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PHYSICO-CHEMICAL AND ANTI-CANCER PROPERTIES OF COLLAGEN DERIVED SILVER NANOPARTICLES SYNTHESIZED FROM LUTJANUS FULVUS

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Abstract:

Acid Soluble Collagen (ASC) and Pepsin Soluble Collagen (PSC) were extracted from the skin of the blacktail snapper L.fulvus. The FT-IR analysis revealed the presence of functional groups and from the results obtained it's been confirmed of type I. The effect of physico-chemical parameters namely, denaturation temperature, pH, NaCl on collagen solubility was determined. The morphological structure of collagen was studied using SEM. Furthermore, the collagen-derived silver nanoparticles were evaluated for anti-cancer activity and it showed the inhibition of breast cancer cell lines (HeLa and MCF) growth at IC50 of 45.6µg/mL.

Keywords: Collagen, Silver nanoparticles, Anticancer, SEM, Blacktail snapper

Introduction:

Collagen is the richest structural polypeptide found in living organisms, accounting for approximately 30 percent of the overall protein in the body (Shoulders and Raines, 2009). Collagen is an important component that serves as a natural substrate for new tissue generation and is involved in hemostasis, inflammatory processes, proliferation, and repair mechanisms (Silver, 2009). Collagen is rapidly expanding as well as widely used in therapeutic formulations, notably cartilage restructuring, scar tissue remodeling, collagenous implants, and drug carriers (Wang et al., 2009). Because collagen deterioration causes freckles, loose skin, joint stiffness, and dryness, it is essential to discover collagen fibers supplies for tissue regeneration (Aguda et al., 2014).

Numerous reports have recently stated that the global market for collagen has indeed been growing over time. Tropocollagen, the basic structure of collagen, is made up of three polypeptide chains that are snapped in a left-handed helix and knotted around each other to form a right-handed triple helix structure (Mario Hiram et al., 2010). Each amino acid sequence is distinguished by the triplet (Gly-X-Y)n repetitive framework, where glycine is the systemic basic requirement for the triple helix (Wang et al., 2009). X and Y, on the contrary hand, are frequently proline (Pro) and hydroxyproline (Hpy), combined (Palpandi et al., 2010). Just after its unique features such as its high structural integrity, and low immunogenicity; it would be progressively becoming a commonly used biocompatible material both in bio linked and other commercial related industries (Singh et al., 2011).

Exploitation of marine byproducts as a different source of collagen is gaining popularity because of their simple extraction, greater collagen composition, and uptake by the human body despite their small molecular weight and biocompatibility (Silvipriya et al., 2015). Despite the fact that a number of studies have been conducted on the attributes of fish skin collagen fibers, See et al. (2010) stated that they differs significantly and

diverse between fish species. For all biomedical application, collagen is focused solely on the resilience of the triple helix. Upon absorbing of excessive heat, the hydrogen bonds are being rapidly destroyed, resulting in the steric shift of collagen. At a certain temperature, thermal degradation would occur. In broad sense, denaturation temperature (Td) and shrinkage temperature (Ts) are used to represent the thermal stability of collagen (Ts). The greater the Td or Ts, the greater would be the thermal stability (Xiaoxia Zhang et al., 2020).

Collagens from fish has therefore been extensively studied in many different species namely cartilage of Chiloscyllium punctatum and Carcharhinus limbatus (Kittiphattanabawon et al., 2010), abalone (Dong et al., 2012), and rainbow trout (Tabarestani et al., 2010). Lutjanus fulvus (Blacktail snapper) is found in bays and reefs of Indo-Pacific Ocean. It is harvested profitably within those areas and thus is normally seen in fresh fish markets. Juveniles are occasionally spotted in shallow mangrove forest and in the lower reaches of river systems (Allen, 1985). Since, there is no information on collagen extracted from this fish species, this study is carried out to explore the presence of collagen and its bioactivity.

Materials and methods

Preparation of collagen sample:

The muscle of L. fulvus was extracted with 0.1M NaOH to eliminate non-collagenous protein. The deproteinized muscles were then lyophilized and cleaned with distilled water for additional research.

Isolation of Acid Soluble & pepsin soluble Collagen:

For three days, 0.5M acetic acid (1:3 w/v) was subjected to the lyophilized muscle, and the samples were separated by centrifugation at 5000 rpm for 30 minutes at 4°C. The residue was extracted for two days with the same solution and centrifuged under the same conditions after the supernatant was obtained (Zhang et al., 2009). NaCl was added to each solution at a final concentration of 2.3 M and a neutral pH of 7.5 to combine and salt it out. The resulting precipitates were collected and redissolved in 0.5 M acetic acid. After three days of dialyzing with acetic acid and distilled water, the finished product was lyophilized. The dried substance was dubbed acid soluble collagen (ASC). The acid-extracted insoluble residue was used to extract the pepsin-soluble collagen (Sigma, India). The residue was thoroughly rinsed with distilled water before even being solubilized in 0.5 M acetic acid with 0.1% (w/v) pepsin for three days. The mixture was centrifuged for 60 minutes at 5000 rpm at 4°C. To salt out the extract's supernatant, NaCl was added, resulting in a final concentration of 2.3 M at a neutral pH of 7.5. After rotating for 30 minutes at 5000 rpm and 4°C, the precipitate was separated using centrifugation. The precipitate was then dissolved in 0.5 M acetic acid, and the resulting solution was dialyzed against water for three days (Huang et al., 2011).

