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ANTIBACTERIAL ACTIVITY OF CHITOSAN FROM HARUAN *(Channa striata)* FISH SCALES AGAINST THE GROWTH OF *Porphyromonas gingivalis*

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ABSTRACT
Background: *Periodontal disease is one of the dental and oral diseases that is often found in people in Indonesia. RISKESDAS (2018) stated that the prevalence of people who have dental and oral health problems in Indonesia is 57.6%. The prevalence of oral and dental problems in South Kalimantan is 60%. Periodontal disease is damage to the supporting tissues of the teeth caused by specific microorganisms such as Porphyromonas gingivalis which are gram negative bacteria. The growth of these bacteria can be inhibited and killed by chitosan from haruan fish scales. Chitosan is a natural product of chitin, chitosan is the basic ingredient of fish scales. Chitosan has advantages and more safety levels, because it has an active group that will bind to microbes so chitosan can inhibit and kill microbial growth.* ***Objective***: *to analyze the antibacterial activity of chitosan scales (Channa striata) on the growth of Porphyromonas gingivalis*. ***Methods:*** *This research used true experimental with randomized pre and post test with only control group design with eight treatments. Chitosan of haruan fish scales with liquid dilution method to obtain MIC and solid dilution test to obtain MBC.* ***Results:*** *The results showed that Minimum Inhibitory Concentration (MIC) of chitosan haruan scales was found at a concentration of 2.5% characterized by a decrease in absorbance value of 0.092 and Minimum Bactericidal Concentration found at a concentration of 20% characterized by the absence of Porphyromonas gingivalis.* ***Conclusion:*** *There is an inhibitory activity and a bactericidal activity from chitosan of haruan fish scales on Porphyromonas gingivalis.*

Keywords: Antibacterial, chitosan from haruan fish scales, *Porphyromonas gingivalis*

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INTRODUCTION

Periodontal disease is one of the dental and oral diseases that is often found in people in Indonesia. RISKESDAS (2018) mentions that 57.6% of Indonesian population were presented with dental and oral health problems. The prevalence of oral and dental problems in South Kalimantan is 60%. Periodontal disease is damage to the supporting tissues of the teeth caused by specific microorganisms such as *Porphyromonas gingivalis* which are gram negative bacteria. [1,2]

The initial process of periodontitis begins with a shift in the normal bacterial flora in the oral cavity to become a pathogen. Some bacteria in the oral cavity can stick and colonize to the surface of the teeth, then become a place for colonization of other bacteria, such as the bacteria *Streptococcus sanguis.*[1.3] *Streptococcus sanguis* is the first type of coccus capable of attaching to the teeth and facilitating other bacteria to colonize the pellicle and form biofilms.[4] Primary colonization on the tooth surface is dominated by aerobic bacteria, then followed by adhesion of *Porphyromonas gingivalis* bacteria which is a secondary colony.[5,6] *Porphyromonas gingivalis* will produce virulence factors such as Lipopolysaccharide that will bind to CD14 macrophages which will be recognized by TLR-4, then macrophages will release cytokines and activate host inflammatory responses such as TNF-α, IL- 1β, IL-6 and IL-8 which will cause vasodilation and permeability of blood vessels.[7] High levels of these cytokines can cause damage to periodontal tissues. [8]

The treatment of periodontitis is focused on controlling the progression of the disease and changes in the periodontal environment of the periodontal microbial flora of pathogens to compatible flora for healthy gingiva, while other alternatives, such as natural medicine, have recently become more popular in society because they have side effects of at least. [8.9] Adults This utilization of waste is increasingly popular in various fields, the

scales of haruan fish consist of two layers, namely the thin outer layer is the epidermis which is formed by epithelial cells and the layers below are the dermis, kutin and korium.[10]

Under the dermis there is a layer containing chitin. The nature of chitin which is non-toxic and easily degraded encourages the modification of chitin with the aim of optimizing usability and expanding the field of chitin application. One of the many chitin derivative compounds which is developed because of its extensive plastics is chitosan. Chitosan is a natural product of chitin, the basic ingredients oftaralain chitosan from fish scales. Chitosan has advantages and more safety levels, because it has an active group that will bind to microbes, chitosan is able to inhibit and kill microbial growth.[11]

The results of Akca et al., 2018 stated that commercial chitosan from blue crabs has a inhibitory power of 1.25% against bacteria *Porphyromonas gingivalis*. *Porphyromonas gingivalis* is the dominant microorganism that causes periodontal disease. Based on the description above it is known that blue crab chitosan can inhibit *Porphyromonas gingivalis* growth, but it is not yet known the antibacterial activity of chitosan scales (*Channa striata)* extract against *Porphyromonas gingivalis* bacteria with a concentration of 1.25%, 2.5%, 5%, 10%, 20%, 40%.

MATERIALS AND METHODS

Before the research was conducted, it had been submitted to the ethics commission at the Faculty of Dentistry, University of Lambung Mangkurat and was declared feasible based on the letter of ethics eligibility number: 109 / KEPKG-FKGULM / EC / I / 2019. This research used pure research (true experimental) with randomized pre and post with only control group design with 8 treatment groups. The sample used in this study were chitosan scales of haruan fish with a concentration of 1.25%, 2.5%, 5%, 10%, 20%, and 40%. The positive control used was 0.2% chlorhexidine and the negative control used was 1% acetic acid. The number of samples from Federer formula for each group comprised of 4 repetitions.

