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Bioactivity of Protein Fraction in Brown Algae, Turbinaria decurrens, as Antibacterial Agent

Bioaktivitas Fraksi Protein dalam Alga Coklat, Turbinaria Decurrens*, sebagai agen anti bakteri*

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ABSTRAK

Penelitian ini bermaksud mengeksplorasi dan mengkarakterisasi fraksi protein dari alga cokelat *Turbinaria decurrens* di Sulawesi Selatan. Senyawa protein bioaktif diperoleh melalui serangkaian proses ekstraksi, fraksionasi dengan kejenuhan amonium sulfat 0-20%, 20-40%, 40-60%, dan 60-80% yang dilanjutkan dengan proses dialisis lalu ditentukan kadar proteinnya dengan metode Lowry. Fraksi protein yang diperoleh diuji bioaktivitasnya terhadap bakteri *Staphylococcus aureus* dan *Salmonella thypi* dengan metode difusi agar pada medium MHA. Hasil penelitian menunjukkan bahwa kadar protein tertinggi ditemukan pada fraksi 0-20% yaitu sebesar 5,1 mg/mL. Hasil uji bioaktivitas antibakteri terbesar ditemukan pada fraksi protein 0-20% terhadap *Staphylococcus aureus* dan *Salmonella thypi* dengan zona daerah hambatan masing-masing sebesar 18,33 mm dan 13,30 mm yang bersifat bakteriostatik. Hasil penelitian awal ini akan dikembangkan pada cakupan penelitian yang lebih luas untuk mengkloning protein bioaktifnya dan uji pre-klinis dari protein antibakteri yang diperoleh.

Kata Kunci: Antibakteri, Metode Lowry, Fraksi protein, Alga cokelat.

INTRODUCTION

Indonesia, known as maritime country has an abundant source of marine biota, among others are a lot of variety of algae species. Some species were reportedly containing bioactive compounds that have been widely applied in pharmaceutical industries (Ahmad *et al*., 2006).

In parallel with the trend of disease pattern changes such as the resistant germs towards a certain medicine, the effort to seek new medicines is deemed important to carry out. Hitherto, marine natural resources have not been optimally utilized so that identifying potential bioactive compounds from marine natural resources will be of a great interest (Caraan, 1994 and Nybakken, 1993).

Marine plants like algae, due to its environment, are capable producing secondary metabolites as the part of self defence system from other organism as well as maintaining their sustainability. Such compounds have a promising prospect to be extracted, isolated and utilized as new potential medicines (Sardjoko, 1996). Some secondary metabolite compounds derived from bioorganisms have been used as popular medicines such as asphirine, morphine, digitalys, peniciline, and taxon (Anonim, 2003). Nybaken (1993) reported that sponges have ability to screen the bacteria in its environment up to 77 % through the utility of digested food enzymatically. Bioactive compounds in algae, on ther other hand, have advantage in digesting process such that the bioactive compounds yielded from such process will be varied according to the consumption habit of each algae. This study was intended to explore and characterize the bioactive compounds of this type of brown algae *Turbinaria decurrens* in South Sulawesi. It was found that the bioactive protein from this species at 0-20% saturation of ammonium sulfate has the strongest activity of inhibiting the growth of *Staphylococcus aureus*, with inhibition zone of 14,43 mm.

MATERIAL AND METHOD

Materials used are brown algae *Turbinaria decurrens*, pure bacterial fertile of *Staphylococcus aureus*, *Salmonella typhi*, MHA (Muller Hinton Agar) medium, DMEM medium, buffer 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 %), buffer B (Tris-HCl 0,1 M pH 8,3, NaCl 0,2 M, $CaCl_2$ 0,01 M), buffer C (Tris-HCl 0,01 M pH 8,3, NaCl 0,2 M, $CaCl_2$ 0,01 M), Bovine Serum Albumin (BSA) 4 mg/mL, chloramphenicol, and aluminium foil.

