The antibacterial inhibition test of gonad sea urchin \((diadema setosum)\) against the growth of \(staphylococcus aureus\)

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Abstract. A The sea urchin is sea animal the rich in vitamin A, vitamin B complex and minerals to the smooth functioning of the nervous system and the metabolism of the human body. Gonad sea urchin contains flavonoids, steroids, triterpenoids, and saponins. This research was conducted to determine inhibition of antibacterial ointment sea urchin gonads against Staphylococcus aureus. The type of research conducted is experimental. This research used a cylinder cup method to measure inhibition zone of gonad sea urchin gonad. After incubation for 24 hours, measured inhibitory zone diameter around the cylinder cup. The results showed that the sea urchin gonads ointment have inhibitory effect on the growth of Staphylococcus aureus bacteria with broad average inhibition zone produced at a concentration of 2% at 2.47 mm, 4% of 1.62 mm, and a concentration of 6% has inhibitory zone Of 1.12 mm, and a positive control of 11.55 mm and the negative control does not provide a drag zone. Based on the ANOVA test results can be concluded that the F test = 0.691 < F table = 3.48 to 0.05, this means significant treatment 3 repetitions in each of the samples showed that there was no significant difference for F count is smaller than F table.

Keywords: Antibacterial, Sea Urchin Gonad Ointment, \(staphylococcus aureus\)

1. Introduction

Sea urchin \((Diadema setosum)\) is a marine animal that is rich in vitamin A, vitamin B complex and minerals that can facilitate the functioning of the nervous system and metabolism of the human body [1]. Bacteria are a group of organisms that do not have a cell nucleus membrane. This organism is very small (microscopic). The bacteria that most often cause infections, one of which is \(Staphylococcus aureus\). \(Staphylococcus aureus\) is a normal bacterium in the skin and mucous membranes in humans. \(Staphylococcus aureus\) can be a cause of infection in both humans and animals [2].

\(Staphylococcus aureus\) is a normal bacterium in the skin and mucous membranes in humans. However, in certain conditions \(Staphylococcus aureus\) can be a cause of infection in both humans and animals. \(Staphylococcus aureus\) bacteria when infecting body tissues will produce pus which then tends to become an abscess. Some diseases caused by \(Staphylococcus aureus\) such as pneumonia, meningitis, arthritis, ulcers and others such as burns [3]. Until now, the prevention of diseases caused by bacteria including \(Staphylococcus aureus\) bacteria still relies on various types of antibiotics [4]. This raises concerns about the emergence of new strains of bacteria that are resistant to antibiotics [5].
Until now, the prevention of diseases caused by bacteria still relies on various antibiotics. This raises fears that new antibiotic resistant bacteria will emerge. Akerina et al (2015) in research entitled "Exploration of Antimicrobial Compounds and Antioxidants from Sea urchins (Diadema setosum)" Stating that active compounds produced by sea urchins have the potential to be used as natural antimicrobial compounds. Antibacterial activity showed that gonad extract of sea urchins had the highest antibacterial activity with inhibition zones 1.5 ± 0 mm against *Staphylococcus aureus* bacteria.

Sea urchin gonads contain flavonoids, steroids, triterpenoids and saponins. Steroids and triterpenoids are one component of the terpenoid group which has potential as an antibacterial. Bacterial growth will be inhibited by inhibiting protein synthesis and causing changes in the components of the bacterial cell itself [6].

Some previous studies related to the anti-bacterial inhibition test using sea urchin, only with shell extract from sea urchin. The novelty in this study is how to see the inhibition of sea urchin gonads from three levels of concentration by 2%, 4% and 6% using positive control and legative control and determine the best concentration in inhibiting bacteria. Besides the test bacteria in this study only focus on *Staphylococcus aureus* bacteria.

2. Materials and Methods

2.1. Materials

2.1.1. Tools used.

The tools used in this research were autoclaves, aluminum foil, stirring rods, petri dishes, 100 mL erlenmeyers, 50 mL chemical beakers, 10 mL and 50 mL measuring cups, hot plates, incubators, calipers, flannelette, rubber suckers, rubber lamps, lamps spiritus, laminar Air Fow (LAF), round loop, oven, 10 mL measuring pipette, 0.1 mL pipette, test tubes, digital scales.

2.1.2. Material used.

The ingredients used are *Staphylococcus aureus*, NA Media (Nutrient Agar), NaCl 0.9%, Ointment of sea urchin gonadal extract and Twen 20.

