

Isolation And Characterization Of Some Kind Bioactive Proteins Sponge As Antibacterial Agent

Asri Salehi, Rauf Patong, Ahyar Ahmad

Abstract: Has conducted research on the bioactivity of antibacterial protein fractions isolated using a polar solvent of some sponge species on the island of Barang Lompo, South Sulawesi. The protein concentration was determined by Lowry method showed concentrations of the four species of sponge proteins in the crude extract in a row is 4.008 mg / ml, 5.376 mg / ml, 3.292 mg / ml and 1.404 mg / ml of each 500 grams of fresh weight sponge BLS 01, BLU 02, BLS 03 and BLU 04. Purification of proteins with ammonium sulfate fractionation method followed by the process of dialysis gives the result that all the ammonium sulfate fraction containing bioactive proteins where the highest inhibition zone found in BLS03 species (20-40%), reaching 9.85 mm and (60-80%) reaching 10.2 mm against *E. coli* and *Staphylococcus aureus*. Testing inhibition in several variations number of bioactive proteins showed that the maximum inhibitory zone activity present in a concentration of 4000 mg / mL protein of BLS03 (20-40%) and (60-80%). Pengujian daya hambat pada beberapa variasi jumlah protein bioaktif menunjukkan bahwa aktivitas zona hambat maksimum terdapat pada konsentrasi 4000 µg/mL protein dari BLS03 (20 – 40 %) dan (60 – 80 %). From the results of this study indicate that the bioactive proteins from sponges potential as a base for new antibacterial drugs, especially against *E. coli*.

Indeks Terms: Bioactivity, sponge, bioactive proteins, antibacterial, inhibition zone

1. INTRODUCTION

Indonesia is known as a maritime nation with a wide 75% is in the oceans, has a wealth of biological resources are abundant, among others found various types of sponges. Some types of them, reportedly have bioactive compounds that can be used in the pharmaceutical field [1]. Along with the change in the pattern of diseases such as the presence of drug resistance in certain bacterial diseases, then attempt the discovery of new drugs continue to be made. The current study tended to be developed into the ocean because most of the natural resources have not been exploited to its full potential [2, 3]. Marine biological resources consist of plants (eg algae) and animals (eg fish, molluscs, soft corals, sponges, echinoderms, and tunicates askidin). Some of certain animal species is a source of vitamins, proteins and minerals. Moreover, there are also several types of animals that synthesize and store the toxin compounds are commonly called marintoksin on the body or released into the environment [4]. These compounds are secondary metabolites that are used in self-defense system, to preserve life and avoid interference from other organisms in the environment, and farmakologiknya activity. Therefore, the compound has the prospect to be isolated and used in medicine [5]. An understanding of the magnitude of potential marine often limited to makroflora and exploitation of marine fauna such as fish, shrimp, shellfish, and seaweed are categorized as tangible resources or resources that can be harvested for subsequent be commercialized directly.

Other marine resources such intangible: microflora and fauna with the content of primary and secondary metabolites are relatively undisturbed [6] Natural medicines are the result of secondary metabolites from organisms that have a distinctive chemical compounds. Secondary metabolites is an element that is used as an antidote to disease and survival of organisms. Secondary metabolites were collected, processed, and used as a new drug formula. Some of the secondary metabolites of bio-organisms has become famous drugs, such as aspirin, morphine, digitalis, penicillin, and taxol [7]. Sponges have the ability to capture bacteria in the surrounding up to 77% through the use of food that enzymatic digested. Bioactive owned by beneficial sponge in the digestive process, so that the obtained bioactive course will vary according to the eating habits of each type of sponge [3]. Based on the results of the study reported that some sponge species contain compounds that are antimicrobial secondary metabolites (extracted with chloroform). Sponges were reported to have bioactive sesterpen derived from *Hyatella intestinalis* [8], methyl steroids obtained from *Agelas flabelliformis* [9], sesterpen, terpenoids and variabilin obtained from *Hipospongia comunis*, *Spongia officinalis*, *Ircinia variabilis*, and *Spongia gracilis*, ketosteroid obtained from *Erylus lendenfeldi* and *Dyctionella insica* [10], short peptides and cyclo peptides obtained from *Theonella* sp. and *Microscleroderma* sp. [11, 12]. All of this bioactive can be utilized in the field of pharmacy and treatment of diseases in humans and animals. However, so far no research data to explore group protein compound from sponge as raw material for medicine in human and animal diseases. Proteins as antibacterial drugs has several advantages that are very promising because it can be received with a good body, and causes fewer side effects. Therefore, the study of medication was developed using proteins [13], and also gene of protein compound can be cloned, therefore can be produced on a large scale on an industrial scale through genetic engineering techniques. This study was conducted to explore and characterize several bioactive protein fractions from several types of sponges in South Sulawesi. the bioactive proteins tested as antibacterial activity. The results of this study are expected emergence of knowledge and a better understanding of the

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protein components of bioactive sponge, where it has an optimal inhibition and effective against the growth of bacteria that can be used as a base for new antimicrobial drugs.

