes of 0.5M acetic acid for one minute and the same was stirred over a magnetic stirrer for 24 hours. The supernatant after

centrifugation (3000 rpm, 20 min) was collected. The residue was once again extracted with acid as anove and the combined supernatant was taken as acid soluble collagen.

Crystalline sodium chloride was added to supernatant to the level of 10% and stirred for 24 hours to precipitate the collagen. The precipitate was suspended in Tris-glycine buffer (50 mM containing 0.2M NaCl, pH 7.4) and dialyzed against the same buffer for 24 hours and then centrifuged. The collagen obtained was spray dried to get fine powder.

2.3 Antioxidant activity

DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined by the method of Binsan *et al* [16]. The collagen samples were prepared in a concentration of 20 – 100 µg/ml and 0.1 mM of DPPH in 95 % (v/v) methonal was added to the sample. The mixture was mixed vigorously using a vortex mixture and allowed to stand at room temperature in the dark for 30 mins. The absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. The blank was prepared in the same procedure, except that deionized water was used instead of the sample. A standard curve was prepared using ascorbic acid in the range of 20 to 100 µg/ml. The capability to scavenge the DPPH radical was calculated using the formula:

DPPH Scavenging activity (%)=$(A_0 – A_1 / A_0) \times 100$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the sample extract or standards.

2.4 Anticancer activity

2.4.1 Cell lines used

To find out the anticancer activity of the acid solubilized collagen of snakehead fish, human colon cancer (HT-29) were used

MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) assay, is based on the ability of a mitochondrial dehydrogenase enzyme of viable cells to cleave the tertrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilization of cells by the addition of detergents

(DMSO) results in the liberation of crystals which are solubilized. The number of surviving cells is directly proportional to the level of formazan product created. The color can be quantified using a multi-well plate reader.

2.4.2 Cell culture

HT-29 (human colon cancer) cell line were cultured in liquid medium (DMEM) supplemented 10% Fetal Bovine Serum (FBS), 100 µ/ml penicillin and 100 µg/ml streptomycin, and maintained under an atmosphere of 5% CO_2 at 37^{o}C.

2.4.3 MTT Assay

The fish collagen was tested for *in vitro* cytotoxicity, using HT-29 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured HT-29 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^4 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the fish samples in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value was calculated using GraphPad Prism 6.0 software (USA).

3. Results

3.1 Antioxidant property of fish collagen

The free radical scavenging potential of collagen extracted from freshwater snakehead fish *C. striatus* was tested by DPPH assay. The radical scavenging ability of collagen was significantly increased

with increasing in concentration. The maximum inhibitory concentration of the fish collagen was obtained at a concentration of 60 µg/ml, whereas L-ascorbic acid showed an IC_{50} of 43 µg/ml. (Fig 1.)

