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Activity of *Caulerpa racemosa* Against *Helicobacter pylori* Bacteria

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ABSTRAK

Anggur Laut (Caulerpa racemosa) terdapat berbagai macam metabolit sekunder dengan senyawa bioaktif yang berfungsi sebagai antibakteri. Penelitian ini bertujuan untuk menguji aktivitas antibakteri dari ekstrak etanol Anggur Laut (Caulerpa racemosa) terhadap bakteri Helicobacter pylori. Metode yang digunakan adalah metode difusi agar dengan kertas cakram pada empat variasi konsentrasi ekstrak yaitu 500 µg, 600 µg, 700 µg, dan 800 µg per cakram. Ekstraksi dilakukan secara maserasi menggunakan pelarut etanol 96%, dan aktivitas antibakteri diuji berdasarkan pembentukan zona hambat di sekitar cakram. Hasil penelitian menunjukkan bahwa semua konsentrasi ekstrak memberikan efek hambat yang sangat kuat terhadap pertumbuhan H. pylori, dengan zona hambat berturut-turut sebesar 27,23 mm; 28,51 mm; 29,91 mm; dan 31,51 mm. Semakin tinggi konsentrasi ekstrak, semakin besar pula diameter zona hambat yang terbentuk. Berdasarkan kategori aktivitas antibakteri, ekstrak Caulerpa racemosa menunjukkan potensi sebagai agen antibakteri kuat terhadap Helicobacter pylori.

Kata kunci: *Caulerpa racemosa, antibakteri, Helicobacter pylori, difusi agar, zona hambat.*

ABSTRACT

Sea grapes (Caulerpa racemosa) contain various secondary metabolites with bioactive compounds that function as antibacterial agents. This study aimed to evaluate the antibacterial activity of ethanol extract from sea grapes (Caulerpa racemosa) against Helicobacter pylori. The method used was the agar diffusion technique with paper discs at four different extract concentrations: 500 µg, 600 µg, 700 µg, and 800 µg per disc. Extraction was carried out through maceration using 96% ethanol as the solvent, and antibacterial activity was assessed based on the formation of inhibition zones around the discs. The results showed that all extract concentrations exhibited very strong inhibitory effects on the growth of H. pylori, with inhibition zones of 27.23 mm, 28.51 mm, 29.91 mm, and 31.51 mm, respectively. The higher the extract concentration, the larger the inhibition zone formed. Based on antibacterial activity categorization, Caulerpa racemosa extract demonstrates potential as a strong antibacterial agent against Helicobacter pylori.

Keywords*: Caulerpa racemosa, antibacterial, Helicobacter pylori, agar diffusion, inhibition zone.*

1. INTRODUCTION

Indonesia, as a megabiodiversity country, has around 30,000 plant species, about 7,500 of which have medicinal properties[1]. One potential marine plant that contains bioactive compounds is Sea Grapes (Caulerpa racemosa), known to contain secondary metabolites such as phenols, saponins, tannins, and flavonoids, and has cytotoxic properties[2,3]. In addition to its use in the food and cosmetics industries, sea grapes have also been reported to have antifungal activity[4] and antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*[5]. On the other hand, *Helicobacter pylori* is a Gram-negative bacterium that infects nearly 50% of the global population and can cause serious diseases such as gastritis and even stomach cancer if not treated promptly[6]. The high prevalence of infection in developing countries highlights the need to search for new antibacterial agents. Based on this background,

this study was conducted to evaluate the antibacterial activity of Caulerpa racemosa extract against *Helicobacter pylori*.

2. RESEARCH METHODS

Tools and Materials

The tools used include: IKA RV 10 rotary evaporator, diving equipment set, autoclave, incubator, chamber, micropipette, scale, gloves, scissors, knife, Erlenmeyer flask, measuring glass, beaker, 15 cm petri dishes, spatula, tweezers, microtubes, stirring rod, refrigerator, urine pots/tubes, caliper, vials, inoculating loop, lab coat, glass container, test tubes, 8 mm Advantec paper discs, camera. Materials used in this study include: sea grapes (*Caulerpa racemosa*), 95% ethanol, 70% alcohol, *Helicobacter pylori* bacteria, 8 mm diameter paper discs, Nutrient Agar (NA), and Nutrient Broth (NB).

