Biofarmasetikal Tropis
(The Tropical Journal of Biopharmaceutical) 2025, 8(2), 24-30
e-ISSN 2685-3167
p-ISSN 2828-6685

Inhibitory Activity of Sea Grapes (*Caulerpa racemosa*) Against the Growth of *Propionibacterium acnes*

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Accepted: 26 September 2025; Approved : 16 Oktober 2025

ABSTRAK

Penelitian ini bertujuan untuk mengetahui aktivitas daya hambat anggur laut (*Caulerpa racemosa*) terhadap pertumbuhan bakteri *Propionibacterium acnes*, yaitu bakteri yang dapat menyebabkan jerawat pada kulit manusia. Anggur laut merupakan salah satu rumput laut yang mengandung senyawa bioaktif seperti flavonoid dan alkaloid yang berpotensi sebagai antibakteri. Penelitian ini dilakukan secara eksperimental di laboratorium dengan metode difusi agar menggunakan kertas cakram. Sampel anggur laut diekstraksi dengan metode maserasi menggunakan etanol 96%, kemudian diuji pada beberapa konsentrasi yaitu 100, 200, 300, dan 400 µg/disk. Aquades digunakan sebagai kontrol negatif. Hasil penelitian menunjukkan bahwa ekstrak anggur laut mampu menghambat pertumbuhan bakteri *Propionibacterium acnes* dengan diameter zona hambat antara 33,9–41,2 mm. Zona hambat terbesar diperoleh pada konsentrasi 400 µg/disk. Berdasarkan hasil tersebut, dapat disimpulkan bahwa anggur laut (*Caulerpa racemosa*) memiliki aktivitas antibakteri yang sangat kuat terhadap bakteri *Propionibacterium acnes* dan berpotensi sebagai bahan antibakteri alami.

Kata kunci: *Anggur laut, Caulerpa racemosa, daya hambat, Propionibacterium acnes, antibakteri*

ABSTRACT

This study aimed to determine the inhibitory activity of sea grapes (Caulerpa racemosa) against the growth of Propionibacterium acnes, a bacterium that can cause acne on human skin. Sea grapes contain bioactive compounds such as flavonoids and alkaloids which may act as natural antibacterial agents. This research was conducted experimentally in the laboratory using the agar diffusion method with paper discs. The sea grape sample was extracted by maceration using 96% ethanol and tested at concentrations of 100, 200, 300, and 400 µg/disc. Distilled water was used as a negative control. The results showed that the extract was able to inhibit the growth of Propionibacterium acnes, with inhibition zone diameters ranging from 33.9 to 41.2 mm. The largest inhibition zone was found at the concentration of 400 µg/disc. In conclusion, sea grapes (Caulerpa racemosa) have very strong antibacterial activity against Propionibacterium acnes and have potential as a natural antibacterial source.

Keywords: *Sea grapes, Caulerpa racemosa, inhibition activity, Propionibacterium acnes, antibacterial*

1. INTRODUCTION

Indonesia, particularly the coastal region of North Sulawesi Province, is endowed with abundant marine biodiversity. One of the marine resources commonly found in this area is sea grapes. Sea grapes are known by various local names, such as *Latoh* in Java, *Bulung Boni* in Bali, and *Lawi-lawi* in Sulawesi. Sea grapes (*Caulerpa racemosa*) are characterized by a green thallus resembling seaweed, consisting of upright branches with small spherical structures at the tips that resemble grapes[1].

Sea grapes (*Caulerpa racemosa*) are widely utilized as a food source, often consumed

as fresh vegetables, and they also possess potential medicinal properties, including antibacterial activity. However, particularly in Sulawesi, although *C. racemosa* is relatively easy to cultivate, many local communities remain unaware of its beneficial properties and the bioactive compounds contained within this marine algae[1].

Propionibacterium acnes is a Gram-positive bacterium that constitutes part of the normal flora of human skin, as well as the oral cavity, large intestine, conjunctiva, and external ear canal. This bacterium predominantly inhabits skin follicles and may contribute to the development of acne when it infects the skin[2].

Previous studies have reported that extracts of sea grapes (*Caulerpa racemosa*) exhibit inhibitory activity against bacterial growth[3]. Research has shown that the active compounds present in *C. racemosa* extract are capable of suppressing the growth of *Bacillus cereus* and *Escherichia coli* at concentrations of 20 µg, 40 µg, and 60 µg.

Considering this potential, the present study was conducted to evaluate the inhibitory activity of sea grapes (*Caulerpa racemosa*) against the growth of *Propionibacterium acnes*.