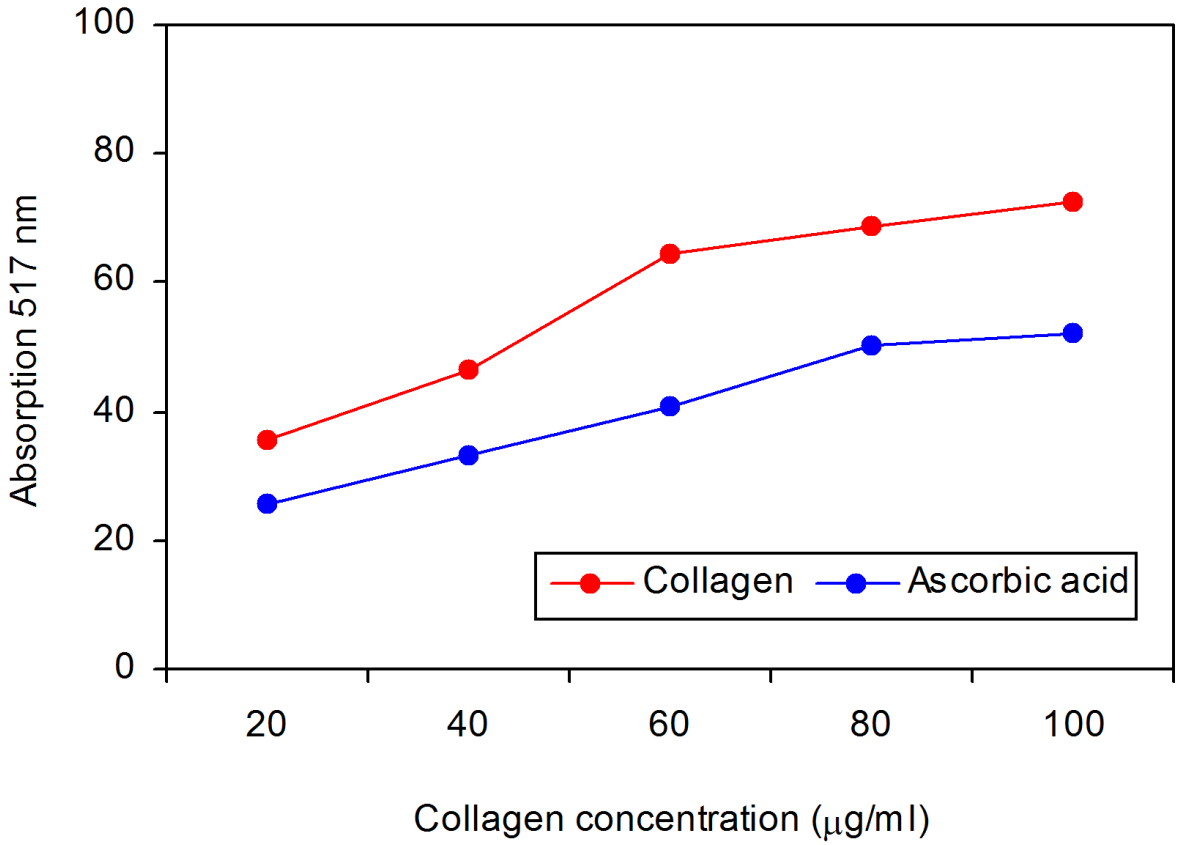


Fig 1. DPPH free radical scavenging activity of fish collagen

3.2 Anticancer activity of fish collagen

The collagen extracted from the freshwater snakehead fish exerts a strong anticancer activity which controls the growth of tested cancer cells (human colon cancer (HT-29) and human breast adenocarcinoma) at different concentration.

3.2.1. Effect of fish collagen on human colon cancer HT-29

Anticancer activity of collagen extracted from snakehead fish was studied against the colon cancer cells. The fish collagen was tested for its cytotoxicity on human colon cancer (HT-29) cell line and the cell viability at different concentrations are shown in Table 2. The collagen extraction of snakehead fish dose-dependently inhibited HT-29 cells proliferation with an IC_{50} value of 42.7 µg/ml (Graph) at which 50 % of

the cancer cell death was observed (Plate). Crude epidermal collagen at a concentration less than 25 µg/ml had less toxicity for HT-29cells, while more than 25 µg/ml significantly inhibited HT-29 cell's proliferation.

3.2.2. Cytotoxicity on normal cell line

The fish collagen was tested against African Green Monkey kidney (Vero) cell line to determine the cytotoxicity. The OD values are shown in Table 1. The cell viability was found to be more in the lower concentration (Table 2.). The high toxicity was observed at a concentration of 100 µg/ml (Graph 5) at which 75 % of the cell death occurred. Whereas in low concentration of crude collagen (5 µg/ml), 95 % of live cells are observed.

Table1 OD Value at 570 nm

S. No	Tested sample concentration (µg/ml)	OD Value at 570 nm (in triplicates)		
1.	Control	0.636	0.667	0.649
2.	100 µg/ml	0.163	0.150	0.133
3.	50 µg/ml	0.321	0.355	0.312
4.	25 µg/ml	0.520	0.565	0.554
5.	10 µg/ml	0.541	0.618	0.589
6.	5 µg/ml	0.610	0.643	0.609

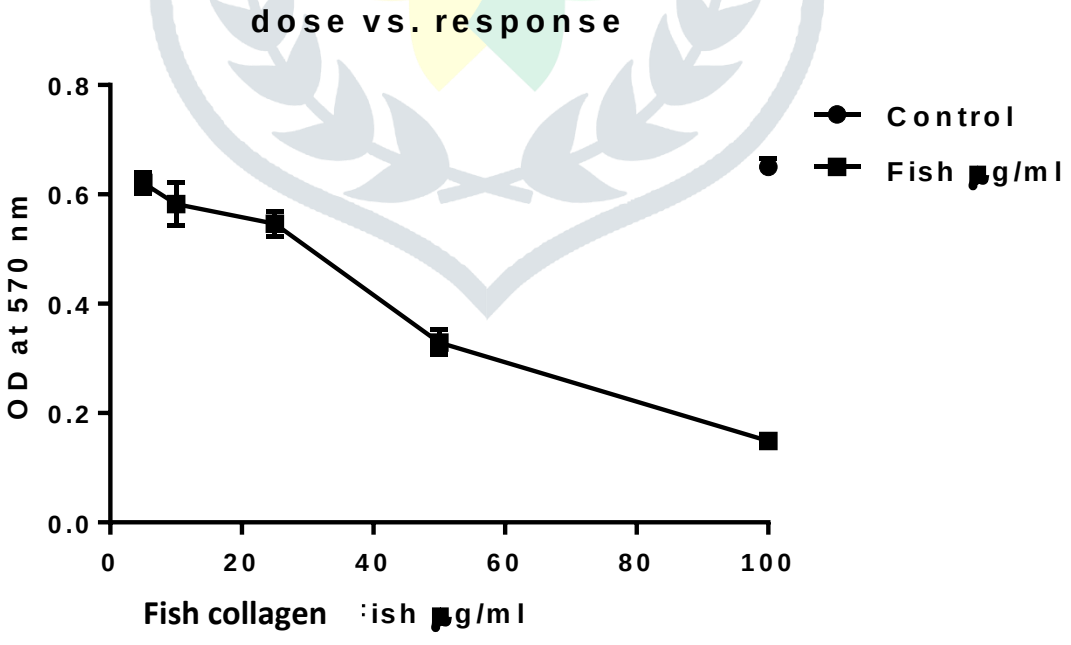
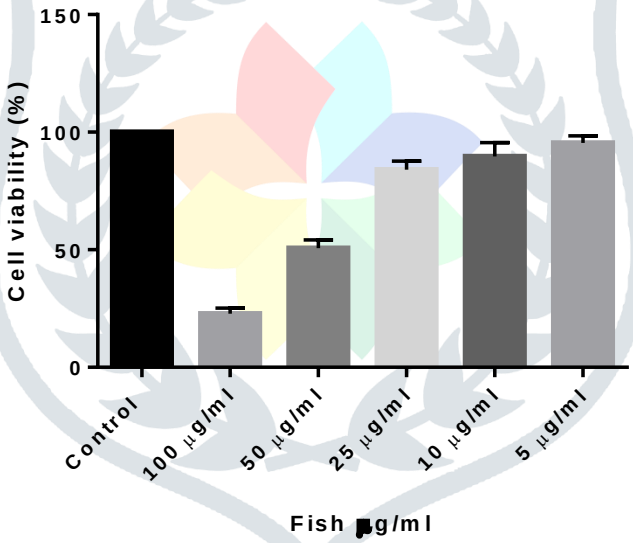


