

Activity Test of Squid Ink (*Loligo Sp.*) As Antibacterial Against *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* Bacteria

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Abstract.

Squid (Loligo sp.) is an invertebrate animal, part of the cephalopod class. The squid has a distinctive black ink fluid for self-defense. Squid ink contains melanin compounds that can be used as an antibacterial. Enterococcus faecalis is a bacterium that causes endodontic infection. While Pseudomonas aeruginosa is a bacterium that causes infection in patients with compromised immune system, and also who using a urinary catheter or intravenous catheter. This study aims to determine whether squid ink can be used as an antibacterial that can suppress the growth of Enterococcus faecalis and Pseudomonas aeruginosa bacteria. This research was conducted with experimental method, true experimental Post-Test Only Control Group research design. The research by using squid ink was carried out by inserting extract with various concentration (20%, 40%, 60%, 80% and 100%) into a petri dish which containing bacterial culture. The final showed that between concentrations of 20%, 40%, 60%, 80% and 100% were no clear zones, which had been repeated 3 times against Enterococcus faecalis and Pseudomonas aeruginosa bacteria. From the results obtained, it can be concluded that there is no bacterial inhibition caused by squid ink against Enterococcus faecalis and Pseudomonas aeruginosa.

Keyword: *Squid ink extract (Loligo sp.), Enterococcus Faecalis, Pseudomonas Aeruginosa, Antibacterial and Bacteria.*

I. INTRODUCTION

Enterococcus Faecalis, according to basic microbiology, is a normal flora that is commonly found growing in the gastrointestinal and oral cavities. *Enterococcus Faecalis* has a high enough resistance against pulp tissue defense procedures and is found in endodontic infections. These bacteria can replicate well, survive in root canals without other bacteria, and can produce toxic substances (toxins) that are released directly through inflammatory stimuli [1]. *Enterococcus Faecalis* is now a circulating and growing pathogen in general hospital areas that is resistant to Antibiotics have become a global health problem in many countries. *Pseudomonas Aeruginosa* is widely distributed in nature and usually occurs in moist environments in hospital areas. Although not part of the normal human microbiome, *Pseudomonas Aeruginosa* is capable of colonizing various parts of the body (e.g., mucous membranes, respiratory tract, and GI tract). It is known to cause disease in humans, especially in persons with altered and decreased host defenses (e.g., neutropenia, chemotherapy, and burns) [2]. Infections resulting in blood, pneumonia, urinary tract infections, and postoperative infections leading to severe infections and can be fatal leading to death [3]. There are two reasons why antimicrobial therapy in serious *P. Aeruginosa* infections can be challenging: patients with *P. Aeruginosa* infections are usually immunocompromised, and in addition the organisms themselves are often resistant to several different classes of antimicrobial agents.

Resistance and lack of sensitivity of an antibiotic can be caused by misuse of antibiotics. It should be noted that antibiotics must be given with adequate time and dose limits to prevent and treat bacterial infections without causing changes in resistance [4]. Apart from plants, products from the sea can also be used as alternative medicine. One of the marine products that can be used is squid. Squid has ink that has been proven to play a role in alternative medicine [5]. The highest content of squid ink is occupied by

melanin. According to the journal Nasution (2017), the remaining content in melanin consists of eumelanin; 5,6-dihydroxyindole (DHI) and 5,6-dihydroxy indole-2-carboxylic acid (DHICA); 2-carboxyl indole can bind Fe²⁺ to slow down the oxidation process in blood lipids [6]. The content in melanin which is rich in protein has anti-tumor properties that can kill cancer, as an antioxidant, antiradiation, and antirotavirus [7][12].

II. METHODS

Tool Sterilization

The equipment to be used must be prepared as much as possible before being put into the autoclave. Sterilize tools at a temperature of 120°C, so that all tools can be exposed to steam evenly for 15-20 minutes. After the tools are removed from the autoclave, all tools must be packed so that they are not contaminated and remain sterile after leaving the autoclave.

