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Abstrak. This study aimed to find out the antibacterial and cytotoxic activity of n-hexane, ethyl acetate, acetone, and ethanol extracts from macroalgae *Halimeda cylindracea* that was collected from Gulf of Boni. The antibacterial activity was evaluated against *Stapilococcus aureus*, *Escherichia coli,* and *Salmonella typhi* with the Kirby Bauer method. The cytotoxic activity was evaluated with a Brine Shrimp Lethality test. Extraction was performed successively with n-hexane, ethyl acetate, acetone, and ethanol for 3 x 24 hours with maceration method and evaporated with a rotary evaporator to obtain dry extract. Antibacterial activity was evaluated by inhibition zone where the cytotoxic was observed by LC_{50} value of each tested extract. The result indicated that ethyl acetate extract showed active to all bacteria test which acetone extract was active against *S. aureus* and *E. coli*, n-hexane extract was active against *S. aureus*, and ethanol extract was not active to all bacteria test. N-hexane, ethyl acetate, acetone, and ethanol extracts of macroalgae *H. cylindracea* were toxic toward *Arthemia salina* Leach with moderate toxicity category with LC_{50} value 134.90, 281.84, 338.84 and 295.12 µg/mL respectively.

1. Introduction

Macroalgae as marine plants have a reasonably high species diversity more than 10,000 species that have been identified and described as 6,500 red algae, 2,000 brown algae, and 1,500 green algae [1]. In Indonesia, more than 1000 species have been identified [2]. Macroalgae have phytochemical potential such as antibacterial and anticancer [1,3,4].

Cancer and antibacterial medicine resistance are the main problems in health. Cancer is health problem worldwide because it is the leading cause of death (9.6 million people) [5]. The diseases caused by bacteria develop a resistance mechanism to antibacterial drugs through genetic mutations [6]. Bacteria resistance are dominated by MRSA bacteria, *Enterobacteriaceae*, *Carbapenemase*, *Acinetobacter*, *Pseudomonas* and including twelve priority resistant bacteria for developing new antibiotics [7,8]. This problem will continue to develop, which is suspected as a result of the interaction between micro molecule medicines with biological molecules in disease sources that are not static or develop continuously [9]. This issue needs continuous effort to overcome this problem. One of which is finding new chemical compounds as antibacterial and anticancer from natural sources. Macroalgae such

as *H. cylindracea* as part of the marine ecosystem offers excellent potential as a productive natural pharmacopeia to find antibacterial and anticancer [10].

The macroalgae *H. cylindracea* in the bay of Boni including non-consumption macroalgae has not been studied. From the macroalgae, there is an opportunity to find chemical compounds that could be used as antibacterial and anticancer. Phytochemical examination of other species in this macroalgae genus (*Halimeda* genus) shows that there are antibacterial and anticancer compounds, for example halimedatrial and halimedatetraacetic groups that are toxic to predatory fish and have cytotoxic and antimicrobial properties [11,12]. Diterpenoid as 4-hydroxydictyolactone, dictyol E, and 8α-11-dihydroxypachydictyol A were active against tumor cells SF-268, MCF-7, H460, and HT-29 with GI_{50} values of 16-88 μM found in *H. stuposa* [13]. In addition, alkaloid compounds are also found such as caulerpin with antibacterial and antitumor activities that are found in *H. incrassate* and halimedin alkaloid are found in *H. xishaensis* which is active against *S. aureus* bacteria and *E. coli* bacteria [14,15]. Several compounds from the steroid group have also been reported [16-19]. Methanol, ethyl acetate and acetone extracts of *H. macrolaba, H. gracilis* and *H. opuntia* were toxic to *A. salina* and active against *S. pneumoniae, S. aureus, P. mirabilis, P. aeruginosa, E. feacalis*, and *E. coli* bacteria with MIC values of 0.5-2 mg/mL [20-22]. Dichloromethane:methanol extract of *H. tuna* and *H. incrassate* is active against HeLa cells, HepG2, and KB cells with LC_{50} values of 29-59 μg/mL [1,23,24]. Based on these assumptions at this early stage, the authors are interested to examine the antibacterial and toxicity levels of *H. cylindracea* extract which is taken from Gulf of Boni, South Sulawesi, Indonesia.

