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Antibacterial Activity of Methanol Extract from Seagrass of *Halodule Uninervis* in the Coastal of Lampung

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ABSTRACT

The purpose of the research was to analyze bacterial activity from methanol extract of Halodule UninervisagainstGram-negative bacteria (Aeromonas hydrophila and Vibrio harveyi) and Gram-positive bacteria (Bacillus subtilis and Listeria monocytogenes).The result showed content of phenolic was 20.17ppm and content of tannin was 1,223 ppm.Antibacterial activity of an extract showed that seagrass can inhibit several types of Gram-negative bacteria (Aeromonas hydrophila and Vibrio harveyi) and Gram-positive bacteria (Bacillus subtilis and Listeria monocytogenes). As for the highest inhibitory activity present in the type of Gram-positive bacteria (Bacillus subtilis and Listeria monocytogenes) between 7 mm to 9 mm.

Keywords *:Seagrass, Haloduleuninervis, antibacterial*

INTRODUCTION

Seagrasses are the marine flowering plants. They are the only angiosperms that successfully growth in tidal and sub tidal marine environment[1]. The seagrasses was found to show prominent effect against the human bacterial pathogens [2-5]and hence they were tested for the effect against the pathogenic bacteria by using their crude extract of *Halodule Uninervis*seagrass.

However reports on the antibacterial of seagrasses of the coastal of Lampung, Indonesia are limited with the exception of few studies. Therefore the present study was undertaken to evaluate the antibacterial, and phytochemical constituents of methanol extract of *Halodule Uninervis* seagrasses.

MATERIALS AND METHODS

Leaves of *Halodule Uninervis*were collected from Coastal of Lampung the and immediately brought to the laboratory in sterile plastic bags containing water to prevent evaporation. Sea grasses were washed thoroughly with distilled water to remove extraneous materials and shade-dried for 10 days at room temperature until constant weight obtained. The dried Sea grasses were powdered and stored in refrigerator for future use.

Preparation of Sea grass extract

Sea grass powder were soaked in 2L organic solvents with methanol (1:4 w/v), and kept for 10 days in a shaker. The extraction was repeated thrice and pooled. Each filtrate was concentrated to dryness under reduced pressure using a rotary flash evaporator. The dry aqueous extracts were stored in a refrigerator until further analysis.

The total phenolic content

The total phenolic content was determined by the Folin-Ciocalteu method [6]. The methanol extracts 250 µL was mixed with 125 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 250 ml of a 7% Na_2CO_3 solution was added to

the mixture followed by the addition of 1250 ml of deionized distilled water and mixed thoroughly. The mixture was kept in the dark at room temperature for 1 h. Absorbance was measured at 725 nm. The content of phenolic compounds was standardized with gallic acid and defined as mg of gallic acid equivalents per 1 g of sample

The total tannin content:
A total of 0.2 g of sample is introduced into the erlenmeyer containing 10 ml of methanol, and then stirred using a mechanical shaker for 1 hour. Taken 1.0 ml of the supernatant then mix them with distilled water in a test tube. Added 0.3 ml of 0.1 M $FeCl_3$ and shake. Added 0.3 ml of 0,008 M $K_3Fe(CN)_6$. Then the mixture was allowed to stand for 10 minutes, the sample absorbance is read at a wavelength of 720 nm. Documents used is the whole solution, but without the sample. Tannin content is calculated by the following formula:

$$\text{Tanin (ppm)} = \frac{\text{absorbance sample}}{\text{absorbance blanko}} \; x \; \frac{100}{\text{Weight of sample(g)}} \; x \; 100$$

Antibacterial test
Pathogens bacterial (*Aeromonas hydrophila*, *Vibrio harveyi*, *Bacillus subtilis* and *Listeria monocytogenes*) used in this study. Antibacterial activity was evaluated using diffusion method[7]. Actively growing lag phase cultures of bacteria were mixed in Nutrien agar (Nutrien broth with 1.5% agar) and plated. The various extract (500 ppm, 1.000 ppm, 1.500 ppm and 2.000 ppm) were loaded onto different paper discs (Whatman no. 1 filter paper). The discs were placed on the agar medium containing cultures incubated for 24 h at 37^0C. Zone of inhibition was recorded in millimeters and mean values were reported.

