COLLAGEN EXTRACTION FROM BONE OF *Lutjanus sp.* AND TOXICITY ASSAY

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Abstrak. Indonesia adalah negara maritim dengan potensi sumber daya perikanan. Namun, pemanfaatan hanya berkisar pada daging, sementara bagian lain tidak digunakan secara optimal, terkhusus tulang ikan yang berpotensial menghasilkan kolagen, sehingga hal ini perlu untuk dikembangkan. Tujuan dari penelitian ini adalah mengekstraksi kolagen dari *Lutjanus sp.* dan menentukan apakah terdapat aktivitas antikanker. Kolagen diektraksi dengan menggunakan metode hydroextraction dan identifikasi dengan FTIR. Penaringan awal kegiatan antikanker dilakukan dengan menggunakan metode Brine Shrimp Lethality Test (BSLT) untuk uji toksisitas. Hasil penelitian menunjukkan bahwa kolagen yang dihasilkan berkisar 4,535% dengan konsentrasi protein berkisar 8.815 mg/mL. Kolagen yang diidentifikasi memiliki spectrum Amida A, B, I, II, and III pada serapan 3421,72; 2926,01; 1651,07; 1541,12; 1240,23 cm^{-1}. Tes Toksisitas ditunjukkan dalam nilai LC_{50} sebesar 8,760 µg/mL. Kolagen dari tulang *Lutjanus sp.* dapat digunakan sebagai agen antikanker alami.

Kata Kunci : Kolagen, *Lutjanus sp.*, Tulang, Antikanker, Hidroextraction, BSLT.

Abstract. Indonesia is a maritime country with potential fisheries resources. However, utilization only revolves around the meat, while other parts have not been used optimally, especially fish bones which have the potential to produce collagen, so it needs to be developed. The aims of this study ware extracted collagen from bone of *Lutjanus sp.* and determine its Anticancer activity. The collagen was extracted by using hydroextraction method and identification by FTIR. The initial screening anticancer activity was done by using Brine Shrimp Lethality Test (BSLT) method for toxicity assay. The results showed that the yield of collagen was 4.535% with protein concentration was 8,815 mg/mL. Identified collagen from spectrum of amide A, B, I, II, and III at 3421.72; 2926.01; 1651.07; 1541.12; 1240.23 cm^{-1}. The toxicity test was shown in LC_{50} values of 8.760 µg/mL. The collagen from *Lutjanus* sp. bone can be used as natural anticancer agent.

Keywords : Collagen, *Lutjanus sp.*, Bone, Anticancer, Hidroextraction, BSLT.

INTRODUCTION

Collagen is a fibrous protein with three polypeptide chains that form a triple helix. collagen has characteristics, composed of repetitive tripeptides (Gly-Xaa-Yaa) which are stabilized by hydrogen and intermolecular bonds (Gelse & Aigner, 2003; Duarte et al, 2016). Xaa and Yaa can be occupied by other amino acids, including proline (28%) hydroxyproline (38%) and generally 10.5% are arranged in the ProHypGly sequence (Shoulders & Raines, 2009). Collagen is found in vertebrate animals and accounts for 30% of the total protein contained in the body. Collagen is an organic structure found in connective tissue such as bones, tendons, blood vessels, skin, teeth and muscles (Silva & Penna, 2012).

Collagen extracted from marine animals is one of the alternative sources of collagen replacing collagen from land animals such as cattle, goats, chickens and pigs which are feared for issues of illness or prohibition in some religions. Marine animal collagen has several advantages including free of zoonoses such as Bovine Spongiform Encephalopathy (BSE), Transmissible Spongiform Encephalopathy (TSE) and Foot and Mouth Disease (FMD), easily absorbed, accepted by many religions, mild regulatory problems and quality control, low inflammatory response, and in accordance with the metabolic system (Silvipriya et al, 2015)

At present, collagen has various applications in various sectors, namely foods, cosmetics and the pharmaceutical. In biomedical application, collagen is used as shield, Sponges for burns, Nanoparticles for gene delivery, matrix for protein and drug delivery (Lee et al, 2001). This is because of the presence of the functional groups, amino and carboxylic acid, which helps in its modification that suits for various end uses (Muthukumar, 2018) . In addition, the study found that collagen had an inhibitory effect on the growth of human cancer cells (Han, 2011).

Indonesia has potential fisheries resources, one of which is red snapper. However, utilization only revolves around meat, while other parts have not been used optimally, especially fish bones that have the potential to produce collagen. However, little information about collagen from red snapper (*Lutjanus sp.*) Has been reported. The purpose of this study was to produce collagen with anticancer activity of red snapper bones (*Lutjanus sp.*).