2. Materials and Methods

2.1 Materials

Macroalga *H. cylindracea* (Figure 2) were collected by extracting it from the growing place on the sandy seabed using hand (mini scuba) from various depths (0.5-2 m). Samples were collected when seawater at the lowest ebb conditions, around 08.00 - 11.00 a.m on the coral islands of the Gulf of Boni (Figure 1), South Sulawesi, Indonesia on November 23-25, 2018. Species names and sample taxonomies were determined in the Waters Productivity and Quality Laboratory, Hasanuddin University with identification number 13UML/Lab.Air/VIII/2018.

Chemicals for extraction use technical quality organic solvents which have been distilled, namely; n-hexane, ethylacetate, acetone and ethanol. For the antibacterial test, Nutrient Agar (NA) and Mueller Hinton Agar (MHA) were used as bacterial growth media and chloramphenicol standard antibiotics. While the material for the cytotoxic activity test uses DMSO and seawater taken from the laboratory of the Brackish Water Center in Kab. Barru, Department of Maritime Affairs and Fisheries, South Sulawesi, Indonesia. Standard bacterial strains from Gram-negative *E. coli, S. typhi* and Gram-positive *S. aureus* bacteria were obtained from Microbiology Laboratory of Hasanuddin University.

2.2 Methods

2.2.1 Sample preparation and Extraction. Fresh macroalgae *H. cylindraceae* in the holdfast section was discarded and the rest of the segment and nodes was washed with sea water until clean, and then it was stored in a cool box, immediately transported to the mainland and dried for three days without being exposed to the sun as well as kept from decaying. The dry samples were cut into small pieces and mashed. The dried macroalgae material was blended into a coarse powder extraction portions of the powdered samples (10.47 kg), packed in macerator apparatus and extracted successively with hexane, ethyl acetate, acetone and ethanol for 3x24 hours for each solvent. Each extract was filtered continually with a Buchner filter and collected in a dark bottle. The extracts were evaporated with a rotary evaporator at 40°C until a viscous extract and then stored in the sample bottle in the refrigerator before use.

2.2.2 Antibacterial Activity Test by Kirby Bauer Method. Each sample (extract) was dissolved in maceration solvents with various concentrations of 12.50, 25.00, 50.00, 100.00, and 1000.00 μg/mL MHA (15 mL) and poured into a petri dish and inoculated with 100 μL of suspension containing 1×105 CFU mL-1 bacteria. Ciprofloxacin was used as a positive control and treated discs with organic solvents.

Each of which was used for maceration as negative controls. Sterile paper discs (7 mm) were soaked for 20 min on each sample which was removed and dried at room temperature in a sterile room and then placed onto the surface of the agar medium containing bacterial suspense. Furthermore, the plates were placed in an incubator at 37°C for 24 hours, and then the diameter of the resistance zone around each disc was measured and noted [25].

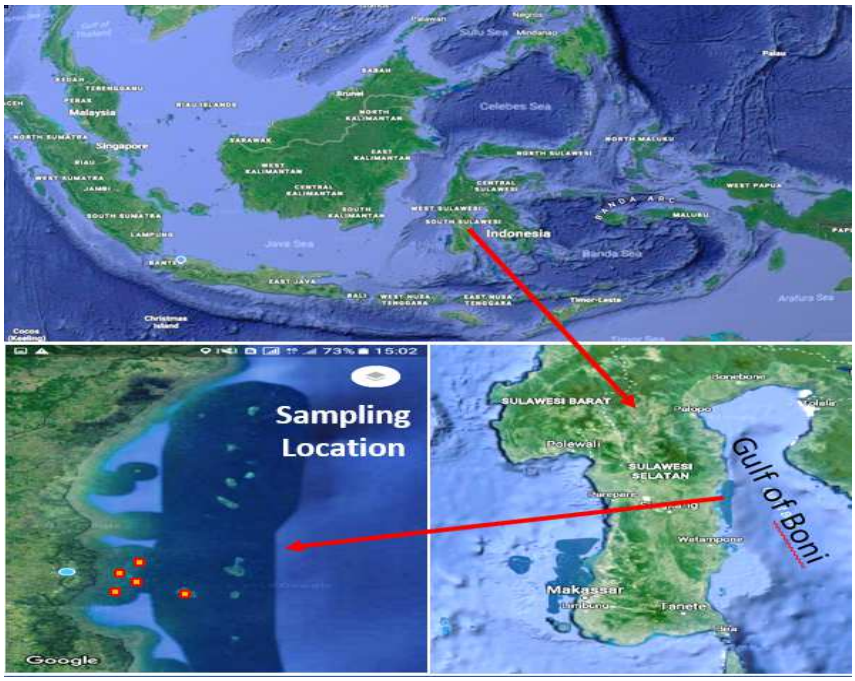

Figure 1. Sampling location in Gulf of Boni, South Sulawesi, Indonesia

(A) **(B)**

Figure 2. *H. cylidracea* viewed from seawater surface (A) and morphology (B).

