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Antibacterial and Wound Healing Activity of Papuyu Fish (*Anabas Testudineus)* Mucus

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ABSTRACT

One of the local fish resources in South Kalimantan is *Anabas testudineus* fish. It is known that *A. testudineus* fish mucus has antibacterial activity, but this study only looked at the inhibitory of fish mucus. Until now, there has been no test for the Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of *A. testudineus* fish mucus. One of the bacteria that infect wounds is *S. aureus*. MIC and MBC obtained are used as a reference in determining the concentration of *A. testedineus* fish mucus gel for wound healing activity test. This study aimed to determine the MIC and MBC of *A. testudineus* fish mucus in *S. aureus* and analyze the wound healing activity of *A. testudineus* fish mucus. MIC and MBC using turbidity test. In this research, 3 fish mucus gel formulas are made. Wound healing activity test on Wistar rats that were given wound initiation with a diameter of 1.5 cm, and were observed after 7 days of treatment. Observations were made covering the area of the wound using image J and histology of rat skin. The results showed that MIC and MBC of *A. testudineus* fish mucus were 21.875%. Fish mucus gel formula was made into concentrations of 10%, 20% and 40%. The results of the wound healing test showed that giving *A. testudineus* fish mucus caused a decrease in the area of the wound but based on histology of the skin, the inflammation still occurred.

Keywords: *Anabas testudineus*, mucus, *Staphylococcus aureus*, Wound healing

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INTRODUCTION

Inland fisheries of South Kalimantan have the highest fish species diversity in Asia (Winemiller et al., 2008). The potential of South Kalimantan's inland fisheries is one million hectares (South Kalimantan Province Government, 2019). The potential of fishery resources is utilized by the community by exploiting them through fishing activities. Fish that are the target of catching (target species) are native fish species of South Kalimantan. Department of Marine and Fisheries of South Kalimantan Province (2019) stated that the level of exploitation of inland fisheries resources in 2018 reached 75,696.71 tons. Fish consumption in South Kalimantan is more than protein consumption from other sources. Based on the data on the level of fish consumption/capita/year (2012-2016), the province of South Kalimantan is classified as a high consumption level (>31.3 kg/capita) (BPS RI, 2017).

Prasetyo and Asyari (2003) stated that types of local fish exploited in South Kalimantan reached 140 species. One of the native fish resources of South Kalimantan which is currently being intensively exploited is the papuyu fish (*Anabas testudineus*). Trend of the total catch of *A. testudineus* fish in the inland fisheries of South Kalimantan from 2011 to 2018 showed an increase (Department of Marine and Fisheries of South Kalimantan Province, 2019).

Based on the research conducted by Al-Rasheed et. al (2018), *A. testudineus* fish has an innate immune component consisting of a layer of mucus secreted on the skin, gills, and gastrointestinal tract. Mucus can able to prevent the growth of *S. aureus, E. coli, Salmonella spp., B. subtilis and A. hydrophila* (Al Rasheed et al., 2018; Subramanian et al., 2007). Midhun et al (2017) stated that *A. testudineus* fish mucus can inhibit the growth of several bacteria that are pathogenic to fish, especially *Aeromonas spp*. and *Pseudomonas spp*.

Results of the research mentioned before have known the inhibitory activity of *A. testudineus* fish mucus against several bacteria, but have not provided information about the minimum inhibitory level (MIC) and minimum killing level (MBC) of *A. testudineus* fish mucus against *Staphylococcus aureus*. *Staphylococcus aureus* is one of the bacteria that can infect wounds. The Information about MIC and MBC is important to know because MIC and MBC are the basic for determining doses for other activities, such as wound healing activities. Until now there has been no research on wound healing activity of *A. testudineus* fish mucus. This study aims to determine the antibacterial activity and wound healing of A. testudineus fish mucus.