2. RESEARCH METHODS

Tools and Materials

The equipment used in this study included tools for sample collection, extraction, and antibacterial activity testing. The instruments consisted of a rotary evaporator (IKA RV 10), diving equipment, scissors, knives, Erlenmeyer flasks, measuring cylinders, beakers, spatulas, forceps, stirring rods, micropipettes, analytical balances, microtubes, test tubes, vials, glass containers, urine pots/tubes, refrigerators, autoclaves, incubators, Petri dishes (15 cm), inoculating loops, calipers, 8 mm paper discs (Advantec), gloves, laboratory coats, and a camera for documentation.

The materials used in this research included sea grape samples *(Caulerpa racemosa)*, the test bacterium *Propionibacterium acnes*, 95% ethanol as an extraction solvent, 70% alcohol for sterilization, Nutrient Agar (NA) as bacterial growth medium, Nutrient Broth (NB) for bacterial rejuvenation, sterile distilled water as a solvent and negative control, and 8 mm paper discs for inhibition testing.

Type and Experimental Design

This study was a laboratory experimental research aimed at determining the inhibitory activity of sea grape extract (*Caulerpa racemosa*) against bacterial growth. Antibacterial testing was performed using the agar diffusion method with paper discs (disc diffusion method), in which the formation of inhibition zones around extract-treated discs was observed.

The experimental design employed four extract concentration variations, with each treatment conducted in triplicate. The concentrations tested 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

Sterile distilled water (50 µL/disc) was used as a negative control. The antibacterial activity was determined based on the diameter of the inhibition zone formed on the bacterial growth medium.

Research Procedures

1. Collection of Sea Grape Samples (*Caulerpa racemosa*)
   Fresh green sea grape samples (*Caulerpa racemosa*) were collected from Basaan Beach, Southeast Minahasa Regency, North Sulawesi Province. The samples were washed under running water to remove sand and impurities, drained to reduce moisture content, and cut into smaller pieces to facilitate the extraction of active compounds. Approximately 2 kg of fresh samples were air-dried to obtain simplicia prior to maceration.
2. Preparation of Sea Grape Extract (*Caulerpa racemosa*)
   A total of 450 g of dried sea grape simplicia was extracted using the maceration method with 96% ethanol as the solvent. The maceration process was conducted for 2 × 24 hours and repeated twice. After the first immersion, the mixture was filtered using filter paper and a funnel to obtain the first filtrate and residue.
   The residue was re-immersed in ethanol and macerated again for 2 × 24 hours until two filtrates were obtained. All filtrates were combined and concentrated using a rotary evaporator at approximately 40°C to produce a thick extract. The extract was transferred

e-ISSN 2685-3167
p-ISSN 2828-6685

into tubes, weighed, and stored in a refrigerator until further antibacterial testing.

3. Preparation of Nutrient Broth (NB) for Bacterial Rejuvenation
   The test bacterium was obtained from the culture stock of the Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Kristen Indonesia Tomohon. Nutrient Broth was prepared by dissolving 0.8 g of NB powder in 100 mL of sterile distilled water in an Erlenmeyer flask and homogenized thoroughly.
   The NB solution was then poured into sterile test tubes. Stock cultures of *Propionibacterium acnes* were inoculated and incubated for 24 hours. Bacterial growth was indicated by turbidity in the broth.
4. Preparation of Nutrient Agar (NA)
   Nutrient Agar medium was prepared by dissolving 6.9 g of NA powder in 300 mL of sterile distilled water. The solution was homogenized using a magnetic stirrer and sterilized in an autoclave at 121°C for 15 minutes. The sterile NA medium was used for antibacterial testing.
5. Antibacterial Activity Assay (Disc Diffusion Method)
   Antibacterial activity was evaluated using the agar diffusion method with paper discs. The rejuvenated bacterial suspension was mixed with sterile NA medium, poured (±50 mL) into Petri dishes, and allowed to solidify.
   Paper discs were soaked in sea grape extract solutions at concentrations of 100 µg, 200 µg, 300 µg, and 400 µg, dissolved in 70% alcohol. The discs were dried for 24 hours in a desiccator.
   The dried discs were placed onto the surface of agar plates inoculated with *P. acnes* and incubated at 34–37°C for 24 hours. Antibacterial activity was assessed by measuring the inhibition zone around each disc. Each treatment was performed in triplicate.
6. Measurement of Inhibition Zone Diameter
   The inhibition zone was measured using a caliper by determining the clear area surrounding the disc. The diameter was calculated as the average of three measurements: vertical, horizontal, and diagonal[4].

