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Inhibitory Activity Test of Sea Grape (*Caulerpa racemosa*) Against *Salmonella typhi* Bacteria

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ABSTRAK

Salmonella typhi merupakan bakteri penyebab utama penyakit tifoid yang masih menjadi masalah kesehatan serius di negara berkembang, termasuk Indonesia. Penelitian ini bertujuan untuk menguji Uji Aktivitas Daya Hambat Anggur Laut Caulerpa racemosa Terhadap Bakteri Salmonella thypi. Ekstrak diperoleh melalui metode maserasi dan diuji menggunakan metode difusi agar dengan kertas cakram pada berbagai konsentrasi (100, 200, 300, dan 400 µg/disc). Hasil penelitian menunjukkan bahwa ekstrak C. racemosa mampu menghambat pertumbuhan S. typhi dengan rata-rata diameter zona hambat sebesar 25 mm (100 µg), 27,3 mm (200 µg), 28,8 mm (300 µg), dan 29,5 mm (400 µg). Kontrol negatif (aquades) tidak menunjukkan aktivitas antibakteri. Berdasarkan klasifikasi, seluruh konsentrasi ekstrak menunjukkan aktivitas antibakteri yang sangat kuat. Semakin tinggi konsentrasi ekstrak, semakin besar daya hambat yang dihasilkan. Temuan ini menunjukkan bahwa C. racemosa berpotensi sebagai sumber antibakteri alami terhadap S. typhi dan dapat dikembangkan lebih lanjut dalam pengobatan alternatif tifoid.

Kata kunci: *Caulerpa racemosa*, antibakteri, *Salmonella typhi*, zona hambat, alga laut.

ABSTRACT

Salmonella typhi is the primary bacterial cause of typhoid fever, which remains a serious health concern in developing countries, including Indonesia. This study aims to evaluate the inhibitory activity of sea grape (Caulerpa racemosa) extract against Salmonella typhi. The extract was obtained through maceration and tested using the agar diffusion method with paper discs at various concentrations (100, 200, 300, and 400 µg/disc). The results showed that C. racemosa extract inhibited the growth of S. typhi with average inhibition zone diameters of 25 mm (100 µg), 27.3 mm (200 µg), 28.8 mm (300 µg), and 29.5 mm (400 µg). The negative control (distilled water) showed no antibacterial activity. Based on classification, all concentrations exhibited very strong antibacterial activity. A higher extract concentration corresponded to greater inhibitory effect. These findings suggest that C. racemosa has potential as a natural antibacterial agent against S. typhi and may be further developed for alternative typhoid treatment.

Keywords*: Caulerpa racemosa, antibacterial, Salmonella typhi, inhibition zone, seaweed.*

1. INTRODUCTION

Salmonella typhi is a Gram-negative bacterium and the primary causative agent of typhoid fever, a systemic infection that poses a significant public health burden, particularly in developing countries such as Indonesia. Transmission of *S. typhi* typically occurs through the consumption of food or water contaminated with feces from infected individuals or asymptomatic carriers, and is often associated with poor sanitation and limited access to clean water[1].

Indonesia is recognized as a megabiodiversity country, hosting more than two-thirds of the world's marine species, which makes it one of the richest regions in terms of marine biodiversity[2]. One of the natural resources that has demonstrated potential as an antibacterial agent is sea grapes (*Caulerpa*

racemosa), a species of green algae commonly found in tropical waters, including those of Indonesia. *C. racemosa* is known to contain various bioactive compounds such as flavonoids, alkaloids, saponins, tannins, and terpenoids, which exhibit antimicrobial properties[3,4]. These compounds are capable of disrupting bacterial cell walls, interfering with protein synthesis, and inhibiting the growth and development of pathogenic bacteria.

Numerous studies have reported that secondary metabolites such as alkaloids, flavonoids, steroids, and saponins possess antibacterial and antioxidant activities[5-7]. Previous research has shown that extracts of *Caulerpa racemosa* exhibit antibacterial activity against several Gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa*[8]. However, studies on the antibacterial activity of this algae against *Salmonella typhi* remain limited. Therefore, this study aims to evaluate the inhibitory activity of *Caulerpa racemosa* extract against the growth of *Salmonella typhi* as part of the effort to explore potential natural antibacterial sources for further development.