2.2.3 Cytotoxic activity test by BSLT method. Cytotoxic activity test was carried out by the Lethality Brine Shrimp Test method. The test procedure was the modification [26]. The concentrations of the test solution used that were 31.25, 62.50, 125.00, 250.00, 500.00, and 1000.00 µg/mL. Preparation of a test solution of 1000 µg/mL was carried out by taking 30 mg of each n-hexane, ethyl acetate, acetone, and methanol extract and then dissolved it in 3.5 mL DMSO and diluted with sea water to a volume of 30

mL. Furthermore, test solutions of 1000 were diluted using sea water to obtain the desired concentration variation. Then, 4 mL of each concentration of sample in vial bottles were inserted by 7-10 shrimp larvae using a micropipette with 1 mL each addition to become 5 mL for all experiments. Mortality of shrimp larvae after 24 hours was observed and the LC_{50} value of the n-hexane, ethyl acetate, acetone and ethanol extract was determined by using a regression curve between log concentration (X) and probity value (Y). The probity value (Y) was obtained by changing the percentage of mortality into the probity table.

The *A. salina* frying process and toxicity criteria for the toxicity assessment of extracts were verified for extracts with LC_{50} above 1000 µg/mL that was not toxic, LC_{50} of 500 - 1000 µg/mL was low toxic, LC_{50} of 100 - 500 µg/mL was moderately toxic and LC_{50} 0-100 µg/mL were very toxic [27].

3. Results and Discussion

3.1. Antibacterial Activity of H. cylindracea ekstract

The results of the antibacterial activity of hexane, ethyl acetate, acetone and ethanol extracts from macroalgae *H. cylindracea* can be seen in Table 1.

Table 1. Antibacterial activity of *Halimeda cylindraceae* extract against bacteria

Concentration (µg/mL)	Inhibition Zone (mm)											
	E. coli				*S. aureus*				*S. typhi*			
	Hxn	Eac	Act	Eth	Hxn	Eac	Act	Eth	Hxn	Eac	Act	Eth
12.50	-	-	-	-	-	-	-	-	-	-	-	-
25.00	-	-	-	-	-	-	-	-	-	-	-	-
50.00	-	7.3	-	-	-	9.6	-	-	-	12.5	-	-
100.00	-	10.5	8.0	-	8.8	11.4	8.9	-	-	14.2	-	-
1000.00	-	10.8	8.8	-	8.8	12.3	10.9	-	-	14.7	-	-
*(-) control		-				-					-	
**(+) control		27.2				28.4					25.4	

Hxn: n-hexane; Eac: ethyl acetate; Act: Acetone; Eth: Ethanol.
* Solvent; ** Chloramphenicol 1 mg/mL

Based on the results of this study, the data described that were the existence of antibacterial potential of macroalgae *H. cylindracea* extract. Antibacterial potential was determined by the presence of inhibition zones at extract concentrations of ≤ 160 µg/mL against standard antibacterial when were tested on the same inoculum [28]. The inhibition zones in the Table 1 showed that chloramphenicol was classified as a strong category for all testing bacteria so that it can be used as a standard in reference to the antibacterial activity of the test extract. The ethyl acetate extract of macroalgae *H. cylindracea* has the most considerable antibacterial potential compared to other extracts because it shows activity against the three test bacteria with MIC value of 50 µg/mL. The acetone extract was active against *S. aureus* and *E. coli*. Hexane extract only active against *S. aureus*, whereas ethanol extract was not active as antibacterial. Based on Table 1, it can also be explained that hexane extract was only active against Gram-positive bacteria compared to ethylacetate extract and acetone were active against Gram-positive and Gram-negative bacteria, while ethanol extract did not show activity against the test bacteria. This showed that ethyl acetate and acetone extract of macroalgae *H. cylindracea* contain antibacterial active compounds, both against Gram-positive bacteria and against Gram-negative bacteria. The results of this study also indicated which chemical compounds that have antibacterial properties in macroalgae *H. cilindracea* that was chemical compounds in the medium polarity category or semipolar.