FTIR analysis:

Collagen sample's amide band patterning was examined using FTIR (BioRad, FTIR 40 model, USA) spectroscopy. Under drying conditions, 100mg of potassium bromide (KBr) and about 0.5mg of lyophilized collagen sample were ground together. With 32 scans per sample, ranging from 4000 to 400 cm-1, the spectrum was acquired. Software called ORIGIN 8.0 was used to analyse the generated spectrum data (Thermo-Nicolet, USA) (Liu et al., 2012).

Determination of collagen denaturation temperature:

Collagen denaturation was carried out by following Pati et al. (2010) with minor modifications. A Brookfield viscometer (Brookfield DV-III, Mecomb Malaysia, Malaysia) was filled with a collagen solution

containing 0.3% (m/v) in 0.05 M acetic acid. The viscometer was then immersed in a 4°C water bath for 15 minutes to enable the collagen solution to reach equilibrium to the water bath temperature. The temperature was gradually increased up to 50°C and held at each temperature for 10 minutes. The viscosity of collagen solution was measured at 2°C intervals from 4°C to 50°C. The following fractional viscosities were calculated for each temperature as follows:

$$\text{Fractional Viscosity} = \frac{\text{measured viscosity} - \text{minim} \quad \text{viscosity}}{\text{measured viscosity} - \text{maximum viscosity}}$$

Thermal denaturation curves for ASC and PSC from the skin of Clarias sp. were then derived by comparing the fractional viscosities against temperature. The temperature upon which fractional viscosity had been 0.5 was chosen as the denaturation temperature.

Collagen solubility:

The solubility of ASC (Singh et al., 2011) and PSC (Kittiphattanabawon et al., 2005) was determined. The collagens were solubilized in 0.05 M acetic acid to achieve a desired concentration of 3 mg/mL, and the components were stirred at 4°C until completely solubilized.

Effect of pH on collagen solubility:

Collagen solution (3 mg/mL; 8 mL) was drawn to a 50 mL centrifuge tube, and the pH was adjusted with 6 M NaOH or 6 M HCl to achieve a pH range of 1 to 10. Distilled water, initially corrected to a similar pH as the collagen solution, was used to make up the volume of solutions to 10 mL. The solution was then centrifuged at 20,000g for 30 minutes at 4°C. The protein content of the supernatant was assessed using the Lowry et al., 1951 method with bovine serum albumin as standard. The relative solubility was calculated by comparing it to the solubility obtained at the highest pH.

Effect of NaCl on collagen solubility:

Collagen solution (6 mg/mL; 5 mL) was mixed with 5 mL of NaCl in 0.05 M acetic acid at varying concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10%). The mixture was continuously stirred at 4°C for 30 minutes before centrifugation at 20,000g for 30 minutes at 4°C. As previously described, the protein content of the supernatant was determined and the relative solubility was calculated.

Synthesis of silver nanoparticles (AgNPs) stabilized with type I collagen (AgNPscol) by chemical reduction method:

The photochemical method was used to create AgNPs from silver nitrate (AgNO3). The reducing agent sodium borohydride (NaBH4) was used in this study. Because NaBH4 is unstable when in contact with water at room temperature, it must be stabilised by using ultra-pure water at a low temperature (4°C) and refrigerating the solution until use. Furthermore, a multilayer film composed of AgNPs and collagen in a layer-by-layer (LbL) configuration was created to stabilise the particles. AgNPs stabilised by collagen designed and synthesised three different AgNPs and NaBH4 formulations as follows: 1:1, 1:6, and 1:15, by varying the concentration of NaBH4 for greater silver (Ag) reduction.

Determination of Percentage of silver in solution:

The atomic absorption spectroscopy was used to quantify the percentage of silver, present in solution at the wavelength of 328.1nm through multi-element lamp. The reading was held in atomic absorption flame with oxygen and acetylene gases.

Microscopic analysis of Collagen:

The cross-section of the materials was imaged using a Scanning Electron Microscope (LEO Electron Microscopy Ltd., Cambridge, UK), and the samples were covered by gold particles. Images were recorded at 2500 times of magnification and the voltage used for the acquisition was 300 kV.

Anticancer activity of collagen based silver nanoparticle:

Cytotoxicity of the collagen based silver nanoparticles was evaluated invitro against HeLa and MCF cell line. The cytotoxic analysis of the sample showed a direct dose dependent relationship as cytotoxicity was increased only at higher concentration. The sample shows a considerable cytotoxicity against HeLa and MCF cell line. The concentration necessary to produce 50% death rate was 45.6µg/mL. Anticancer activities of the silver nanoparticles evaluated against HeLa and MCF cell line were represented in Table 6. As a result, the synthesized collagen based AgNPs induces the dose dependent inhibition for HeLa and MCF7 cells. The percentage growth inhibition and percentage cell protection was calculated using the following formula

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of Individual Test groups}}{\text{Mean OD of Control}} \times 100$$

Results:

Collagen solubility

Effect of pH on solubility

The solubility of ASC and PSC of blacktail snapper reached its maximum levels at pH 4 and 3 respectively. Collagen solubility was greatest at pH levels ranging from 1 to 4. Furthermore, minimum solubility was discovered at pH 7. Hence, both collagens were solubilized to a greater extent in acidic pH 1to 4 as shown in fig.1

