

## Original Research Article

# Minimum inhibitory concentration of squid ink *Loligo sp.* extract on growth of *Staphylococcus aureus* and *Streptococcus mutans* bacteria

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### ABSTRACT

**Background:** Dental caries and periodontal disease can be caused by bacteria that attack hard and soft tissues in the oral cavity like *Staphylococcus aureus* and *Streptococcus mutans* bacteria. One way to prevent infections caused by the bacteria are by using mouthwash. Currently, mouthwash that is widely used is mouthwash containing chlorhexidine, which has side effects in the form of tooth staining when used prolonged. The need for alternative medicines from nature that effectively cope with polymicrobial infections. One of the marine products that has pharmaceutical properties is squid, especially the ink it produces.

**Methods:** This research is a pure experimental study (true experimental design) with a randomized pretest-posttest control group design. Squid ink *Loligo sp.* extract, obtained by extracting squid ink *Loligo sp.*, with maceration method using 96% ethanol solvent. This research uses serial dilution method with spectrophotometric testing method.

**Results:** After measuring the turbidity value in each treatment tube, it was found that at a concentration of 1.56% squid ink *Loligo sp.* extract began to inhibit the growth of *Staphylococcus aureus* and *Streptococcus mutans* bacteria. This is proven because the absorbance value after and before incubation is fixed.

**Conclusions:** The minimum inhibitory concentration of squid ink *Loligo sp.* extract on the growth of *Staphylococcus aureus* and *Streptococcus mutans* at a concentration of 1.56%.

**Keywords:** Minimum inhibitory concentration, Squid ink *Loligo sp.*, *Staphylococcus aureus*, *Streptococcus mutans*

### INTRODUCTION

The human body is a place to live for various kinds of microorganisms that can be commensal and pathogenic. One of the places where these microorganisms live is the oral cavity. This microorganism is a normal flora but can become pathogenic if the balance of the oral cavity is disturbed. *Staphylococcus aureus* and *Streptococcus mutans*, are bacteria that can be found in the oral cavity.

Dental and oral health is often the umpteenth priority for some people, even though as authors know, teeth and mouth are the 'gateway' for the entry of germs and bacteria so that they can interfere with the health of other

organs.<sup>1</sup> Oral and dental health is not only related to aesthetic issues, but it can also cause serious health problems if someone ignores the health of their oral cavity. Therefore, oral health is a part of the body that cannot be separated from one another because it will affect overall body health.

The results of the Basic Health Research (RISKESDAS) data in 2018 show the proportion of dental and oral disease problems in Indonesia at 57.6%.<sup>2</sup> This has increased from the previous data, namely RISKESDAS in 2013 which showed that dental and mouth disease problems in Indonesia amounted to 25.9%.<sup>3</sup> The most common dental and oral diseases experienced by people

in Indonesia are dental caries and periodontal disease. Dental caries and periodontal disease can be caused by bacteria that attack hard and soft tissues in the oral cavity.<sup>4</sup>

*Staphylococcus aureus* is one of the bacteria in the oral cavity that can cause disease in the oral cavity if there is a change in the quantity of microorganisms and eventually cause imbalance. Diseases that can be caused by *Staphylococcus aureus* bacteria include gingivitis, angular cheilitis, parotitis, *staphylococcal mucositis*, denture stomatitis and also abscesses.<sup>5</sup> *Streptococcus mutans* bacteria is one of the cariogenic microorganisms associated with oral diseases namely dental caries.<sup>6</sup> *Streptococcus mutans* bacteria is capable of synthesizing extracellular glucan polysaccharides, which can produce lactic acid through a homo-fermentation process, forming colonies that are firmly attached to the tooth surface and are more acidogenic than other *Streptococcus* species.<sup>7</sup>

One way to prevent infections caused by *Staphylococcus aureus* and *Streptococcus mutans* in the oral cavity is by using mouthwash. The use of mouthwash aims to kill bacteria, as a refresher, and eliminate unpleasant odors, and has a therapeutic effect by curing infections.<sup>8</sup> Currently, mouthwash that is widely used is mouthwash containing chlorhexidine, which has side effects in the form of tooth staining and decreased taste when used prolonged.<sup>9</sup> Therefore, research is needed on new agents as an alternative to antibacterial especially against the bacteria *Staphylococcus aureus* and *Streptococcus mutans*.

