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ANALYSIS OF Fe, Zn, AND Cr AT SOME FRACTIONATED PROTEINS IN SPONGE *AGELAS NAKAMURAI*

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Abstract

This research aims to analyze the concentration of metals Fe, Zn, and Cr in protein fractions of sponge *Agelas nakamurai* from Kapoposang Island, Spermonde archipelago. Protein samples were extracted, fractionated using various saturated ammonium sulphate (SAS) concentration, namely 0 -20%, 20-40%, 40-60% and 60-80%, then purified by dialysis using celophane membranes. Each fraction was digested using microwave before metal analysis using ICP OES (Inductively Coupled Plasma Optical Emission Spectroscopy). The results have shown that highest iron concentration was found in 20-40% fraction, 28.2 µg/L, while lowest one was in 60-80% fraction, 2.39 µg/L. For Zn, its highest concentration were found 20-40% fraction, 9.42 µg/L and the lowest one was 40-60%, 3.66 µg/L. On the other hand, the concentration of Cr was found highest at 20-40% fraction, 2.28 µg/L, while its lowest amount was at 60-80% fraction, 0.238 µg/L. In the meantime, its highest antibacterial activity was at 0-20% fraction examined in *Salmonella thypusa* with inhibition diameter was 7,00 mm, where the amount of essential metals Fe, Zn and Cr are 17,2 µg/L, 6,32 µg/L, 1,47 µg/L. It was concluded that the essential metals investigated were coexisted and distributed in several protein fractions and its bioactive properties.

Keywords : *Agelas nakamurai*, Bioactive protein, essential metals, ICP-OES, Kapoposang Island

INTRODUCTION

Spermonde archipelego has abundant source of marine biota, such as 199 varieties of sponge species (De Voogd, 2005). Sponges are filter feeders species that live in low mobility. Metals which exist in sea water will be accumulated by the biological sponge system. Essential metals are required with trace amounts for sponges metabolism (Netty, A., 2014).

Sponges have high adaptability in their habitats. To sustain life in extreme environments, sponges produce chemical compounds actively (Suparno, 2005). Some sponge species have been reported containing bioactive compounds that have been widely applied in pharmaceutical industries (Ahmad *et al*., 2006).

The presence of metals in sponges can be accumulated in protein and nonprotein part of sponge body (Netty, A., 2014). Metal is one

component that affect the bioactivity of chemical compounds produced by sponges. Generally, essential metal in bioactive proteins of sponges function as active interface on the compounds (Arifin, 2008).

The objective of this research is to know about the level of essential metals in bioactive protein sponge that live in Spermonde archipelago, Makassar.

MATERIALS AND METHODS

Materials used in this research are sea sponge *Agelas nakamurai*, buffer A (Tris-HCl 0.1 M pH 8.3; 2 M NaCl; CaCl2 0.01 M; β-mercaptoetanol 1% Triton X-100 0.5%), buffer B (Tris-HCl 0.1 M pH 8.3; 0.2 M NaCl; CaCl 2 0.01 M), buffer C (0.01 M Tris-HCl pH 8.3; 0.2 M NaCl, 0.01 M CaCl 2), ammonium sulphate (Merck), 65% HNO_3, bidistilled water, tissue roll, microwave disgution and *Shimadzu* ICPE-9000.

Sampling Location

Figure 1. Sampling Location

Samples of sponge were taken in the Kapoposang Island at a depth of 9 meters and coordinate 118° 54'00" E - 119° 10' 00" longitude and 04° 37' 00" LS - 04° 52' 00" LS.

Extraction and isolation of sponge bioactive protein

Extraction and isolation of protein bioactive sponge were conducted using methods (Schroder *et al*., 2003; Reha, *et al,* 2013) as follows sponge ***Agelas nakamurai*** cut into small pieces, and weighed into 500 g of fresh sample, homogenized using buffer solution A (Tris-HCl 0,1 M pH 8,3, NaCl 2 M, $CaCl_2$ 0,01 M, β - merkaptoetanol 1 %, Triton X- 100 0,5 %), filtered with buchner, and the filtrate obtained to be freezed and liquefied between 2 or 3 times, and then centrifuged at 12.000 rpm and 4°C for about 30 minutes, and finally the supernatant obtained was stored in a refrigerator before further purification stage.