Squid Ink Collection

Squid ink is taken from squid obtained from the Belawan sea, Medan City, North Sumatra. The squid for which the ink will be extracted are washed first and then surgically removed to remove the ink sac. The ink bag that has been removed is placed in the container. Then cut and squeezed on a sieve to remove the ink. Then the ink is put into a bottle and stored in a cooler with the aim of preventing damage or spoilage of the squid ink.

Squid Ink Extraction

The step in producing the extract was carried out by maceration using methanol as a solvent. The difference between squid ink and methanol is 1:3 [8]. Then the squid ink and methanol are put into a glass beaker and homogenized using a stirrer at 120 rpm for 30 minutes – 1 hour/day. It was carried out for 7 days, then the solution was filtered and the filtering results were collected and evaporated using a rotary evaporator at a temperature of 80°C. Then to get the squid ink extract which is thicker and free from solvents, it is evaporated again using a water bath at a temperature of 80°C.

Bacteria Suspension Manufacturing

Take a small amount of pure *Pseudomonas Aeruginosa* and *Enterococcus Faecalis* bacteria into the Nutrient Agar (NA) growing media to obtain bacterial cultures. Then incubated in an incubator at 37°C for 1x24 hours. Then make a bacterial suspension by dissolving the cultured bacteria with 0.9% NaCl solution until the turbidity level matches the McFarland standard.

Squid Ink Extract Antibacterial Activity Test

The results of the bacterial suspension of *Pseudomonas Aeruginosa* and *Enterococcus Faecalis* bacteria were applied with a sterile cotton swab on MHA media. Furthermore, the filter paper discs were dipped into the squid ink extract with concentrations of 20%, 40%, 60%, 80% and 100%, respectively, and into the control (+) which was diluted ciprofloxacin and the control (-) was distilled water., then placed on the surface of the MHA media that has been embedded with bacteria. Then the petri dish was put into an incubator at 37°C for 1x24 hours. If the clear zone is visible, the closed zone is measured using a caliper.

III. RESULT AND DISCUSSION

RESULT

From the results of experiments that have been carried out with the aim of knowing the effectiveness of squid ink in inhibiting the development of *Enterococcus Faecalis* and *Pseudomonas Eurigonosa*. If it causes an effect, then this is indicated by the presence of a clear zone (inhibition zone) around the paper disc on the petri dish being tested. The measurement of the zone is carried out with a caliper. With concentration levels of 20%, 40%, 60%, 80% and 100%, the inhibition zones are listed in the following figures and tables:

Table 1. Results of Measurement of Clear Zone Diameter Against *Enterococcus Faecalis*

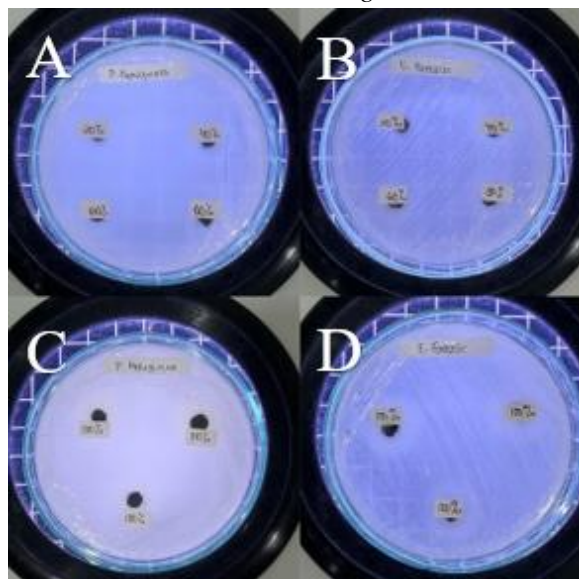
Concentration	Clear Zone Diameter (mm)			Average
	1 st Petri	2 nd Petri	3 rd Petri	
20%	0	0	0	0

40%	0	0	0	0
60%	0	0	0	0
80%	0	0	0	0
100%	0	0	0	0
Control (+)	29,25	29	29,75	29,33
Control (-)	0	0	0	0

Table 2. Results of Measurement of Clear Zone Diameter Against *Pseudomonas Aeruginosa* Bacteria

Concentration	Clear Zone Diameter (mm)			Average
	1 st Petri	2 nd Petri	3 rd Petri	
20%	0	0	0	0
40%	0	0	0	0
60%	0	0	0	0
80%	0	0	0	0
100%	0	0	0	0
Control (+)	27,975	27,025	27,5	27,5083
Control (-)	0	0	0	0

Figure 1. Diameter of the clear zone of squid ink extract (*Loligo Sp.*) on the growth of *E. Faecalis* and *P. Aeruginosa* bacteria.