MATERIAL AND METHODS

Instruments

The instrument used include analytical scales, UV-Vis Spectrophotometer Shimadzu UV-2600, Magnetic Stirrer, drop pipette, erlenmeyer, beaker, measuring flask, stirring bar, spray bottle.

Material

Bones of Red snapper (*Lutjanus sp.*), NaOH, CH_3COOH, KBr, and BSA.

Methods

1. Collagen Extraction

Collagen extracted from bone of *Lutjanus sp.* used by modification of Baehaki et al (2015) method. Pretreatment, the bones, which has been cut into small pieces and washed, were

treated with 0.1 M NaOH at a ratio of 1:10 (w/v) for 6 h, and the solution was changed every 2 h and finally washed with the aquadest. Hydrolysis with 1.5% CH_3COOH at a ratio of 1:2 (w/v) for 24 h, then washed with the aquadest. Collagen was Extracted by using aquadest at a ratio of 2:1 (w/v) for 3 h at 45 °C. Collagen solution was freeze dryed.

2. Infrared Spectroscopy

Fourrier Transform Infra Red spectra were obtained from collagen in approximately potassium bromide (KBr) on a Shimmadzu FT-IR spectrometer in the range of 300–4000 cm^{-1}.

3. Toxicity Assay

Toxicity assay by using Brine Shrimp Lethality Test (BSLT) and shown the LC_{50} value of collagen.

RESULT AND DISCUSSION

Extraction of Bone Collagen

The extraction collagen from *Lutjanus sp.* bone consists of three steps, these are pretreatment using NaOH, hydrolysis using acetic acid and extraction using aquadest. The pretreatment aims to eliminate non-collagenous proteins (Silva et al, 2014) and impurity. it can be seen from the colour which turns white in the material. The hydrolysis aims to break the cross-linked collagen which presents in the connective tissue of animals before extraction. In this cased, the material swells and cleavages the non-covalent inter and intra-molecular bonds (Schmidt, 2016).

The yields of collagen extracted from *Lutjanus sp* found at 4.535%. This value was observed higher than the yield of collagen from Tilapia (*Oreochromis mossambicus*) Bone (3.5%) (Liu & Huang, 2016) and bigeye snapper (*Priacanthus tayenus*) bone (1.59%) by ASC extraction method (Kittiphattanabawon, 2005), but lower than bone collagen of yellow sea bream (*Dentex tumifrons*) (40.1%) (Nagai & Suzuki, 2000) and Pangasius catfish (*Pangasius pangasius*) by hydroextraction method (12.86%) (Baehaki, 2016). Apparently the collagen of *Lutjanus sp.* bones is relatively small. This is thought to be a strong hydrogen bond in the collagen structure so that the break up during extraction is difficult. Besides that, some collagen is lost during neutralization. The difference in yields of collagen might be caused by the difference in extraction methods and the fish species. Furthermore, the protein content obtained is 8.815 mg/mL.

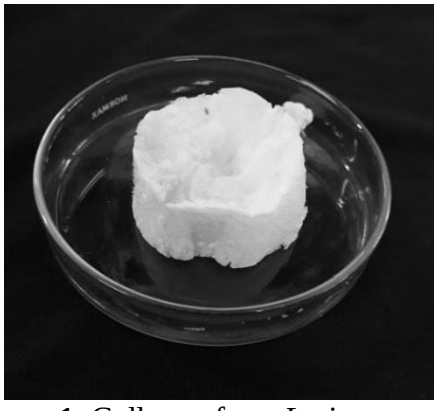

Figure 1. Collagen from *Lutjanus sp. Bone*

Infrared Spectroscopic Analysis

FTIR spectra of extracted collagen is shown in Fig.2 and table 1. The absorption bands of amides I, II, III, A, and B were observed. The amide A

band was observed in 3421.72 cm^{-1}. This band is associated with N-H stretching vibration in the range of 3400-3440 cm^{-1} (Veeruraj et al, 2013). Different from several studies (Liu & Huang, 2016; Tziveleka et al, 2017), where amide A is in the range of 3300 cm^{-1}. This shift is because the NH group binds to hydrogen bonds. The amide B band, related to the asymmetrical stretch of CH_2 was observed at 2926.01 cm^{-1}. The amide I band, resonating in the range of 1600-1700 cm^{-1}, was found in 1651.07 cm^{-1}. This band mainly associated with the C=O stretching vibration which the most intense band in proteins and sensitive marker of the secondary structure of the protein (Veeruraj et al, 2013; Tziveleka et al, 2017). The amide II band, resonating in the range of 1480-1575 cm^{-1}, associated with the N-H bending vibration coupled with the C-N stretching vibration, was found at 1541.12 cm^{-1}. The finally, the amide III band, resonating in the range of 1229-1301 cm^{-1} which attributed to the C-N stretching vibration in combination with the N-H deformation, was observed at 1240.23 cm^{-1}. The amide III band can identicate the triple helix in extracted collagen. The ratio between amide III and the band at approximately 1450 cm^{-1} which approaches one indicates the presence of a triple helix (0.85) (Tziveleka et al, 2017).