Fractionation and Dialysis of Protein

The supernatant (whole extracts) containing protein and having anti-bacterial activities was then fractionated using ammonium sulfate at saturated levels of 0 – 20 %, 20 – 40 %, 40 – 60 % and 60 – 80 %, respectively. The precipitates obtained after fractionation at each saturation level of ammonium sulphate was added 5mL of buffer B (Tris-HCl 0,1 M pH 8,3, NaCl 0,2 M, $CaCl_2$ 0,01 M), and then dialyzed in buffer solution C (Tris-HCl 0,01 M pH 8,3, NaCl 0,2 M, $CaCl_2$ 0,01 M) using selophan pocket (sigma) until obtaining colorless buffer. After dialysis, each protein fraction was then undergoing anti-bacterial testing (Reha, *et al,* 2013) and ready to destructed using microwave disgution.

Destruction Samples and Metal Measurement With ICP-OES

Each dialysis results weighed 0,5 g into the digestion vessels. Then, added 10 mL HNO_3 suprapure 65% (w/w) in each tube. All tubes are ready for destruction by microwave disgution. Radiated for 15 minutes at 100% power (as directed) and at the end of the

program, allowed the vessel tubes to cool at room temperature before it is opened. Aquabidest water added in every destructed fraction and ready to metals analysis by ICP-OES.

RESULTS AND DISCUSSION

General Condition of Sampling Location

The results of analysis of several parameters of sea water quality in Kapoposang Island showed in Table 1.

Table 1. Sea Water Quality in Kapoposang Island, Makassar

Parameter	Result of Measurement
pH	6,8
Salinity	28 – 29 ‰
Temperature	30-31 °C

Source: Results of measurement, September 2013

Table 1 shows the conditions of sea water in Kapoposang Island where in accordance with optimum growth conditions of marine sponge, where the optimum temperature ranges from 26 - 31°C, pH from 6.5 to 8.5 and salinity 29 - 32°/oo (De Voogd, 2005) respectively.

Level of Metal Analysis in Protein Fraction

Preparation of sample that was used in extraction procedure to make distribution level of metals in every fraction. Amonium sulphate as saturation agent give different precipitation that give affect to distribution level of metals.The factors that can make different precipitation of protein are the number and