Table 2.Cell Viability (%)

S. No	Tested sample concentration (µg/ml)	Cell Viability (%) (in triplicates)			Mean value (%)
1	Control	100	100	100	100
2	100 µg/ml	25.07	23.07	20.46	22.86
3	50 µg/ml	49.38	54.61	48.00	50.66
4	25 µg/ml	80.00	86.92	85.23	84.05
5	10 µg/ml	83.23	95.07	90.61	89.63
6	5 µg/ml	93.84	98.92	93.69	95.48

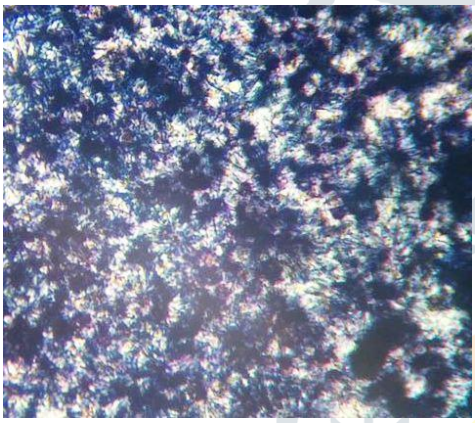


IC50 Value of tested sample: 42.27 µg/ml

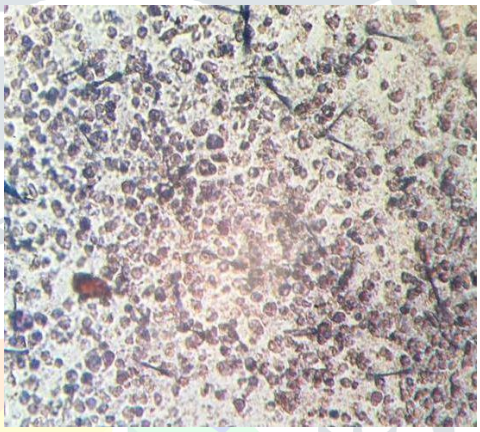
log(inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
LogIC50	1.626
HillSlope	-3.349
IC50	42.27
Std. Error	
LogIC50	0.01818
HillSlope	0.4243
95% Confidence Intervals	

LogIC50		1.587 to 1.665
HillSlope		-4.266 to -2.433
IC50		38.62 to 46.28
Goodness of Fit		
Degrees of Freedom		13
R square		0.9753
Absolute Sum of Squares		542.5
Sy.x		6.460
Number of points		
Analyzed	3	15

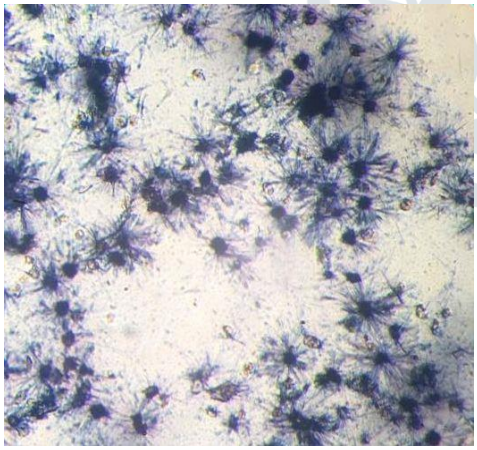
Plate 1 . Formation of formazan crystals in control cells and fish sample treated cells