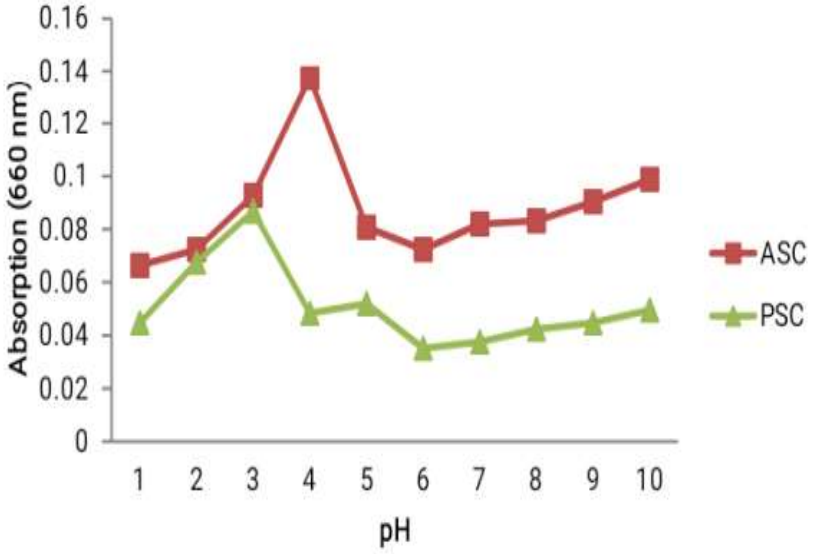

Fig 1: Effect of pH on solubility of ASC and PSC

Effect of NaCl concentration on solubility:

The effect of NaCl on the solubility of isolated ASC and PSC were represented in Fig.2. In 0.5M aceticacid, the solubility of ASC and PSC remained constant upto 2% NaCl concentration. A drastic decrease in the solubility of ASC was observed at 4% NaCl or above. For PSC, a slight decrease in solubility was obtained in the presence of 3% NaCl. A sharp decrease in solubility was observed with 4% NaCl or above. From the result, similar behavior was found for both collagen fractions. However, PSC exhibited a greater solubility than ASC at more than 2% NaCl concentration. A greater solubility of PSC could be due to the partial hydrolysis of high molecular weight cross-linked molecules by pepsin.

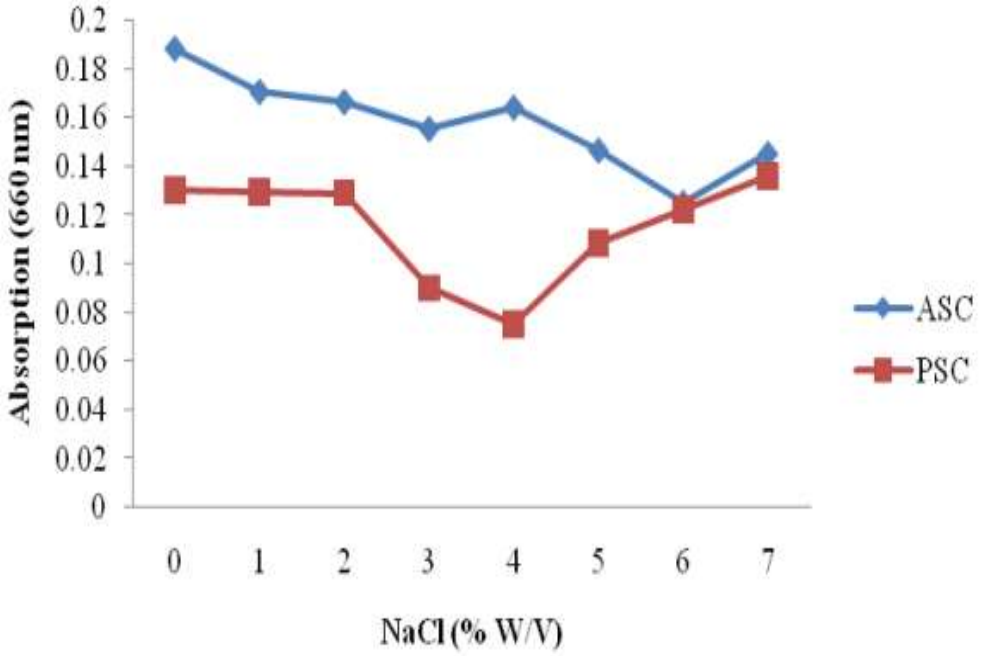

Fig 2: Effect of NaCl (%) on solubility of ASC and PSC

Thermal denaturation temperature:

The thermal denaturation temperature (Td) of L.fulvus skin collagen was calculated using thermal denaturation curve. The Td of isolated skin collagen by ASC and PSC method were 28ºC and 29.5º C as shown in Fig.3. ASC and PSC showed transition curves with maximum denaturation temperatures at 28ºC and 29.5º C respectively.

FT-IR spectral analysis of collagen :

The FT-IR peak details and their corresponding chemical stretches were given in Table 1. In the present study, the ASC and PSC of black tail snapper fish skin showed the characteristic peaks of amide A (3410cm-1), amide B (2854cm-1), amide I (1620cm-1), amide II (1460cm-1) amide III (1087cm-1) bands; whereas the PSC showed the characteristics peaks of amide A (3404cm-1), amide B (2854cm-1), amide I (1616cm-1), amide II (1413cm-1) and amide III (1055cm-1) bands. Amide A band at 3410 cm-1 in ASC and 3404 cm-1 in PSC of blacktail snapper was related to aliphatic secondary amine, N-H stretching vibrations. Amide B band in ASC and PSC (2854 & 2854 cm-1) of blacktail snapper was associated with stretching of methylene C-H asymmetrical / symmetric stretch. Amide I (1620 cm-1) in ASC and (1616 cm-1) in PSC was associated with C=O stretch/hydrogen bond coupled with CN stretch. Amide II (1460cm-1) in ASC and PSC (1413 cm-1)

associated with methylene C-H bend. Amide III (1087cm cm-1) in ASC and (1055 cm-1) in PSC is associated with C-O stretch (fig 5).

