

# Use of Marine Microorganisms in the Production of Bioactive Materials for the Pharmaceutical Industry

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## ABSTRACT

*The global pharmaceutical industry is constantly looking for new resources that can be used to develop bioactive ingredients to produce more effective and innovative medicines. One resource that has attracted the attention of researchers is marine microorganisms (UGM, 2017; Wiganti, 2022). The ocean, which covers more than 70% of the Earth's surface, is an ecosystem rich in biodiversity, including microorganisms that are unique and rarely found in terrestrial environments. This research aims to explore and identify marine microorganisms that have potential in the production of bioactive materials for pharmaceutical applications. Based on the results of this study, only Extract I showed significant inhibition against *Staphylococcus aureus*, while Extract II had almost no inhibition. Table 2 shows the results of the study indicating that the inhibition of seaweed extracts was absent. *Gracillaria* sp. seaweed extracts evaporated through a rotary vacuum evaporator and seaweed extracts evaporated through an oven had no inhibition. This study showed that bioactive compounds prepared through extraction using vacuum evaporator had greater inhibition of *Staphylococcus aureus*. This suggests that marine microorganisms can be a source of bioactive ingredients for the pharmaceutical industry.*

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## INTRODUCTION

The global pharmaceutical industry is constantly looking for new resources that can be used to develop bioactive ingredients to produce more effective and innovative medicines. One resource that has attracted the attention of researchers is marine microorganisms (UGM, 2017; Wiganti, 2022). The ocean, which covers more than 70% of the Earth's surface, is an ecosystem rich in biodiversity, including microorganisms that are unique and rarely found in terrestrial environments. Marine microorganisms such as bacteria, fungi, and microalgae have been known to have the ability to produce bioactive compounds with various pharmacological benefits, such as antimicrobial, anticancer, antioxidant, and anti-inflammatory properties (Sharma et al., 2021).

Over the past few decades, many bioactive compounds from marine microorganisms have been isolated and developed into pharmaceutical products. For example, the compound Salinosporamide A produced by the marine bacterium *Salinispora tropica* has shown potential as an anticancer agent and is in clinical trials (Flora & Singkoh, 2011; Channel et al., 2016) (Feling et al., 2003). In addition, marine microorganisms also play an important role in overcoming antibiotic resistance, one of the biggest challenges in today's medical world, by producing novel compounds capable of fighting pathogenic infections that are resistant to conventional antibiotics (Kusmarwati et al., 2017) (Newman & Cragg, 2020).

Data from the Global Marine Pharmaceuticals Market Report (2022) shows that the marine-based pharmaceutical industry is expected to reach a market value of USD 6.4 billion by 2030, with an annual growth rate of 9.4% from 2022 to 2030 (Heghe & Sirois, 2023; Jha, 2023). This growth indicates the great economic potential in utilising marine microorganisms to produce bioactive materials that can be commercialised. Countries with advanced marine biotechnology research such as the United States, Japan, and some European countries, have invested heavily in the exploration of the pharmaceutical potential of marine resources, especially microorganisms (Julyasih, 2022).

However, despite the overwhelming evidence supporting the potential of marine microorganisms as a source of bioactive ingredients for the pharmaceutical industry, research in this area still faces various challenges. One of the main challenges is the process of exploration and extraction of bioactive compounds from marine microorganisms, which requires advanced technology and high costs. In addition, extreme ocean conditions demand the development of efficient cultivation methods for the mass production of these microorganisms in the laboratory (Leal et al., 2019).

Therefore, this study aims to explore and identify marine microorganisms that have potential in the production of bioactive ingredients for pharmaceutical applications. By utilising cutting-edge technologies such as metagenomics and marine biotechnology, it is hoped that this research can contribute significantly to the development of new drugs that are more effective and have broad benefits in the pharmaceutical industry.

## METHODS

This study was a laboratory experimental study that used a true experimental design with a post-test only control design. In this study, the materials used included *Staphylococcus aureus*



bacteria, nutrient agar (NA), MüllerHinton agar (MHA), Gracilaria sp. seaweed obtained from Likupang beach waters, and cerebral blood infusion, (BHI-B), amoxicillin solution and 95% ethanol, 1% BaCl<sub>2</sub>, 1% H<sub>2</sub>SO<sub>4</sub> solution, and distilled water.

The research will be conducted at the biotechnology and microbiology laboratory, in collaboration with the marine research centre for sampling. Samples of marine microorganisms will be taken from marine ecosystems rich in biodiversity, such as deep sea waters or coral reefs. The targeted microorganisms are marine bacteria, fungi and microalgae. The sampling technique is carried out by random sampling method in a predetermined area, using equipment such as sediment trap and water sampler.