2.2. Methods

This research is an experimental research with Randomized Block Design (RCBD) with the statistical test used is the ANOVA test. This research aims to determine the antibacterial activity and inhibition of sea urchin gonadal ointment (*Deadema setosum*) on the growth of *Staphylococcus aureus* bacteria in several concentrations.

2.2.1. Sterilization of tools.

All heat-resistant devices are washed first, then wrapped in aluminum foil, then sterilized in an oven at 180 °C for 2 hours, while heat-resistant devices are sterilized in an autoclave at 121 °C, with a pressure of 1.5 - 2 atm for 15 minutes. Whereas for rounded nose and tweezers are sterilized by burning using direct fire until incandescent.

2.2.2. Dilution of sea urchin ointment sample 2%, 4%, 6% with a sample ratio 1 : 9.

Dilution of sea urchin samples in a row carried out as follows : weighed 1 gram of sea urchin gonad ointment, put in a beaker then, dissolved with 9mLtwen 20, stir until homogeneous, and labeled, the same thing is done for concentrations of 4% and 6% by adding twen 20 as much as 9 mL.

2.2.3. Making of NA (Nutrient Agar) media.
The making of NA (Nutrient Agar) media is carried out in the following stages: weighed 4.2 grams of nutrient agar (NA) and then put into an erlenmeyer then dissolved with 150 mL aquadest, stirring until dissolved using a stirring rod, moved above the water bath to boiling while stirring, sterilized in an autoclave at 121 °C for 15 minutes and cooled at room temperature.

2.2.4. Bacterial culture.

The bacterial culture is as follows: take one loop of Staphylococcus aureus bacteria using a sterilized loop; scratched on agar nutrient media (NA) by tilting and incubated at 37 °C for 18 hours.

2.2.5. Making of positive control.

The making of positive control is as follows: weighed 1 gram of oxytetracycline and put it in a beaker, dissolved with twen 20 little by little as much as 9 mL and stir until homogeneous and labeled.

2.2.6. Making a bacterial suspension.

In making a bacterial suspension follows the following steps: prepared tools and materials, as much as 1 ose culture of Staphylococcus aureus culture that has been rejuvenated in agar nutrient media (NA) is tilted; put in a test tube that contains 0.9% NaCl as much as 9 mL and shaken until homogeneous until bacterial suspension is obtained.

2.2.7. Antibacterial testing of sea urchin gonadal ointment against the growth of staphylococcus aureus bacteria by the cylinder cup method.

Take a 15 mL pipette of nutrient agar (NA) into a petri dish, then leave it until it solidifies. (Layer 1); Staphylococcus aureus bacteria that have been suspended with NaCl0.9% pipette 1 mL, then spread using a tubular stem over layer 1 (Layer 2); Put the cylinder cup on the surface of the media that has been compacted and then put the ointment extract of sea urchin gonad with concentrations of 2%, 4%, and 6% as much as 0.5 mL, as well as positive and negative controls on each cylinder cup; Incubate for 24 hours at 37 °C in an incubator and is removed from the incubator and observed area of Staphylococcus aureus growth inhibition by using calipers.

3. Results and Discussion

3.1. Observation Results of Antibacterial Inhibition Zones

This research uses the cylinder cup diffusion method which is a method carried out by placing several cylinders made of glass or stainless steel on media so that it has been inoculated with bacteria [7]. The research wanted to observe the presence or absence of zohambat inhibitory antibacterial ointment of sea urchin gonads (Diadema setosum) against Staphylococcus aureus bacteria. The observations of the inhibition zone test using the slinder cup method of three concentration levels, namely concentrations of 2%, 4% and 6% show the average values of inhibition zones varying from 1.12 mm - 2.47 mm (Figure 1).
In Figure 1, it appears that of the three treatments at three different concentrations showed a slight difference in each inhibition zone. From the results of this research it was found that from the three treatments, 2% concentration had a wider average inhibition zone area compared to the average inhibition zone area at concentrations of 4 and 6% of the three each treatment. This research is in accordance with the results of a research conducted by Akerina et al (2015) which stated that gonad extract of sea urchin / sea urchin had the highest antibacterial activity with inhibition zone values of 1.5 mm. Research by Akerina et al (2015) also shows that lower solvent concentrations have wider inhibition zones and high solvent concentrations have smaller tasteless zones.