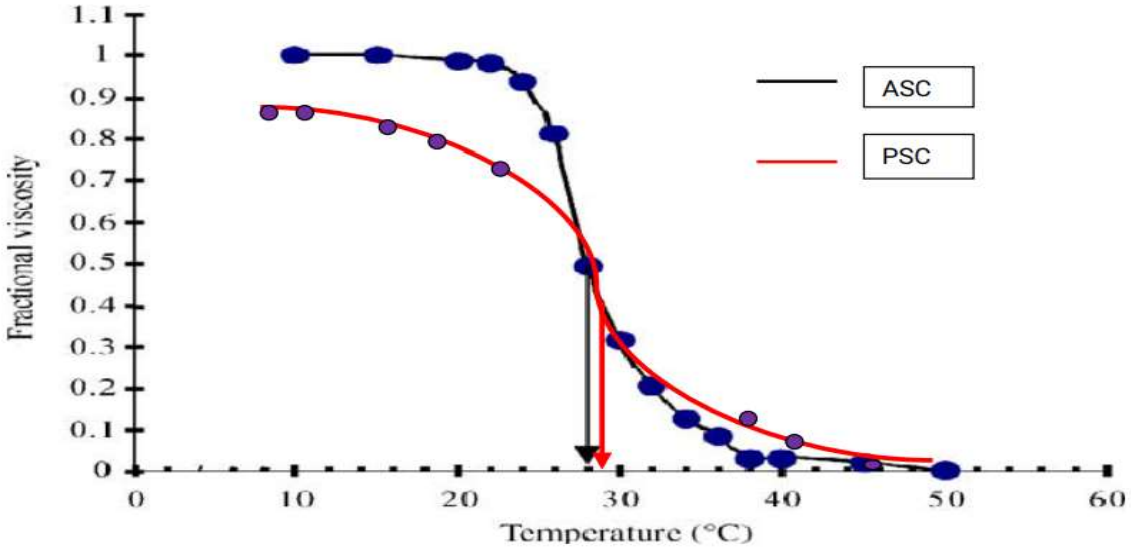

Fig 3: Thermal Denaturation Curve of ASC and PSC

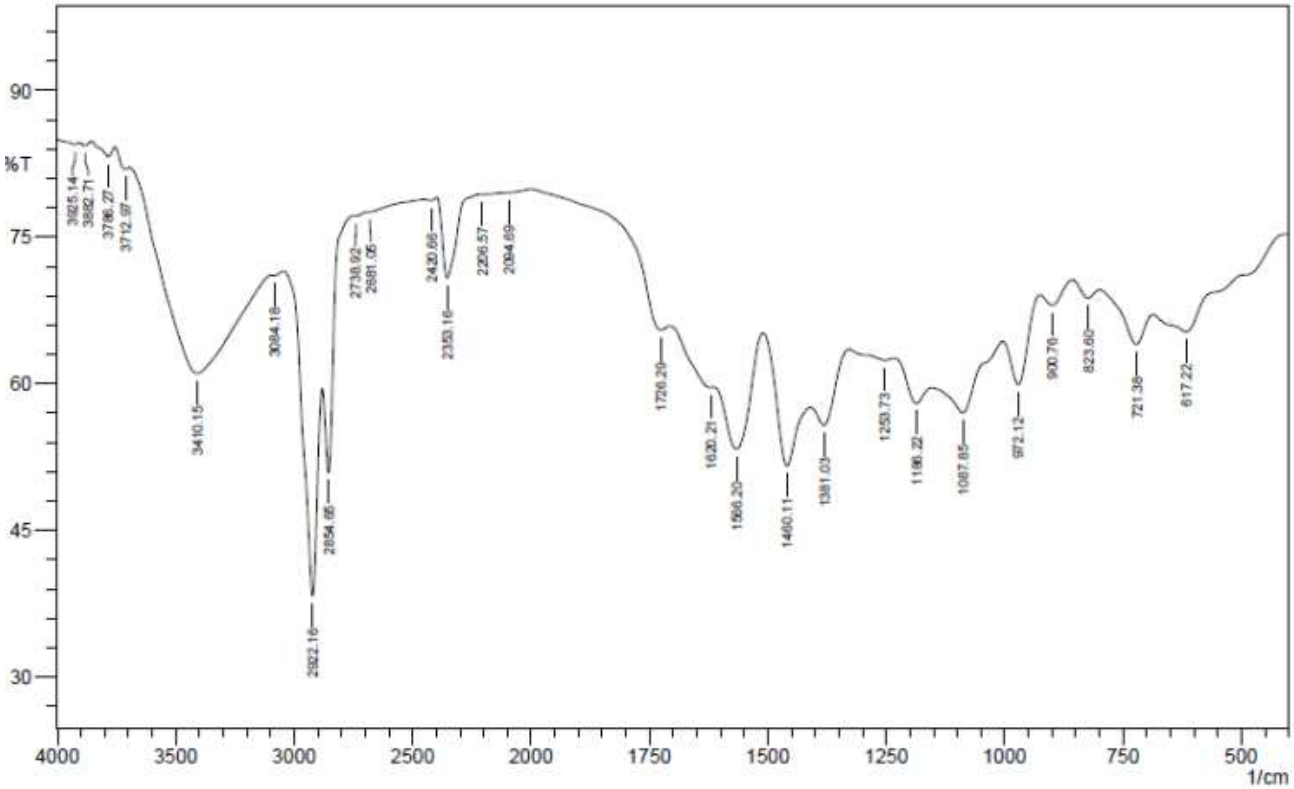

Fig 4: FT-IR spectrum of collagen for ASC

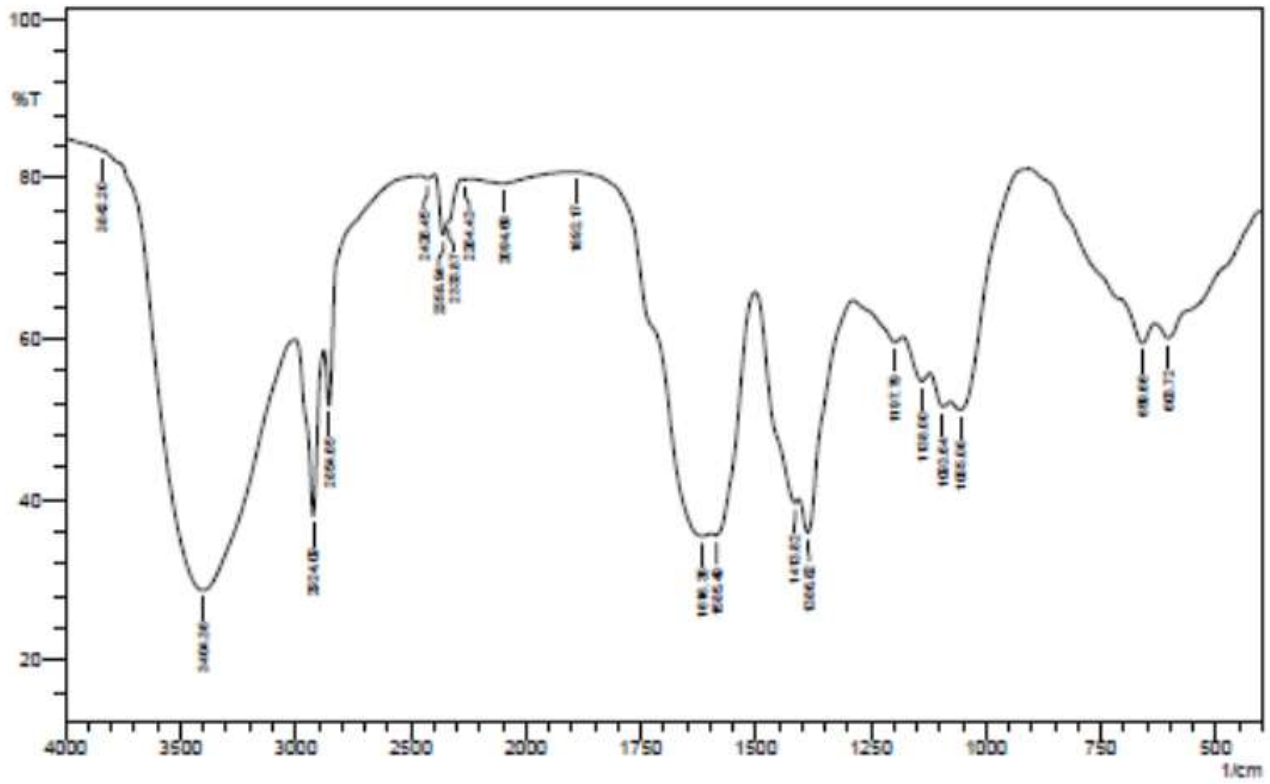

Fig 5: FT-IR spectrum of collagen for PSC

Table 1: FT-IR Characteristic peak obtained for standard collagen, ASC and PSC

Region	Standard Collagen	ASC	PSC	Assignment
Amide A	3520-3310cm-1	3410	3404	Aliphatic secondary amine, NH Stretch
Amide B	2865-2845cm-1	2854	2854	Methylene C-H Asymmetric / symmetric stretch
Amide I	1640-1580cm-1	1620	1616	C=O Stretch/Hydrogen bond coupled with CN stretch.
Amide II	1485-1405 cm-1	1460	1413	Methylene C-H bend
Amide III	1120-1080 cm-1 1060 - 1025cm-1	1087	1055	C-O stretch

Microscopic analysis of collagen:

The scanning electron microscopy images of isolated ASC and PSC from blacktail snapper was shown in Figure 6. The morphological structures of the isolated collagen samples were observed under SEM micro –

photography with lower (50µm) and higher magnification (10µm). The regular porous structure of collagen was clearly visible and the collagen surface was found to be rough and uneven. The fibrillar structures of collagen samples were also observed. The irregular, wavy collagen fibers were found to be alone or in small groups. These collagen fibrils formed bundles which varied in width and thickness and intertwined with each other. The cross-linked fiber networks can be mediated by hydrogen bond, hydrophobic interaction, electrostatic bond, entropic and dispersion forces.