2. RESEARCH METHODS

Tools and Materials

The equipment used in this study included a *rotary evaporator* (IKA RV 10), diving gear, autoclave, incubator, ultraviolet lamp, chamber, TLC plate, micropipette, balance, gloves, scissors, knife, Erlenmeyer flask, measuring cylinder, beaker, 15 cm Petri dishes, spatula, tweezers, microtubes, glass rod, refrigerator, urine collection container, caliper, vials, inoculation loop, lab coat, glass vessels, test tubes, 8 mm Advantec paper discs, and camera.

The materials used were: sea grape (*Caulerpa racemosa*), *Salmonella typhi* bacteria, 95% ethanol, 70% alcohol, n-hexane, ethyl acetate, 8 mm paper discs, Nutrient Agar (NA), Nutrient Broth (NB), and distilled water, which was used as a control.

Type and Design of the Study

The bacterial inhibition test method used in this study employed the agar diffusion technique with paper discs. This research is a laboratory experimental study, using various extract concentrations with three replications for a single bacterial species.

The extract concentrations and control used were as follows:
K1: Sea grape extract at 100 µg/disc
K2: Sea grape extract at 200 µg/disc
K3: Sea grape extract at 300 µg/disc
K4: Sea grape extract at 400 µg/disc
Control: Distilled water at 50 µL/disc

Research Procedure

1. Sample Collection of Sea Grape *(Caulerpa racemosa)*
   The sea grape (*Caulerpa racemosa*) used in this study was fresh green algae collected from Basaan Beach, Southeast Minahasa, North Sulawesi Province. The samples were thoroughly washed with running water, drained to reduce moisture content, and chopped into smaller pieces to facilitate the extraction of active compounds. A total of 2 kg of the sample was weighed, air-dried, and subsequently subjected to maceration.
2. Preparation of Sea Grape Extract *(Caulerpa racemosa)*
   A total of 450 g of *Caulerpa racemosa* was extracted using the maceration method for 2 × 24 hours, repeated twice (2 × 24 hours each). The soaked samples were filtered using filter paper and a funnel, resulting in 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, which were combined. The combined filtrates were evaporated at 40°C to obtain a concentrated extract of *Caulerpa racemosa*, which was stored in tubes, weighed, and kept refrigerated.
3. Preparation of Nutrient Broth (NB) for Bacterial Culture Activation
   A total of 0.8 g of Nutrient Broth (NB) was dissolved in 100 mL of distilled water in an Erlenmeyer flask and stirred until fully dissolved. The solution was then distributed into test tubes. A stock culture of *S. typhi* was added into the test tubes and incubated for 24 hours until turbidity was observed, indicating bacterial growth.
4. *Preparation of Tools and Media for Bacterial Testing*
   For Nutrient Agar (NA), 6.9 g of NA was dissolved in 300 mL of distilled water in an Erlenmeyer flask and homogenized using a magnetic stirrer. The NA solution was used for pour-plate media preparation and

sterilized in an autoclave at 121°C for 15 minutes.

Procedure for Antibacterial Activity Testing

The bacterial suspension was mixed with sterile media and poured into Petri dishes (50 mL per dish), then allowed to solidify. Paper discs were immersed in test solutions of 100, 200, 300, and 400 µg concentrations (dissolved in 70% alcohol), and dried for 24 hours in a desiccator.

The dried discs were placed onto the surface of solidified media in Petri dishes, which had been pre-labeled, and incubated at 34°C–37°C for 24 hours. The formation of inhibition zones around the discs was observed, and tests were conducted in triplicates for each concentration against *S. typhi*.

Inhibition Zone Diameter Calculation[5]:

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

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

Table 1. Antibacterial Activity Classification[6]

Inhibition Zone Diameter (mm) (1)	Antibacterial Activity (2)
2–5 mm	Very weak
5–10 mm	Moderate
10–20 mm	Strong
≥20 mm	Very strong

Data Analysis

Data processing in this study was based on observations and measurements of inhibition zones resulting from antibacterial activity tests of *Caulerpa racemosa* extract. The diameter of inhibition zones was measured after 24-hour incubation using a caliper for precision. The collected data were tabulated and presented in graphical form to facilitate result interpretation.