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MATERIALS AND METHODS

Collection of *A. testudineus* Fish

This research used *A. testudineus* fish. Fish were obtained from rivers in Tungkaran village, Banjar district, South Kalimantan. This fish was identified in the faculty of Fisheries and Marine, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia

Extraction of *A. testudineus* Fish Mucus

Measure the weight of fish, then put fish into container. Place the container in the freezer at a temperature of (-15^0) – (-20^0) for 1 hour. After 1 hour, remove fish and let it rest at room temperature for 30 minutes. Rinse fish with aquadest (ratio 1 : 1). Filter the rinsed water and store it in the freezer (Modification from Al Rasheed et al., 2018).

Determination of Protein

Determination of protein content using the Kjehdahl method. Protein was calculated according to the formulas below (Saputro et al., 2019; (Mariotti et al. 2008):

Determination of Borate Ion:

Borate content = (V HCL x N HCL) – (V $B(OH)^{4-}$ x N $B(OH)^{4-}$)

Determination of Protein Levels:

$$\% \text{ Nitrogen Content} = \frac{\text{Borate Ion Content x BE Nitrogen}}{\text{W (initial weight of sample)}}$$

% Protein = % Nitrogen Content x Conversion Factor (6.25)

Turbidity Test

Minimum Inhibitory Concentration (MIC) was determined using a broth dilution method. Cultures of *Streptococcus aureus* were grown in heart infusion broth (BHI Broth, Oxoid UK) and each culture was adjusted to McFarland standard 0.5. Results of the preliminary test showed that MIC of *A. testudineus* fish mucus was 25%, so mucus concentration used in this test were 12.5%, 15.625%, 18.75%, 21.875% and 25%. Put 0,5 mL a *Streptococcus aureus* into test tube containing 5 mL of various concentration *A. testudineus* fish mucus and 5 mL BHI broth. Test tubes were incubated at 37 °C for 24 h and then observed for growth or turbidity. Tube showing the absence of bacterial growth, which is indicated by a clear solution. MIC is determined by looking at the smallest concentration of the tube which indicates the absence of bacterial growth. A loop full of broth from each test tube was not showing growth, the solution in that tube were inoculated into nutrient agar plate and incubated further for 24 h at 37 ^{0}C. Then, agar plates were examined for growth of bacteria (CLSI, 2012). The procedure was repeated three times. The MBC is the least concentration of antimicrobial agent that prevents microbial growth (Kumari et al., 2019).

Wound Healing Test

Rats were randomly separated into at least 5 cages for adaptation for 1 week. During the adaptation period, rats received food and drank ad libitum. Each cage contained 5 rats. Rat weight between 200 - 250 g. Two groups as a control groups, namely Negative Control (NC), rats were only given gel base, and three groups were treated as gel treatment group using *A. testudineus* fish mucus with

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concentration based on the value of MBC, namely ½ x MBC (MA1), 1 x MBC (MA2) and 2 MBC (MA3). Before wound incision, Rats were given anesthetic ketamine injection at a dose of 40 mg/kg BW. Rats that had received anesthesia were initiated on the back with a diameter of 2 cm until the skin was detached. Once a day, the wound smeared with gel according to the treatment. Observations were made on 7th day (Modification of Liu et al., 2019; IACUC, 2020).

RESULTS DISCUSSION

Results of the measurement of protein content of *A. testudineus* fish mucus averaged 0.32%. Preliminary test results showed that starting at a concentration of 25% there was a decrease in absorbance, so it can be said that MIC of fish mucus was above 12.5% - 25%. To ensure the concentration, another test was carried out by making a dose range between 12.5% - 25%. In this test, it was made into 5 concentrations, so that the concentration were 25%; 21.875%; 18.75%; 15.625% and 12.5%. The results of this research showed that at a concentration of 21.875% it was seen that the solution still looked clear and there was no change in the absorbance value before and after incubation. Test of MBC showed that there was no bacterial growth so it could be concluded that the MIC value of *A. testudineus* fish mucus was 21.875%. This value was be used for the wound healing test, with concentrations of 10%, 20% and 40%.