2. MATERIALS AND METHOD

Material

The materials used are four species of sponge, pure cultures of bacteria *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella pollorum*, *Pasteurella Mulcotida*, distilled water, MHA Medium (Muller Hinton Agar), buffer A (Tris-HCl 0.1 M pH 8.3, NaCl 2 M, 0.01 M CaCl₂, β-merkaptotanol 1%, Triton X-100 0.5%), buffer B (Tris-HCl 0.1 M pH 8.3, 0.2 M NaCl, 0.01 M CaCl₂), buffer C (0.01 M Tris-HCl pH 8.3, 0.2 M NaCl, 0.01 M CaCl₂), BSA (Bovine Serum Albumin) 4 mg / mL, kloramfenicol 30 ppm, cotton, and aluminum foil.

Research Methods

Extraction and isolation of bioactive proteins sponge

Extraction and isolation of bioactive sponge proteins using the procedure from Schroder [14] and Ely [15]. Four species of sponges cut into small pieces, and then weighed as much as 500 g, crushed in a blender using a solvent buffer A (Tris-HCl 0.1 M pH 8.3, 2 M NaCl, 0.01 M CaCl₂, 1% β-merkaptotanol, Triton X-100 0.5%), filtered with a Buchner funnel and then the filtrate was obtained freeze-thawed 2-3 times and then centrifuged at 12,000 rpm, 4 ° C, for 30 minutes. Furthermore, the supernatant was stored in the refrigerator prior to testing antibacterial and subsequent purification process.

Antibacterial Activity Tests

Testing of bioactive protein inhibition on the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella pollorum*, *Pasteurella mulcotida* done by diffusion method [15]. Iron cylinder placed over the seed layer on MHA medium and then samples about 250 ml was added to metal cylinder. Furthermore incubated for 24 h at 37 ° C and then measured of inhibition zone with calipers. Tests carried out in duplo.

Fraksinasi dan dialisis protein

Crude extract containing proteins and have antibacterial activity fractionated using ammonium sulfate at saturation level: 0-20%, 20-40%, 40-60% and 60-80%. The precipitate obtained from the fractionation, dissolved in a buffer B, then in buffer C using a cellophane bag until the buffer solution does not change color. Each protein fraction was analyzed after then tested the antibacterial activity like at a crude extract.

3. RESULTS AND DISCUSSION

Sponge samples were taken from 2 locations, 2 species in BrangLompo Southern (BLS01, BLS03), and 2 species in the BrangLompo northeastern (BLU02) and BLU04. Sponge samples taken from their habitat at a depth of about 5-15 m above sea level with a temperature of 29 °C. Furthermore sponge identification and results identification can be seen in Table 1. Identify the type of sponge is

important to tracing back the source of bioactive compounds produced by marine biota.

Table 1. The results of the identification of sponge species [16]

No.	Species code	Species name
1	BLS 01	<i>Callyspongia sp</i>
2	BLU 02	<i>Agelas clothrides</i>
3	BLS 03	<i>Clathria Reinwardti</i>
4	BLU 04	<i>Aglaophenia cupressina</i>

Main reason for choosing the four sponge species because of the results of research conducted by Razak and Ridhay [17] using 29 species of sponges include the four sponge species (extraction using chloroform) and inhibitory test results showed no antimicrobial activity. From the results of these studies, is suspected other compounds that have antimicrobial activity at four sponge species.

The protein content of the sponge

Protein content of sponge determined by the Lowry method [18] using bovine serum Albumine (BSA) as standard. The results showed that the concentration of protein of sponge crude extract varies with the highest concentration (5.376 mg / mL) was found in BLS02 species with a total of 2956.8 mg protein (Table 2).