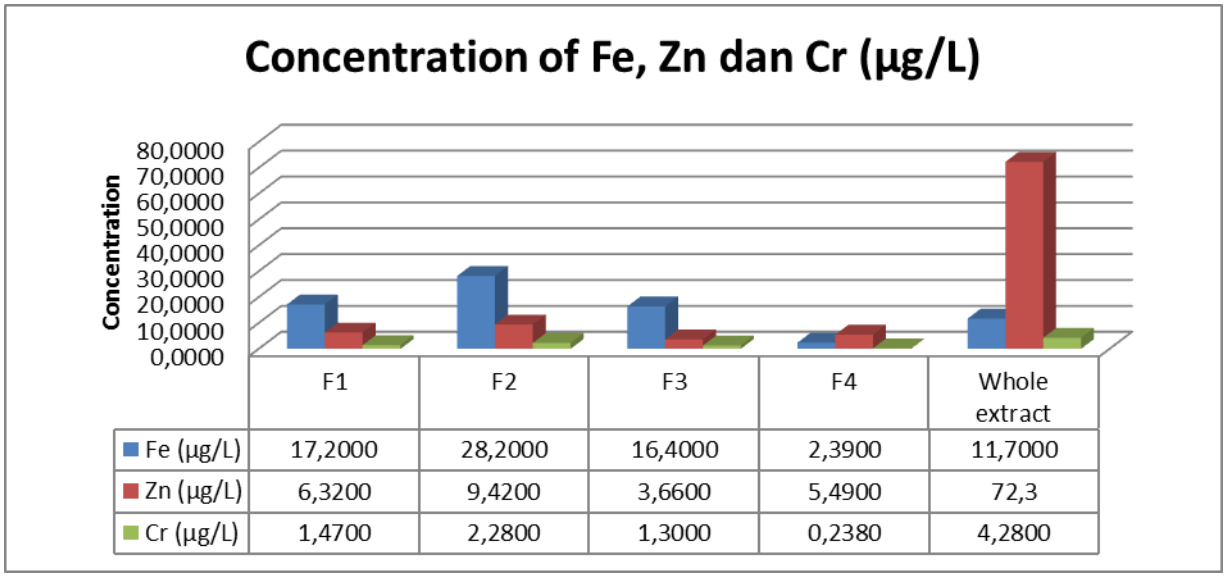

Figure 2. Distribution of essential metals Fe, Zn, Cr in protein fraction of sponge *Agelas nakamurai*

position of polar groups, molecular weight, pH and temperature of the solution (Ismet, 2007).

Figure 2 shows the concentration of essential metals Fe, Zn and Cr in each protein fraction. Measurement of metals using ICP OES instrument because it has advantages such as high accuracy, sensitivity and low detection limit. Another advantage of this instrumentl is can analyze multiple elements at once (Noor, 2014).

The existence of essential metals in each fraction proteins is influenced by the strength of bond that is formed between metals and amino acids. From separation and purification processes of protein fractions, shows that the detected metal have strong binding of these proteins (metaloprotein). Metal bonding in proteins can formed by metal chelates with amino acids or metallic bonding with the alkyls of amino acids (Netty, A., 2014 Poedjiadi, 1994).

Functions of iron in the body are as constituent of living things oxidereductase enzymes and carrier agent in the body. Which is an iron storage protein, among others ferritin, ferredoksin and rubredoksin. The sponge body itself need iron metals in the formation fiber tissues, commonly known as lepidokrosit (Verdenal, 1990).

Zinc is required for the activity of more than 90 enzymes that has function of the carbohydrate and energy metabolism, degradation/protein synthesis, nucleic acid synthesis, heme biosynthesis, the transfer of CO_2 (carbonic anhydrase).

Chromium (Cr) is an essential trace element that very important in the metabolism of glucose, protein and fat in the tissues. Most of the Cr is present in nature in the form of Cr^{3+}.

Analysis of Metals and Bioactivity in Protein Fraction

The diameter of each fraction inhibitory against *Salmonella typhosa* can be seen in Table 2. The highest inhibition diameter in fraction 1 with metal contents of Fe is 17,2 µg/L, Zn 6,32 µg/L and Cr 1,47µg/ L.

In bioactive compounds, metal compounds act on the active side. The reaction form be a metal complexes or an ion exchanges. The factors that can affect the bioactivity of protein fractions are the metal contents in each fraction and the complexity of the protein structure.

Protei nt Fracti	Diameter of Inhibition	Fe (µg/ L)	Zn (µg/ L)	Cr (µg/ L)
F1	7,0 mm	17,2	6,32	1,47
F2	6,8 mm	28,2	9,42	2,28
F3	6,2 mm	16,4	3,66	1,3
F4	5,6 mm	2,39	5,49	0,238

Table 2. Levels Of Essential Metals And The Diameter Of Inhibition Against The Bacteria *Salmonella Thypusa*

CONCLUSION

Protein fractions was isolated from the sponge *Agelas nakamurai* contains the essential metal Fe, Zn and Cr in fairly low levels. Levels of Fe for F1 was 17.2000 µg/L ; F2 28.2000 µg/L ; F3 16.4000 µg/L and F4 2.3900 ug/L. Levels of Zn for F1 was 6.3200 µg/L; F2 9.4200 µg/L; F3 3.6600 µg/L and F4 5.4900 µg/L. Level of Cr for F1 was 1.4700 µg/L; F2 2.2800 µg/L; F3 1.3000 µg/L and for F4 0.2380 ug/L.

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