3.2. Measurement results of inhibition zones
The inhibitory activity of the sea urchin gonadal ointment (Diadema setosum) against Staphylococcus aureus showed that the 2% concentration was the best of the three concentration levels available (Table 1.).

| No | Treatment | Inhibition zones (mm) | Rates (mm) |
|----|-----------|-----------------------|-----------|
|    |           | I        | II       | III      |           |
| 1  | Ecstrac 2%| 1.81     | 3.5      | 2.1      | 2.47      |
| 2  | Ecstrac 4%| 1.52     | 1.55     | 1.8      | 1.62      |
| 3  | Ecstrac 6%| 0.55     | 1.45     | 1.35     | 1.12      |
| 4  | Control (+)| 11.55   | 11.55    | 11.55    | 11.55     |
| 5  | Control (-)| 0       | 0        | 0        | 0         |

Description:  
I = Inhibition zone in the first treatment with extract concentration of 2%, 4%, 6%, positive control and negative control.  
II = Inhibition zone in the second treatment with extract concentration of 2%, 4%, 6%, positive control and negative control.  
III = Inhibition zone in the third treatment with extract concentration of 2%, 4%, 6%, positive control and negative control.

Based on table 1, it can be seen that sea urchin ointment extract with a concentration of 2%, 4% and 6% has different average inhibition zones, ie for 2% it has an average inhibition zone value of 2.47 mm, 4% construction has an average value of inhibition zones of 1.62 mm and a concentration of 6% has an average value of inhibition zones of 1.12 mm. The results of this research indicate that the extract of sea urchin gonadal ointment with a concentration of 2% is the concentration with the best inhibitory zone activity against the test bacteria (Staphylococcus aureus). This is different from the results of a research conducted by Indrawati et al (2018) which found that from various concentrations of sea urchin extract (Diadema setodum), 80, 40, 20, 10, 5, 2.5 and 1.25% had an average inhibition zone values between 7 - 9 mm, but the best inhibition zone is in sea urchin extract with a concentration of 80%. This condition is to be expected because the source and raw material of the extract used (sea
urchin extract or *Diadema setodum*) are different. In this research sea urchin organs used as ointments were gonads while in research from Indrawati et al (2018) the sea urchin organ used was its shell. In addition, both of these studies used different positive control types, where in this research the positive controls used were Oxytetracycline while in the research of the positive control used was Trimethoprim [8].

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Risnauli et al's (2015) research entitled Identification of Secondary Metabolites of Sea Urchin Extract (*Diadema setosum*) and Antibacterial Activity Test for *Escherichia coli* and *Staphylococcus aureus* concluded that sea urchin extract contains alkaloid, phenolic, saponin, and triterpenoid compounds for the ethyl acetate activity test for antibacterial activity. the best antibacterial activity with a clear zone diameter of 12.02 mm at a concentration of 100 mg / mL against *Stapilococcus aureus* bacteria. This research is also in line with this research in the value of concentration and inhibition zones which are directly proportional [9].

The results of this research indicate that the lower the concentration (2%) of the three treatments carried out, the higher the inhibitory power of sea urchin gonadal ointment. This can be caused by the influence of the positive control used (Oxytetracycline) which is indeed a class of antibiotic drugs that also functions as an antibacterial while in the research of Indrawati et al (2018) showed different results, the higher the concentration of sea urchin extract, the greater the inhibition zone.

Sidiqi et al (2019) found something different from this study: the gonad methanol extract from *Diadema setosum* did not have antibacterial activity and formed inhibitory zones at all test concentrations. This can occur with different methods and types of bacteria. Where in siddiki research using the broth microdilution method and *Escherichia coli* bacteria while in this study using a completely randomized design method and using stapilococcal aureus bacteria [10].

Other studies that support the results of this research are research conducted by Karmilah and Badia E. (2019) in their research entitled Comparison Test of Effectiveness of Shell Extract and Sea Urchin Gonad (*Diadema setosum*) Against Burns in Animal Tests Specificity concluded that extract of sea urchin gonad had the highest effectiveness in concentration of 4% heals burns with a wound size of 1.4 cm because sea urchin gonads contain flavonoids that can repair damaged cells and as a broad-spectrum antibacterial that is able to prevent infection in burns so as to accelerate the healing process of burns [11].

