

## Potential of Red Algae *Eucheuma spinosum* as Antibacterial to *Pseudomonas aeruginosa*

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### ABSTRACT

**Introduction:** *Eucheuma spinosum* is one of Indonesia's potential marine resources that can be used as an antimicrobial. The red algae *E. spinosum* contains many secondary metabolites which can inhibit the growth of bacteria. This research aimed to determine the optimum inhibitory power of the red algae extract *E. spinosum* using n-hexane, ethyl acetate, and methanol solvents in inhibiting the growth of *Pseudomonas aeruginosa* bacteria.

**Method:** The extraction process uses the maceration method with n-hexane, ethyl acetate, and methanol as solvents. Antibacterial testing was carried out using the paper disk diffusion method with soaking for 1 hour and incubating for 24 hours. The extract with the largest clear zone diameter was then tested further using Gas Chromatography-Mass Spectrometry (GCMS) to investigate the compound content in the sample.

**Results:** The solvent that produced the largest inhibition zone diameter was ethyl acetate extract, namely 16.1 mm at a concentration of 4%. Analysis of the compounds contained in the ethyl acetate extract using GCMS showed the presence of hexadecanoic acid which is a terpenoid group.

**Conclusion:** The optimum concentration of red algae *E. spinosum* extract to inhibit the growth of *Pseudomonas aeruginosa* was 16.1 mm in 4% ethyl acetate solvent.

**Key words:** Antibacterial, *Eucheuma spinosum*, *Pseudomonas aeruginosa*



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## **Introduction**

Indonesia is a country whose vast territory is surrounded by ocean and is the country with the largest number of islands in the world. The huge potential of marine biota has made scientists and antibiotic producers in several countries interested in developing it as a potential antibiotic. This is due to the lack of maximum utilization of marine biota while the need for new types of antibiotics is increasingly urgent because the current antibiotics being marketed are decreasing in their effect on some bacteria which are increasingly becoming resistant to these antibiotics. One of the marine biota that can be utilized is red algae (*Eucheuma spinosum*).<sup>1</sup>

*E. spinosum* belongs to the Rhodophyceae group which is capable of producing carrageenan in large quantities.<sup>2</sup> This type of algae is used for traditional medicine because it does not cause side effects on health.<sup>3</sup> The ability of red algae *E. spinosum* to produce bioactive secondary metabolites are very likely due to extreme environmental conditions such as high salinity or used to defend itself from predators. Red algae *E. spinosum* contains bioactive compounds such as flavonoids, alkaloids, steroids, and triterpenoids which can treat various diseases in humans but are toxic to all bacteria.<sup>4,5</sup>

Bioactive compounds can inhibit the growth of bacteria due to inhibition of protein synthesis because they accumulate and cause changes in the components that make up the bacterial cell, which ultimately results in toxic effects on the bacteria.<sup>6</sup> Inhibition of bacterial growth can be seen by the size of the diameter of the inhibition zone produced on the paper disc which is then measured using a caliper or ruler to determine antibacterial effectiveness. The diameter of the inhibition zone is the diameter where there is no growth around the disc paper minus the diameter of the disc paper. The inhibitory activity against bacterial growth increases with the concentration extract.<sup>7</sup>

Cultivation is carried out in places where the current conditions are relatively calm so that productivity can be increased. Based on the background above, this research aims to determine the inhibitory power of the red algae *Eucheuma spinosum* extract against *Pseudomonas aeruginosa* bacteria.

## **Methods**

This research was conducted from January 25 to April 13, 2023 at the Biochemistry Laboratory and Analytical Chemistry Laboratory of Science and Technology at Universitas Islam Negeri Alauddin Makassar. The materials used are samples of red algae (*E. spinosum*), *Pseudomonas aeruginosa* bacteria, dimethyl sulfoxide (DMSO), methanol (CH<sub>3</sub>OH), n-

hexane, ethyl acetate, physiological sodium chloride (NaCl), Nutrient Agar (NA), Nutrient Broth (NB) and Oxoid disc paper.