Wound Healing

A. testudineus fish naturally has immune components in the form of a layer of mucus that is secreted on the skin, gills, and gastrointestinal tract. Mucus can able to prevent the growth of *S. aureus, E. coli, Salmonella spp., B. Subtili, A. Hydrophila, Aeromonas spp. and Psudomonas spp* (Midhun et al, 2017; Al Rasheed et al, 2018; Subramanian et al., 2007).

Mucus is composed of water and gelling macromolecules including mucins and other glycoproteins. Mucus layer on the surface of fish has several functions, namely in maintaining resistance to disease, respiration, ion and osmotic regulation, movement, reproduction and communication (Wei et al., 2010). *A. testudineus* fish mucus has several enzyme compounds in the form of protease, lysozyme, alkaline phosphatase (ALP), and esterase (Al-Rasheed et al., 2020). Protease works by degrading mucus and biofilm, so that it can penetrate the bacterial cell wall and cause lysis of bacterial cells (Bhaskar et al., 2007). N-Acetyl Neuramide Glycan Hydrolase (Lysozyme) is a hydrolyzing enzyme that can kill pathogens by causing bacterial cell lysis (Melani et al., 2013; Al Rasheed et al., 2018). Alkaline phosphatase or ALP functioned to reduce inflammation by dephosphorylate compounds and detoxify endotoxins (lipopolysaccharides), which are *S. typhi* virulence factors and important mediators of sepsis (Authman et al., 2018).

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a b c d

Figure 1. Pictures of rat wound after being treated for 7 days. (a) negative control, (b) mucus gel 10%, (c) mucus gel 20%, (d) mucus gel 40%

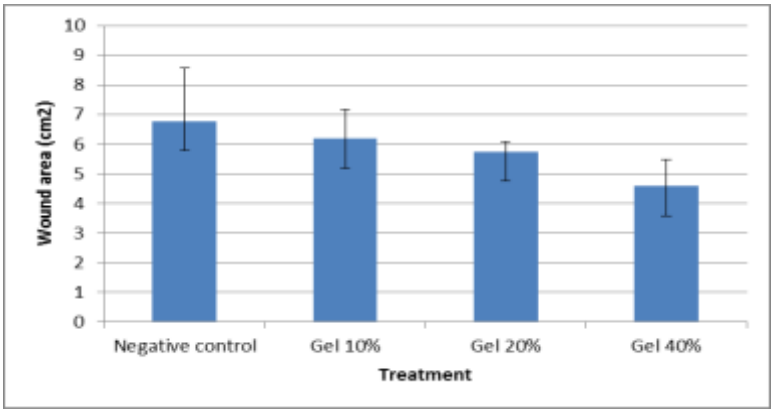

Figure 2. Graph of wound area by treatment. The area of the wound were calculated using the image J

Magnification	NC	MA 1	MA 2	MA 3
40 x				
100 x				

Figure 3. Histology of rat skin under various treatments and magnifications. Histological results showed that all samples were still in the ulcer phase, there were no improvement.

In this study, it was seen that mucus gel of *A. testudineus* fish could accelerate wound healing seen from the area of the wound, but when viewed from the histology of the skin, it was seen that in all treatments there were still no change because all samples were still in an ulcer or inflammatory condition. The wound healing process itself consists of 4 phases that occur including haemostasis, inflammation, proliferation and remodeling. Haemostasis is the process of wound matrix formation and the release of cytokines and growth factors from the wound. Inflammation is a process mediated by neutrophils and macrophages that

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will kill bacteria and denature matrix components that inhibit wound healing. Proliferation is the process of forming new cells that will cover the wound. Remodeling is the process of removing the initial scar tissue that will be replaced with normal skin (Schutz et al., 2011; Gonzales et al., 2016). The presence of antibacterial activity in the mucus of *A. testudineus* fish can accelerate the inflammatory phase, but the length of the study which was only 7 days caused the wound healing process was still in the inflammatory phase. Further research is needed with a longer duration of research.

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