

**BIOACTIVITY OF PROTEIN FRACTION IN BROWN ALGAE, *Turbinaria decurrens*, AS ANTIBACTERIAL AGENT**

**BIOAKTIVITAS FRAKSI PROTEIN DALAM ALGAE COKLAT, *Turbinaria decurrens*, SEBAGAI AGEN ANTI BAKTERI**

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**ABSTRACT**

A research on the ability of protein fraction from chocolate algae *Turbinaria decurrens* in territorial of South Sulawesi to inhibit the growth of *Salmonella thypi* and *Staphylococcus aureus* has been conducted. Proteins was fractionated from the whole extract using salting out method with 0-20%, 20-40%, 40-60% and 60-80% ammonium sulphate saturation. Protein was purified by a dialysis method using a selophan membrane. The protein level was determined by a Lowry method, the highest protein concentration, 5.1 mg/mL was found in the 0-20% fraction of the chocolate algae. Antibacterial activity test was performed using a layered jelly diffusion method on the MHA medium. The highest antibacterial activity to *Staphylococcus aureus* was found in whole extract and the 0-20% fraction with the inhibition diameter of 18.63 mm and 13.30 mm, respectively.

**Keywords:** Antibacterial; Lowry method; Protein fraction; Brown algae.

**INTRODUCTION**

Indonesia, known as a maritime country with ocean area of 75% covering the country, has abundant source of marine biota, among others are a lot variety of algae species. Some species have been reported 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 resistance germs towards a certain medicine, the efforts to find new medicines is therefore necessarily to carry out. Hitherto, marine natural resources have not been optimally

utilized. Therefore, any effort to identify potential bioactive compounds from marine natural resources will be of a great interest (Caraan, 1994 and Nybakken, 1993).

It is widely accepted that marine natural resources can be classified into 2 classes i.e., marine biota or plants such as algae, seagrass and marine animal such as fish, molusca, soft coral, sponge, echinodermata, aschidin, and tunichata. Some marine animal species have a good resource of vitamin, protein, and mineral compounds. In addition to that, several certain species have a capability to synthesize and accumulate toxic compounds in a certain part of the body (Rachmaniar, 1996). Such compounds are considered as secondary metabolite compounds that are usually used as a self defence system from other organism in its environment and pharmacologic activities. Therefore, such compounds have a promising prospect to be extracted and isolated and utilized as new potential medicines (Sardjoko, 1996).

In general, the natural medicines are derived from secondary metabolite compounds having a specific chemical characteristic. The compounds resulted from secondary metabolite reactions compounds in the living organisms are used as a defence mean against disease attacks as well as to defend the existence of the living organisms. These secondary metabolite compounds are then collected, processed, and used as a new drug formula. 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 have advantage in digesting process such that the bioactive compounds yielded from such process will be varied according to the eating habit of each algae.

The study was conducted to explore and characterize the bioactive compounds of this type of chocolate algae *Turbinaria decurrens* in South Sulawesi. Derived

bioactive compounds to be tested its effect on anti-bacterial. The results showed that the bioactive protein from the chocolate algae *Turbinaria decurrens* 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 in this research is 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<sub>2</sub> 0,01 M,  $\beta$ -merkaptoetanol 1 %, Triton X-100 0,5 %), buffer B (Tris-HCl 0,1 M pH 8,3, NaCl 0,2 M, CaCl<sub>2</sub> 0,01 M), buffer C (Tris-HCl 0,01 M pH 8,3, NaCl 0,2 M, CaCl<sub>2</sub> 0,01 M), Bovine Serum Albumin (BSA) 4 mg/mL, chloramfenicol, and aluminium foil.

#### **Extraction and isolation of bioactive protein from algae**

Extraction and isolation of bioactive algae were conducted using previous methods (Schroder *et al.*, 2003; Ely *et al.*, 2004 modified) as follows; brown algae *Turbinaria decurrens* that have been collected were cut into small pieces, and each species was weighed into 500 g of fresh sample, homogenized using buffer solution A (Tris-HCl 0,1 M pH 8,3, NaCl 2 M, CaCl<sub>2</sub> 0,01 M,  $\beta$ -merkaptoetanol 1 %, Triton X- 100 0,5 %), filtered with buchner, and the filtrate obtained was then freezed and liquefied between 2 or 3 times, and then centrifugized at 12.000 rpm and 4°C for about 30 minutes, and finally the supernathan obtained was stored in a refrigerator before being tested for anti bacterial and further purification stage.