### ***Preparation and Extraction of Red Algae (*E. spinosum*) Samples***

20 kg of red algae *E. spinosum* was washed with water and then sliced into small pieces. After that, let it air dry and grind it using a grinder to get the red algae powder *E. spinosum*. The weight of the dry red algae sample obtained was 273 gr. The samples were put into 3 different containers and then macerated using each solvent n-hexane, ethyl acetate, and methanol. Each container is then evaporated using a rotary evaporator. The thick extract obtained was diluted to various concentrations of 2%, 4%, and 6% using DMSO solvent.

### ***Making Nutrient Agar (NA) and Nutrient Broth (NB) Media***

The tools used were sterilized in an oven at 1700°C for  $\pm$  1 hour while the media was sterilized in an autoclave at 121°C for 15 minutes. Weigh 2.5 g of NA, then dissolve it in 100 mL of warm H<sub>2</sub>O, then put all the ingredients in an Erlenmeyer and sterilize in an autoclave for 15 minutes at 121°C and 1 atm pressure. Weigh 0.8 grams of NB in a beaker then dissolve it in 100 mL of warm distilled water. Sterilize in an autoclave at 121°C for 15 minutes and 1 atm pressure.

### ***Bacterial Rejuvenation Test***

The test bacteria used is *Pseudomonas aeruginosa*. Rejuvenation of bacteria is carried out by taking 1 cycle of pure culture and then transferring it to a petri dish containing NA. Incubation for 24 hours at 37°C.

### ***Preparation of Bacterial Suspension***

The bacterial culture resulting from rejuvenation was taken 1 cycle on NA media. Suspend it in NB media. Incubation for 24 hours at 37°C. Bacterial growth is characterized by turbidity in the suspended media.

### ***Making Antibacterial Positive and Negative Control Solutions***

A positive control was made by weighing 1 gram of amoxicillin and then diluting it in 2 mL of physiological NaCl then homogenizing. Negative control was made from 2 mL of distilled water

### ***Inhibitory Power Test of Red Algae *E. spinosum* Extract***

A total of 15 mL of agar media was poured into a sterile petri dish. Take 100  $\mu$ L of the bacterial culture in the suspension and pour it into a sterile petri dish and then homogenize it with agar media. Each extract at various concentrations of 2%, 4%, and 6% (w/v) and 100  $\mu$ L of the control solution were dropped onto sterile disc paper and left for 30 minutes. Place the dry paper discs regularly on the agar medium containing the test bacteria

and then label them. Incubate the petri dish containing the media and paper discs for 24 hours at 37°C. The inhibitory power of the extract was determined by subtracting the diameter of the inhibition zone formed from the diameter of the paper disc (6 mm).

### Compound Analysis using Gas Chromatography-Mass Spectrometry (GCMS)

1 µL of red algae extract *E. spinosum* was injected into GCMS. Identify the graph peaks at each retention time from the initial peak to the final peak and match them with the reference in the GCMS program by selecting a similar search.

### Results

The measurement of the diameter of the inhibition zone in the study used *Pseudomonas aeruginosa*, which is a pathogenic bacteria. The incubation process for 24 and 48 hours used the solvents n-hexane (Figure 1), ethyl acetate (Figure 2), and methanol (Figure 3). Figure 1 shows that at a concentration of 2% the initial diameter of 4.00 mm increased to 6.8 mm; concentration of 4% initial diameter of 4.3 mm increased to 6.2 mm; while at a concentration of 6% the initial diameter of 4.3 mm increased to 6.5 mm.