   Formula:

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

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

Table 1. Classification of Antibacterial Activity[5]

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

Data Analysis

The inhibition zone diameter data obtained from the antibacterial activity test of sea grape (*Caulerpa racemosa*) extract against *Propionibacterium acnes* were statistically analyzed. If the data were normally distributed and homogeneous, a parametric One-Way ANOVA test was applied. However, if the data were not normally distributed or not homogeneous, a non-parametric Kruskal–Wallis test was used. All statistical analyses were performed using SPSS software version 22 with a 95% confidence level ($\alpha = 0.05$)[6].

3. RESULTS AND DISCUSSION

Preparation of Sea Grapes Extract (*Caulerpa racemosa*)

Sea grapes (*Caulerpa racemosa*) samples were collected from Basaan Beach, Southeast Minahasa Regency. The samples were then transported to the Laboratory of the Faculty of Mathematics and Natural Sciences for the extraction process. Prior to extraction, the samples were washed under running water, drained, and cut into small pieces to increase the surface area, thereby facilitating the extraction of bioactive compounds.

A total of 450 g of the sample was macerated using 4 L of 96% ethanol for four days, with two remaceration cycles. The maceration method was selected because it is simple and can prevent the degradation of heat-sensitive active compounds. Ethanol was used as the solvent due to its ability to dissolve both polar and non-polar compounds.

The maceration filtrate was filtered and concentrated using a rotary evaporator at 40°C until a thick, dark green extract was obtained, weighing 45 g. Evaporation at low temperature was performed to prevent decomposition of active compounds, as high temperatures may reduce the concentration of bioactive substances in the extract.[7] The extraction yield was calculated using the following formula:

$$\%\text{yield} = \frac{extract\ weight}{simplisia\ weight} \times 100\%$$

Table 2. Extraction Yield of Sea Grapes Extract

Sample	Sample Weight	Solvent	Extract Weight	Yield (%)
(1)	(2)	(3)	(4)	(5)
Sea grapes	450 g	96% ethanol	45 g	0.1

Antibacterial Inhibitory Activity Against *Propionibacterium acnes*

The antibacterial activity of sea grapes (*Caulerpa racemosa*) extract against *Propionibacterium acnes* was evaluated using the agar disc diffusion method. Paper discs with a diameter of 8 mm were impregnated with extract solutions at different concentrations and then dried for 24 hours in a vacuum desiccator.

After the Nutrient Agar (NA) medium inoculated with a suspension of *P. acnes* solidified in 15 cm Petri dishes, the extract-loaded discs were placed on the surface of the medium. The plates were then incubated at 34–37°C for 24 hours. Antibacterial activity was determined by the formation of a clear inhibition zone surrounding the discs.

In this study, four extract concentrations were tested: 100 µg/disc, 200 µg/disc, 300 µg/disc, and 400 µg/disc. Distilled water (aquadest) at 50 µL/disc was used as a negative control.[8]

Table 3. Inhibitory Activity of *Caulerpa racemosa* Extract Against *Propionibacterium acnes*

Extract Concentration	Replicate I (mm)	Replicate II (mm)	Replicate III (mm)	Mean (mm)
(1)	(2)	(3)	(4)	(5)
100 µg/disc	33.9	33.9	33.9	33.9
200 µg/disc	36.2	36.5	36.2	36.3
300 µg/disc	38.7	38.0	38.0	38.2
400 µg/disc	40.0	42.1	41.7	41.2
Control (Aquadest)	0	0	0	0

Based on Table 3, the ethanol extract of sea grapes (*Caulerpa racemosa*) exhibited very strong antibacterial activity against *Propionibacterium acnes*. The smallest inhibition zone was observed at 100 µg/disc with a mean diameter of 33.9 mm, while the largest inhibition zone was recorded at 400 µg/disc with a mean diameter of 41.2 mm.

These findings indicate that higher extract concentrations resulted in larger inhibition zones. The negative control (aquadest) showed no inhibition zone, confirming that the antibacterial effect was derived from active compounds present in the sea grapes extract. According to antibacterial activity classification, inhibition zones ≥20 mm are categorized as very strong.[5]

e-ISSN 2685-3167
p-ISSN 2828-6685

Figure 1. Petri Dish Showing Inhibition Zones of *Propionibacterium acnes* Treated with Crude Extract

Based on the results presented in Table 3 and Figure 1, the sea grapes extract effectively inhibited the growth of *Propionibacterium acnes* at all tested concentrations. The mean inhibition zone diameter increased from 33.9 mm at 100 µg/disc to 36.3 mm at 200 µg/disc, 38.2 mm at 300 µg/disc, and reached the highest value of 41.2 mm at 400 µg/disc. Meanwhile, the negative control showed no inhibition zone.

