Antibacterial Activity of Sea Cucumber (Stichopus ocellatus) Against Bacillus cereus and Escherichia coli Bacteria

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Abstract. Sea cucumber (Stichopus ocellatus) is one of the bioresources that has not been utilized optimally. Samples were collected in the coastal sea of Lengkang Island, Batam. This study was aimed to determine the bioactive compound and antibacterial activity of sea cucumber extract against Bacillus cereus and Escherichia coli. The research method used was an experiment with a series of experiments, namely the extraction of sea cucumbers with ethanol as a solvent. Parameter analysis were identification of bioactive compound (phytochemical) and antibacterial activity against Bacillus cereus and Escherichia coli using well diffusion method. The treatment for the antibacterial activity test used various extract concentrations consisting of 125, 250, 375, and 500 g/mL with three replications. The results of the identification of the bioactive compounds of sea cucumber extract showed that the ethanolic extract of sea cucumbers contained saponins, alkaloids, and phenolics. The analysis of the antibacterial activity of the sea cucumber extract showed that the diameter of the inhibition zone of the ethanol extract at a solvent ratio of 1: 5 (w/v) and at a concentration of 500μg/mL against Bacillus cereus was 14.66 ± 0.37mm and Escherichia coli was 15.45 ±0.17mm. The results showed that the highest antibacterial activity of sea cucumber extract against Escherichia coli bacteria.

1. Introduction
Sea cucumber (Stichopus ocellatus) is one of the marine animals containing bioactive compounds from secondary metabolites that are antibacterial, antioxidant, and anticoagulant. Sea cucumber bioactive compounds are used as functional food for health and the pharmaceutical industry as medicinal raw materials. Bioactive compounds are antibacterial, act as food preservatives, reduce the risk of food poisoning and inhibit bacterial pathogens [1]. The bioactive compounds in sea cucumber extract that act as antibacterial compounds are saponins (triterpene glycosides) [2]. Stichopodidae species such Stichopus vastus had potential as antifungal and antibacterial activity of fraction methanol extract [3]. Antifungal and Antibacterial potential is caused by several compounds including saponins, terpenoids and steroids.
[4]. Fraction n-hexane of Sea cucumber Holothuria atra extract had high antibacterial activity on Pseudomonas aeruginosa [5].

Extraction using ethanol solvent can identify more metabolites than water solvents, because ethanol has the same level of polarity with the compounds obtained [6]. Pathogenic bacteria such as Bacillus cereus and Escherichia coli that can be a source of disease at humans and B. cereus and E. coli bacteria produce enterotoxins that cause diarrhea which are toxic. Bacillus sp is resistant to the antibiotic erythromycin [7]. Furthermore E. coli bacteria is resistant to the antibiotic ampicillin, gentamicin, streptomycin, erythromycin and tetracycline [8]. The results showed that the ethanolic extract of sea cucumbers had potential as an antibacterial against B. cereus and E. coli is still rare. Therefore, the authors were interested in examining the antibacterial activity of S. ocellatus extract against B. cereus and E. coli. This study aims to determine the bioactive compounds in sea cucumbers and the antibacterial activity of sea cucumbers against bacteria B. cereus and E. coli.

2. Materials and Methods

2.1. Materials

The material used is sea cucumber (Stichopus ocellatus). Samples were taken from the coastal sea on Lengkang Island, Batam. The microbes used were pure cultures of Bacillus cereus and Eschercia coli from the Bogor Agricultural University. The agar media used consisted of Nutrient Agar (Merck, Germany), nutrient broth media (Merck, Germany), chloramphenicol (Pharos, Indonesia), ethanol organic solvent (Merck, Germany), dimethyl sulfoxide/DMSO, whatmat whatman 1 filter paper. The equipment used were vacuum rotary evaporator (Heidolph WB 2000), laminar (Kleanzone air system, India), incubator (Isuzu incubator SSJ-115, Japan), petri dish (Pyrex), inoculation loop, oven (Yamato DV 41).