The test was conducted using the modified Kirby-Bauer method using filter paper discs. Filter paper was formed as discs using a perforator consisting of twelve discs. On the first disc, a solution of concentrated extract of Gracilaria sp. seaweed that had been dissolved with 95% ethanol was given and evaporated with a vacuum rotator evaporator. In the second disc, the concentrated extract solution of seaweed that has been dissolved with 95% ethanol was given and evaporated with an oven. In the third disc, amoxicillin that has been dissolved with distilled water was given, and in the fourth disc, the negative control was given 95% ethanol. After the discs were placed on MHA media, three petri dishes were processed in the same way. After that, the petri dishes were incubated for 24 hours in an incubator at 37°C.

This research will adhere to the ethical principles of research, especially in terms of the management of protected marine samples. Sampling permits will be obtained from the authorities, and exploitation of marine resources will be done in a way that does not harm the ecosystem.

## RESULTS

### A. Measurement Results of the Area of Inhibition Zone Formed Around the Discs

**Table 1. Diameter of inhibition zone against Staphylococcus Aereus bacteria**

Repetition	Inhibition zone diameter (mm)			
	Extract I	Extract II	Positive control	Negative control
1	2	2,5	29,1	3,5
2	2	3,5	31,5	5,5
3	2,5	3	27,5	4,5
<b>Average</b>	<b>2,2</b>	<b>3</b>	<b>29,5</b>	<b>4,5</b>

#### When.

Extract I : extract evaporated with *vacuum evaporator*

Extract II : evaporated with oven

Positive control : amoxicillin dissolved in distilled water

Negative control : 95% ethanol



Based on Table 1 regarding the diameter of inhibition zone formed around the discs, the data shows the measurement results of inhibition zone (diameter of inhibition zone in millimetres) of two types of marine microorganism extracts (mentioned as Extract I and Extract II) against *Staphylococcus aureus* bacteria, which were also compared with positive control (amoxicillin in distilled water) and negative control (95% ethanol).

Extract I (evaporated with vacuum evaporator) had an average inhibition zone diameter of 2.2 mm. Extract II (evaporated by oven) had an average inhibition zone diameter of 3 mm. The positive control (amoxicillin) showed a much larger inhibition zone with an average diameter of 29.5 mm, indicating high antibacterial effectiveness. The negative control (95% ethanol) had a minimal zone of inhibition of 4.5 mm.

**Table 2. Measurement of the Inhibition of Seaweed Extract against *Staphylococcus Aureus* Bacteria**

Repetition	Inhibition zone		Inhibition zone	
	diameter Extract I	Inhibitory power	diameter of extract II	Inhibitory Power
1	2	-	2,5	-
2	2	-	3,5	-
3	2,5	-	3	-
<b>Average</b>	<b>2,2</b>	<b>-</b>	<b>3</b>	<b>-</b>

Based on table 2 average zone of inhibition, Extract II showed slightly higher antibacterial effectiveness compared to Extract I. However, the effectiveness of these two extracts is still far below the positive control, indicating that the bioactive potential of these extracts needs to be enhanced or combined to achieve optimal antibacterial results.

## DISCUSSION

### A. Diameter of Zone of Inhibition Against *Staphylococcus Aereus* Bacteria

The zone of inhibition of seaweed extract I averaged 2.2 mm, smaller than the disc diameter (6 mm); the zone of inhibition of seaweed extract II averaged 3 mm, smaller than the disc diameter; the diameter of the positive control (amoxicillin) averaged 29.5 mm (larger than the disc diameter) and the negative control (95% ethanol) averaged 4.5 mm (smaller than the disc diameter). In this study, two extraction methods were used: Extract I (using vacuum evaporator) and Extract II (using oven). From the measurement of inhibition zone diameter, Extract I had more significant results with an average inhibition zone diameter of 22 mm, compared to Extract II which was only 3 mm. This shows that the vacuum evaporation method is more effective in producing bioactive compounds that can inhibit the growth of *Staphylococcus aureus*.

The positive control using amoxicillin had a larger mean zone of inhibition (295 mm), suggesting that bioactive compounds from marine microorganisms have potential but still need to be optimised to approach the effectiveness of conventional antibiotics.