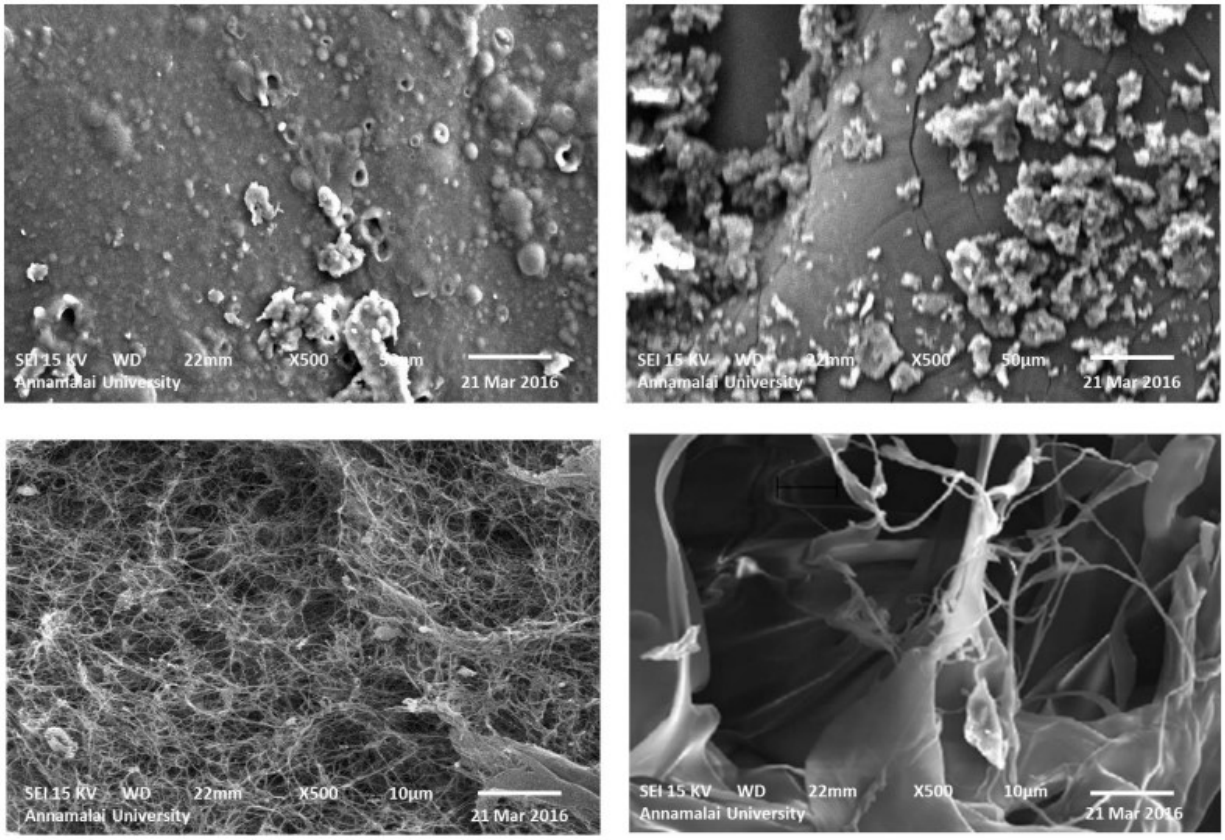

Fig.6: SEM Morphology of ASC and PSC from blacktail snapper

Synthesis of silver nanoparticles (AgNPs) stabilized with type I collagen (AgNPs collagen) by chemical reduction method:

The AgNO3/NaBH4 molar ratio of 1:6 resulted at the Ag concentration of 0.64mM (Table5) confirmed by the atomic absorption test. In the ratio of 1:1between $AgNO_3$ and $NaBH_4$, the amount of reducer was not sufficient to reduce all silver molecules. At the same time, the1:6 ratios was obtained the maximum concentration for the chemical reaction probably the molecules amount of AgNO3 and NaBH4 reached at their optimum value for reduction. However, the ratio was 1:15 excess NaBH4 causing release of ions in solution and forming nanoparticles with hydrodynamic diameter higher by aggregation. Hence, the maximum ratio (1:6) was selected for anticancer activity.

Physicochemical characterization of AgNPcol

Determination of Percentage of silver in solution:

Molar concentration of silver in the silver nanoparticle solution at different ratios were calculated and presented in the table 2.

Table 2: Different ratios of AgNPs collagen and its molar concentration of silver in the solution

Ratios of (AgNPs collagen)	[Ag](mM)
AgNP col (1:1)	0.434
AgNP col (1:6)	0.645
AgNP col (1:15)	0.345

Anticancer activity of collagen based silver nanoparticle:

Cytotoxicity of the collagen based silver nanoparticles was evaluated invitro against HeLa and MCF cell line. The cytotoxic analysis of the sample showed a direct dose dependent relationship as cytotoxicity was increased only at higher concentration. The sample shows a considerable cytotoxicity against HeLa and MCF cell line. The concentration necessary to produce 50% death rate was 45.6µg/mL. Anticancer activities of the silver nanoparticles evaluated against HeLa and MCF cell line were represented in Fig 7. As a result, the synthesized collagen based AgNPs induces the dose dependent inhibition for HeLa and MCF7 cells