3.2. Cytotoxic Activity of H. cylindracea extract

The results of cytotoxic activity test from *H. cylindracea* extract by using the Brine Shrimp Lethality Test (BSLT) can be seen in Tables 2, 3, 4 and 5.

Table 2. BSLT Results of Hexane Extract of *Halimeda cylindraceae*

Concentration (µg/mL)	Log Concentration	% Mortality Average		% Mortality Correction	Probity Value	LC_{50} (µg/mL)
		Sample	control			
31.25	1.49	16.67	4.00	12.67	3.87	134.90
62.50	1.80	33.33	4.17	29.17	4.45	
125.00	2.10	73.91	13.64	60.28	5.25	
250.00	2.40	84.00	17.39	66.61	5.44	
500.00	2.70	100.00	13.04	86.96	6.13	
1000.00	3.00	100.00	16.00	84.00	5.99	

Table 3. BSLT Results of Ethyl Acetate Extract of *Halimeda cylindraceae*

Concentration (µg/mL)	Log Concentration	% Mortality Average		% Mortality Correction	Probity Value	LC_{50} (µg/mL)
		Sample	control			
31.25	1.49	12.00	4.00	8.00	3.59	281.84
62.50	1.80	18.52	4.17	14.35	3.92	
125.00	2.10	20.83	13.64	7.20	3.52	
250.00	2.40	51.85	17.39	34.46	4.59	
500.00	2.70	100.00	13.04	86.96	6.13	
1000.00	3.00	100.00	16.00	84.00	5.99	

Table 4. BSLT Results of Acetone Extract of *Halimeda cylindraceae*

Concentration (µg/mL)	Log Concentration	% Mortality Average		% Mortality Correction	Probity Value	LC_{50} (µg/mL)
		Sample	control			
31.25	1.49	5.00	4.00	1.00	2.67	338.84
62.50	1.80	7.41	4.17	3.24	3.12	
125.00	2.10	20.00	13.64	6.36	3.44	
250.00	2.40	48.15	17.39	30.76	4.50	
500.00	2.70	100.00	13.04	86.96	6.13	
1000.00	3.00	100.00	16.00	84.00	5.99	

Table 5. BSLT Results of Ethanol Extract of *Halimeda cylindraceae*

Concentration (µg/mL)	Log Concentration	% Mortality Average		% Mortality Correction	Probity Value	LC_{50} (µg/mL)
		Sample	control			
31.25	1.49	15.00	4.00	11.00	3.77	295.12
62.50	1.80	20.00	4.17	15.83	4.01	
125.00	2.10	40.00	13.64	26.36	4.36	
250.00	2.40	50.00	17.39	32.61	4.56	
500.00	2.70	76.92	13.04	63.88	5.36	
1000.00	3.00	100.00	16.00	84.00	5.99	

The LC_{50} value of each extract was determined based on a straight line equation with X is the antilog of the sample concentration and the probit value Y. The obtained probit value from the percentage of the average mortality of *A. salina* (triplo treatment) was transformed to the probit value table by entering the value of Y = 5 (death of 50%) so that the value of LC_{50} can be determined. The LC_{50} values listed in tables 2, 3, 4 and 5 showed the highest toxicity level of extracts namely n-hexane extract that was followed by extract of ethyl acetate, ethanol and finally acetone extract with LC_{50} values of 134.90, 281.84, 295.12, 338.84 µg/mL respectively. Based on the level of toxicity category, the four macroalgae extracts of *H. cylindracea* were in the category of moderate toxicity. The results of this data indicate that the extracts have the highest possible cytotoxic properties of chemical components and toxicity in n-hexane and ethylacetate extract which showed that chemical compounds that have stronger cytotoxic activity in macroalgae *H. cylindracea* were non-polar.

4. Conclusion

The ethylacetate extracts of macroalgae *H. cylindracea* showed significant antibacterial action. From these preliminary investigations need further investigation, a detailed study on the principle compound in this macroalga which is responsible for antimicrobial activity. The four different solvent of *H. cylindracea* extract used in the present study showed moderate cytotoxic level toward *A. salina* Leach.

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