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Antibacterial activity of red algae (*Gracilaria verrucosa*) extract against *Escherichia coli* and *Salmonella typhimurium*

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Abstract. Red alga was widely used in several fields, including food, feed, pharmacy and industrial point of view. The chemical analysis showed that red alga contained terpenoid, acetogenic, and aromatic compounds, which have a wide range of biological activities, such as anti-microbial, anti-inflammatory and anti-viral. The objectives of this research was to evaluate the effect of extraction solvent and time on antibacterial activity of red alga (*Gracilaria verrucosa*), and to explore the bioactive compound contained within *Gracilaria verrucosa*. The method in this study used descriptive research. These findings revealed that the highest inhibition activity among all extracts was obtained with the ratio of methanol:aquades (75:25) and extraction time around 72 hours against *Escherichia coli* and *Salmonella typhimurium*. The bioactive compounds of *Gracilaria verrucosa* tested by phytochemical analysis consisted of flavonoid, alkaloid, and saponin. Those secondary metabolites may be approximated as antibacterial substances.

1. Introduction

Currently, the use of seaweeds have been extensively studied in many fields, including food and pharmacy. they are also used as a new material for cosmetics, herbal medicines and fertilizers [1]. In the animal industry point of view, seaweeds can improve nutritional value of animal feed. One of the most potential sources of seaweeds is red algae. It has a brilliant color due to the pigment phycoerythrin and phycocyanine. This algae can live at greater depths than brown and green algae because it absorbs blue light.

Red algae is considered as a source of bioactive compounds as it is able to produce secondary metabolites [2] characterized by a wide spectrum of biological activities, such as antiviral, antibacterial and antifungal [3]. Previous studies have suggested that red algae has a great variety of secondary metabolites than brown and green algae. Hence, red algae provides an alternative approach to the use of the synthetic antimicrobial agents. However, the potent antimicrobial effect of red algae resides in the efficiency of the extraction method [4], the red algal species, and the solvents being used [5].

According to Shekhar [6], red algae (*Gracilaria edulis*) had antibacterial activity extracted by ethanol against *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*. On the other hand, the research of *Gracilaria verrucosa* as an antibacterial substance and had bioactive compounds especially isolated from Ponjuk, Talango, Madura (East Java) are poorly documented yet. Therefore, in this study we focus on evaluating the effect of extraction solvent and time on antibacterial activity of *Gracilaria verrucosa*, and to explore the bioactive compounds contained within *Gracilaria verrucosa*.



2. Material and Methods

2.1. Read algae collection and preparation

The red algae used as raw material in this research was *Gracilaria verrucosa*, which was obtained from Ponjuk Village, Talango Island, Madura. *Gracilaria verrucosa* was washed by seawater to clean impurities material. *Gracilaria verrucosa* was put into a polyethylene bag. Then, it was transferred into cool box. *Gracilaria verrucosa* was dried, then cleaved into small pieces using a grinding machine. The extraction was conducted by maceration technique in which *Gracilaria verrucosa* powder have been dried was subsequently soaked (maceration). The maceration technique was used to extract the bioactive compounds within *Gracilaria verrucosa*.

In this study used descriptive method, which can be described as a statement of affairs as they are at present with the researcher having no control over variable. We applied 60 g of each treatment macerated by solvent with different extraction time in the range of 24, 48, and 72 hours, respectively. The first treatment was conducted with solvents ratio (ethanol with different water content or ethanol : distilled water) were 75 % : 25 %; 50 % : 50 %; and 25 % : 75 %. The second treatment used methanol extraction in the ratio of 75 % : 25 %; 50 % : 50 %; and 25 % : 75 % distilled water.

2.2. Phytochemical analysis

The phytochemical analysis used Nurdiani *et al.* [7]. It was conducted to investigate a wide range of active compound contained in the fraction. Alkaloids, flavonoids, saponins, and terpenoids were analyzed. The data analysis was described elsewhere.