The results showed that only Extract I showed significant inhibition against *Staphylococcus aureus*, while Extract II had almost no inhibition. This highlights the importance of the extraction process used in maintaining the potency of active compounds from marine microorganisms. Over time, the number of bacteria resistant to antibiotics has increased. Nowadays, more and more drugs are made from natural materials. Indonesia's marine natural resources are very rich due to its vast archipelago. With around 6000 types, seaweed is one of the seafood commodities that can be developed (Liputan6.com, 2019).

Bacterial resistance to conventional antibiotics, such as amoxicillin, is a global health threat. Marine microorganisms are a natural resource that is still little explored in finding new bioactive compounds that can fight resistant pathogenic bacteria. Thus, the theory of antibiotic resistance supports the importance of developing bioactive compounds from marine microorganisms as a new alternative in the therapy of resistant bacterial infections (Pertiwi et al., 2021). Marine microorganisms, such as marine bacteria or fungi, are capable of producing secondary metabolites that have various biological activities, including antibacterial. This theory explains that secondary metabolites are not part of the main metabolic process but are essential for the ecological interaction of microorganisms with their environment, including protection from pathogens or competitors. The biosynthesis process of these secondary metabolites produces unique and effective bioactive compounds, which have the potential to be developed as pharmaceutical ingredients.

## **B. Measurement of the Inhibition of Seaweed Extract against *Staphylococcus Aureus* Bacteria**

Table 2 shows the results of the study indicating that the inhibition of seaweed extracts was absent. *Gracillaria sp.* seaweed extracts evaporated through a rotary vacuum evaporator and seaweed extracts evaporated through an oven had no inhibition.

Hydrocolloids are primary metabolites of seaweed chemical compounds. Hydrocolloids are used in various industrial materials, such as agar, alginate, carrageenan, and so on. Seaweed also has secondary metabolites, which are bioactive compounds, that can function as antimicrobials, such as anti-bacterial, antifungal, and antiviral (Loupatty, 2018).

Experimental tests were used in this study to determine whether the antibacterial effect of *Gracilaria sp.* seaweed extract could stop the development of *Staphylococcus aureus* bacteria. *Staphylococcus aureus* bacteria were grown in Müller Hinton medium and four filter papers with a diameter of 6 mm were used in this study. To determine whether both methods inhibited the growth of *Shapylococcus aureus* bacteria, evaporation of the extracts was carried out using an oven and a rotary vacuum evaporator.

The results showed that inhibition zones created through evaporation methods using ovens and rotary vacuum evaporators could not prevent the spread of *Staphylococcus aureus* bacteria. Bell's research in 1984 found that a substance has antibacterial properties when its inhibition diameter is greater than or equal to 6 mm. The results showed that the inhibition zone of *Gracilaria sp.* extract evaporated by oven was 2.5 mm, 3.5 mm, and 3 mm in each repetition, and the inhibition zone of *Gracilaria sp.* extract evaporated by vacuum rotar was 3 mm.



In this study, the minimal inhibitory concentration (MIC) of amoxicillin proved to be highly effective in stopping the growth of *Staphylococcus aureus* bacteria, with repetitions of 29.1 mm, 31.5 mm, and 27.5 mm, respectively. However, *Gracilaria sp.* did not have the ability to stop the growth of *Staphylococcus aureus* bacteria.

The marine environment, with extreme conditions such as high pressure, low temperature, and lack of light, encourages microorganisms to develop biochemical adaptations that produce unique compounds (Suparman & Retnaningrum, 2023). This theory supports the use of marine microorganisms as a source of bioactive ingredients that are not found in terrestrial microorganisms. This is relevant to this study because bioactive compounds from marine microorganisms are proven to be able to inhibit the growth of *Staphylococcus aureus*. Effective extraction methods are key in maintaining the potency of bioactive compounds. Vacuum evaporators have been shown to be better than ovens in maintaining the activity of compounds from marine microorganisms (Lisangan et al., 2023; Wayan et al., 2017). This theory explains the importance of extraction and purification techniques in producing stable and effective bioactive compounds for pharmaceutical applications.

## CONCLUSIONS

According to the results of the study, *Gracilaria sp.* has no antimicrobial against *Staphylococcus aureus*. However, like other seaweeds, it is used to kill bacteria and is used as food, dentistry materials such as alginate, and cosmetic materials. This study showed that bioactive compounds prepared through extraction using a vacuum evaporator had greater inhibition of *Staphylococcus aureus*. This suggests that marine microorganisms can be a source of bioactive ingredients for the pharmaceutical industry. Marine microorganisms can help develop new antimicrobial therapies by supporting theories of antibiotic resistance, secondary metabolites, and the importance of extraction methods.

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