Antibacterial activity of *Escherichia coli* from squid ink (*Loligo* sp.) n-Hexane extracts

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Abstract. Squid is one of the export commodities in Indonesia. In general, the use of squid meat, while the ink is only as waste. In fact, Squid ink contain bioactive compound that potential as anti-inflammatory, anti-hypertensive, anti-diabetic, anti-microbial and anti-malaria agents. The purpose of the study is to determine the types of secondary metabolite compounds contained in n-hexane extract of *Loligo* sp. ink using maceration method to determine its antibacterial activity against *Escherichia coli*. The results of secondary metabolite compounds obtained from the n-hexane extract of *Loligo* sp. ink are alkaloid, saponins, glycosides and phenol. The results of antibacterial test against *E. coli* using the disc method obtained the average of inhibition zone diameter at the concentration of 4% is 6.3 mm (intermediate), concentration of 8% is 7.83 mm (intermediate), concentration of 16% is 14.5 mm (susceptible) and concentration of 32% is 10.83 mm (intermediate). The antibacterial activity in n-hexane extract of *Loligo* sp. ink is optimal at the concentration of 16% against *E. coli* bacteria.

1. Introduction

Squid is a remarkable product of fisheries which is exported by many countries to be used as industrial production or for consumption. In export scale, so a lot of waste and unused Production materials (e.g. squid ink) [1]. Squid ink contains melanoprotein consisting of as much as 15% melanin of the total wet weight of ink and proteins as much as 5-8%. Squid ink has anti-retroviral, anti-tumor, Antioxidants and the ability to protect cells from chemotherapy and antibiotic damage Squid ink's inhibitory effect on pathogens (e.q biofilm bacteria) [2,3]. Squid ink contains secondary metabolites like phenols, flavonoids, tannins, steroids, terpenes, alkaloids, glycosides, and saponin [4]. Squid ink (*Loligo duvaucelli*) was extracted using ethyl acetate has antibacterial activity against *E. coli* and *K. pneumoniae* bacteria [5]. Meanwhile the extract of methanol from *Sepioteuthis lessoniana* had antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria [6].

Antibacterial works by damaging cell walls, interfering with protein synthesis, inhibiting enzymes and changing membrane permeability. Furthermore, antibacterial has a mechanism in forming complexes with cellular extract proteins and dissolved together with the walls of microorganisms. Secondary metabolite compounds such as flavonoids, alkaloids and phenols have an important role in
damaging bacterial cell walls. Some of these metabolites have potential as natural antibacterial against pathogenic bacteria as *Escherichia coli* [7]. *Escherichia coli* is a bacterium that exists in the human or animal body but when it is out of its habitat it will become a pathogenic bacterium for humans and animal. *Escherichia coli* will cause various diseases, namely abdominal cramps, disrupting the work system of the stomach organs, urinary tract infections, diarrhea, bile duct infections and other places in the abdominal cavity [7,8]. Based on background above, the objective of the present study was inhibitory power of n-hexane ink squid extract (*Loligo* sp.) against *Escherichia coli*.

2. Research Methods

2.1. Location and Place
Sampling of squid ink (*Loligo* sp.) of medium size (15-20 cm) was carried out at Lampulo fish market in Banda Aceh, February 2021. Phytochemical tests and antibacterial tests on the extraction of squid ink (*Loligo* sp.) were carried out at the Laboratory of Marine Chemistry and Biotechnology, Faculty of Marine and Fisheries, Universitas Syiah Kuala.

2.2. Sample Extraction
Fresh squid was medium size (15-20 cm) and the same physical appearance were taken according to the needs of the analysis and stored in plastic containers with ice cubes to prevent damage to the squid samples. Then, 25 ml of squid ink was extracted using n-hexane as a solvent with a sample ratio of 1:3 using the maceration method for 3 x 24 hours. The results of the extraction were filtered using Whatman filter paper then dried using a rotary evaporator at 40 °C and the yield was calculated for further tests.

2.3. Phytochemical screening test
Analysis of phytochemical (Alkaloid, Flavonoid, steroid, saponin, glicoside and phenol)

2.4. Antibacterial Activity
Drying of n-hexane extract *Loligo* sp. weighed as much as 1 mL dissolved in 3.125 mL of 2% DMSO to obtain a concentration of 32% as stock solution. Furthermore, the dilution of the stock solution was carried out to obtain concentrations of 16%, 8% and 4%. Nutrient Agar (NA) media was made by dissolving 2.8 g of NA powder into 100 ml of distilled water. Furthermore, the NA media was sterilized by autoclave at 121 °C at a pressure of 1 atm for 15 minutes along with a petri dish and an Erlenmeyer.

The bacterial suspension was put in a few ml in a cuvette tube and its absorbance was measured using a UV-Vis spectrophotometer according to the standard 0.5 Mc.Farland by determining the OD of 0.1 at 625 nm for E.coli bacteria. Then the bacterial suspension was put into a petri dish as much as 1 ml and 15 ml of NA medium was added, allowed to solidify. Then, 3 sterile paper discs with the same diameter and thickness were placed on the surface so that they were far from each other and at the same distance. Then the disc paper was dripped with 25µL extract of n-hexane ink *Loligo* sp. at each concentration (4%, 8%, 16% and 32%), negative control (n-hexane and 2% DMSO) and positive control (amoxicillin). Incubated at 37 °C for 24 hours. The formation of a clear area (halo) around the paper disc indicates the presence of antibacterial activity. The diameter of the inhibition area was measured using a caliper.