2.3. Antibacterial activity test

Antibacterial activity was carried out using agar diffusion method. Pure cultures of *E. coli* and *S. typhimurium* were grown in liquid media nutrient broth and incubated at 35°C for 3 h, then the suspension was cultured using TSA media. The fraction was dissolved in DMSO 10 %, then 10 µL of the fraction was impregnated into a paper disc and was put on TSA media consisted of bacteria. It was kept for 24 h at temperature of 30°C. Zone of inhibition was measured.

3. Results and Discussion

3.1. Extraction yields of *Gracilaria verrucosa*

The yields of the crude extraction of *Gracilaria verrucosa* using distilled water, ethanol, and methanol were presented in table 1.

Table 1. Extraction yield of *Gracilaria verrucosa*.

Treatment		Weight of sample (g)	Average of weight extract (g)	Yield (%) ± SD
Solvent	Extraction time			
Ethanol	24	60	3.37	5.61±0.55
	48	60	4.33	7.22±0.80
	72	60	4.81	8.01±1.05
Methanol	24	60	4.86	8.09±1.50
	48	60	5.54	9.23±0.88
	72	60	5.55	9.26±1.13

The extraction yield of *Gracilaria verrucosa* treated by the different concentration of solvents and extraction time revealed that highest yield of *Gracilaria verrucosa* extraction was 9.26 in the ratio of 75 : 25 (methanol : distilled water) for 72 h of extraction time. According to Wijaya *et al.* [8], this solvent mixture will result in a maximum amount of yield. Mustafa and Turner [9] added that methanol solvent can result in the highest yield. Siregar *et al.* [10] stated that the longer extraction time between solvent and sample can get more extract.

3.2. Qualitative phytochemical analysis

The qualitative phytochemical analysis of *Gracilaria verrucosa* extract was conducted to find out the types of secondary metabolites existing in the extract. The secondary metabolites assessed were alkaloids, flavonoids, saponins, and terpenoids. The results of secondary metabolite screening from *Gracilaria verrucosa* can be seen in table 2.

Table 2. Qualitative phytochemical analysis of *Gracilaria verrucosa* extract.

Secondary Metabolite	Ethanol : Distilled water ^a			Methanol : Distilled water ^a		
	75:25			75:25		
Time (h)	24	48	72	24	48	72
Flavonoid	-	-	-	-	+	+
Alkaloid	+	+	+	+	+	+
Saponin	-	-	+	-	-	+
Terpenoid	-	-	-	-	-	-

^a (+) = Contains test compound; and (-) = Does not contain test compounds

The results of the phytochemical analysis showed that the highest number of secondary metabolite compounds was in the ratio of methanol-distilled water around 75 : 25 with extraction time of 72 h. The metabolite compounds detected in the phytochemical analysis were flavonoid, alkaloid, and saponin. It means that the highest yield of metabolite compounds is suggested by using 75 % of methanol as a extraction solvent. Phytochemicals can be extracted with an appropriate solvent [11]. The degree of polarity affects the components of the extracted phytochemicals. Nafisyah *et al.* [12] stated that the secondary metabolite compounds, such as flavonoids, alkaloids, and saponins have capability in dissolving in the polar solvents.

3.3. Antibacterial activity of *Gracilaria verrucosa* against *E. coli* and *S. typhimurium*

The results of the antibacterial activity of *Gracilaria verrucosa* extract against *E. coli* and *S. typhimurium* can be seen in figure 1 and figure 2.