Ointment contains a hydrocarbon base, namely vaseline album. Where the base of hydrocarbons shows faster antibacterial power and the base of hydrocarbons has better physical properties than other bases. More clearly the area of inhibition zones for each treatment of the three concentration levels in this research can be seen in Figure 2 below:
Figure 2. Treatment of the three concentration levels

From Figure 2 above it appears clearly that the sea urchin gonadal ointment (*Diadema setosum*) is the best and most effective in inhibiting the activity of *Staphylococcus aureus* bacteria from three concentration levels, 2, 4 and 6% are sea urchin gonadal ointment (*Diadema setosum*) with a concentration of 2%.

Data Table 1 shows that the results of several concentrations with positive and negative controls have differences, for ointments with a concentration of 2% have an average inhibition of 2.47 mm, the ointment with a concentration of 4% has an average inhibition of 1.62 mm, the ointment with a concentration of 6% has an average inhibition of 1.12 mm, while for positive control has an average inhibition of 11.55 mm and for negative control does not indicate the existence of inhibition. This shows that the ointment with a concentration of 2% is the concentration that has the best inhibition against the growth of *Staphylococcus aureus* bacteria compared to ointments with a concentration of 6%. Ointment preparations contain a hydrocarbon base namely vaseline album. Where the hydrocarbon base shows antibacterial power faster and the hydrocarbon base has better physical properties compared to other bases [9]. So it can be concluded that the concentration of 2% has an effective effect as an antibacterial. This research is in accordance with the research of Marimuthu et al (2015) who found that the ovary extract of *Diadema setosum* has excellent antimicrobial properties against a vast variety of pathogenic and non-pathogenic bacteria [13].

Figure 1 shows that the three treatments at three different concentrations showed a slight difference in each inhibition zone. From the results of this research it was found that from the three treatments, 2% concentration had a wider average inhibition zone area compared to the average inhibition zone area at concentrations of 4 and 6% of the three each treatment. This research is in accordance with the results of a research conducted by Akerina and et al (2015) which stated that sea urchin / sea urchin gonad extract had the highest antibacterial activity with inhibition zone values of 1.5 mm. Research by Akerina et al (2015) also shows that lower solvent concentrations have wider inhibitory zones and high solvent concentrations have smaller inhibitory zones [6].

From the description above it can be seen that the ointment with a concentration of 2% is the concentration that has the best inhibition against the growth of *Staphylococcus aureus* bacteria compared to ointments with a concentration of 6%. The graph also shows that positive control has a significantly high inhibitory activity, because the positive control used in this research is Oxytetracycline) which is a broad-spectrum group of antibiotics that have a fairly good ability to prevent the occurrence or expansion of infection areas in tissues caused by bacteria. The workings of Oxytetracycline are interfering with the ability of bacteria to produce essential proteins [12]. The research of ) also revealed that additional sea urchin shells and gonads can be developed as potential antimicrobial drugs [14].

To see the results of the inhibitory test of sea urchin gonadal ointment (*Diadema setosum*) against *Staphylococcus aureus* bacteria, anova test was carried out (Table 2).

**Table 2. Test results of inhibitory ANOVA test of sea urchin gonadal ointment (Diadema setosum) against Staphylococcus aureus bacteria**
### Table 1

| Source of Variation | Free Degrees | Number of Squares | Middle of Squares | \(F_{\text{hitung}}\) | \(F_{\text{table}}\) |
|---------------------|--------------|-------------------|-------------------|----------------|----------------|
| Treatment           | 4            | 25.167            | 6.292             | 0.691          | 3.48           |
| Error               | 10           | 91.102            | 9.110             |                |                |
| Total               | 14           | 116.269           |                   |                |                |

From table 1 it can be seen that \(f_{\text{arithmetic}} = 0.691\) and \(f_{\text{table}} = 3.48\) with a significant 0.05, this means that the treatment of 3 repetitions in each sample shows that there is no significant difference in average because \(F\) count is smaller than \(F\) the table means that the average treatment does not have a very significant effect on the growth of *Staphylococcus aureus* bacteria. This is possible because each concentration level is almost the same.

### 4. Conclusions

Sea urchin gonadal ointment has antibacterial inhibition against the growth of *Staphylococcus aureus* with an average inhibition zone of 2.47 mm at a concentration of 2%, 1.62 mm at a concentration of 4% and 1.12 mm at a concentration of 6%.

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