Extraction and isolation of bioactive protein from algae

Extraction and isolation of bioactive algae were conducted according to previous methods (Schroder *et al*., 2003; Ely *et al*., 2004 modified) where the collected brown algae were cut into small pieces, weighed 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. The filtrate obtained was then freezed and liquefied, 2 - 3 times, and then centrifuzed at 12.000 rpm and 4^{o}C for about 30 minutes. Finally the supernathan obtained was stored in a refrigerator ready for anti bacterial testing and further purification.

Fractionation and Dialysis of Protein

The supernathan (whole extracts) containing protein and having anti bacterial activities was then fractionated using ammonium sulfate at saturated levels of 0 – 30 %, 30 – 40 %, 40 – 60 % and 60 – 80 %, respectively. The precipitates obtained after fractionation at each saturation level of ammonium sulfate was then suspended in a certain amount of buffer B (Tris-HCl 0,1 M pH 8,3, NaCl 0,2 M, $CaCl_2$ 0,01 M), and then dialysed in buffer solution C (Tris-HCl 0,01 M pH 8,3, NaCl 0,2 M, $CaCl_2$ 0,01 M) using celophane pocket (sigma) until becoming colorless buffer. After dialysis testing, each protein fraction was then undergoing anti-bacterial testing similar to the previous testing on the whole extract protein.

Protein Concentration Determination

The calculation of bioactive protein concentration in the buffer 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 %) was determined based on *Lowry* method (Colowick and Kaplan, 1957) using *bovine serum albumine* (BSA) as a standard.

Assay of Anti Bacterial Activity

Antibacterial activity assays against *Staphylococcus aureus, Salmonella typhi*, was conducted using diffusion method. All about 20 µL of samples (whole extract and protein fractions, approximately 4 µg), were applied into sterile paper disc (diameter 6 mm), then put on the agar surface of the bacterial test culture. After 24 h incubation at 37^{0}C, the diameter of inhibition zone was determined in millimeter. The same procedure was applied to 20 µL BSA alone (approximately 4 µg) and 20 µL chloramphenicol (approximately 30 ppm) as negative and positive control, respectively.

RESULTS AND DISCUSSION

Isolation and Determination of Protein Level of Brown Algae *Turbinaria decurens*

Data on distribution pattern of raw extract and protein fraction at fractionation of various complete saturation levels of ammonium sulfate as shown in Table 1. Table 1 showed that protein concentration from whole extract of brown algae *Turbinaria decurens* is 0.3 mg/mL with the total amount of protein 138.0 mg from the whole extract volume 460 mL. The highest protein concentration was

found at fraction of ammonium sulfate 0-20% is 5.1 mg/mL, whereas the lowest protein concentration was found at ammonium sulfate fraction 60-80% is 0.2 mg/mL.

Antibacterial bioactivity of protein fraction of brown algae to tested bacteria *Salmonella typhi*.

The measurement results of inhibition diameter of protein fraction from brown algae *Turbinaria decurens* against *Salmonella typhi* after the incubation periods 24 hours and 48 hours as shown in Table 2. Table 2 shows that the bioactivity and the greatest effectiveness is obtained at whole extract and protein fraction from brown algae *Turbinaria decurens* against *Salmonella typhi* bacteria after incubation period 24 hours at whole extract is 14.24 mm, then on protein fractions 40-60% (10.97 mm), 20-40% (10.54 mm), 0-20% (10.46 mm) respectively; whereas the smallest inhibition diameter shown at protein fraction 60-80% (F4) is 10.10 mm. The average inhibition diameter shown at the whole extract and the four protein fractions do not exceed the measurement of positive control inhibition diameter is 19.33 mm. This indicates that the whole extract sample of brown algae *Turbinaria decurens* (14.24 mm) is effective as an antibacterial material against *Salmonella typhi* due to its inhibiting power ≥ 14 mm, whereas the four protein fractions have an antibacterial effect which tends to be ineffective due its inhibition diameter 10-11 mm.