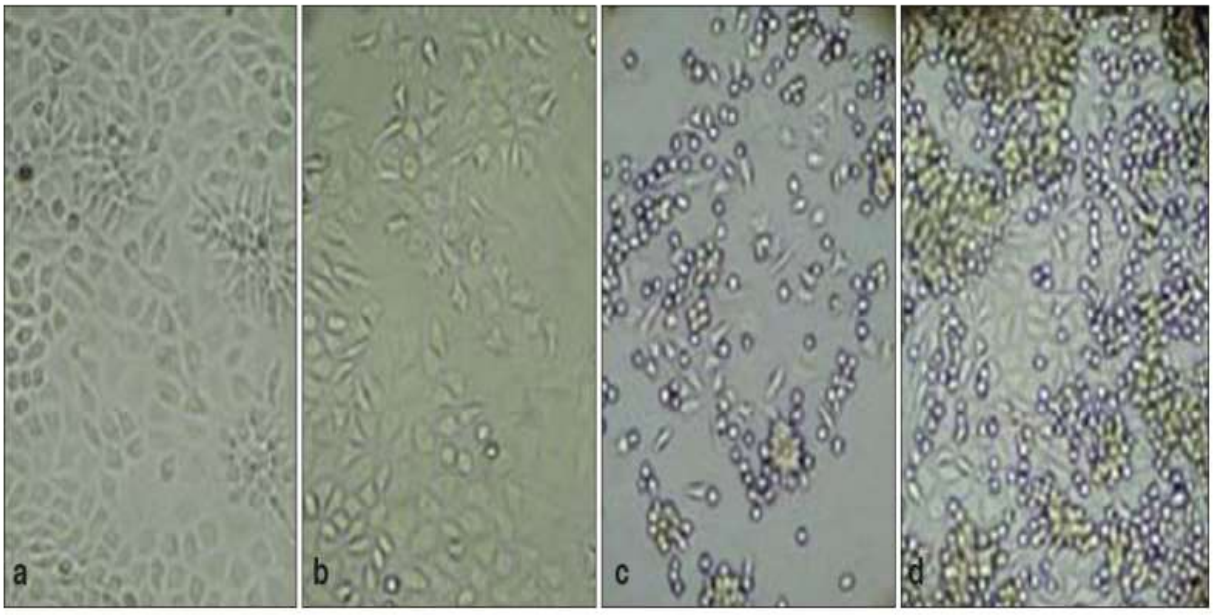

Fig.7.Cell line showing live cells and dead cells: (a=100µg/ml, b=75µg/ml,c=50µg/ml,d=25µg/ml)

Discussion:

Determination of Blacktail snapper fish skin Collagen denaturation temperature:

The thermal denaturation temperature (Td) of Blacktail snapper fish skin collagen was calculated using thermal denaturation curve. The Td of Blacktail snapper fish skin collagen was 28ºC as shown in Fig.4.Thermal denaturation temperature of porcine skin collagen was 37ºC (Nagaietal., 1999). When comparing with Td of porcine skin collagen, Td of Blacktail snapper fish skin collagen was about 9ºC lower than the Td of porcine skin collagen because Td of collagen was correlated with their body temperature and environment temperature (Rigby, 1968).

With thermal treatment, both the collagens obtained from ASC and PSC treatment, lost viscosity hastily. This phenomenon could be associated to collagen degradation (Muyonga et al., 2004). Collagen typically denatures at temperature rise of about 40ºC, resulting in a combination of random-coil single, twofold, and tri helices (Kittiphattanabawon et al., 2005). The thermal denaturation temperature (Td) of Blacktail snapper fish skin collagen treated with ASC and PSC in this study was estimated to be about 28 and 29.5ºC, respectively. This suggested that Td of collagen from Blacktail snapper fish skin were consistent with collagens isolated from warm-water species such as balloon fish (ASC 29.1º C, PSC 30.1º C), and carp (ASC 28.0ºC) (Zeng et al., 2012), Td of carpfish scale collagen (ASC 32.9°C) and the denaturation temperature of Chrysaora collagen was showed 29˚C in 60 minutes (Krishnan et al., 2013). The Td levels found in this study, however, were higher than those observed in cold water species of fish skin. Td of cod collagen were previously reported to be 15ºC, Alaska Pollack (16.8º C), and for chum salmon Td was found to be 19.4ºC (Muyonga et al., 2004).

The proportion of proline and hydroxyproline, as suggested throughout many studies published in the literature, was related directly to the thermal properties of collagen via intermolecular hydrogen bonding (Senaratne et al., 2006). According to Zeng et al. (2012), proline and hydroxyproline conferred constraints mostly on structure of a polypeptide chain as well as assisted in strengthening collagen's triple helical shape. As a result, the greater the imino concentration, ever more consistent the collagen's helical structure, resulting in greater Td (Senaratne et al., 2006).

Effect of pH on solubility of Blacktail snapper ASC and PSC:

The solubility of ASC and PSC from the skin of Blacktail snapper was investigated in relation to pH. Both ASC and PSC demonstrated high solubilization in quite acidic pH ranges from 2 to 5. Both collagens' dissolution rate has been drastically decreased at pH levels above 5. When the pH was allowed to raise 6 and 7, the diffusion coefficient of ASC and PSC were at their lowest. At the neutral and basicity pH ranges, low solubility was observed (Matmaroh et al., 2011).

The relationship between collagen solubilization and pH was discovered to be reciprocal, i.e., collagen solubility rise when pH decreases. The ASC had higher solubility than the PSC during the solubilization evaluation at different pH ranges (except 1 and 2). This difference was attributed to the presence of a larger molecular weight protein in ASC collagen than the protein in PSC collagen. Subsequently, the solubilization of collagen with pH variance in bigeye snapper skin and bone (Kittiphatanabawon et al., 2005) has already been observed over pH ranges of 1-10. Thus, the above result suggests that the ASC fraction has more predominance bonds or a higher degree of molecular crosslinking than the PSC fraction (Montero et al., 1999).

The fact that collagen has isoelectric points ranging from pH 6 to 9 backed up the study's findings (Huang et al., 2011). When the pH was lower or higher than the isoelectric point, the net positively and negatively charged residues of proteins increased, resulting in increased solubility due to electrostatic repulsion among the chains

(Zeng et al., 2012). Similar results were obtained using collagen from bigeye snapper (Nalinanon et al., 2007), sea chub (Bae et al., 2008), and balloon fish (Huang et al., 2011).