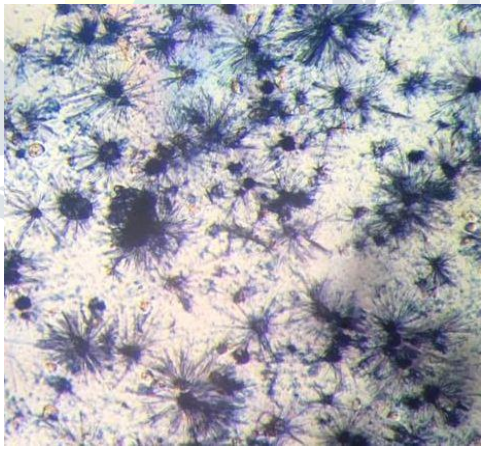
Control cells



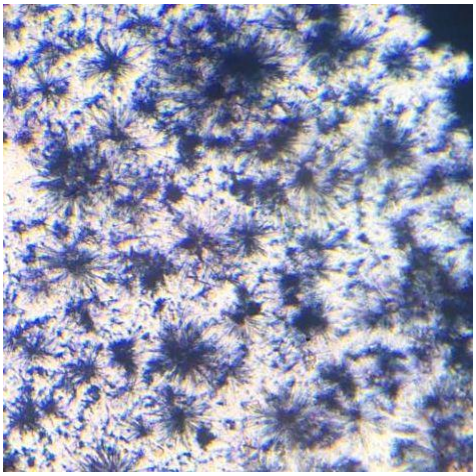
Treated 100 µg/ml

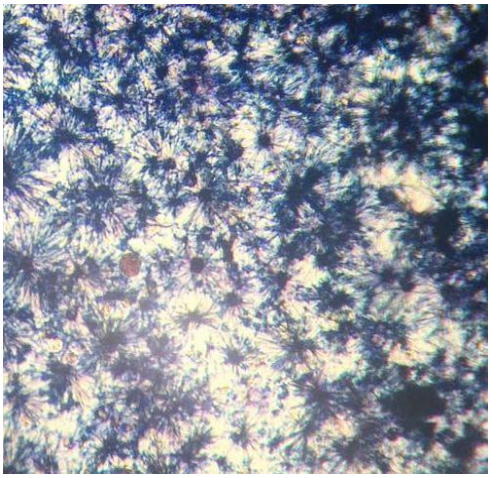


Treated 50 µg/ml



Treated 25 µg/ml





Treated 10 µgml **Treated 5 µg/ml**

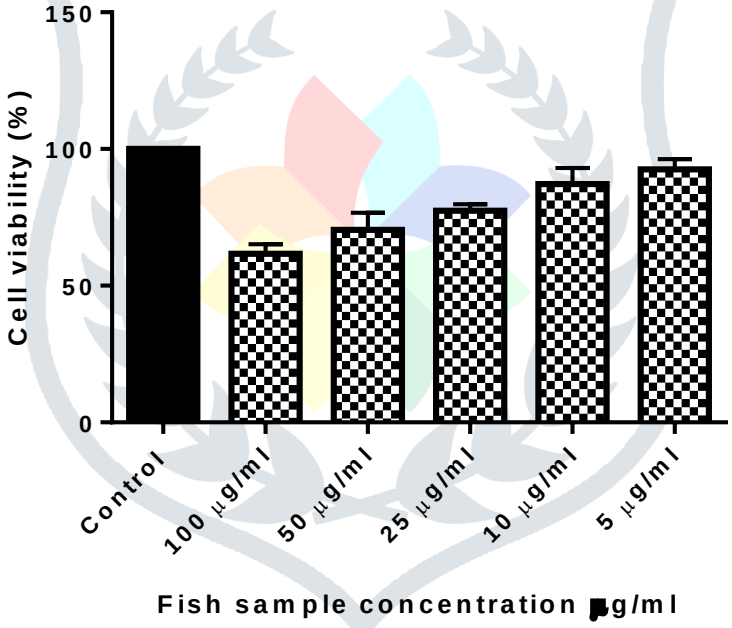
Plate 1 represents the formation of formazon crystals in the control cells fish collagen treated on human colon cancer cells (HT-29). The cultured cells are observed under microscope for the presence of needle shaped formazon crystals which indicates the metabolically active cells. In control cells almost all the cells are having needle shaped formazon crystals. Where as in the collagen treated cells the formation of needle shaped formazon crystals is rapidly decreased with increasing concentration. In 100 µg of collagen treated cells, the needle shaped formazon crystal was not seen and the colour is changed from dark blue to pale violet which shows the inhibition of the growth of colon cancer cell.

5.8.3. Effect of fish collagen on human breast adenocarcinoma

Anticancer activity of collagen extracted from snakehead fish was studied against the human breast adenocarcinoma cells. The fish collagen was tested for its cytotoxicity on human breast adenocarcinoma cell line and OD values in different concentration are shown in Table 3. The collagen extraction of snakehead fish dose-dependently inhibited HT-29 cells proliferation with an IC_{50} value of 26.15 µg/ml at which 50 % of the cancer cell death occurred (Plate). Crude epidermal collagen at a concentration less than 25 µg/ml had less toxicity for human breast adenocarcinoma, while more than 25 µg/ml significantly inhibited human breast adenocarcinoma cells proliferation.