2.5. Analysis of data
Inhibition zone diameter formula using disc diffusion method according as follow:

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\text{Inhibition zone diameter} = \frac{Dv+Dh}{2} \quad (1)
\]

Note:
\(Dv = \text{Vertical diameter of clear zone on media (mm)}\)
\(Dh = \text{Diameter of the clear zone on the media (mm)}\)
3. Result and Discussion

3.1. Phytochemical screening test

Extract n-hexane of squid ink (*Loligo* sp.) contains four secondary metabolites that were alkaloids, saponins, glycosides and phenols. The results of phytochemical screening test in extract, can be seen at Table 1.

| Secondary metabolite | Methods       | Yield | Colour                  | Results (+)                  |
|----------------------|---------------|-------|-------------------------|------------------------------|
| Alkaloid             | Wagner        | +     | Brown                   | Brown to reddish brown       |
| Flavonoid            | Shinoda       | -     | Colourless              | Red to dark red              |
| Steroid              | Salkowski     | -     | Colourless              | Pattern of red ring          |
| Glycoside            | Foam          | +     | Foam is formed at the top with a height of 0.5cm which lasts more than 10 minutes | A foam layer is formed that lasts more than 10 minutes |
| Glycoside            | Keller-Kiliani| +     | Forming 2 layers with a thin brown ring between the two layers | Pattern of brown ring         |
| phenol               | Iron (III) Chloride | +     | Cerulean black         | Cerulean black               |

3.2. Antibacterial activity

Antibacterial is a compound with selective toxicity which is not harmful to the host but is harmful to the parasite so that it can kill pathogenic bacteria in the host's body. Some research reports claim that cephalopod ink has antibacterial activity [8,9]. It is reported that the crude extract of *Loligo duvauceli* has antifungal and antibacterial activity against *Candida albicans*, *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* [9]. The antibacterial activity can be determined by observing zones of bacterial growth inhibition. Inhibitory zone that contain in squid ink extract (*Loligo sp.*) can be form because there is melanin level can make inhibit growth the bacteria.

The result of inhibitory zone in various concentration can be seen in Table 1 and Figure 1.

Based on antibacterial test on n-hexane extract from squid ink *Loligo* sp. against *E. coli* bacteria showed that the average inhibition zone diameter of 6.3 mm at a concentration of 4%, 7.83 mm at a concentration of 8%, 14.5 mm at a concentration of 16% and 10.83 mm at a concentration of 32%. The lowest diameter of the inhibition zone was found at a concentration of 4% and the highest diameter of the inhibition zone was found at a concentration of 16%. The concentration of 16 % has strong antibacterial activity. The diameter of the inhibition zone obtained in this study is lower than the diameter obtained by [4], where the methanol extract of *Loligo duvauceli* ink has an inhibition zone diameter of 21 mm against *E. coli* bacteria at a concentration of 200 µL. *Loligo sp.* ink extraction use 0.5M HCl has a relatively small diameter of the inhibition zone against *E. coli* bacteria, which is 4.69 mm at a concentration of 32% [10], smaller than the inhibition zone produced in the n-hexane extract of *Loligo sp.*

The alkaloid compounds contained in squid ink play an important role in antibacterial activity. Alkaloids contain nitrogen and its derivatives, such as phenol, amine, amide, and methoxy, which can be used as antibacterial compounds [2,14]. The mechanism of the compound's antibacterial activity is to inhibit the growth of bacteria by disrupting cell metabolism. Antibacterial mechanism by attack the cytoplasmic membrane, loses stability on protons and electrons on components cell constituents. In
addition, it interferes with the peptidoglycan component in the bacterial cell, so that the cell wall layer is not completely formed and leads to cell death [10,11]. Moreover, the halide dependent peroxidase enzyme present in ANG of squid ink is responsible for the antimicrobial activity [12]. Increasing content of biologically active compounds sterilization, and lower levels are usually just bacteriostatic [13,14].

Figure 1. Antibacterial activity test of n-hexane extract of ink *Loligo* sp. (a) 4%, (b) 8%, (c) 16%, (d) 32%.

| Concentration   | Diameter of inhibitory zone (mm) |
|-----------------|----------------------------------|
| 4%              | 6.3                              |
| 8%              | 7.83                             |
| 16%             | 14.5                             |
| 32%             | 10.83                            |
| N-hexane (-)    | -                                |
| DMSO 2% (-)     | -                                |
| Amoxicillin 4% (+) | 10                               |

Table 2. Inhibitory zone in various concentration.

4. Conclusion
The group of secondary metabolites that contained in the n-hexane extract of squid ink (*Loligo* sp.) are alkaloids, saponins, glycosides and phenols. The optimum concentration of antibacterial activity from extracts to against *E. coli* was 16% with an inhibitor zone of 6.3-14.5 mm.

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