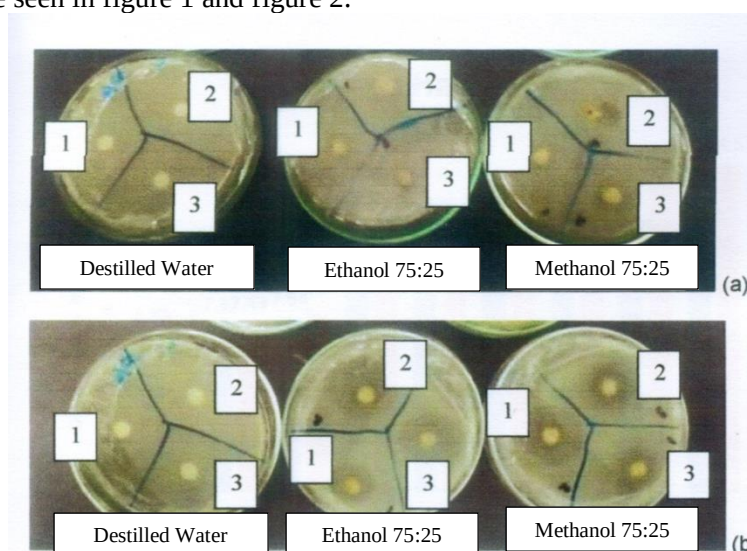


Figure 1. The results of *Gracilaria verrucosa* extract against *E. coli* and *S. Typhimurium*. The code a and b indicated *E. coli* inhibition and *S. typhimurium* inhibition, respectively. The number '1, 2, 3' showed the extraction time for 24 h, 48 h, and 72 h, respectively.

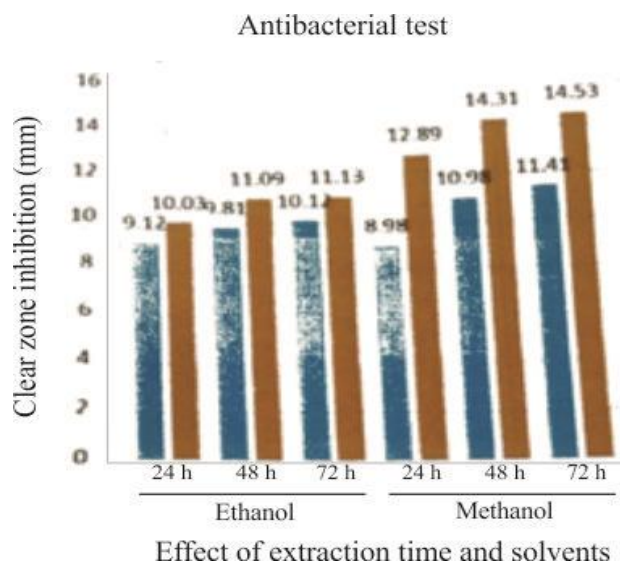


Figure 2. The Inhibition zone of *Gracilaria verrucosa* extract.

The figure 2 above showed that the activity of antibacterial compounds extracted from *Gracilaria verrucosa* had positive effect to inhibit pathogenic bacteria such as *E. coli* and *S. typhimurium*. The antibacterial inhibition formed clear zones around the disc test through the paper treated by *Gracilaria verrucosa* extract. However, the inhibition activity of antibacterial compounds has different inhibition zone.

The lowest inhibition zone of antibacterial activity against *E. coli* and *S. typhimurium* was displayed in the methanol extraction with the range of time for 24 h and the concentration of methanol around 25 %. On the contrary, the highest inhibition zone was performed in the concentration of methanol 75 % for 72 h of extraction time. The lowest antibacterial activity was due to the presence of only one secondary metabolite compound (alkaloid), and the highest antibacterial activity was caused by the activity of three phytochemicals, they are alkaloid, saponin dan steroid. The extraction of natural sources like red algae is suggested to use more methanol concentration as an agent of extracting secondary metabolites compounds that will obtain higher antibacterial activity [13]. Secondary metabolites extracted by using methanol will produce better inhibitory power than that of produced by chloroform [14].

4. Conclusion

The conclusions of this research are the qualitative physicochemical screening test showed that only appropriate secondary metabolites could be extracted by ethanol and methanol solvents. the highest inhibition activity among all extracts was obtained with the ratio of methanol: aquades (75:25) and extraction time around 72 hours against *Escherichia coli* and *Salmonella typhimurium*.

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