Table 1. Distribution of Protein Concentration in Whole Extract and ammonium sulfate fraction from brown algae *Turbinaria decurens*

No	Protein Fraction	Fraction Volume (mL)	Protein Concentration (mg/mL)	Total Protein (mg)
1	Whole Extract	460,0	0,3	138,0
2	0-20%	20,0	5,1	102,0
3	20-40%	19,5	2,3	44,8
4	40-60%	23,0	0,5	11,5
5.	60-80%	19,0	0,2	3,8

Based on the data on measurement to tested bacteria *Salmonella typhi* at the interval 48 hours, the inhibiting diameter of each treatment has a significant decrease from whole extract at the beginning (24 hours) of 14.24 mm diameter decreases to 14.1 mm. Likewise for the protein fraction 0-20% (F1) at the beginning has 10.46 mm diameter decreases to 10.13 mm. Further for the protein fraction 20-40% (F2) from 10.54 mm diameter decreases to 10.36 mm, and protein fraction 40-60% (F3) from 10.97mm decreases to 10.46 mm. Also for protein fraction 60-80% (F4) decreases from 10.10 mm to 9.66 mm, and for the positive control of 19.33 mm diameter also decreases to 15.53 mm.

Based on the results, it can be concluded that the inhibition diameter shown by whole extract to the growth of *Salmonella typhi* bacteria is probably due to the accumulation of various polar compounds including protein in whole extract.

Antibacterial bioactivity of protein fraction of brown algae to tested bacteria *Staphylococcus aureus*

The results of inhibition diameter measurement of whole extract and protein fraction of green algae *Turbinaria decurens* against *Staphylococcus aureus* after the incubation periods 24 hours and 48 hours can be seen in Table 3. Table 3

Table 2. Bioactivity of whole extract and protein fraction from brown algae *Turbinaria decurens* against *Salmonella typhi* bacteria after incubation period 24 and 48 hours.

No	Protein Fraction	Inhibition Zone (mm)	
		Salmonella typhi	
		24 h	48 h
1	Whole Extract	14.24	14.10
2	0- 20%	10.46	10.13
3	20- 40%	10.54	10.36
4	40- 60%	10.97	9,46
5	60- 80%	10,10	9,66
6	Positive control (+) cloramphenicol	19,33	15,53
7	Negative control (-) BSA	6,0	6,0

shows that the biggest inhibition diameter formed by the protein fraction of brown algae *Turbinaria decurens* against *Staphyloccus aureus* bacteria after the incubation period 24 hours at the raw extract is 18.63 mm, and then at the protein fraction 0-20% (13.3 mm), 40-60% (11.53 mm), 20-40% (11.03 mm) respectively; whereas the smallest inhibiting diameter at the incubation period 24 hours of protein fraction 60-80% (F4) is 9.83 mm.

The average inhibiting diameter shown by the whole extract and the four protein fractions of brown algae *Turbinaria decurens* against *Staphylococcus aureus* bacteria is smaller than the inhibiting zone of positive control that is 22.57 mm. This indicates that the raw extract of brown algae *Turbinaria decurens* (18.63 mm) ≥ 14 mm, whereas the four protein fractions have an antibacterial effect which tends to less effective due to its inhibiting power 10-11 mm. According to Cappucino (1978), an antibiotic which is effective to inhibit the growth of bacteria is when the inhibiting diameter is ≥ 14 mm, and it is ineffective when the inhibiting diameter is ≤ 96 mm.

Based on the data on measurement to tested bacteria *Staphylococcus aureus* at the interval of 24 hours, the inhibiting zone diameter of each treatment decreases. At the raw extract at the beginning (incubation period 24 hours), the diameter is 18.63 mm and after incubation for 48 hours, it decreases to 17.1 mm. Also at the protein fraction 0-20% at the beginning the diameter is 13.3 mm after incubation for 48 hours, it decreases to 9.7 mm. Further the protein fraction 20-40% (F2) of diameter 11.03 mm at the incubation period 24 hours decreases to 9.47 mm, and protein fraction 40-60% (F3) decreases from 11.53 mm to 10.73 mm. As for the protein fraction 60-80% (F4), the inhibiting zone decreases from 9.83 mm at the incubation period 24 hours to 9.2 mm after the incubation period 48 hours. As for the positive control, the diameter 22.57 mm decreases to 16.6 mm, and for the negative control, there is no inhibiting zone at all (size 6.mm on data is a paper disc diameter).