The need for alternative medicines from nature that effectively cope with polymicrobial infections is increasingly urgent to improve the quality of human life. Natural bioactive substances have more minimal side effects than synthetic substances, so they are safer for the host body. Indonesia is the largest archipelagic country in the world, has a coastline of 81,000 km with an area of fisheries in the sea around 5.8 million km<sup>2</sup> consisting of 3.1 million km<sup>2</sup> of territorial waters and 2.7 million km<sup>2</sup> of Indonesia's Exclusive Economic Zone.<sup>10</sup> Many natural materials come from the sea has contributed to creating alternative medicines that are considered safer and have relatively small side effects. One of the marine products that has pharmaceutical properties is squid, especially the ink it produces. Squid ink has been shown to play a large role in the world of alternative medicine and has a wide reach in therapeutic applications.<sup>11</sup> Squid ink contains melanoprotein, which is melanin as much as 15% of the total wet weight of ink and protein as much as 5%-8%.<sup>12</sup> Test for minimum inhibitory concentration aims to determine the smallest concentration of antibacterial that is effective to inhibit the growth of certain microorganisms.<sup>13</sup> This test needs to be done to develop the utilization of a new material to the next test stage before it can be used by humans.

This is in accordance with the road map of the leading fields in the development of health technology and

medicines, namely improvement of health and nutrition status and disease control and strengthening access to health service.

## METHODS

This research is a pure experimental study (true experimental design) with a randomized pretest-posttest control group design. This research was conducted at the Laboratory of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sam Ratulangi for the manufacture of squid ink *Loligo sp.*, extract the experiment was conducted in May-August 2019.

The variables in this study are Minimum Inhibition Concentration squid ink *Loligo sp.* extract, *Staphylococcus aureus* and *Streptococcus mutans* bacteria.

The method used in study is the turbidimetry method or turbidity test, then proceed by using a UV-Vis spectrophotometer to see the absorbance value as an accurate turbidity determinant. To test the turbidity, a bacterial suspension media taken which was equalized with McFarland 1 turbidity standard as much as 0,5 ml was put into a test label 1 test tube and then measured the initial absorbance value using a UV-Vis spectrophotometer. After that, the same thing is done on the 2-9 label treatment tube. Concentration in this study was obtained by multilevel dilution or serial dilution using a ratio of 1: 2 (w/ v), so that the concentrations obtained 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.39%.

Each tube that has known the initial absorbance value is then put into an incubator and incubated for 1x24 hours. In this study, the treatment and testing were repeated twice.

After the media the treatment tubes were incubated for 1x24 hours, all treatment tubes measured the absorbance value with a spectrophotometer UV-Vis as the final absorbance value. If the final absorbance value (after incubation) of each tube is greater than the initial absorbance value (before incubation), it is concluded that bacterial growth still occurs. However, if on the contrary there is no change in absorbance value between absorbance values end with the initial absorbance or the final absorbance value is smaller than the value initial absorbance then it was concluded that bacterial growth was inhibited. Minimum Inhibition Concentration determined by the concentration of the smallest extract on the treatment tube that has been begins to inhibit bacterial growth. The research data is calculated manually, then processed computerized. Existing data is processed and presented in the form of images, tables and writing.

## RESULTS

Squid *Loligo sp.* is taken from the water/sea of Siladen, *Staphylococcus aureus* and *Streptococcus mutans* bacteria which were stored in agar media, were made

rejuvenation by implanting them on slanted media then incubated in an incubator at 37°C for 1x24 hours.

The results of the minimum inhibitory concentration squid ink *Loligo sp.* extract on *Staphylococcus aureus* and *Streptococcus mutans* can be seen in the Table 1 and 2. The total of 11 sterile test tubes were prepared, each test tube is labeled 1-9, that the concentrations obtained 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, 0.78%, 0.39%.

Tube 10 is labeled K (+) which is a positive control, that contains of *Staphylococcus aureus*, which is equivalent to McFarland 1 turbidity standard. Tube 11 is labeled K (-) which is a negative control, that containing extract of squid ink *Loligo sp.*, with a concentration of 100%. Measurements were made 2 times, namely before and after incubation, where each repetition was carried out 2 times (Table 1).

The Minimum Inhibitory Concentration is determined by comparing the absorbance after the incubation treatment minus the absorbance before treatment. If there is the lowest concentration that inhibits bacterial growth, indicated by the absence of turbidity (bacterial OD is  $\leq 0$ ), the Minimum Inhibitory Concentration is obtained.

Results of *Staphylococcus aureus* Minimum Inhibitory Concentration tests showed at a concentration of 1,56% had the lowest absorbance value (Table 1). This means that bacterial growth is inhibited at these concentrations, so this concentration is determined as the minimum inhibitory concentration of squid ink *Loligo sp.* extract on the growth of *Staphylococcus aureus* using the UV-Vis spectrophotometer method.

The same study was conducted to determine the minimum inhibitory concentration of squid ink *Loligo sp.* Extract on *Streptococcus mutans* bacteria (Table 2).