Table 3 **OD Value at 570 nm**

S. No	Tested sample concentration (µg/ml)	OD Value at 570 nm (in triplicates)			Mean OD value
7	Control	0.509	0.535	0.497	**0.513**
8	100 µg/ml	0.296	0.330	0.323	**0.316**
9	50 µg/ml	0.335	0.353	0.397	**0.361**
1	25 µg/ml	0.390	0.391	0.411	**0.397**
1	10 µg/ml	0.462	0.467	0.413	**1.342**
1	5 µg/ml	0.493	0.455	0.477	**1.425**

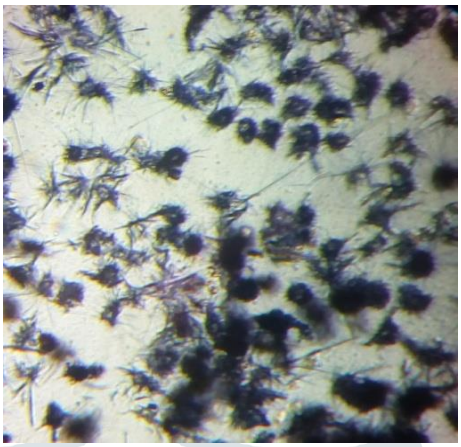


IC50 Value of tested sample: 26.14

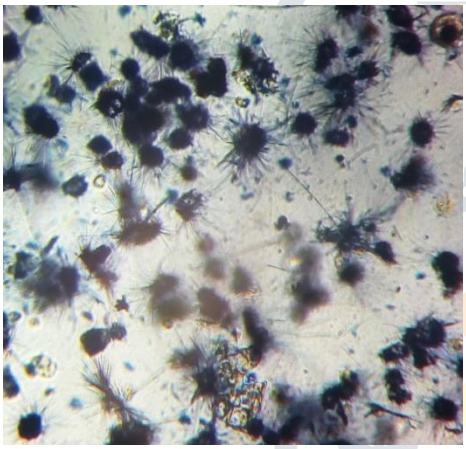
log(inhibitor) vs. normalized response -- Variable slope		
Best-fit values		
LogIC50		1.417
HillSlope		-1.863
IC50		26.14
Std. Error		
LogIC50		0.05554
HillSlope		0.4002
95% Confidence Intervals		
LogIC50		1.297 to 1.537
HillSlope		-2.727 to -0.9983
IC50		19.83 to 34.45
Goodness of Fit		
Degrees of Freedom		13
R square		0.8832
Absolute Sum of Squares		2528
Sy.x		13.95
Number of points		
Analyzed	3	15

Plate Formation of Formazan crystals in control cells and fish collagen treated breast adenocarcinoma cells

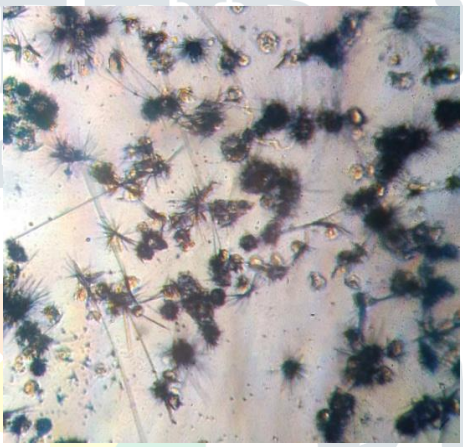
Control cells



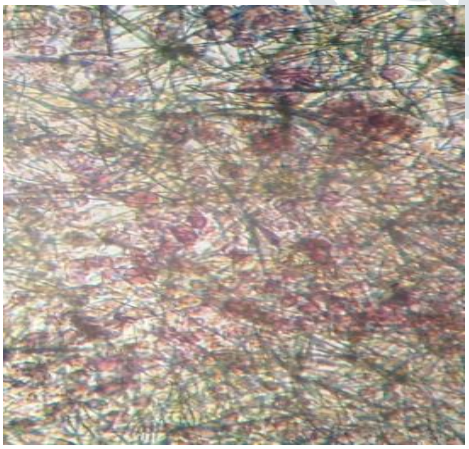
Treated 5 µg/ml



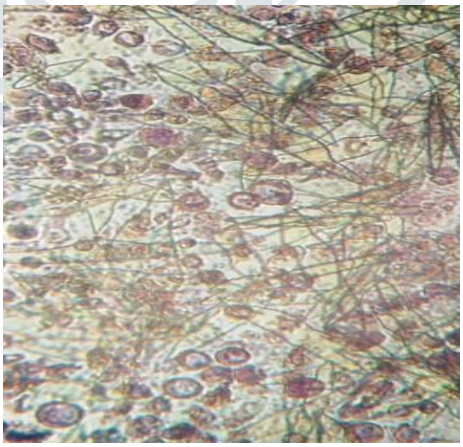
Treated 10 µgml



Treated 25 µg/ml



Treated 50 µg/ml



Treated 100 µg/ml

Plate represents the formation of formazon crystals in the control cells fish collagen treated on human breast adenocarcinoma cells (HT-29). The cultured cells are observed under microscope for the presence of needle shaped formazon crystals which indicates the metabolically active cells. In control cells almost all the cells are having needle shaped formazon crystals. Where as in the collagen treated

cells the formation of needle shaped formazon crystals is rapidly decreased with increasing concentration. In 100 µg of collagen treated cells, the needle shaped formazon crystal was not seen and the colour is changed from dark blue to pale violet which shows the inhibition of the growth of colon cancer cell.