Effect of NaCl on Blacktail Snapper fish skin ASC and PSC:

In the 0.5M acetic acid dissolution studies, Blacktail Snapper fish ASC was more soluble at 1% concentration and less dissolved at 5% composition, whereas PSC remained insoluble up to 2% concentration. PSC dissolution rate decreased dramatically at 3% NaCl or higher. PSC collagen dissolution rate reduced up to 4% but rather increased slightly at 6% concentration. In the circumstance of ASC, the dissolution rate was gradually reduced up to 3% NaCl, with a sudden minimum at 4% NaCl. According to the literature, the dissolution rate of collagen from the skin of trout, bigeye snapper (Priacanthus tayenus and Priacanthus marcracanthus), and hake in acetic acid solution decreases with NaCl concentration. Montero et al., 1999; Kittiphatanabawonetal., 2005; Jongjareonraketal., 2005). The salting phenomenon, which existed at remarkably low NaCl concentrations, could explain the decreases in collagen solubility (Asgharand Henrickson, 1982). Increased ionic strength reduces protein solubility because of increased hydrophobic interactions between polypeptide chains and ionic salts competing for water, resulting in protein precipitation (Damodaran, 1996; vojdani, 1996). However, at NaCl concentrations greater than 2%, PSC was more soluble than ASC. PSC's increased solubility could be attributed to pepsin's partial hydrolysis of high molecular weight cross-linked molecules. Furthermore, the disparity in compositions and molecular species between the ASC and PSC fractions may result in such inequities. Reich et al. (1962) also stated that the component contained both collagenous and non-collagenous matter. Furthermore, PSC with distinct amino acid combinations may be less amenable to the 'salting out' impacts than ASC.

FT-IR spectral analysis of Blacktail snapper fish skin collagen:

Amide A band at 3410cm-1 in ASC and 3404 cm-1 in PSC of Blacktail snapper was much related to aliphatic secondary amine, N-H stretching vibrations. This is in accordance with research findings by Abe and Krimm, 1972. Amide B band in ASC and PSC (2854 & 2854 cm-1) of Blacktail snapper is associated with stretching of methylene CH asymmetrical / symmetric stretch. Amide I in ASC (1620 cm-1) and PSC (1616 cm-1) is associated with C=O stretch/hydrogen bond coupled with CN stretch. The obtained peaks were in agreement with other research findings that stated that peaks ranging from 1600 to 1700 cm-1, were primarily associated with carbonyl stretching mode all along polypeptide backbone (Singh et al., 2011). Amide II (1460 cm-1) ASC and PSC (1413 cm-1) associated with methylene C-H bend. Amide III (1087 cm-1) in ASC and (1055 cm-1) in PSC is associated with C-O stretch. The skin collagen of young nileperch showed the amide bands of A, B, I, II and III at the wavelengths of 3434, 2924, 1650, 1542 and 1235 cm-1, respectively and that of the adult nileperch skin collagen were at 3458, 2926, 1654, 1555 and 1238 cm-1, respectively (Muyonga et al., 2004). Correspondingly the collagen from S. inermis also recorded the bands meant for amide regions of A, B, I, II, III at 3442, 2923, 1646, 1542 and 1249 cm-1 in ASC and 3440, 2923, 1648, 1542 and 1246 cm-1 in PSC respectively. The amide A band is associated with the N-H stretching frequency. According to Doyle et al. (1975), a free N-H stretching vibration occurs in the range of 3400 to 3440 cm-1, and when the NH group of a peptide is involved in a hydrogen bond, the position was shifted to lower frequencies. When the NH group of a peptide is involved in a hydrogen bond, the position is shifted to lower frequencies. Amide B band of both collagens was observed at 2921 to 2925 cm-1, in agreement with that reported by Nagai et al., (2001). The amide A band of skin collagen of Sebastesmentella was at 3425 cm-1, while those of scale and bone were at 3296 and 3300 cm-1, respectively,

which indicate that more NH groups of scale and bone were involved in hydrogen bonding than in skin (Wang et al., 2008). Comparing to this, in the present investigation also the amide A band of ASC and PSC were located at 3442 and 3440 cm-1, respectively. It also indicates the involvement of more NH groups in hydrogen.

Microscopic analysis of collagen:

SEM revealed that the sponge skeleton had a collagenous fibrous network with interconnecting channels. Figure 10 shows scanning electron microscopy images of ASC and PSC from blacktail snapper. Collagen samples' fibrillar structures were observed. The collagen fibres were discovered to be arranged singly or in small groups. These collagen fibrils intertwined and formed bundles that varied in width and thickness. The hydrogen bond, hydrophobic interaction, electrostatic bond, entropic and dispersion forces can all be used to mediate cross-linked fibre networks. The collagen's regular porous structure was clearly visible, and the collagen surface was observed to be rough and uneven. Structures with pores will promote the cellular adhesion, aggregation, and growth. The SEM images of sponge collagen in this study revealed that all composites had an open and interrelated pore structure, and the spicules had a rod-like structure. Collagen's chemotactic properties have numerous advantages in tissue engineering scaffolds (Postlethwaite et al., 1978). Because of their connective tissue, sponge collagenous poriferans provide a natural habitat for cellular attachment and aggregation; it should be more complex organisms and is analogous to collagen type XIII (Green et al., 2003).

Conclusion:

The skin of the blacktail snapper L.fulvus was utilized to extract Acid Soluble Collagen (ASC) and Pepsin Soluble Collagen (PSC). The thermal denaturation threshold in fish collagen was moderate (28 C). The presence of functional groups, as determined by FT-IR analysis, also indicated that the isolated collagen is type I. Furthermore, the collagen-derived silver nanoparticles demonstrated significant anticancer activity against breast cancer cell lines (HeLa and MCF). Initially, isolated collagen was suitable for pharmaceutical agents and plays an important role in research as it possess pharmacognostic properties. As a result, the current study illuminates the path for the development of natural therapeutic agents derived from marine fishes.

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