Activity Test of Chitosan Haruan (Chana Striata) Fish Scales as Antibiofilm Agent Against Biofilm of Porphyromonas Gingivitis

by Rosihan Adhani

Activity test of chitosan haruan (*Channa striata*) fish scales as antibiofilm agent againts biofilm of *Porphyromonas gingivalis*

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1. Introduction

The prevalence of people who have dental and oral health problems in Indonesia based on [1] is 57,6%. One of them is periodontal disease, a chronic inflammation due to the inflammatory response of the supporting tissues. The main cause of this inflammation is the biofilm of subgingival bacterial plaque with its virulence factors. Initial biofilm formation begins with the attachment of bacterial adesin to tooth surface receptors containing glycoprotein which then facilitates other bacteria to colonize by producing a highly sticky extracellular polymeric matrix to strengthen the integrity of the biofilm [2,3]

Biofilms have been known to be involved in a wide variety of microbial infections. In dentistry,

biofilm is very influential on the formation of dental plaque. Plaque contains a collection of bacteria bound in an organic matrix and tightly attached to the tooth surface so that it becomes a major problem that can cause infectious diseases in the oral cavity. *Porphyromonas gingivalis*, a secondary biofilm-forming bacteria, is the dominant bacteria in plaque that can cause infection in periodontal tissues. *P.gingivalis* colonization of the periodontal sulcus or pocket is the first step in the development of periodontal disease. Its virulence factors include lipopolysaccharide which will bind to CD14 macrophages which in turn will release proinflammatory cytokines. High levels of these cytokines will cause damage to the periodontal tissue and cause periodontitis [4]

Periodontitis can be prevented by inhibiting the biofilm formation of *P.gingivalis* bacteria using chlorhexidine gluconate mouthwash, which is the gold standard in periodontal treatment. The mouthwash has been shown to have bactericidal and bacteriostatic properties against a wide variety of bacteria, including the bacteria that make up biofilms. However, if used for a long period of time, chlorhexidine will cause black stains on the teeth and disrupt the ecology of the normal flora in the oral cavity [5].

Traditional medicine is very popular in South Kalimantan, including using Haruan Fish [6] Haruan fish is known to have the property of accelerating wound healing and has been shown to have anti-inflammatory effects. Many studies have been developed on chitosan derived from freshwater fish scales, including Haruan fish scales. According to Dania 2020, chitosan of haruan fish scales with concentrations of 2.5%, 10%, 20% and 40% was proven to be able to inhibit and kill the growth of *P. gingivalis* bacteria with 2.5% as MIC and 20% as MBC. Costa (2014) said that commercial chitosan was proven to be able to inhibit biofilms. Based on the results of Archana (2013) research, chitosan with a concentration of 0.5% was able to inhibit the biofilm of several oral bacteria such as *S. sanguis* and *S. mutant*, but there was no specific study on the biofilm of gram-negative bacteria such as *Porphyromonas gingivalis*. Therefore, the researchers wanted to test the activity chitosan of haruan fish scales against *P.gingivalis* biofilm.

2. Materials and Methods

This research has obtained a research permit and ethical feasibility issued by the Health Research Ethics Commission of the Faculty of Dentistry, Lambung Mangkurat University No.016/KEPKG-FKGULM/EC/1/2020.

The research design used was a true experimental design with a post test with control group design. The population in this study used a pure isolate of the bacterium *Porphyromonas gingivalis* ATCC 33277 obtained from the Microbiology Laboratory of Airlangga University. The bacteria were diluted according to the standard Mc Farland scale of 0,5 and were cultured on TSB Glu media in 96 well plates and incubated for 5 days at 37° to form a biofilm. After the biofilm was formed on the 5th day, then rinsed with PBS solution 3 times, given Crystal violet staining for 5 minutes in each well, then rinsed again with PBS solution, then added 0.1 ml of chitosan suspension of concentrations 2.5%, 10%, 20%, 40% and positive control chlorhexidine gluconate 0.2% to each well, then incubated for 1 hour in an incubator at 37°. Measured with a Microplate Reader Spechtrophotometer and seen Optical density with a wavelength of 570 nm. The data obtained were analyzed by the One Way Anova Test and continued with the Posthoc Games Howell Test.

3. Results

Activity Test of Chitosan Haruan Fish Scales and 0.2% Chlorhexidine gluconate against *Porphyromoas gingivalis* biofilm was carried out by measuring Optical density using a Microplate Reader Spechtrophotometer.

Table 1. Value of optical density of Chitosan Haruan Fish Scales and 0.2% Chlorhexidine Gluconate against Porphyromonas gingivalis biofilm.