4. Discussion

Fish is leaner than meat that is lower fat and contains amino acids and minerals that are vital to the body. The importance of protein in human nutrition and fish as a source of animal protein in maintenance of health has been known from time immemorial. It is also believed that as people become increasingly aware of the association between diet and good health, the consumption of fishery products will most likely increase [17]. Fish have often been called the poor man's source of animal protein. The systematic use of fish concentrate has long been proven advantageous in animal husbandry and there is growing recognition of its potential for improving human nutrition and health. Recent studies have shown that fish derived bioactive peptides play a vital role in human health and nutrition and they can be a part of the human diet for several years. The main site of action of the majority of microbes is believed to be the plasma membrane, where they cause pore formation or membrane lysis [18]. The selectivity for foreign substances such as those of bacteria and fungi is believed to result from high content of anionic lipids on the surface of microbes, high potential gradient across the membrane and lack of cholesterol. Virtually all fishes are covered with integumental mucus that is involved in many aspects of their biology ranging from disease resistance to rearing of young ones to shelter and locomotion. It was reported that epithelial tissue produces antimicrobial molecules which serve as the first line of host defense against microbial invasion in a variety of vertebrates including humans [19].

Oxidation is a vital process in aerobic organisms, particularly in vertebrates and humans. However, oxidation leads to the formation of reactive oxygen species including free radicals and non-free radical species. Oxidation primarily occurs on unsaturated fatty acids by a free radical mediated process. The antioxidant effect of some collagen derived peptides has been observed in culture cells. The exact mechanism by which peptides display antioxidant activity is not fully understood. Some peptides derived from hydrolysed proteins would exert antioxidant activities like, free radical scavengers, lipid peroxidation

inhibitors or chelating agents [20]. The radical scavenging activity of some peptides could be ascribed to the presence of determined amino acids within their sequence which could donate protons to electron deficient radicals [21,22]. In this sense, the high Glycine and Proline content in collagen could be related to the antioxidant activity of some collagen-derived peptides. The presence of several residues of glycine in a peptide sequence may confer high flexibility on the peptide structure, while the pyrolidine ring of Proline could impose certain conformational consrtaints in the secondary structure of the peptide.

Kim *et al* [23] have isolated two antioxidant peptides from a hydrolysate of Alaska Pollack skin, both containing a Glycine residue at the C-terminus and the repeating motif Gly-Pro-Hyp. These gelatin derived peptide has found to exhibit valuable free radical quenching capacity. The high content of specified amino acids could exhibit the antioxidant activity of the collagen. Various antioxidant compounds are identified in many natural sources including some protein compounds. The antioxidant activity of collagen is an essential property for the oral tolerance mechanism in autoimmune diseases [24]. Few studies have been conducted on the antioxidant activity of collagen and gelatin isolated from the aquatic animals including squid skin [25] and jellyfish skin [26].

The antioxidant activity of collagen has been linked to the high content of hydrophobic amino acids, which could increase their solubility in lipids and therefore enchance their anti-oxidative activity. Composition of amino acid plays an important role in antioxidant activities of protein hydrolysate. High content of hydrophobic amino acid could increase the solubility of collagen peptides in lipid which enhance their antioxidant activities [27]. Falling in line with the above finding in the present study also the acid soluble collagen extracted from the freshwater snakehead fish *C. striatus* exhibit strong antioxidant acitivity.

Cancer is an abnormal growth of the cells that divided uncontrollably and may spread to other parts of the body. There are many kinds of cancer which can involve just about any part of the body. Cancer is a broad term describing a wide group of diseases sharing the common characteristic of abnormal cellular division that is not subject to normal growth controls. It is one of the most feared diseases due to general perception that it is an indiscriminate and incurable affliction that insidiously attacks people of all cultures and ages.

Nearly 95% of cancer is initiated by severe chronic rather than a single insults, whereas the inherited cancers accounts for only 5% of human cancers [28].

MTT assay originally developed for cell viability testing is now used in cancer cell cytotoxicity testing [29]. Antiproliferative activity was considered to be the effect produced when the hydrolysates were incorporated before cell growth started. The peptides from fish collagen affected the viability of both cell lines depending on the time of hydrolysis used. Three peptides from a fish source have been described as having antitumor activity. The hydrophobic peptides was able to induce apoptosis in human U397 lymphoma cells by increasing caspase-3 and caspase-8 activity [30].

Molecules isolated from marine animals such as corals, sponges and ascidians produces substances (to protect themselves from predators), which are shown to posses anticancer properties belong to classes polyketides, terpenes, gleroids and peptides [31]. Among the antimicrobial peptides many show antibacterial activity on gram positive and gram negative bacteria, whereas few have been shown to exhibit anticancer and antiviral properties [32].

Vinodh kumar *et al* [33] examined the effect of collagen peptides on the growth, and viability of COLO320 cells. The cytotoxicity effect exhibited by collagen peptides ranged between 17-50% on COLO320 cells. They added that the cell viability inhibition by CP-5 was relatively higher than that expressed by CP-25 and CP50. The maximum activity exhibited by CP-5 was 49.78% at 1 mg/mL concentration, while those expressed by Collagen Peptide-25 and Collagen Peptide -50 were 32.67% and 29.92%, respectively. The positive control, cyclophosphamide expressed much higher activity of 75.74% at the same concentration. Picot *et al*. [34], revealed that fish peptides prepared using Protomex and alcalase exhibited 33.3% growth inhibition on HeLa cells, but 81.7% inhibition on HCT166 cells at 1mg/mL concentration. In an another study the collagen peptide isolated from the skin of salmon exhibited the highest cytotoxicity activity of 65% and 50% at 0.2 mg/mL concentration against HepG2 and HeLa cells, respectively [35]. In the same study they stated that bluefin tuna collagen reduced the growth of HepG2 and HeLa cells by 50% and 38%, respectively. The collagen peptides extracted from milk fish (*Chanos chanos*) exhibited 70% and 18% inhibition in HeLa cells and HCT-166 cells at 1 mg/mL concentration, respectively [36]. Much lower