Caption Figure 1: 20%, 40%, 60%, 80% and 100% concentrations of *Pseudomonas Aeruginosa* bacteria (Fig. A & C). Concentrations of 20%, 40%, 60%, 80% and 100% in *Enterococcus Faecalis* bacteria (Fig. B & D).

Based on table 1 and table 2, the data that has been collected, the clear zone size of the squid ink extract against *Enterococcus Faecalis*, the highest average diameter of the clear zone was found at the control concentration (+), with an average of 29.333 mm. The same was true for *Pseudomonas Aeruginosa*, with the highest average clear zone diameter at the control concentration (+); but with a lower average than *Enterococcus Faecalis*, which is 27,500 mm. While the average value of the clear zone diameter of the squid ink extract at the concentration levels of 20%, 40%, 60%, 80%, 100%, and Control (-) was the lowest average value of the clear zone diameter, with an average an average of 0 mm in both types of bacteria.

Table 3. Results of Normality Test and Homogeneity Test

Inhibition Zone Diameter	Concentration	Normality Test	Homogeneity Test
<i>Enterococcus Faecalis</i> bacteria	20%	-	0.001
	40%	-	
	60%	-	
	80%	-	
	100%	-	
	Control (+)	0.637	
	Control (-)	-	
<i>Pseudomonas Aeruginosa</i> bacteria	20%	-	0.015
	40%	-	
	60%	-	
	80%	-	
	100%	-	
	Control (+)	1.000	
	Control (-)	-	

Based on the results of the normality test, the data on the diameter of the inhibition zone of *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* were normally distributed. With p value = 0.637 > 0.05 in control concentration of *Enterococcus Faecalis* bacteria and with p value = 1,000 > 0.05 in control concentration of *Pseudomonas Aeruginosa* bacteria (+). However, based on the results of the homogeneity test, the diameter of the inhibition zone on *Enterococcus Faecalis* with p value = 0.001 < 0.05 and *Pseudomonas Aeruginosa* with p value = 0.015 < 0.05, so the two data are not homogeneous. So that the next test uses the Kruskal-Wallis test and the Mann-Whitney test.

Table 4. Kruskal-Wallis Test Results

Inhibition Zone Diameter	Concentration	Kruskal-Wallis Test
<i>Enterococcus Faecalis</i> bacteria	20%	0.003
	40%	
	60%	
	80%	
	100%	
	Control (+)	
	Control (-)	
<i>Pseudomonas Aeruginosa</i> bacteria	20%	0.003
	40%	
	60%	
	80%	
	100%	
	Control I (+)	
	Control (-)	

The results of the Kruskal-Wallis test showed a p-value = 0.003 ($p < 0.05$). It can be concluded that there is a significant difference in the diameter of the inhibition zone between *Enterococcus Faecalis* and *Pseudomonas Aeruginosa*, between concentrations of 20%, 40%, 60%, 80%, 100%, control (+), and control (-).

Table 5. Mann Whitney Test Results

Inhibition Zone Diameter	Concentration	Mann-Whitney Test
<i>Enterococcus Faecalis</i> bacteria	20%	1.000

	40%	
	60%	
	80%	
	100%	
	Control (+)	0.037
	Control (-)	1.000
Pseudomonas Aeruginosa bacteria	20%	1.000
	40%	
	60%	
	80%	
	100%	
	Control (+)	0.037
	Control (-)	1.000

Based on the Mann-Whitney test, the results showed that there was no significant difference in the diameter of the inhibition zone from all concentrations of squid ink extract as antibacterial against *Enterococcus Faecalis* and *Pseudomonas Aeruginosa*, with $p = 1,000 > 0.05$. The control (+) ciprofloxacin showed that there was a significant difference in the diameter of the inhibition zone as antibacterial against *Enterococcus Faecalis* and *Pseudomonas Aeruginosa*, p value = $0.037 < 0.05$. The value of $p = 1,000 > 0.05$ in the control (-) aquadest indicated that there was no significant difference in the diameter of the inhibition zone as an antibacterial against *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* bacteria.