Research Method

The antibacterial testing method used in this study is the agar diffusion technique with paper discs. This type of research is a laboratory experimental study using extract concentrations with three replications for testing one type of bacterium. The extract concentrations used are as follows:

K1: Sea grape extract at 500 µg/disc
K2: Sea grape extract at 600 µg/disc
K3: Sea grape extract at 700 µg/disc
K4: Sea grape extract at 800 µg/disc

Research Procedure

1. Sample Collection of Sea Grapes (*Caulerpa racemosa*)
   The sea grapes (*Caulerpa racemosa*) used were fresh and green, collected in February 2024 at Basaan Beach in Southeast Minahasa Regency, North Sulawesi Province, from the coastal area at low tide, approximately 1 meter below sea level. The collected samples were washed with running water and drained to reduce moisture. They were then chopped into smaller pieces to ease the extraction process. A total of 2 kilograms of the sample was weighed, air-dried, and then macerated.
2. Preparation of Sea Grape Extract (*Caulerpa racemosa*)
   A 450 g sample of *Caulerpa racemosa* was extracted using the maceration method for 2 × 24 hours and repeated twice (2 × 24 hours). The soaked samples were filtered using filter paper and a funnel to produce Filtrate 1 and Debris 1. Debris 1 was then re-soaked in 96% ethanol until fully submerged and macerated again for 2 × 24 hours. This process was repeated to obtain two filtrates and two sets of debris, after which all filtrates were combined. The combined filtrate was evaporated at 40°C to produce a thick extract, which was placed in a tube, weighed, and stored in a refrigerator.
3. Preparation of Nutrient Broth (NB) Solution for Culture Activation
   To prepare the NB solution, 0.8 g was dissolved in 100 mL of distilled water in an Erlenmeyer flask and stirred until completely dissolved. The solution was then transferred into test tubes. A stock culture of *H. pylori* was inoculated into the test tubes and left for 24 hours until bacterial growth, indicated by turbidity, was observed.
4. Preparation of Nutrient Agar (NA) Solution
   To prepare the NA solution, 3.9 g was dissolved in 150 mL of distilled water in an Erlenmeyer flask and homogenized using a magnetic stirrer. The NA solution was used to prepare pour plates. It was sterilized in an autoclave at 121°C for 15 minutes.
5. Antibacterial Activity Test Procedure
   The bacterial suspension was mixed with sterilized media, then 50 mL was poured into each petri dish and allowed to solidify. Paper discs were dipped into the test solutions with concentrations of 500 µg, 600 µg, 700 µg, and 800 µg dissolved in 70% alcohol, and then dried in a desiccator for 24 hours. The dried paper discs were placed on the testing media in labeled petri dishes and incubated at 34°C–37°C for 24 hours. The presence or absence of inhibition zones around the discs was observed. This test was repeated three times for each *Helicobacter* treatment in five treatments per petri dish.

Data Analysis

The research data were processed based on observations and measurements, which were tabulated in a table showing the antibacterial activity of *Caulerpa racemosa*. Data collection involved observing and measuring the inhibition zones after 24 hours of incubation. The diameter of the inhibition zones was measured using a caliper. The data were presented in descriptive form using tables and images.

The calculation of the inhibition zone diameter[7]:

$$\textbf{Formula: D} = \frac{A + B + C}{3}$$

Description:
A = vertical diameter
B = horizontal diameter
C = diagonal diameter
D = inhibition zone diameter

3. RESULTS AND DISCUSSION

This test used the agar diffusion method with paper discs. The paper discs were pre-treated by applying the sample extract and leaving them for 24 hours in a vacuum desiccator. The discs were then placed onto solid media in petri dishes that had been inoculated with *Helicobacter pylori* bacteria. The diameter of the paper discs used was 8 mm, and the diameter of the petri dishes was 15 cm. The test was conducted on *Helicobacter pylori* bacteria using five treatments, which consisted of sea grape (*Caulerpa racemosa*) ethanol extracts at concentrations of 500 µg, 600 µg, 700 µg, and 800 µg.