cytotoxicity activity in HaCaT cells was recorded as 19.9±1.9% at 1 mg/mL concentration by fish scale collagen peptide [37]. Falling in line with the above evidences, in the present study also the acid soluble collagen extracted from the freshwater snakehead fish *C. striatus* exhibit a strong anticancer activity against the human colon cancer and human breast cancer. The acid soluble collagen shows a maximum inhibition on increasing the concentration on the both tested cancer cell lines, whereas the acid soluble collagen has 95% of cell viability in lower concentration on the vero cell line. Further our study was supported by the findings of Izabela Dobrzynska *et al*. [38] who reported that the AMPs kill cancer cells without damaging the healthy cells. This is due to the electrostatic interaction between AMPS and components of cell membrane. This study supports that the acid soluble collagen is not cytotoxic for normal cells and toxic for the cancer cells.

5. Conclusion

The snakehead fish Channa striatus can be used as a natural source of collagen. The acid soluble collagen extracted from C. striatus have good source of antioxidant activity (DPPH radical scavenging activity) and comparable biological impact on anticancer human cells tested by MTT assay. The results of this study show that the snakehead fish collagen appears to be a good material for the biomedical device that could be used as functional ingredient in pharmaceutical industry and food industries.

Acknowledgement

Authors are thankful to the authorities of Annamalai University and Head, Department of Zoology, Annamalai University for providing facilities to carry out this study.

References

1. Newman DJ, and Cragg GM. Natural products as source of new drugs over the last 25 years. J Nat Prod., 2007; 70: 461-477.

2. Venugopal V. Marine products for healthcare: functional and bioactive nutraceutical compounds from the ocean. CRC press 2008; 13.

3. Samanta S Khora. Therapeutic benefits of Omega-3 fatty acids from fish. Int J Drug Dev & Res, 2013; 5(2): 99-102.

4. Rajanbabu VCJ. Applications of antimicrobial peptides from fish and perspectives for future. Peptides, 2011; 32: 415-420.

5. Bailey AJ, Paul RG and Knott L. Mechanism of maturation and ageing of collagen. Mech Ageing Dev, 1998; 56: 249-258

6. Leitinger B, Hohenester E. Mammalian collagen receptors. Matrix Biol, 2007; 26: 146-155.

7. Cunniffe GM, Brien FJO. Collagen scaffolds for orthopedic regenerative medicine. The journal of the Minerals, Metals and materials Society, 2011; 63(4): 66-73.

8. Osman H, Suriah AR, Law EC. Fatty acid composition and cholesterol content of selected marine fish in Malaysian waters. Food Chem., 2001; 73: 55-60.

9. Negi CS, Palyal VS. Traditional uses of animal and animal products in medicine and rituals by the Shoka Tribes of District Pithoragarh, Uttaranchal, India. Studies on Ethnomedicine., 2007; 1:51–53.

10. Haniffa MA, Arun Sing SV, Dhanaraj M, Muthu Ramakrishnan C, Manikandaraja D, James Milton M. Antibacterial activity of skin and intestinal mucus of different freshwater species viz, *Channa striatus, Channa micropietes, Channa marulius, Channa punctatus* and *Channa gachua.* J Malay Sci., 2009; 28(3): 257-262.

11. Loganathan K, Muniyan M, Arulprakash A, Gunasekaran G, Senthilraja P, Prakash M. Studies on the role of mucus from *Clarias batrachus* (*Linn*.) against selected microbes. Int J Pharma Appl., 2011; 2(3): 202-206.

12. Loganathan K, Arulprakash A, Prakash M, Senthilraja P. Lysozyme, protease, alkaline phosphatase and esterase activity of epidermal skin mucus of freshwater snakehead fish Channa striatus. Int. J. of Res. In Pharmaceutical and bioscience, 2013; 3(1): 17-20.

13. Kuppulakshmi C, Prakash M, Gunasekaran G, Manimegala G, Sarojini S. Antibacterial properties of fish mucus from *Channa punctatus* and *Cirrhinus mrigala*. Eur Rev Med Pharmacol Sci., 2008; 12: 149-153.

14. Mohanty IR, Maheshwari U, Joseph D, Deshmukh Y. Tribulus teresstris modulates heat shock protein and key anti-apoptotic proteins in the Langendorff model of myocardial ischemia and reperfusion injury. Afri J Pharma Pharmacol., 2011; 5(2): 165-174.

15. Hema GS, Shyni K, Suseela Mathew, Anandan R, George Ninan, Lakshmanan PT. A simple method for isolation of fish skin collagen-biochemical characterization of skin collagen extracted from Albacore tune (*Thunnus alalunga*), dog shark (*Scoliodon sorrakowah*) and Rohu (*Labeo rohita*). Anals of Biological Research, 2013; 4(1): 271-278.