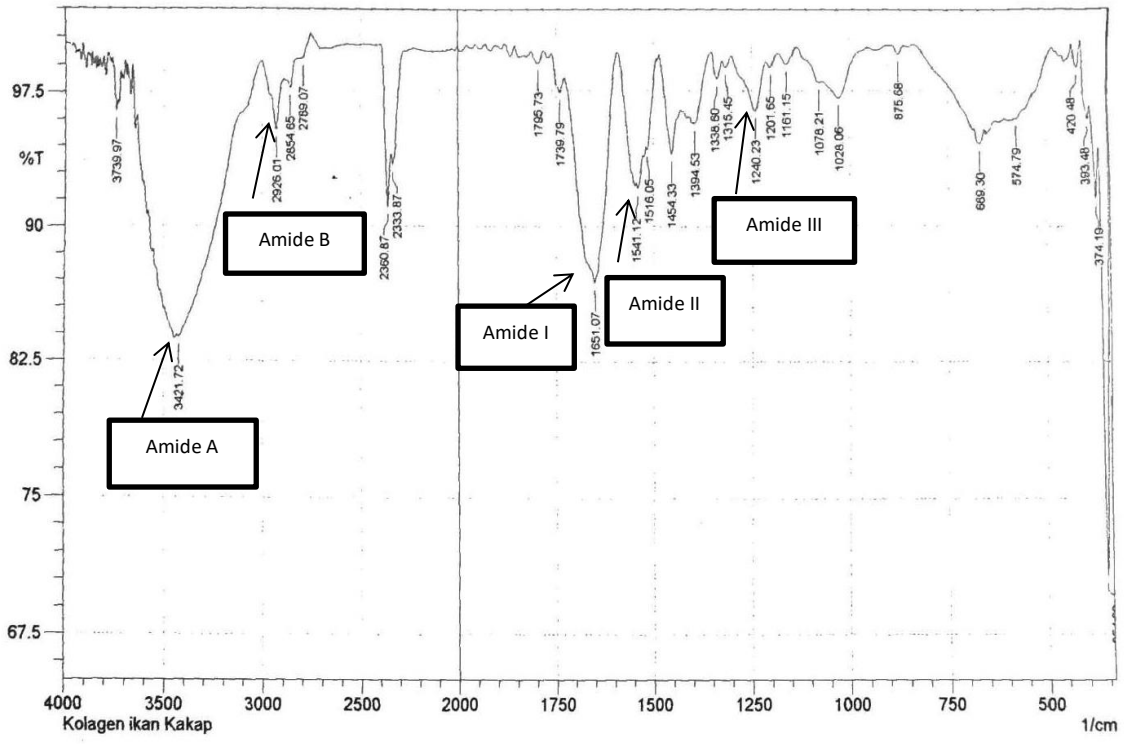

Figure 2. IR Spectra of collagen from *Lutjanus sp. Bone*

Table 1. IR Spectra Peak Position and assignments for collagen from *Lutjanus sp. Bone*

Region	**Peak Wavenumber(cm^{-1})**	**Absorption Region**	**Region Peak Assignment** (Veeruraj et al, 2013)
Amide A	3421.72	3400-3440	N-H str
Amide B	2926.01	2922-2924	asymmetrical of CH_2 str
Amide I	1651.07	1600-1700	C=O str
Amide II	1541.12	1480-1575	N-H bending/ C-N str
Amide III	1240.23	1229-1301	C-N stretching with combination with N-H def

Toxicity Assay

The BSLT method is a simple toxicity test on bioactive compounds based on the cytotoxic ability of the test compounds to kill zoological organisms, *Artemia salina* Leach. The results of the BSLT method are shown in the form of LC_{50} in µg/ml, which is the ability of the compound which causes 50% mortality of test animals.

The LC_{50} value for fish bone collagen was 8.760 µg/mL. Based on Clarkson's toxicity criterion for the toxicity assessment, extracts with LC50 of 0 - 100 µg/ml are highly toxic (Hamidi, 2014) Bone collagen has high toxicity to be used as a natural anticancer agent.

CONCLUSION

Collagen can be extracted from Lutjanus sp. bone by using hidroextraction method. The yield of collagen was 4.535% with protein concentration was 8,815 mg/mL. Identified collagen can be known from spectrum of amide A, B, I, II, and III. The toxicity test shown in LC_{50} values of 8.760 µg/mL.

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