3. RESULTS AND DISCUSSION

This test employed the agar diffusion method using paper discs. The paper discs were pre-impregnated with the test extract and allowed to dry in a vacuum desiccator for 24 hours. The dried discs were then placed onto solid media inoculated with *Salmonella typhi* in Petri dishes. The paper discs used had a diameter of 8 mm, and the Petri dishes measured 15 cm in diameter. This test was specifically conducted against *Salmonella typhi*.

Five treatments were applied in this study, involving ethanol extracts of the sea grape (*Caulerpa racemosa*) at concentrations of 100 µg, 200 µg, 300 µg, and 400 µg per disc, with distilled water (aquadest) used as the negative control[7].

The negative control consisted of 50 µg of distilled water applied to the paper disc.

Table 2. Antibacterial activity of *Caulerpa racemosa* extract against *Salmonella typhi*

Extract	Concentration	*Salmonella thypi*			
		Rep. I	Rep. II	Rep. III	Average
(1)	(2)	(3)	(4)	(5)	(6)
Caulerpa racemosa	100 µg	25.2 mm	24.0 mm	22.0 mm	25.0 mm
Caulerpa racemosa	200 µg	27.5 mm	27.5 mm	27.0 mm	27.3 mm
Caulerpa racemosa	300 µg	29.1 mm	28.5 mm	28.0 mm	28.8 mm
Caulerpa racemosa	400 µg	29.8 mm	29.8 mm	29.0 mm	29.5 mm
Control (distilled water)	50 µg/disc	0 mm	0 mm	0 mm	0 mm

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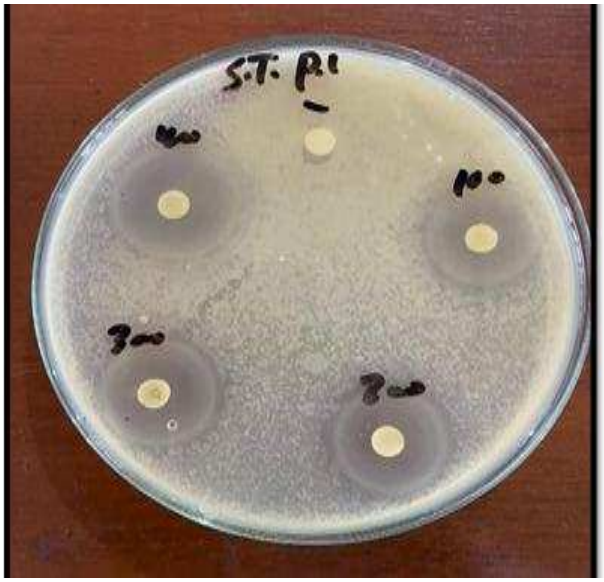

Figure 1. Inhibition zone results of *Salmonella typhi* treated with crude extract

The results shown in Table 2 and Figure 1 indicate that at a concentration of 100 µg, the average inhibition zone was 25 mm; at 200 µg, 27.3 mm; at 300 µg, 28.8 mm; and at 400 µg, 29.5 mm. The control treatment (distilled water) showed no inhibition zone. These findings demonstrate that each concentration produced a different level of antibacterial activity, with the zone of inhibition increasing as the extract concentration increased.

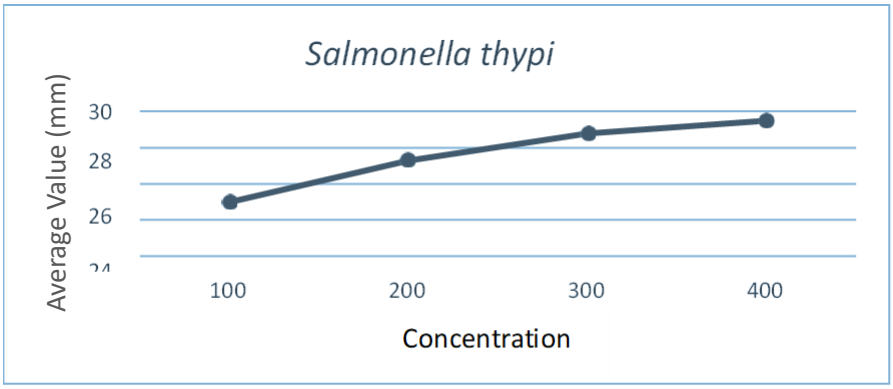

Figure 2. Inhibition zone activity curve of *Salmonella typhi*

The results of the inhibition zone activity test in Figure 2 show that all concentrations of sea grape extract have inhibitory activity. The smallest inhibition zone was observed at 100 µg/disc (25 mm), and the largest at 400 µg/disc (29.5 mm), indicating a positive correlation between extract concentration and inhibition zone diameter. In contrast, the distilled water control exhibited no inhibitory activity. According to antibacterial activity classification, the inhibition zones observed in Figure 2 confirm that *Caulerpa racemosa* extract demonstrates very strong antibacterial activity against *Salmonella typhi*[5].