#### **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<sub>2</sub> 0,01 M), and then dialysed in buffer solution C (Tris-HCl 0,01 M pH 8,3, NaCl 0,2 M, CaCl<sub>2</sub> 0,01 M) using selofan pocket (sigma) until obtaining 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<sub>2</sub> 0,01 M,  $\beta$ -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  $\mu$ L of samples (whole extract and protein fractions, approximately 4  $\mu$ g), 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<sup>0</sup>C, the diameter of inhibition zone was determined in millimeter. The same procedure was applied to 20  $\mu$ L BSA alone (approximately 4  $\mu$ g) and 20  $\mu$ L 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.

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

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

The measurement results of inhibition diameter of protein fraction from chocolate 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 chocolate 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 chocolate algae *Turbinaria decurens* (14.24 mm) is effective as an antibacterial material against *Salmonella typhi* due to its inhibiting power  $\geq 14$  mm, whereas the four protein fractions have an antibacterial effect which tends to be ineffective due its inhibition diameter 10-11 mm.

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

No	Protein Fraction	Inhibition Zone (mm)	
		<i>Salmonella typhi</i>	
		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 (+) cloramfenicol	19,33	15,53
7	Negative control (-) BSA	6,0	6,0

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 chocolate 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 shows that the biggest inhibition diameter formed by the protein fraction of chocolate algae *Turbinaria decurens* against *Staphylococcus 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 chocolate 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 chocolate algae *Turbinaria decurens* (18.63 mm)  $\geq$  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  $\geq$  14 mm, and it is ineffective when the inhibiting diameter is  $\leq$  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).

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

No	Protein Fraction	Inhibition Zone (mm)	
		<i>Staphylococcus aureus</i>	
		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 (+) cloramfenicol	22,57	16,60
7	Negative control (-) BSA	6,0	6,0

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 chocolate 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 bioactive compound in the raw extract and all fractions of chocolate 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 teichoic 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* bory stongest activity with inhibition zone of 13,30 mm toward *Salmonella typhy*.

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### **REFERENCES**

Ahmad, A., Prastawa, B. dan Salama, D. 2006. Bioaktivitas antimikroba dan antikanker fraksi protein yang diisolasi dari beberapa spesies makro alga

di pulau Barang Lompo Sulawesi Selatan. Proseding Seminar Nasional  
*Research Grant TPSDP Batch II*, Bali.

- Anonymous. 2003, "Seaweed is a beneficial alternative. Science Store Wesite PKT – BPPT.
- Brooks, G.F., Butel, J.S., dan Ornston, L.N., 1996. *Medical Microbiology*. Twentieth edition, Translation Edi Nugroho, R.F. Maulany, EGC. Jakarta.
- Cappuccino, J G., dan Sherman, N. 1978. *Microbiology A Laboratory Manual*. Rockland Community College. Suffern, New York.
- Caraan, G.B., Lazaro, J. E., Concepcio, G. P., 1994. Biological Assays for Screening of Marine Sampels, Second Marine Natural Product Workshop, Marine Science Institute and Institute of Chemistry, University of the Philippines.
- Chan, Y. Y., Ong, Y. M., and Chua, K. L., 2006. Synergistic Interaction between Phenothiazines and Antimicrobial Agents Against *Burkholderia Pseudomallei*. *Antimicrob. Agents Chemother.* 51 (2) 623-630.
- Ely, R., Supriya, T, and Naik, C. G., 2004. *Antimicrobial Activity of Marine Organisms Collected of The Coast of South East India*, L. of Exp. Marin Biol. Eco. 1-7.
- Masduki, I., 1996. Antibacterial Effects of Area Seed Extract (Area Catechu) Against *Staphylococcus aureus* and *Escherichia coli* in vitro. Journal of Research Result UGM, Yogyakarta.
- Pelczar, M. J. and Chan, E. C. S. translated by Hadioetomo, R. S., Imas T., Tjitrosomo, S. S. and Angka, L. A., 1988. *Fundamental of microbiology*. Jilid I, UI Press, Jakarta, 138.o the New Perspective on Day Seminar. Makassar, October 26,
- Rahmaniar. 1966. Marine Natural Products as Lead Compound for Pharmaceuticals and Agriculture. Brought to the New Perspective on Day Seminar. Makassar, October 26, 1966.
- Schroder, H.C. Ushijima, H. Krasko, A., Gamulin, V., Thakur, N.L., Diehl-Seifert, B., Muller, I.M., and Muller, W.E.G., 2003. Emergence and Disappearance of An Immune Molecule, An Antimicrobial Lectin, In Basal Metazoan, *J. Biol. Chem.* 278. 32810-32817.