16. Binsan W, Benjakul S, Visessanguan W, Roytrakul S, Tanaka M, Kishimura H. Antioxidative activity of mungoong, an extract paste from the cephalothorax of white shrimp (liptopenaeus vannamel). Food Chem, 2008; 106: 185-193.

17. Kadam S, Prabhasankar P. Marine foods as functional ingredients in bakery and pasta products. Food Res Int, 2010; 43: 1975-1980.

18. Hancock REW, Lehrer R. Cationic peptides: a new source of antibiotics. Trends Biotechnol., 1998; 16: 82-88.

19. Shephard KL. Functions for Fish Mucus. Rev Fish Biol Fisher., 1994; 4: 401-429.

20. Erdmann K, Cheung BW, Schroder H. The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. J Nutri Biochem, 2008; 19(10): 643-654.

21. Suetsuna K, Ukeda H, Ochi H. Isolation and characterization of free radical scavenging activities peptides derived from casein. J Nutri Biochem, 2000; 11: 128-131.

22. Sarmadi BH, Ismail A. Antioxidative peptides from food proteins: A review. Peptides, 2010; 31: 1949-1956.

23. Kim SK, Kim YT, Byun HG, Nam KS, Joo DS, Shahidi F. Isolation and characterization of anti-oxidative peptides from gelatin hydrolysate of Alaska Pollack skin. J Agric Food Chem, 2001; 49: 1984-1989.

24. Youn J, Hwang SH, Ryoo ZY, Lynes MA, Paik DJ, Chung HS. Metallothionein suppresses collagen-induced arthritis via induction of TGF-beta and down regulation of proinflammatory mediators. Clin Exp Immunol, 2002; 129: 232-239.

25. Mendis E, Rajapakse N, Byun HG, Kim SK. Investigation of jumbo squid (Dosidicus gigas) skin gelatin peptides for their in vitro antioxidant effects. Life Sci, 2005; 77: 2166-2178.

26. Zhuang Y, Hou H, Zhao X, Zhang Z, Li B. Effects of collagen and collagen hydrolysate from jellyfish (Rhopilema esculentum) on mice skin photoaging induced by UV irradiation. J Food Sci, 2009; 74: 138-183.

27. Arulvasu C, Michael Jennifer S, Prabhu D, Chandhirasekar D. Toxicity Effect of Silver Nanoparticles in Brine Shrimp Artemia. The Scientific world Journal, 2014; Article ID 256919, 10 pages.

28. Balmain A, Derynck R, Akhurst RJ. TGF-beta signaling in tumor suppression and cancer progression. Nat Genet. 2001 Oct;29(2):117-29.

29. Alley MC, Scudiero DA, Monks A, Hursey ML, Czerwinski MJ, Fine DL, Abbott BJ, Mayo JG, Shoemaker RH, Boyd MR. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. Cancer Res. 1988; 48(3): 589-601.

30. Ace Baehaki , Rodiana Nopianti, Shella Anggraeni. Antioxidant activity of skin and bone collagen hydrolyzed from striped catfish (Pangasius pangasius) with papain enzyme. Journal of Chemical and Pharmaceutical Research, 2015; 7(11): 131-135

31. Mayer AM, Gustafson KR. Marine pharmacology in 2005-2006: Antitumour and cytotoxic compounds. Eur J Cancer, 2008; 44(16): 2357-2387.

32. Zasloff M. Antimicrobial peptides of multicellular organisms. Natl., 2002; 415: 389-395.

33. Vinothkumar L, Jeya shakila R, Jeyasekaran G. *In vitro* Anti-Cancer, Anti-Diabetic, Anti-Inflammation and Wound Healing Properties of Collagen Peptides Derived from Unicorn Leatherjacket (*Aluterus Monoceros*) at Different Hydrolysis. Turk. J. Fish.& Aquat. Sci, 2018; 19(7): 551-560.

34. Picot L, Bordenave S, Didelot S, Fruitier-Arnaudin I, Sannier F, Thorkelsson G, Piot JM. Antiproliferative activity of fish protein hydrolysates on human breast cancer cell lines. Process Biochemistry, 2006; 41(5): 1217-1222.

35. Han, SH, Uzawa Y, Moriyama T, Kawamura Y. Effect of collagen and collagen peptides from bluefin tuna abdominal skin on cancer cells. Health, 2011; 3(03): 129.

36. Baehaki A, Suhartono MT, Sukarno Syah D, Setyahadi S. Collagen peptides from fish skin with angiotensin I-converting enzyme (ACE) inhibitor and cancer antiproliferative activity. Research Journal of Pharmaceutical Biological and Chemical Sciences, 2016; 7(1): 1994-2000.

37. Subhan F, Kang HY, Lim Y, Ikram M, Baek SY, Jin S, Yoon S. Fish scale collagen peptides protect against CoCl2/TNF-α-induced cytotoxicity and inflammation via inhibition of ROS, MAPK, and NF-κB pathways in HaCaT cells. Oxidative Medicine and Cellular Longevity, 2017; 1-17.

38. Izabela Dobrzyńska, Barbara Szachowicz-Petelska, Stanislaw sulkowski, Zbigniew Figaszewski. Changes in electric charge and phospholipids composition in human colorectal cancer cells. Molecular and Cellular Biochemistry, 2005; 276,(1-2): 113–119