

Antibacterial Activity of Macroalgae (*Hypnea charoides*) from Lumintang Coastal Waters, Southeast Minahasa Regency Against *Helicobacter pylori*

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ABSTRAK

Makroalga merupakan salah satu sumber daya hayati laut yang kaya akan senyawa bioaktif dan berpotensi dimanfaatkan sebagai sumber antibakteri alami. Salah satu jenis makroalga yang tumbuh di Perairan Pantai Lumintang, Kabupaten Minahasa Tenggara, adalah *Hypnea charoides*. Makroalga ini diketahui mengandung berbagai metabolit sekunder, seperti alkaloid, flavonoid, tanin, saponin, dan senyawa fenolik yang memiliki potensi dalam menghambat pertumbuhan bakteri patogen. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri ekstrak *Hypnea charoides* terhadap bakteri *Helicobacter pylori*. Proses ekstraksi sampel dilakukan dengan metode maserasi menggunakan etanol 95% sebagai pelarut. Pengujian aktivitas antibakteri dilakukan dengan metode difusi cakram pada empat variasi konsentrasi, yaitu 100, 200, 300, dan 400 µg/disk, masing-masing dengan tiga kali pengulangan. Diameter zona hambat yang terbentuk diukur menggunakan jangka sorong dan selanjutnya dianalisis secara deskriptif. Hasil pengujian menunjukkan bahwa seluruh konsentrasi ekstrak mampu menghambat pertumbuhan *H. pylori* dengan rata-rata diameter zona hambat masing-masing sebesar 10,71 mm; 11,37 mm; 11,37 mm; dan 11,38 mm. Sementara itu, kontrol negatif berupa akuades tidak menunjukkan adanya zona hambat. Temuan penelitian ini membuktikan bahwa ekstrak *Hypnea charoides* memiliki aktivitas antibakteri terhadap *Helicobacter pylori*. Oleh karena itu, makroalga ini berpotensi untuk dikembangkan lebih lanjut sebagai sumber senyawa antibakteri alami yang berasal dari ekosistem laut.

Kata kunci: *Hypnea charoides*, makroalga, aktivitas antibakteri, *Helicobacter pylori*, difusi cakram.

ABSTRACT

Marine macroalgae represent a valuable source of bioactive compounds and have attracted increasing attention as potential natural antibacterial agents. One of the macroalgal species inhabiting the coastal waters of Lumintang Beach, Southeast Minahasa Regency, is *Hypnea charoides*. Previous studies have reported that this species contains a variety of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which are associated with antibacterial properties. The present study was conducted to investigate the antibacterial activity of *H. charoides* extract against *Helicobacter pylori*. Extraction was carried out using the maceration technique with 95% ethanol as the extraction solvent. Antibacterial activity was evaluated by the disc diffusion method using extract concentrations of 100, 200, 300, and 400 µg/disc, with each treatment performed in triplicate. Inhibition zone diameters were measured using a caliper and subsequently analyzed descriptively. The findings revealed that all tested concentrations exhibited inhibitory activity against *H. pylori*, with mean inhibition zone diameters of 10.71 mm, 11.37 mm, 11.37 mm, and 11.38 mm, respectively. No inhibition zone was observed in the negative control treatment containing distilled water. These results indicate that *H. charoides* extract possesses antibacterial activity against *H. pylori*. Accordingly, this macroalga may serve as

a promising marine-derived source of natural antibacterial compounds for further pharmaceutical exploration and development.

Keywords: *Hypnea charoides*, macroalgae, antibacterial activity, *Helicobacter pylori*, disc diffusion method.

1. INTRODUCTION

Indonesia is recognized as one of the countries with the richest marine biodiversity in the world, including a wide diversity of macroalgae distributed throughout its coastal regions. Macroalgae play important ecological roles in maintaining the balance of marine ecosystems, serving as primary producers, providing habitats for various marine organisms, acting as food sources for herbivorous biota, and contributing to nutrient cycling and carbon sequestration in aquatic environments^{1,2}. In addition to their ecological functions, macroalgae are also known to possess significant potential as sources of bioactive compounds that can be utilized in the food, cosmetic, and pharmaceutical industries.

Macroalgae produce a variety of primary and secondary metabolites with biological activities. Primary metabolites such as polysaccharides, vitamins, minerals, agar, alginates, and carrageenans have been widely utilized in various industries. Meanwhile, secondary metabolites including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds are known to exhibit antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, and anticancer activities^{3,4}. Numerous studies have demonstrated that these compounds can inhibit the growth of pathogenic microorganisms through different mechanisms, making macroalgae a promising source of natural antibacterial agents.