2.2. Methods

This research consisted of several stages, namely conducting a series of experiments by extracting Stichopus ocellatus by maceration with ethanol as solvent. The comparison of the sample with the solvent is 1:1, 1:3, 1:5 (w/v). The extract results were analyzed for bioactive components and antimicrobial analysis.

2.2.1. Extraction

500 g of sample was dissolved in ethanol and macerated for 3x 24 hours. After 72 hours of filtering, the crude extract of sea cucumber was produced. The crude extract was evaporated, aiming to remove all the remaining solvent. The viscous extract was analyzed for secondary metabolites/phytochemicals and antibacterial analysis using the disc diffusion method.

2.2.2. Analysis of bioactive compound [9]

The analysis of bioactive components (alkaloids, flavonoids, terpenoids, saponins and phenols)

2.2.3. Analysis of antibacterial activity

100μL of bacterial suspension was pipetted and put into a sterile petri dish and added 12 mL of nutrient agar. The cup that already contains the culture and media is stirred by rotating it slowly so that it is homogeneous. 10 L of chloramphenicol as a positive control of the test solution was dropped into a sterile paper disc, incubated for 24 hours at 37oC. Samples of sea cucumber extract were made in several concentrations consisting of 125, 250, 375, and 500 g/mL, to be tested for their antibacterial activity.
3. Result and Discussion

3.1. Yield of sea cucumber *Stichopus ocellatus*

The yield of sea cucumber extract is presented in Table 1.

Table 1. The yield of sea cucumber extract

| Solvent Ratio (b/v) | Samples (g) | Weight of extract (g) | Yield (%) |
|--------------------|-------------|-----------------------|-----------|
| 1 : 1              | 500         | 5.834                 | 1.1668    |
| 1 : 3              | 500         | 7.920                 | 1.5840    |
| 1 : 5              | 500         | 10.082                | 2.0164    |

The yield of sea cucumbers in this study was highest at a ratio of 1: 5 b/v of 2.1%. Another study stated that the yield of *Stichopus hermanii* was higher than that of *Stichopus variegatus* and reported that the yield of dry matter to liquid was 2.15% in *S. hermanii* and 1.06% in *S. variegatus* extract [10]. The yield of ethanol extract of *Holothuria edulis* sea cucumber fraction was 2.59%.

Yield of sea cucumber in this research Table 1 shows that sea cucumber *Stichopus ocellatus* has a greater result of yield at ratio 1 : 5 w/v. The result of sea cucumber *Stichopus ocellatus* greater than the result of yield *S. vastus*. *Stichopus hermanii* produces more yield than *Stichopus variegatus* and reported that the yield of dry matter to liquid material was 2.15 % on *S. hermanii* and 1.06 % on *S. variegatus* extract [10]. The yield of ethanol extract from the *Holothuria edulis* sea cucumber fraction was 2.59% [11].

3.2. Bioactive compound

The results of the analysis of the bioactive components of the extract *Stichopus ocellatus* was extracted with ethanol as solvent are presented in Table 2. The bioactive components produced from the ethanol extract solvent consist of Phenol, Terpenoid, and Saponin.

Table 2. Bioactive compounds of sea cucumber *Stichopus ocellatus*

| Bioactive Compounds | Reagent                | Result   | Color standard                |
|---------------------|------------------------|----------|-------------------------------|
| Alkaloid            | Mayer Dragendorff      | -        | Not form white precipitate,   |
| Flavonoid           | Cyanidin test          | -        | Not form red solution         |
| Phenol              | FeCl₃ 1%               | +        | blue/purple solution          |
| Terpenoid           | Liebermann-Burchard    | +        | greenish blue                 |
| Saponin             | H₂O                    | +        | Formed foam                   |

Information: + Detected, - Not detected

Sea cucumber extract *S. ocellatus* contains phenols, terpenoids and saponins (Table 2). Steroid compounds, terpenoids and saponins have antibacterial activity in sea cucumbers [3]. The role of phenolic compounds can damage cell membranes, inactivate enzymes, and denature proteins so that cell permeability decreases. As a result of changes in the permeability of the cytoplasmic membrane, the transportation of important organic ions into the cell is disrupted, so that cell growth becomes inhibited and the cell dies. Other bioactive compounds are terpenoids that can inhibit transport through thicker
bacterial cell membranes. Saponins are a group of compounds that function to kill microbes by reacting with sterols in the membrane. Saponins cause bacteria to be released from proteins and enzymes from within the cell so that saponins are compounds that actively inhibit cell growth [13].