The curve shown in Figure 2 demonstrates that increasing extract concentration corresponded with an increase in inhibition zone diameter. This suggests a direct relationship between extract concentration and antibacterial activity against *P. acnes*. According to the classification criteria, the inhibition zones observed in this study fall within the very strong antibacterial activity category (≥20 mm).[5]

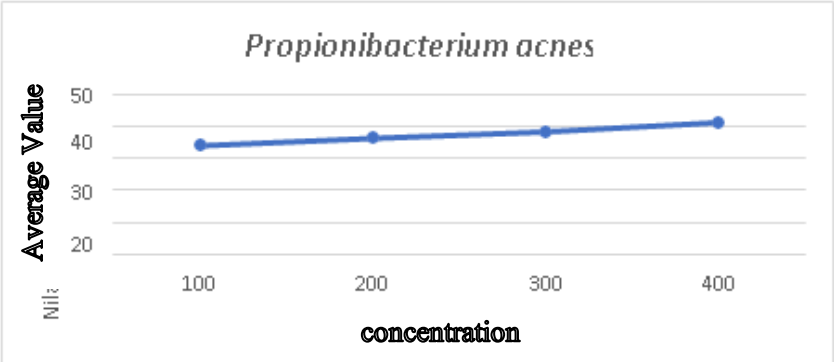

Figure 2. Inhibition Zone Activity Curve Against *Propionibacterium acnes*

Data Analysis (*Propionibacterium acnes*)

Statistical analysis of the inhibition zone data was preceded by normality and homogeneity tests. Data are considered normally distributed and homogeneous when the significance value is >0.05. However, the results indicated a p-value of 0.000, suggesting that the data were not normally distributed and not homogeneous ($p < 0.05$). Therefore, further analysis was conducted using the non-parametric Kruskal–Wallis test.

Table 4. Normality Test Results for *P. acnes*

Test Method	Statistic	df	Sig.
(1)	(2)	(3)	(4)
Kolmogorov–Smirnov	0.141	12	0.200
Shapiro–Wilk	0.923	12	0.310

e-ISSN 2685-3167
p-ISSN 2828-6685

The homogeneity test showed that the inhibition zone data were not homogeneous, with a significance value of 0.007 ($p < 0.05$). Consequently, the Kruskal–Wallis test was selected as an alternative to ANOVA because it does not require equal variances.[9]

The Kruskal–Wallis analysis yielded a significance value of $p = 0.014$, indicating a statistically significant difference among treatment groups.

Table 5. Kruskal–Wallis Test Results for *P. acnes*

Test Statistics[a,b]	
	Inhibitory Power of P. acnes
(1)	**(2)**
Chi-Square	10,607
Df	3
Asymp. Sig.	
a. Kruskal Wallis Test	0,014
b. b. Grouping Variables: Treatment	

Table 6. Mann–Whitney Post Hoc Test Results

Treatment	100 µg/disc	200 µg/disc	300 µg/disc	400 µg/disc
(1)	**(2)**	**(3)**	**(4)**	**(5)**
100 µg/disc	—	0.034	0.034	0.037
200 µg/disc		—	0.043	0.046
300 µg/disc			—	0.046
400 µg/disc				—

The Mann–Whitney post hoc test revealed significant differences between all concentration groups ($p < 0.05$). Thus, it can be concluded that the inhibitory activity of *C. racemosa* extract against *P. acnes* differed significantly across all treatments.

Bioactive Compounds and Antibacterial Mechanism

The antibacterial activity of *Caulerpa racemosa* is attributed to the presence of flavonoids and alkaloids. Flavonoids inhibit bacterial growth by disrupting nucleic acid synthesis, damaging cytoplasmic membrane function, and interfering with bacterial energy metabolism. Alkaloids, on the other hand, affect peptidoglycan synthesis, preventing proper cell wall formation and leading to bacterial cell death.[10,11]

The inhibition zones produced at each concentration confirm that *Caulerpa racemosa* extract has potential as an anti-*Propionibacterium acnes* agent.

4. CONCLUSION

Based on the results of this study on the inhibitory activity of ethanol extract of sea grapes (*Caulerpa racemosa*) against *Propionibacterium acnes*, it can be concluded that:

1. *Caulerpa racemosa* extract exhibited very strong antibacterial activity against *Propionibacterium acnes* at all tested concentrations, with inhibition zone diameters ranging from 33.9 mm to 41.2 mm.
2. A clear correlation was observed between increasing extract concentration and increased inhibition zone diameter. The highest inhibitory effect was achieved at 400 µg/disc.
3. Statistical analysis demonstrated significant differences among concentration treatments. The Kruskal–Wallis test produced $p = 0.014$ ($p < 0.05$), and the Mann–Whitney post hoc test confirmed that each concentration differed significantly from the others.

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