The Making Of Chitosan Fish Scales

Scales were obtained from industrial waste of haruan cracker craftsmen, then scales were collected and washed using running water. Wet scales were weighed with digital scales and obtained 3000 grams of scales and were stored in an ice box to keep them fresh. The scales were dried at 50°C in an oven for 24 hours. Furthermore, the scales that had been dried were weighed again and obtained a weight of 1003 grams. The scales were then crushed by blending to powder and stored in an airtight container.

Chitin Isolation

Deproteination. At this stage the chitin isolation of the fish scales that had been destroyed was washed. Scales were placed in 1000 ml of glass and were soaked in boiling sodium hydroxide (4% b / v) in one hour which aimed to dissolve protein and sugar and obtained 4% NaOH with a ratio of powder and solution, which was 1: 4 b / v used for chitin preparation. Samples were boiled in sodium hydroxide, beaker containing fish scales samples. The samples were then cooled for 30 minutes at room temperature and filtering sample residues were performed. The samples were washed repeatedly using distilled water to get a neutral pH. Next, the sample residue was filtered back and put into the oven to be dried for 24 hours at 50°C. Then the weighing was done again and the deproteination sample acquired 620 grams.

Demineralization. Scales were demineralized with 1% HCl with a ratio of powder and solution, which was 1: 4 b/v and soaked for 24 hours to remove minerals (especially calcium carbonate). The sample residues were filtered and the samples were washed repeatedly by using distilled water to get a neutral pH. Furthermore, the neutral samples were put into the oven for 24 hours with a temperature of 50°C and were re-weighed to obtain a demineralization sample of 342 grams. Converting chitin to chitosan was preceded by deacetylation process.

Deacetylization. The deacetylation process used 50% NaOH solution with the ratio 1: 4 b / v of powder and solution. It was then boiled for 2 hours at 100° on the hot plate. The samples were cooled for 30 minutes at room temperature. Then, the samples were washed continuously with 50% NaOH which had been cooled and filtered the residue. Then, the samples were washed with distilled water repeatedly until the pH was neutral. The samples was filtered to maintain its solidity, namely chitosan. The samples were dried in the oven for 24 hours at 50°C and weighed again and obtained a deacetylation sample of 82.43 grams. Chitosan obtained from the results of deacetylation in the form of white powder.

Cultivation of *Porphyromonas gingivalis* Bacterial Isolates

Breeding of *Porphyromonas gingivalis* ATCC 33277 bacteria used 30 ml BHIB media and incubated for 2x24 hours at 37^{o}C in anaerobic condition using anaerobic jar. In a sterile test tube, an aquadest was added until the turbidity was equal as 0.5 McFarland standard.

Minimum Inhibitory Concentration Test (MIC)

Chitosan scales of fresh fish in the form of 500 mg powder were dissolved in 1% acetic acid. Chitosan solution which had been diluted according to the concentration were made each 1 ml for

positive control of chlorhexidine 0.2% and negative control of 1% acetic acid which then added to the culture of *Porphyromonas gingivalis* bacteria, The samples were covered with sterile cotton then homogenized with vortex mixer to be incubated at 37°C for 24 hours in anaerobic conditions. Chitosan solution of fragrant fish scales and *Porphyromonas gingivalis* bacteria which were incubated were measured using a Biobase BK-D 560 UV-Vis spectrophotometer with a wavelength of 450 nm to obtain MIC.

Minimum Bactericidal Concentration (MBC)

Chitosan scales of Haruan fish and *Porphyromonas gingivalis* bacteria were taken 5 µl from the inoculation and spread on Nutrient Agar (NA) media with micropipets which had been sterilized with 96% alcohol, then incubated 24 hours at 37^{o}C under anaerobic conditions, then *Porphyromonas gingivalis* bacteria growth was seen directly to get MBC.

RESULTS

Based on the results of the study, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are as follows:

Table 1. Minimum Inhibitory Concentration Test Result from Chitosan Haruan Fish Scales (*Channa Striata*) on *Porphyromonas gingivalis* Growth

Concentration	N	Incubation 0 hours	Incubation 24 hours	Difference	Information
1,25%	4	0.218	0.234	0.016	Up
2,5%	4	0.358	0.266	-0.092	Down
5%	4	0.529	0.286	-0.243	Down
10%	4	1.875	0.755	-1.120	Down
20%	4	2.322	0.860	-1.462	Down
40%	4	2.412	0.949	-1.463	Down
Chlorhexidine 0,2%	4	2.070	1.044	-1.026	Down
Acetic acid 1%	4	0.216	0.318	0.102	Up

Information:

Up : Cannot inhibit the growth of *Porphyromonas gingivalis*

Down : Can inhibit the growth of *Porphyromonas gingivalis.*

Table 1 illustrated the results of the research using the Biobase BK-D 560 UV-Vis spectrophotometer. The minimum inhibitory concentration (MIC) of haruan fish scales was obtained at a concentration of 2.5% on the growth of *Porphyromonas gingivalis* which was characterized by a decrease in absorbance value of 0.092.