**Table 1: Results of *Staphylococcus aureus* minimum inhibitory control tests.**

Tubes	Sample concentration (%)	Treatment (before incubation)		Average before incubation	Treatment (after incubation)		Average after incubation	Average before and after incubation
		1	2		1	2		
1	100	1.357	1.098	1.2275	1.179	1.406	1.2925	1.26
2	50	0.564	0.544	0.554	1.399	1.295	1.347	0.9505
3	25	0.288	0.304	0.296	0.995	1.079	1.037	0.6665
4	12.5	0.276	0.242	0.259	1.07	1.09	1.08	0.6695
5	6.25	0.273	0.177	0.225	1.045	0.96	1.0025	0.61375
6	3.125	0.157	0.168	0.1625	0.961	0.986	0.9735	0.568
7	1.56	0.156	0.143	0.1495	0.972	0.944	0.958	0.55375
8	0.78	0.161	0.133	0.147	0.928	1.02	0.974	0.5605
9	0.39	0.166	0.163	0.1645	1.073	0.917	0.995	0.57975
10	Control +	0.135	0.137	0.136	0.142	0.139	0.1405	0.13825
11	Control -	0.089	0.076	0.0825	0.928	1.201	1.0645	0.5735

**Table 2: Results of *Streptococcus mutans* minimum inhibitory control tests.**

No.	Sample concentration (%)	Treatment (before incubation)		Average before incubation	Treatment (after incubation)		Average after incubation	Average before and after incubation
		1	2		1	2		
1	100	1.101	1.097	1.099	1.016	1.164	1.09	1.0945
2	50	0.473	0.524	0.4985	1.03	1.051	1.0405	0.7695
3	25	0.273	0.319	0.296	0.831	0.942	0.8865	0.59125
4	12.5	0.193	0.211	0.202	0.927	0.982	0.9545	0.57825
5	6.25	0.127	0.128	0.1275	0.987	0.866	0.9265	0.527
6	3.125	0.13	0.121	0.1255	0.832	0.866	0.849	0.48725
7	1.56	0.122	0.105	0.1135	0.852	0.762	0.807	0.46025
8	0.78	0.132	0.124	0.128	0.995	0.941	0.968	0.548
9	0.39	0.146	0.143	0.1445	1.073	0.915	0.994	0.56925
10	Control +	0.165	0.167	0.166	0.152	0.149	0.1505	0.15825
11	Control -	0.089	0.076	0.0825	0.928	1.201	1.0645	0.5735

The results of the minimum inhibitory control of squid ink extract in *Streptococcus mutans* bacteria. showed at a concentration of 1.56% have the lowest absorbance value. This means that bacterial growth is inhibited at these concentrations, so that this concentration is determined as the minimum inhibitory concentration of squid ink extract *Loligo sp.* on the growth of *Streptococcus mutans* using the UV-Vis spectrophotometer method.

## DISCUSSION

Measurement of absorbance value is done before and after incubation to get the Minimum Inhibitory Concentration determined from the difference between the measurement results of the final absorbance value (after incubation) with the initial absorbance value (before incubation). If the final absorbance value (after incubation) of each tube is greater than the initial absorbance value (before incubation) then it is concluded that bacterial growth still occurs. However, if the reverse absorbance value does not change between the final absorbance value and the initial absorbance value or the final absorbance value is smaller than the initial absorbance value, it is concluded that bacterial growth is inhibited.<sup>14</sup>

After measuring the turbidity value in each treatment tube, it was found that at a concentration of 1.56% squid ink *Loligo sp.* extract began to inhibit growth of *Staphylococcus aureus* and *Streptococcus mutans* bacteria. This is proven because the absorbance value after and before incubation is fixed. A decrease in absorbance is found at a concentration of 50% which means that there can be inhibition of bacterial growth at that concentration. If there is an increase in absorbance at lower concentrations, this is not entirely due to bacterial growth, but may be influenced by the concentration density that occurs at higher concentrations, so that it can affect the absorption of light by bacteria cells that die in the solution.

In a previous study by Rahayu PM, Pangemanan DHC and Mintjelungan C found that extract of squid ink *Loligo sp.* had inhibitory effect on the growth of *Staphylococcus aureus* bacteria, with the average inhibition zone that was formed at 11.22 mm. Likewise, with other studies on the inhibitory test of *Streptococcus mutans* by Mangindaan R, Pangemanan DHC and Mintjelungan C found that squid ink extract (*Loligo sp.*) has inhibitory properties against *Streptococcus mutans*. The average inhibition zone of squid ink extract (*Loligo sp.*) against *Streptococcus mutans* bacteria formed by 12.32 mm.

The ability of squid ink *Loligo sp.* extract to inhibit bacterial growth can be caused by the content of squid ink, namely melanin which has the ability to absorb Cd (II) and Pb (II) by functional groups contained in melanin molecules. The functional groups are phenolic hydroxyl (OH), carboxyl (COOH) and amine (NH). The ability of melanin to absorb metal ions is what will be observed by testing the activity against bacterial cell growth.<sup>8</sup>

In addition, squid ink is alkaloid, which is one of the antibacterial compounds. Alkaloids are amino acid derivatives that are antibacterial. The mechanism of action of alkaloids is inhibiting the work of enzymes that are useful for synthesizing bacterial proteins, so that bacterial metabolism is disrupted and can damage the constituent components of peptidoglycan which causes bacterial cell death.<sup>15</sup>

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