DISCUSSION

Test the effectiveness of this squid ink using experimental research methods with in vitro laboratories to determine whether there is an inhibitory power of squid ink extract (*Loligo Sp.*) against microorganisms *Enterococcus Faecalis* and *Pseudomonas Aeruginosa*. The test was carried out by culturing *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* bacteria in Muller-Hinton Agar (MHA) growing media. A filter paper disc was placed where it had previously been dipped in squid ink extract (*Loligo Sp.*), distilled water, and a filter paper disc containing ciprofloxacin as a positive control, and then put into the incubator. Incubation was carried out at a temperature of 37°C for a period of 1×24 hours. The activity of squid ink extract against bacteria can be indicated by the appearance of a clear zone on the MHA media. Based on the data collected, it was found that the squid ink extract did not have sufficient effectiveness in inhibiting the growth of *Enterococcus Faecalis* and *Pseudomonas Aeruginosa*. This can happen because it is influenced by several factors such as the method used or the bacteria used in the test. The activity test of squid ink (*Loligo Sp.*) in inhibiting the growth of bacteria with the types of microorganisms *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* has never been studied before. However, tests on the activity of squid ink (*Loligo Sp.*) against other pathogens have been carried out, for example research conducted by Rocky and Christy in Manado regarding the test of squid ink extract (*Loligo sp.*) in inhibiting the growth of *Streptococcus Mutans* bacteria [7][14][15]. The results of the test showed that squid ink had sufficient effect in inhibiting *Streptococcus Mutans* bacteria with an inhibition zone of 10.50 mm.

Mayangsari in Manado conducted a study on the activity of squid ink extract (*Loligo Sp.*) against *Staphylococcus Aureus* bacteria [9], revealing the effectiveness of squid ink as an antibacterial against *Staphylococcus Aureus* microorganisms with an inhibition zone of 11.22 mm. The research on the inhibitory activity of melanin in squid and cuttlefish ink against *Esherichia Coli* microorganisms that was carried out by Yuspihana in South Kalimantan, said that the melanin contained in cuttlefish and squid ink had an effectiveness in inhibiting the growth of *Esherichia Coli* [10]. that squid ink extract can also prevent plaque formation, where squid ink can neutralize the causative bacteria such as *Lactobacillus Acidophilus*, *Actinomyces Viscosus*, *Candida Albicans* and *Streptococcus Mutans* [11][13]. This trial used a positive control of ciprofloxacin as an antimicrobial against *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* bacteria. From the results obtained, the size of the clear zone caused by ciprofloxacin was 29.33 mm against gram-positive bacteria *Enterococcus Faecalis* while the gram-negative bacteria *Pseudomonas Aeruginosa*

was 27.5083mm. Aquades was used for negative control, the results showed no antibacterial activity against *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* bacteria.

IV. CONCLUSION

Based on the research above, it can be seen that the concentration of squid ink extract (*Loligo Sp.*) with levels of 20%, 40%, 60%, 80% and 100% did not have an inhibitory effect on the gram-positive pathogen *Enterococcus Faecalis* and the gram-negative *Pseudomonas Aeruginosa*. It can be concluded that there is no antibacterial activity of squid ink extract (*Loligo Sp.*) against *Enterococcus Faecalis* microorganisms and *Pseudomonas Aeruginosa* bacteria. Squid ink extract (*Loligo Sp.*) which has no definite effect in inhibiting the growth of *Enterococcus Faecalis* and *Pseudomonas Aeruginosa* bacteria and its effectiveness as an antibiotic cannot be determined in vitro.

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