Table 2. Minimum Bactericidal Concentration Test Results from Chitosan Haruan Fish Scales (*Channa Striata)* on *Porphyromonas gingivalis* Growth

Concentration	N	Mean (CFU/µL)
KSH 2,5%	4	149.2
KSH 5%	4	44.2
KSH 10%	4	31.8
KSH 20%	4	0
KSH 40%	4	0
CHX 0,2%	4	0.5
Acetic acid 1%	4	1565

Information:

N : Many repetitions
2.5% KSH : Chitosan Haruan Scales for 2.5%
5% KSH : Chitosan Haruan Scales for 5%
10% KSH : Chitosan Haruan Scales for 10%
20% KSH : Chitosan Haruan Scales for 20%
40% KSH : Chitosan Haruan Scales for 40%
0.2%CHX : Chlorhexidine gluconate 0.2%
1% As asetat : 1% acetic acid

Table 2 obtained results in each sample group through manual calculation using colony counter to determine the minimum bactericidal concentration (MBC) on the growth of *Porphyromonas gingivalis*. Minimum Bactericidal Concentration (MBC) was found in 20% chitosan concentration with an average colony growth of 0 CFU/ml.

Data analysis in this study used the Saphiro-Wilk normality test and obtained results, namely the data in this study were normally distributed with a significance value (P> 0.05). Homogeneity test was conducted with Levene's Test. Dunnet's post hoc test results revealed differences in the antibacterial activity of chitosan scales of haruan fish against the growth of *Porphyromonas gingivalis*.

DISCUSSION

The results of the research on the antibacterial activity of chitosan scales (*Channa striata*) on the growth of *Porphyromonas gingivalis* showed that chitosan scales were haruan (*Channa striata*) with a concentration of 1.25%; 2.5%; 5%; and 10% can inhibit the growth of *Porphyromonas gingivalis* bacteria and at a concentration of 20%; 40% can kill the growth of *Porphyromonas gingivalis* bacteria. The inhibition of *Porphyromonas gingivalis* growth can be seen by decreasing the absorbance value in each treatment and the lowest absorbance value at a concentration of 10%, which means that the concentration is the most effective concentration. After observing the inhibitory power, it was followed by observing the power of killing.

Observation of killing power was seen in the concentration of Haruan chitosan scales of 20% and

40%. The absence of colonies of *Porphyromonas gingivalis* bacteria formed in Nutrient Agar after incubation for 1x24 hours under anaerobic conditions showed that the concentration of Haruan chitosan scales 20% and 40% had the power to kill *Porphyromonas gingivalis* bacteria. At a concentration of 2.5% the minimum inhibitory concentration (MIC) can inhibit the growth of *Porphyromonas gingivalis* bacteria. Chitosan has a specific form that contains an amino group in its carbon chain that is positively charged, so that in a liquid state it will be sensitive to high ionic strength.

Chitosan has a highly reactive, positively charged amine (–NH2) functional group, so it can bind to the negatively charged cell wall of *Porphyromonas gingivalis*. As an antibacterial, chitosan has an inhibitory mechanism, where chitosan will bind to cell membrane proteins, namely glutamate which is a component of cell membranes. In addition to binding to membrane proteins, chitosan also binds to membrane phospholipids, especially choline, thereby increasing the inner permeability of the membrane. Increasing permeability of the inner phosphatidyl membrane will facilitate the release of cell fluids in the cytoplasm (lysis) so that it will inhibit cell division (regeneration).[12,13]

The concentration of 20% was claimed as the Minimum Bactericidal Concentration (MBC) because the mechanism possessed by chitosan as an antibacterial is depicting a very strong affinity with microbial DNA. Thus, it can bind to DNA which then interferes with mRNA and protein synthesis. The interaction between the chitosan polymer chain which is positively charged and the negatively charged cell surface of *Porphyromonas gingivalis* cell can change bacterial cell permeability and cause leakage of protein components and other intracellular components, which then causes bacterial cell death. The antimicrobial affinity of chitosan in fighting bacteria or microorganisms depends on molecular weight and degree of deacetylation. Greater degrees of deacetylation show greater antimicrobial activity.[14,15]

According to Aliasghari et al., 2016 and Costa et al., 2013, it was stated that the greater the concentration of chitosan, the higher the ability to inhibit and kill bacteria. This can be expected to occur because of the difference in the number of amine groups (-NH2) which are positively charged on chitosan which will bind to the surface of the negatively charged bacterial cell. The greater the concentration of chitosan, the more the number of amine groups (-NH2) which can bind to the surface of bacterial cells, making it more effective in inhibiting and killing bacteria. It can be concluded that chitosan scales (*Channa striata*) at 20% and 40% concentration demonstrates a superior killing ability against *Porphyromonas gingivalis* bacteria compared to 0.2% Chlorhexidine gluconate, because the antibacterial content contained in chitosan scales of haruan fish works in a complex mechanism against the growth of *Porphyromonas gingivalis.*

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