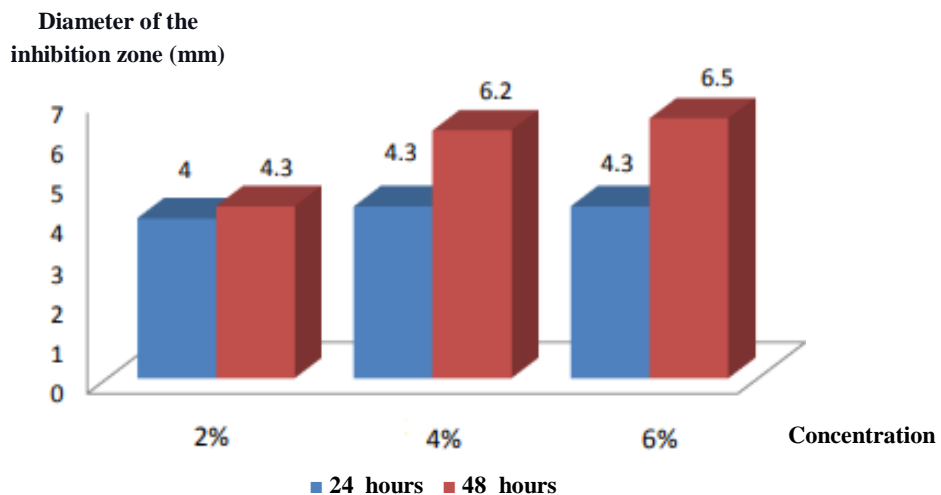


Figure 1. Diameter of the inhibition zone of n-hexane extract

Figure 2 shows the diameter of the inhibition zone of *E. spinosum* ethyl acetate extract produced with an incubation period of 24 and 48 hours. The largest diameter of the inhibition zone was at a concentration of 4%, namely 16.1 mm. Figure 3 shows the results of the inhibition zone diameter of the methanol extract of *E. spinosum* which was produced at a concentration of 2%, the initial diameter of 6.3 mm decreased to 4.7 mm, at a concentration of 4%, the initial concentration of 6.7 mm decreased to 4.1 mm and the concentration of 6. % initial diameter of 6.3 mm reduced to 4.6 mm.