RESULTS AND DISCUSSION

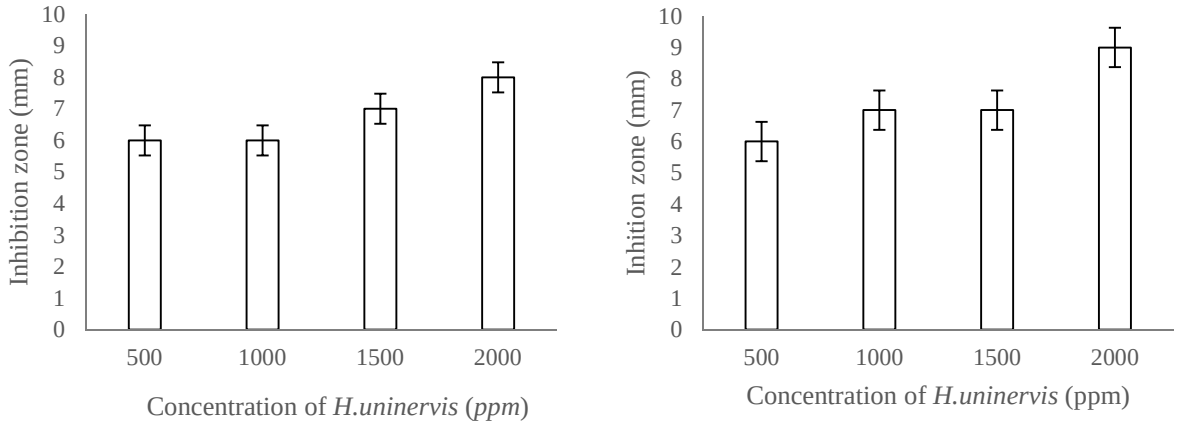
The total phenolic and tannin content

Table 1. The total phenolic and tannin content of *Halodule uninervis*

Extract of *Halodule uninervis*	Total Phenolic (ppm)	Total tannin (ppm)
	20.17	1,223

Our study shows a small content of phenolic compared to total tannin which is around 20.17 ppm (20.17 ppm to 1,223 ppm, respectively).The phytochemical compounds detected such as tannins and phenolic have previously been reported to have antimicrobial and antioxidant activity[8].

Antibacterial activity from*H. uninervis*againstGram-positive bacteria
Antibacterial activity from*H. uninervis*with different concentration of extract againstGram-positive bacteriaare depicted in Fig. 1. The Antibacterial activity from*Halodule uninervis*toward *Bacillus subilis* and *Listeria monocytogenes* were peaked after 2.000 ppm of extract concentration.



A **B**

Figure 1. Bacterial activity of *H. uninervis*againstGram-positive bacteria (A=*Bacillus subtilis* and B=*Listeria monocytogenes*)

In antimicrobial assay the *H. uninervis* extracts have has shown good antimicrobial activities against the *Bacillus subtilis* and *Listeria monocytogenes* with a maximum inhibitory effect at 9mm and minimum inhibitory effect at 6 mm.

Antibacterial activity from*H. uninervis*against Gram-negative bacteria

Antibacterial activity from*H. uninervis*with different concentration of extract againstGram-negative bacteriaare depicted in Fig. 2.

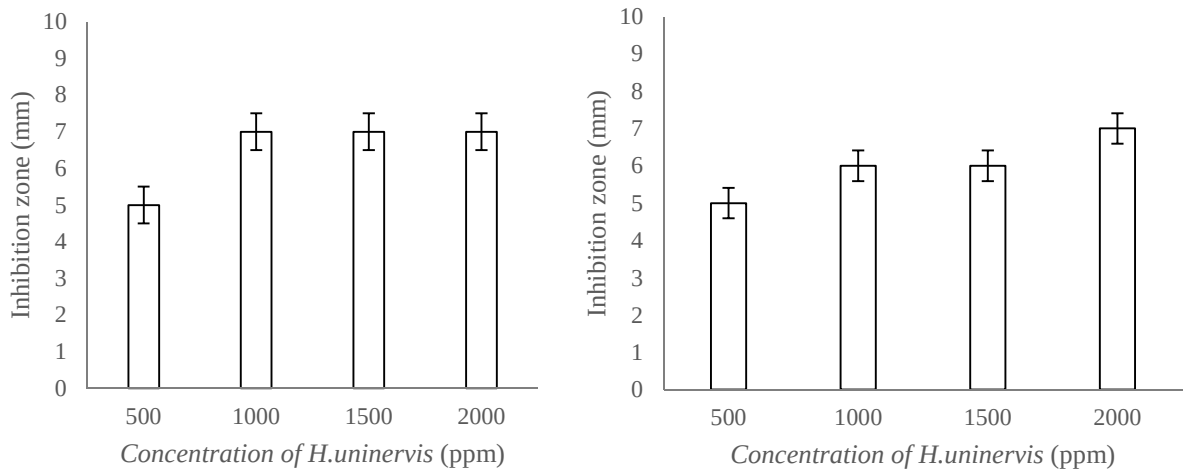


Figure 2. Bacterial activity of *Halodule uninervis* against Gram-negative bacteria (A= *Aeromonas hydrophila* and B= *Vibrio harveyi*)

Bacterial activity of *Halodule uninervis*against *Bacillus subtilis*was from 6 mm to 8 mm and the extract of *Halodule uninervis*had the highest bacterial activity, which was 8 mm at the concentration was2,000 ppm. While bacterial activity of *Halodule uninervis* against *Listeria monocytogenes* was from 6 mm to 9 mm and the extract had the highest bacterial activity which 9 mm at concentration was 2,000 ppm.

These findings suggest that antimicrobial activity of *H. uninervis* extract may be primarily due to the presence of tannins and phenolic compound. The inhibition of microorganisms by phenolic compounds may also be due to iron deprivation or hydrogen binding with vital proteins such as microbial enzymes [9]. Tannins have been traditionally used for treatment of catarrh, hemorrhoids and diarrhea [10].

The bacterial activity of *H. uninervis* extract againstGram-positive bacteria higher than that of these Gram-negative bacteria. A small activity of *uninervis* extract against Gram-negative bacteria due to Gram-negative bacteria contain three layer : liposacharide, peptidoglican and proteinso that compounds of seagrasses can not damage the cell of bacteria.

CONCLUSION

From the present study theextractofseagrass(*Haloduleuninervis*) hasanti-bacterial effect and effective ongram-positive(*Bacillus subtilisandListeriamonocytogenes*).

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