One species of macroalgae found in the coastal waters of Lumintang Beach is *Hypnea charoides*. This macroalga belongs to the red algae group (Rhodophyta) and commonly grows on coral and rocky substrates in coastal areas. Previous studies have reported that *H. charoides* contains various secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which possess potential antibacterial properties^{5,6}. Alkaloids are known to interfere with peptidoglycan synthesis in bacterial cell walls, flavonoids can disrupt cell membranes and inhibit nucleic acid synthesis, whereas tannins

are capable of precipitating cellular proteins, thereby inhibiting bacterial growth⁷⁻⁹.

In addition to its potential as a source of antibacterial compounds, *H. charoides* has also been reported to possess significant anti-inflammatory and antioxidant activities¹⁰. Beyond these biological benefits, macroalgae play a crucial role in maintaining aquatic ecosystem stability as primary producers that support marine food webs and nutrient cycling.

One pathogenic bacterium that remains a major global health concern is *Helicobacter pylori*. This Gram-negative, spiral-shaped bacterium colonizes the human gastric mucosa and is recognized as a primary causative agent of chronic gastritis, peptic ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma^{11,12}. The World Health Organization (WHO) has classified *H. pylori* as a Group I carcinogen due to its association with the development of gastric cancer. It is estimated that more than 50% of the world's population is infected with *H. pylori*, with a higher prevalence observed in developing countries than in developed nations¹¹.

The treatment of *H. pylori* infection generally involves a combination of antibiotics and proton pump inhibitors (PPIs). However, the increasing resistance of *H. pylori* to commonly used antibiotics, such as clarithromycin, metronidazole, and levofloxacin, has led to declining eradication success rates in many countries^{12,13}. This situation highlights the need to explore alternative antibacterial sources derived from natural products, including marine organisms rich in bioactive compounds.

Various studies have demonstrated the antibacterial activity of macroalgae against several pathogenic bacteria. However, information regarding the antibacterial potential of *Hypnea charoides* against *Helicobacter pylori* remains limited. Therefore, this study was conducted to evaluate the antibacterial activity of *H. charoides* collected from the coastal waters of Lumintang Beach, Southeast Minahasa Regency, against *H. pylori* using the disc diffusion method. The findings of this study are

expected to provide scientific evidence regarding the potential of *H. charoides* as a natural antibacterial source that may be utilized in the development of pharmaceutical and healthcare products.

2. RESEARCH METHOD

Time and Location of the Study

This study was conducted from March to June 2025. Samples of the macroalga *Hypnea charoides* were collected from the coastal waters of Lumintang Beach, Bentenan Satu Village, Pusomaen District, Southeast Minahasa Regency (0°59'57"N; 124°53'29"E). All extraction procedures and antibacterial activity assays were carried out at the Laboratory of the Faculty of Mathematics and Natural Sciences (FMIPA), Indonesian Christian University Tomohon.

Instruments and Materials

The equipment used in this study included diving equipment, an IKA RV 10 rotary evaporator, an analytical balance, test tube racks, test tubes, inoculating loops, pipettes, micropipettes, sterile cotton, beakers, an autoclave, forceps, 10-cm Petri dishes, stirring rods, an incubator, a Bunsen burner, a caliper, a laminar air flow (LAF) cabinet, and a camera.

The materials used consisted of *Hypnea charoides* macroalgae, 95% ethanol, *Helicobacter pylori* bacterial cultures, Nutrient Agar (NA), Nutrient Broth (NB), sterile distilled water, and sterile paper discs with a diameter of 6 mm.

Research Design

This study employed a laboratory experimental design aimed at evaluating the antibacterial activity of *Hypnea charoides* extract against *Helicobacter pylori* using the disc diffusion method. Four extract concentrations and one negative control were tested, with each treatment performed in triplicate.

The treatments were as follows:

K1: *H. charoides* extract at 100 µg/disc

K2: *H. charoides* extract at 200 µg/disc

K3: *H. charoides* extract at 300 µg/disc

K4: *H. charoides* extract at 400 µg/disc

K(-): Sterile distilled water at 50 µL/disc

Sample Preparation and Macroalgal Extraction

Fresh *Hypnea charoides* samples collected from the study site were washed

thoroughly under running water to remove sand, salt, and attached organisms. The samples were then cut into small pieces and dried. After drying, the samples were extracted by maceration using 95% ethanol for 48 hours, with the extraction process repeated twice.

The resulting filtrate was filtered and concentrated using a rotary evaporator at 40°C until a viscous crude extract was obtained. The extract was subsequently stored in a refrigerator until further use in the antibacterial activity assay.