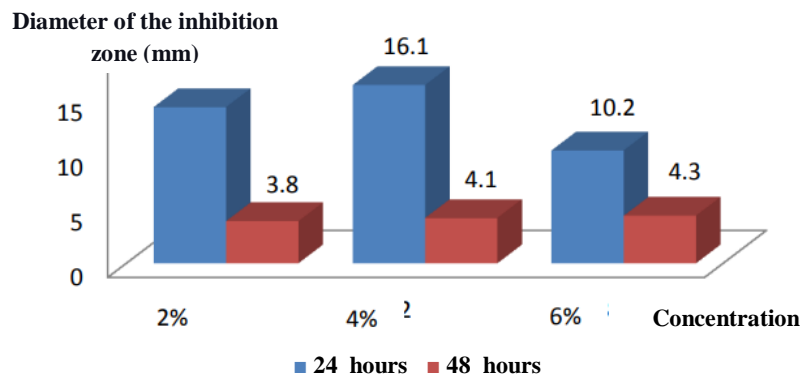


Figure 2. Diameter of the Inhibitory Zone of Ethyl Acetate Extract

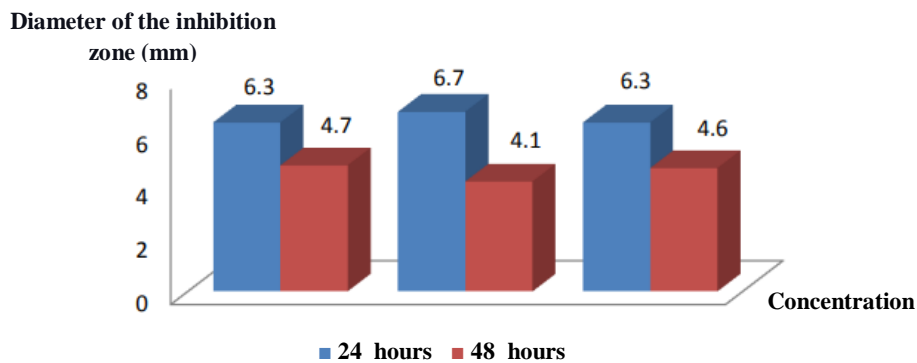


Figure 3. Diameter of the Inhibitory Zone of Methanol Extract

Analysis with GCMS can be divided into two groups, namely: qualitative and quantitative. Both analyses use a mass spectrometer as a detector. The mass spectrum of the results of the analysis of the mass spectroscopy system is an illustration of the type and number of molecular fragments formed from a chemical component (each peak on the chromatogram). The chemical content of the red algae *E. spinosum* was analyzed using KGSM as shown in Figure 4. The chromatogram results showed that the 7th peak had the highest percent area, namely 31.40% with a retention time of 20.23. The mass spectrum in Figure 5 shows the presence of molecular ion peaks, namely 256, 43, 60, 85, 15, 143, and a basic peak of 73, and is indicated as a hexadecanoic acid compound.

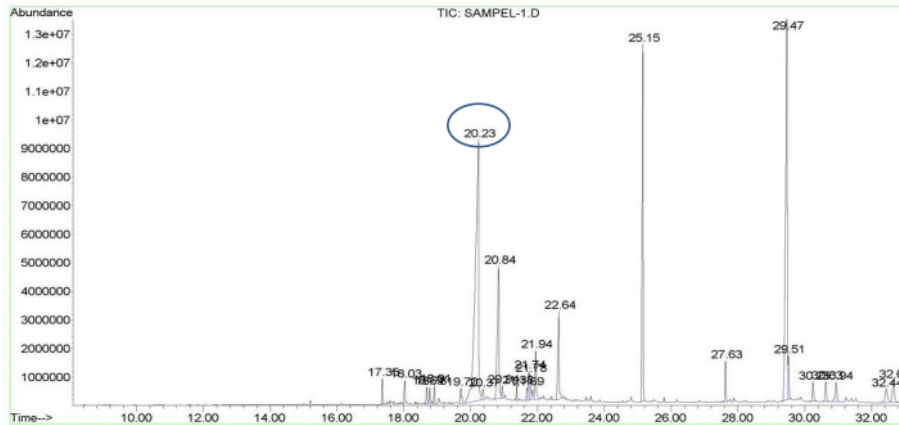


Figure 4. GCMS chromatogram of Ethyl Acetate Extract of Red Algae *E. spinosum*

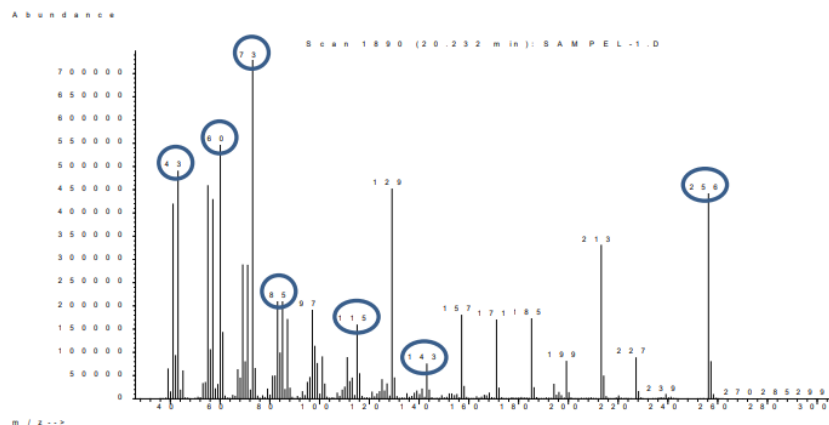


Figure 5. Mass spectrum of hexadecanoic acid at a retention time of 20.23

## Discussion

Red algae contain secondary metabolites with different polarities, namely non-polar, semi-polar and non-polar, so solvents with different polarities are also used to extract these compounds, such as n-hexane, ethyl acetate and methanol. The method used to extract these compounds is the maceration method.<sup>9</sup>