4. CONCLUSION

The results showed that the ethanolic extract of sea grapes (*Caulerpa racemosa*) has very strong antibacterial activity against *Salmonella typhi*. All concentrations tested (100, 200, 300, and 400 µg/disc) produced inhibition zones with diameters that increased with increasing extract concentration, namely 25 mm, 27.3 mm, 28.8 mm, and 29.5 mm, respectively. n contrast, the negative control (distilled water) showed no antibacterial activity. These findings suggest that the higher the concentration of the extract, the greater its inhibitory effect on the growth of *S. typhi*.

5. BIBLIOGRAPHY

1. World Health Organization. (2018). *Typhoid fever*. Retrieved from https://www.who.int/news-room/fact-sheets/detail/typhoid
2. Hutomo, M., & Moosa, M. K. (2005). Indonesian marine and coastal biodiversity: Present status. *Indonesian Journal of Biology*, 2(2), 7–14.
3. Sudarmi, M., Fadli, A., & Fitriani, D. (2019). Aktivitas antibakteri ekstrak rumput laut *Caulerpa racemosa* terhadap bakteri patogen. *Jurnal Ilmiah Mahasiswa Biologi*, 4(3), 233-239.
4. Manoppo, H., Pontoh, J., & Mantiri, D. (2021). Kandungan fitokimia dan aktivitas antibakteri ekstrak *Caulerpa racemosa*. *Pharmacon*, 10(2), 97-103.
5. Rasyid A. (2012). Identification Of Secondary Metabolites Compounds, Antibacterial And Antioxidant Activities On The Methanol Extract Of Sea Cucumber *Stichopus hermanii*. *Jurnal Ilmu Dan Teknologi Kelautan Tropis*, *4*(2). https://doi.org/10.29244/jitkt.v4i2.7799
6. Mewengkang, T. T., Lintang, R. A., Losung, F., Sumilat, D. A., & Lumingas, L. J. L. (2022). Identification of Bioactive Compounds and Antibacterial Activity of Sea Cucumber, Holothuria (*Halodeima*) atra Jaeger 1833 Flesh Extract from Kalasey Coastal Waters, Minahasa District. *Jurnal Ilmiah Platax*, *10*(2), 355–363. https://doi.org/10.35800/jip.v10i2.42271
7. Zahki M. (2023). Efektifitas Antibakteri Senyawa Metabolit Sekunder Pada Beberapa Tanaman Obat Terhadap Pertumbuhan Bakteri *Staphylococcus Aureus*. Jurnal Integrasi Obat Tradisional, 2(2), 25-30
8. Putri, D. W., Yuliani, S., & Nurhayati, T. (2020). Uji aktivitas antibakteri ekstrak rumput laut terhadap bakteri gram negatif. *Jurnal Bioteknologi Tropis*, 8(1), 12-20.
9. Tethool, A.M., Tulandi S.S., Tulandi H. V., Paat V.I., Potalangi N.O. 2017. Pengaruh Daya hambat Sediaan Salep Ekstrak Daun Katuk (*Sauropus androgynus* L.) terhadap pertumbuhan Bakteri (*Stphylococcus aureus*). Tesis Program Studi Farmasi Fakultas Matematika Dan Ilmu Pengetahuan Alam Universitas KristenIndoenesia Tomohon.
10. Mpila, D. A. 2012. Uji Aktivitas Antibakteri Ekstrak Etanol Daun Mayana (*Coleus atropurpureus* (L) Benth) Terhadap *Staphylococcus aureus*, *Escherichia coli* dan *Pseudomonas aeruginosa* Secara In Vitro.
11. Suheri L. F., Agus Z., Fitria I. Perbandingan Uji Resistensi Bakteri *Staphyloccocus aureus* terhadap Antibiotik Ampisilin dan Tetrasiklin. Andalas Dental Journal, 3(1):25-33