Table 2. The protein concentration of the crude extract of the sponge

No	Sponge species	Crude extract volume (ml)	Protein Content (mg/ml)	Total protein (mg)
1	BLS 01	550	4.008	2204.4
2	BLU 02	550	5.376	2956.8
3	BLS 03	600	3.192	1915.2
4	BLU 04	600	1.404	842.4

Test the antibacterial activity of the crude extract protein

Testing the inhibition of protein crude extract of the sponge to the growth of the bacterium *Escherichia coli*, *Staphylococcus aureus*, *Salmonella pollorum*, and *Pasteurella mulcotida* performed by diffusion method. Protein crude extract from fourth the sponge showed a very strong antibacterial activity, as seen in the amount of bacterial growth inhibition zone test as shown in Table 3. Table 3 shows that the zone of inhibition of crude extract protein from the sponge in BLS03 against *Staphylococcus aureus* higher than BLS01, BLU02 and BLU04, where the zone of inhibition of 12.65 mm, and does not exceed chloramphenicol inhibition zone. This happens because of suspected bacterial pathogens in test medium already are resistant to chloramphenicol (positive control) or the concentration needs to be improved.

Table 3. The test results of antibacterial activity of crude extract protein

Sponge Species	Bacteria Species			
	SA (mm)	SP (mm)	EC (mm)	PM (mm)
BLS 01	-	-	10.45	11.20
BLU 02	9.10	-	-	-
BLS 03	12.65	-	-	11.10
BLU 04	-	-	-	-
Control (+)	31.45	26.20	26.10	20.25
Control (-)	-	-	-	-

Note : SA = *Staphylococcus aureus*
 SP = *Salmonella pollorum*
 EC = *Escherichia coli*
 PM = *Pasteurella multocida*

Protein crude extract sponge from BLS03 has inhibition zone a very strong. This is presumably because these species live in the harsh environment, which is in shallow water, located in tidal areas with a depth of 0-5 m above sea level. In the biological environment is open and big choppy, can cause physical growth of the sponge becomes shorter. The condition causes resistance to the environment very well, maybe this which stimulates sponges produce secondary metabolites that much, so it can inhibit the growth of pathogenic bacteria very well especially against *Staphylococcus aureus*. The concentration of very high protein crude extract not always showed antibacterial activity is strong. The data in Table 2 shows that BLS03 sponge species have a protein concentration are 3.192 mg / mL, but showed very strong antibacterial activity. This is presumably because of all the proteins contained in crude extract of sponge at BLS03 serves as an antibacterial protein. however, sponge species BLS01 many accumulated antimicrobial proteins in high amounts, so that the antibacterial activity showed strong inhibitory power than others. This may occur because in species BLS01 not only the fraction of proteins that possess antibacterial activity but there are nonprotein polar compounds also inhibit the growth of bacteria.

Protein fraction containing the antibacterial activity

Crude extracts protein that have antibacterial activity of fractionated using ammonium sulfate with saturation at level: 0-20%, 20-40%, 40-60% and 60-80%. The addition of ammonium sulfate salts from low concentration to high concentration causes difference of protein type that precipitated at each fraction level. The addition of ammonium sulfate with high concentrations caused many hydrophobic groups neutralized by ammonium salts, so that water can not binding again. As a result, protein solubility in water decreases and causes the protein is precipitate. The distribution pattern of proteins of sponge in BLS03 can be seen in Table 4. Bioactive proteins (fraction 20-40%) were isolated from sponge in BLS03 and bioactive proteins showed strong activity (inhibition zone is 9.85 mm). To determine the effect of protein concentration on the fraction on antibacterial activity, performed inhibition assay at various protein concentrations of 4000, 400, 200, 100 and 40 mg / mL. The results showed that the zone of inhibition of several concentrations of bioactive proteins on *E. coli* showed maximum activity at a protein concentration of

4000 mg / mL. The decrease in antibacterial activity when compared with other concentrations may be caused by the storage, so that of bioactive protein to become unstable. Furthermore, at a lower protein concentration (40-400 mg / mL) showed a relatively low antibacterial activity.

Table 4. The distribution pattern of proteins of sponge in BLS03

Sponge species	Purification step	The volume of each fraction (ml)	The protein concentration (mg/ml)	Total protein (mg)
BLS 03	0-20%	600	7.5	4500.00
	0-20%	610	4.8	2928.00
	0-20%	582	5.3	3072.96
	0-20%	576	5.4	3110.40

4. CONCLUSION

Based on the research and discussion that has been done, we can conclude some of the following:

1. Three species of sponges were isolated, containing bioactive proteins capable of inhibiting the growth of pathogenic bacteria *Pasteurella multocida*, *Staphylococcus aureus*, and *Escherichia coli*.
2. Bioactive proteins in ammonium sulfate saturation of 20-40% and 60-80% from sponge species BLS03, had inhibition zone are 9.85 mm and 10.2 mm against *Escherichia coli* and *Staphylococcus aureus*.
3. Bioactive proteins that have the highest antibacterial activity against *E. coli* showed maximum activity at a protein concentration of 4000 g / mL.

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