The maceration extraction method extracts a sample using an organic solvent with several simple stirs. The maceration method does not use heating so that the natural material samples used do not decompose or become damaged.<sup>10</sup> The nonpolar solvent used is n-hexane. The n-hexane solvent can attract nonpolar compounds found in the red algae *E. spinosum* extract, namely terpenoids.<sup>11</sup> The semi-polar solvent used is ethyl acetate. Ethyl acetate solvent can extract active antibacterial compounds. Ethyl acetate can attract semi-polar active compounds in the red algae *E. spinosum*, namely tannins. Tannins have antibacterial properties related to their ability to inactivate microbial cell adhesion, inactivate

enzymes, and interfere with protein transport in the inner layer of cells.<sup>12</sup> Meanwhile, the polar solvent used is methanol. Methanol can dissolve almost all organic compounds in polar and nonpolar compound samples. Methanol is volatile, so it is easily released from the extract. Red algae samples also contain polar secondary metabolite compounds, namely flavonoids. Flavonoids are a chemical compound in the red algae *E. spinosum*, which is bacteriostatic.<sup>9</sup> The thick extract obtained in each solvent was diluted at concentrations of 2%, 4% and 6% using DMSO.

According to the data above, a concentration of 2% makes the initial diameter 4.00 mm increased to 6.8 mm; at a concentration of 4%, the initial diameter of 4.3 mm increased to 6.2 mm; while at a concentration of 6%, the initial diameter of 4.3 mm increased to 6.5 mm. This is because at this concentration the extract is bacteriostatic against the test bacteria. An antimicrobial is bactericidal if the diameter of the inhibition zone increases at 48 hours of incubation, this is because the compound can kill and increase the physiological activity of the bacteria, even though the administration of the compound is stopped.<sup>11</sup> The size of the inhibition area is influenced by several factors, namely the growth rate of microorganisms, the ability and rate of diffusion of active ingredients in the medium, the sensitivity of microorganisms to the active substance, and the thickness of the viscosity of the medium.<sup>13</sup>

The 4% ethyl acetate extract has the optimum level of polarity. A compound that has an optimum level of polarity has maximum antibacterial activity because the interaction of antibacterial compounds with bacteria requires a balance (HLB: Hydrophilic Lipophilic Balance). Ethyl acetate extract at concentrations of 2% and 6% had an inhibition zone diameter of 14.1 mm and 10.2 mm smaller than 4%. This shows that higher concentrations will not always increase the inhibition zone formed. The diameter of the inhibition zone is not always directly proportional to the concentration of the extract.<sup>15</sup>

The chromatogram of the ethyl acetate extract of the red algae *E. spinosum* shows 25 peaks. However, there was one peak with a relatively high abundance that was analyzed by the mass spectrometer, namely at a retention time of 20.23 with an area percent (abundance) of 31.40%. Retention time shows how long it takes for a compound to move through the column to the detector. Hexadecanoic acid is a carboxylic acid derivative compound and is a saturated fatty acid composed of 16 carbon atoms ( $C_{16}H_{32}O_2$ ). The mechanism of action of hexadecanoic acid in inhibiting bacterial growth is that it can absorb nutrients in bacteria and can inhibit water and inhibit the enzyme systems of some bacteria.<sup>15</sup> The compound analysis carried out still focused on n-hexane, ethyl acetate, and methanol extracts so the chromatogram results showed many peaks.

## Conclusion

The optimum concentration of red algae *E. spinosum* extract to inhibit the growth of *Pseudomonas aeruginosa* bacteria was 16.1 mm in 4% ethyl acetate solvent.

## Conflicts of Interest

Nothing to declare

## Funding sources

Nothing to declare

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