Preparation of Media and Bacterial Culture

All laboratory equipment was sterilized in an autoclave at 121°C for 15 minutes. Nutrient Broth (NB) was used for bacterial culture rejuvenation, whereas Nutrient Agar (NA) was used as the growth medium for antibacterial activity testing.

Nutrient Broth (NB) was employed for the maintenance and propagation of bacterial cultures prior to antibacterial testing, while Nutrient Agar (NA) served as the culture medium for antibacterial assays using the disc diffusion method. Nutrient Agar is a general-purpose medium widely used in microbiological studies to support the growth of various bacterial species and to facilitate the observation of inhibition zone formation during antibacterial testing^{15,16}.

As a commonly used medium in antibacterial activity assays, Nutrient Agar supports the growth of a broad range of bacteria and allows inhibition zones to be clearly observed using the disc diffusion method^{15,16}. Nutrient Broth was used to cultivate and propagate bacterial cultures prior to antibacterial testing.

Helicobacter pylori cultures obtained from the laboratory collection were rejuvenated in Nutrient Broth and subsequently inoculated onto Nutrient Agar for antibacterial activity evaluation.

Antibacterial Activity Assay

The antibacterial activity of *Hypnea charoides* extract against *Helicobacter pylori* was evaluated using the disc diffusion method. The bacterial culture was evenly inoculated onto the surface of sterile Nutrient Agar plates, after which sterile paper discs (6 mm in diameter) containing different concentrations of the extract were placed on the agar surface.

Following incubation at 34–37°C for 24 hours, antibacterial activity was determined based on the formation of inhibition zones surrounding the discs. The diameter of the inhibition zone indicated the ability of *H. charoides* extract to inhibit the growth of *H. pylori*.

Measurement of Inhibition Zone Diameter

The inhibition zone diameter was measured using a caliper in three directions: vertical, horizontal, and diagonal. The average inhibition zone diameter was calculated using the following formula according to the referenced method¹⁷:

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

Where:

D = Average inhibition zone diameter (mm)

A = Vertical diameter (mm)

B = Horizontal diameter (mm)

C = Diagonal diameter (mm)

Classification of Antibacterial Activity

The antibacterial activity of *Hypnea charoides* extract was classified according to the following criteria¹⁸:

Table 1. Classification of Antibacterial Activity

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 diameters obtained from each treatment were averaged and analyzed descriptively. The results were presented in tables and graphs to illustrate differences in inhibition zone diameters among the various concentrations of *Hypnea charoides* extract against *Helicobacter pylori*. The antibacterial activity was interpreted based on the inhibition zone classification criteria¹⁹.

3. RESULTS AND DISCUSSION

The antibacterial activity of *Hypnea charoides* macroalgal extract against *Helicobacter pylori* was evaluated using the disc diffusion method on Nutrient Agar (NA) medium. Antibacterial activity was indicated by the formation of a clear zone (inhibition zone) surrounding the discs containing the extract. A larger inhibition zone diameter reflects a greater ability of the extract to inhibit bacterial growth.

Table 1. Antibacterial Activity of *Hypnea charoides* Extract Against *Helicobacter pylori*

Treatment	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean (mm)
(1)	(2)	(3)	(4)	(5)
100 µg/disc	10.05	10.03	12.06	10.71
200 µg/disc	10.05	12.00	12.06	11.37
300 µg/disc	10.01	10.03	12.07	11.37
400 µg/disc	10.02	10.05	12.07	11.38
Distilled water (Control)	0	0	0	0

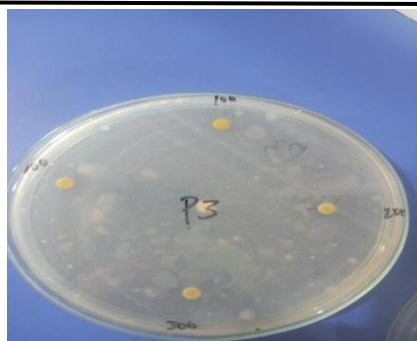


Figure 1. Tested Petri Cup Bacterial Inhibition Zone

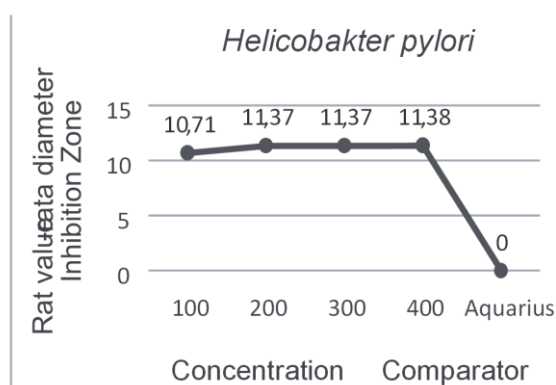


Figure 2. Inhibition Zone Diameter of *Hypnea charoides* Extract Against *Helicobakter pylori*