Table 1. *Antibacterial activity test results of sea grape (Caulerpa racemosa) extract against Helicobacter pylori*

Extract	Concentration	*Helicobacter pylori*			
		P.I	P.II	P.III	Average
(1)	(2)	(3)	(4)	(5)	(6)
Caulerpa racemosa	500 µg	27.25 mm	25.2 mm	29.33 mm	27.23 mm
	600 µg	29.15 mm	26.21 mm	30.18 mm	28.51 mm
	700 µg	30.80 mm	27.50 mm	31.44 mm	29.91 mm
	800 µg	31.30 mm	31.00 mm	32.21 mm	31.51 mm
Control	50 µg/disc	0	0	0	0

Figure 1. *Petri dish showing inhibition zones against Helicobacter pylori using Caulerpa racemosa extract.*

From the test results shown in Table 1 and Figure 1 above, it can be observed that the 500 µg concentration had an average inhibition zone of 27.23 mm; the 600 µg concentration had an average inhibition zone of 28.51 mm; the 700 µg concentration had an average of 29.91 mm; and the 800 µg concentration had an average of 31.51 mm. This indicates that each concentration resulted in a different inhibition zone.

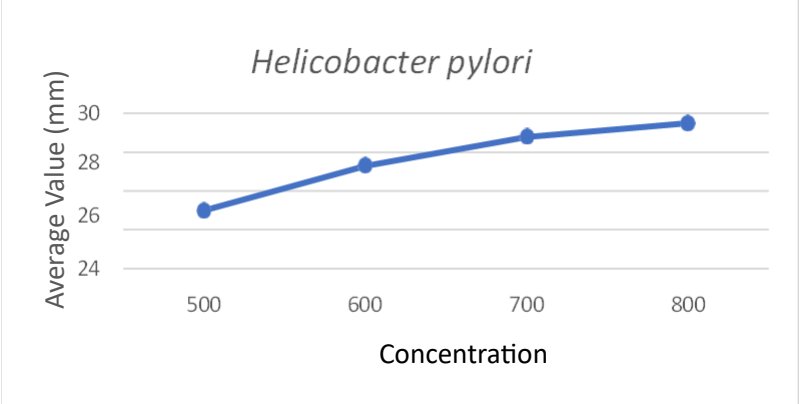

Figure 2. *Graph of Antibacterial Activity Test on Helicobacter pylori.*

From the inhibition zone activity results shown in Figure 2, it is evident that all concentrations of the sea grape extract exhibited antibacterial activity. The average inhibition zone for each concentration was as follows: 500 µg — 27.23 mm; 600 µg — 28.51 mm; 700 µg — 29.91 mm; and 800 µg — 31.51 mm. Thus, the higher the concentration, the greater the inhibition zone formed. Figure 2 shows that *Caulerpa racemosa* has a very strong antibacterial activity against *Helicobacter pylori*[8].

The measured inhibition zones from the *Caulerpa racemosa* extract, ranging from 27 mm to 31.51 mm, fall into the very strong category for antibacterial activity against *Helicobacter pylori*, based on standard inhibition zone diameter classifications. These results show that sea grape (*Caulerpa racemosa*) provides strong antibacterial activity at all tested concentrations: 500, 600, 700, and 800 µg/disc.

The inhibition zones produced by each concentration show a clear effect against *Helicobacter pylori*. In addition to concentration, the type of material used can also influence the inhibitory activity of *Caulerpa racemosa* against the growth of *Helicobacter pylori*, due to differences in the diffusion rate of the active compounds in the agar media. Therefore, the extract from sea grape (*Caulerpa racemosa*) has the potential to act as an anti-*Helicobacter pylori* agent.

4. CONCLUSION

Based on the research conducted, sea grape (*Caulerpa racemosa*) exhibits strong antibacterial activity and is highly effective at inhibiting *Helicobacter pylori* at all tested concentrations.

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