The size of inhibiting zone diameter shown by raw extract to the growth of *Staphylococcus aureus* bacteria is probably due to the accumulation of various polar compounds and protein compound at the raw extract. This effect can be additive or synergic (inhibit) between compound in the whole extract. Besides that this is also probably due to resistance of the bacteria to bioactive substance, level of active substance, and amount of bacterial inoculum or density of tested bacteria.

Data on Table 3 showed that the tested bacteria *Staphylococcus aureus* at the incubation period 24 hours, the protein fraction of brown algae *Turbinaria decurens* forms an inhibiting zone marked by clear zone around the paper disc. But after the incubation period 48 hours, the inhibiting zone becomes smaller. This indicates that the

Table 3. Bioactivity of whole extract and protein fraction of brown algae *Turbinaria decurens* against *Staphylococcus aureus* after the incubation periods 24 hours and 48 hours

No	Protein Fraction	Inhibition Zone (mm)	
		Staphylococcus aureus	
		24 jam	48 jam
1	Whole Extract	18,63	17,10
2	0- 20%	13,3	9,70
3	20- 40%	11,03	9,47
4	40- 60%	11,53	10,73
5	60- 80%	9,83	9,20
6	Positive control (+) cloramphenicol	22,57	16,60
7	Negative control (-) BSA	6,0	6,0

bioactive compound in the raw extract and all fractions of brown algae *Turbinaria decurens* tend to inhibit the bacteria growth in a short time or is called bacteriostatic. According to Cappucino et al. (1978), there are several factors causing the difference of the inhibiting power: 1) the growth of microorganism, 2) the ability and flow of active material diffusion on medium, and 3) thickness and viscosity of medium. Besides those factors, other factors which might affect the effectiveness of a bioactive compound are physiological and chemical characteristics of each type of bacteria (Pelczar, 1988).

The biological ability of each bacterium varies in responding antibacterial substance. One of the most dominant factors is the difference of cell walls between Gram-negative and positive bacteria. The specific components the positive gram bacteria have are teichoat acid, teichuronic acid, and polysaccharide, whereas the specific components of Gram-negative bacteria are lipoprotein, outside membrane, and lipopolysaccharide. Outside membrane of Gram-negative bacteria cell wall is a double phosphor lipid mostly replaced by lipopolysaccharide molecule. The outside membrane is permeable to dissolved low molecule substance so that the active matter cannot get into the bacteria cell resulting in the bacteria is hard to destroy or is inhibited its growth (Masduki, 1996).

Brook et al. (1996) state that the outside membrane of Gram-negative bacteria has a specific channel from the protein molecule called porin that can allow passive diffusion from several molecules with low weight such as glucose, amino acid, and particular ion. The great antibiotic molecule penetrates the

outside membrane slowly so that the negative gram bacteria are more resistant to antibiotic. Ajizah et al. (2007) also state that *Staphylococcus aureus* is a Gram-positive bacterium which has osmotic pressure 3-5 times greater than the negative gram bacterium so that this bacterium undergoes lysis easily when there is a damage to the cell wall.

Based on several studies above, it can be said that both Gram-positive and negative bacteria have a different structure and mechanism in protecting itself from outside interference. In one side the Gram-positive bacteria has a thick structure of cell wall but has only one layer consists of peptidoglycan with density and less areas repeatedly. Whereas the Gram-negative bacteria has a thinner structure of cell wall but the cell wall has three layers consists of lipoprotein layer (outside layer), polysaccharide layer (intermediate layer), and less peptidoglycan layer (inside layer) compared to a Gram-positive bacteria (Pelczar, 1988 and Chan, 2006). Therefore, the response to antibacterial compound is also different in each tested bacteria.

CONCLUSION

Based on the result and discussion described previously, it can be concluded as follow. Bioactive protein at saturation level of ammonium sulfate of 0-20% derived from brown algae species *Turbinaria decurens* showed strongest activity with inhibition zone of 13,30 mm toward *Salmonella typhy.*

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