Based on the results presented in Table 1, Figure 1, and Figure 2, all tested concentrations of *H. charoides* extract exhibited inhibitory activity against *H. pylori*, as indicated by the formation of inhibition zones around the discs. The inhibition zone diameters ranged from 10.71 to 11.38 mm. The concentration of 100 µg/disc produced a mean inhibition zone diameter of 10.71 mm, while the concentrations of 200 µg/disc and 300 µg/disc yielded identical mean inhibition zone diameters of 11.37 mm. The largest inhibition zone was observed at the concentration of 400 µg/disc, with a mean diameter of 11.38 mm. In contrast, the negative control consisting of sterile distilled water did not produce any inhibition zone, confirming that the observed antibacterial activity originated from the bioactive compounds present in the *H. charoides* extract.

According to the antibacterial activity classification, inhibition zone diameters ranging from 10 to 20 mm are categorized as strong antibacterial activity¹⁸. Therefore, all tested concentrations of *H. charoides* extract demonstrated strong antibacterial activity against *H. pylori*. These findings indicate that the extract possesses the ability to inhibit the growth

of this pathogenic bacterium, which is associated with various gastric disorders.

An increase in extract concentration tended to be accompanied by a slight increase in inhibition zone diameter. Although the increase was not substantial, this trend suggests that higher extract concentrations resulted in greater diffusion of active compounds into the medium, thereby enhancing bacterial growth inhibition. Similar findings have been reported in previous studies, indicating that antibacterial activity is influenced by extract concentration and the quantity of bioactive compounds present within the extract⁹.

The antibacterial activity of *H. charoides* is likely associated with its secondary metabolite content. Previous phytochemical screening studies have demonstrated that this macroalga contains alkaloids, flavonoids, tannins, saponins, and phenolic compounds⁵. Alkaloids are known to inhibit peptidoglycan synthesis in bacterial cell walls, leading to structural defects and eventual cell damage¹⁹. Flavonoids exert antibacterial effects by inhibiting nucleic acid synthesis, disrupting cytoplasmic membranes, and interfering with bacterial energy metabolism⁷. In addition, flavonoids can form complexes with extracellular proteins and

bacterial cell walls, thereby enhancing their antibacterial efficacy.

Tannins present in *H. charoides* are also believed to contribute to its antibacterial activity. These compounds can bind to essential proteins and enzymes within bacterial cells, inhibiting metabolic processes and cellular replication. Furthermore, tannins may interfere with bacterial genetic material, thereby suppressing microbial growth and development⁷. Saponins have been reported to increase cell membrane permeability, resulting in leakage of intracellular components and ultimately bacterial cell death⁶. Meanwhile, phenolic compounds function as antimicrobial agents through protein denaturation and disruption of microbial cell membrane integrity.

The antibacterial activity demonstrated by *H. charoides* extract in this study indicates that this macroalgae has promising potential as a natural resource for inhibiting the growth of *H. pylori*. Considering the increasing incidence of antibiotic resistance in *H. pylori*, the utilization of bioactive compounds derived from marine resources such as macroalgae represents a promising alternative approach. The findings of this study further support the biological potential of *H. charoides* as a natural source of antibacterial compounds obtained from the coastal waters of Lumintang Beach, Southeast Minahasa Regency.

4. CONCLUSION

Based on the results of this study, it can be concluded that the macroalgal extract of *Hypnea charoides* collected from the coastal waters of Lumintang Beach, Southeast Minahasa Regency, exhibited antibacterial activity against *Helicobacter pylori*. This activity was demonstrated by the formation of inhibition zones at all tested extract concentrations, namely 100, 200, 300, and 400 µg/disc. The mean inhibition zone diameters obtained were 10.71 mm, 11.37 mm, 11.37 mm, and 11.38 mm, respectively. The largest inhibition zone diameter was observed at a concentration of 400 µg/disc, whereas the smallest inhibition zone diameter was recorded at a concentration of 100 µg/disc.

The findings of this study indicate that *Hypnea charoides* has promising potential as a natural source of antibacterial compounds capable of inhibiting the growth of *Helicobacter pylori*. Therefore, further studies are warranted

to identify and characterize the bioactive compounds responsible for its antibacterial activity and to evaluate their potential applications in the development of pharmaceutical and healthcare products.

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