3.3. Antibacterial activity.
The results of the analysis of the antibacterial activity of the sea cucumber extract *S. ocellatus* against *B. cereus* bacteria based on the diameter of the clear zone was presented in Table 3.

| Concentration (μg/mL) | Inhibitory Zone (mm) | Control |
|-----------------------|----------------------|---------|
|                       | 1:1                  | 1:3     | 1:5     | Positive (Chloramphenicol) | Negative (DMSO) |
| 125                   | 0.84± 0.16           | 1.52± 0.20 | 3.17± 0.20 | 22.40                  | 0 |
| 250                   | 2.65± 0.12           | 4.78± 0.25 | 5.47± 0.11 | 22.60                  | 0 |
| 375                   | 6.04± 0.10           | 6.52± 0.14 | 7.70± 0.21 | 22.76                  | 0 |
| 500                   | 7.28± 0.14           | 13.12±0.40 | 14.66±0.37 | 22.80                  | 0 |

Table 3, shows that the sea cucumber extract with a ratio (1:5 w/v) had an inhibition zone diameter of 14.66 ± 0.37 mm at a concentration of 500 g/mL. Anti-bacterial activity in this treatment was the highest compared to other treatments. The results of this study have a high activity of *H. scabra* extract against *B. cereus* bacteria is 2.30±0.58 mm at a concentration of 450μg/mL[14]. Another study showed that the minimum inhibitory concentration of *H. edulis* extract with ethyl acetate against *B. cereus* was 2.80 ± 0.20 mm at a concentration of 450 g. incubated for 48 hours [15].

| Concentration (μg/mL) | Inhibitory Zone (mm) | Control |
|-----------------------|----------------------|---------|
|                       | 1:1                  | 1:3     | 1:5     | Positive (Chloramphenicol) | Negative (DMSO) |
| 125                   | 3.26± 0.47           | 4.31± 0.08 | 9.78± 0.20 | 27.39                  | 0 |
| 250                   | 3.56± 0.28           | 5.64± 0.07 | 9.83± 0.33 | 27.41                  | 0 |
| 375                   | 3.86± 0.08           | 5.92± 0.06 | 12.24±0.10 | 27.54                  | 0 |
| 500                   | 4.00± 0.07           | 6.63± 0.27 | 15.45±0.17 | 27.60                  | 0 |

The antibacterial activity of sea cucumber extract had an inhibition zone diameter of 15.45 ± 0.17 mm at a concentration of 500μg/mL (Table 4). The results of another study showed that the minimum inhibitory concentration of sea cucumber (*S. vastus* extract) against *E. coli* was 10.33±0.00 mm at a concentration of 1000μg/mL [3]. The minimum inhibitory concentration of the aqueous methanolic extract of *S. variegatus* against *E. coli* was 12.26 ±0.00 mm at a concentration of 8 mg/mL [16]. The minimum inhibitory concentration of *S. variegatus* extract on the growth of *Streptococcus viridans* was in
the strong category of 10.8 ± 0.00 mm at a concentration of 2.5% [17]. Antibacterial test showed that the methanol extract of sea cucumber *Stichopus* has potential as antibacterial against *E. coli* bacteria, at a concentration of 156.25 ppm with a minimum inhibitory concentration of 7.35 mm±0.00 or 36.21% against tetracycline and 30.75 against ciprofloxacin [18]. The minimum inhibitory concentration of the ethanolic extract of the *H. edulis* fraction against *E. coli* was 2.65 ±0.00 mm at a concentration 250 μg [11].

4. Conclusions
Secondary metabolite compounds identified in *S. ocellatus* extract were terpenoids, phenolic, and saponins. *S. ocellatus* extract had high antibacterial activity against *B. cereus* and *E. coli* bacteria.

5. References.
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