No	CHFS 2,5%	CHFS 10%	CHFS 20%	CHFS 40%	Control (+) Chlorheksidine Gluconate 0,2%
1.	2,2475	1,8854	1,4560	0,8250	0,3448
2.	2,2284	1,8684	1,4688	0,8290	0,3455
3.	2,2115	1,8790	1,4690	0,8285	0,4209
4.	2,2090	1,8860	1,4715	0,8215	0,4018
5.	2,2532	1,8785	1,4554	0,8245	0,4226

Description:
CHFS2,5%: Chitosan Haruan Fish Scales 2,5%
CHFS 10%: Chitosan Haruan Fish Scales 10%
CHFS 20%: Chitosan Haruan Fish Scales 20%
CHFS 40%: Chitosan Haruan Fish Scales 40%

Table 1 shows that there are differences in optical density values of each treatment in destroying the *Porphyromonas gingivalis* biofilm which was carried out 5 times.

Data from the activity test of the chitosan haruan fish scales against the *Porphyromonas gingivalis* biofilm were then carried out statistical analysis tests using SPSS 26.0. The data normality test was carried out with first using the Saphiro-Wilk Test, then the data homogeneity test with Levene's Test. The normality test results showed that the data were normally distributed $p = 0.351$ ($p > 0.05$). Furthermore, the results of the homogeneity test showed that the data was not homogeneous, $p = 0.011$ ($p < 0.05$) so that it was continued with the One Way Anova parametric analysis and continued with the Post hoc Games Howell significance test.

The results of the One Way Anova statistical test showed the value of $p = 0.000$ ($p < 0.05$), which means that H0 is rejected. Then continued with the Post hoc Games Howell significance test.

Tabel 2. Games-Howell Post hoc test Chitosan Haruan Fish Scales and 0.2% Chlorhexidine Gluconate against *Porphyromonas gingivalis* biofilm.

Perlakuan	CH 0,2%	CHFS 40%	CHFS 20%	CHFS 10%	CHFS 2,5%
CHFS 0,2%		0,000*	0,000*	0,000*	0,000*
CHFS 40%			0,000*	0,000*	0,000*
CHFS 20%				0,000*	0,000*
CHFS 10%					0,000*
CHFS 2,5%					

*Significance p<0,05)

Table 2 shows the p<0.05 in each treatment of haruan fish scale chitosan compared to 0.2% chlorhexidine gluconate. Each treatment has a significant difference when compared to other treatments.

4. Discussion

The research on the activity of chitosan Haruan fish scales (*Channa striata*) against *Porphyromonas gingivalis* biofilm showed that chitosan Haruan fish scales (*Channa striata*) with concentrations of 2.5%, 10%, 20%, 40% can damage *P.gingivalis* biofilm. When measuring the optical density value using a Microplate Reader Spectrophotometer with a wavelength of 570 nm according to the wavelength that can be absorbed by crystal violet.[6] It was found that chitosan Haruan fish scale with concentrations

2.5%, 10%, 20% and 40% had an effect on damaging *P.gingivalis* biofilm which was better when given higher concentrations.

The ability of the chitosan Haruan fish scale to damage *P.gingivalis* biofilm can be seen by the decrease in Optical density value in each treatment and the lowest is found at a concentration of 40%, which means that this concentration is the most effective concentration to damage *P.gingivalis* biofilm. This can be seen from the treatment test media, that if the concentration is higher, the turbidity of the test media will also decrease, in line with research conducted by Aviantina 2018 which states that this level of turbidity indicates how much biofilm is still present.

Biofilms can be formed and developed through three stages, namely adhesion, maturation, and differentiation [7] Compounds from chitosan can act as antibiofilm by interfering with the processes of these stages [8] Yasir in his research in 2018 proved that the antibiofilm compounds present in chitosan were able to degrade polysaccharide components and the biofilm matrix and disrupt the communication system between bacterial cells.

Warroka 2016 in her research stated that the higher the concentration of the chitosan used, the higher the activity of chitosan in damaging the biofilm because the content of molecules that act as antibiofilm in chitosan is getting bigger. It was shown from the 40% concentration of chitosan Haruan fish scale was more effective in destroying *P.gingivalis* biofilm than the 2.5%, 10% and 20% concentration of the chitosan Haruan fish scale. The 20% concentration of chitosan Haruan fish scale was also more effective than the 10% and 2.5% concentrations and so on. Thus, the ability of haruan fish scale chitosan to damage *P.gingivalis* biofilm was influenced by its concentration. This is in accordance with research conducted by Costa 2013 and Aliasghari 2016 that the greater the concentration of chitosan, the higher its ability to damage biofilms.

This is presumably due to the difference in the number of positively charged amine groups ($-NH_2$) in chitosan which will bind to the negatively charged bacterial cell surface. The greater the concentration of chitosan, the more the number of amine groups present. This also can be related to the degree of deacetylation of chitosan. Putri in her research in 2020 proved that chitosan Haruan fish scale (*Channa Striata*) which went through a deacetylation process with 50% sodium hydroxide at 80^0C had a degree of deastilization of 85.25% higher than the SNI chitosan ($\geq$75%). The higher the degree of deacetylation of chitosan, the greater the number of positively charged amine groups formed so that its ability to bind to the bacterial cell wall also increases. The number of amine groups is directly proportional to the amount of chitosan concentration, the greater the concentration of chitosan, the greater the number of amine groups.

The mechanism of chitosan Haruan fish scale in damaging the *P.gingivalis* biofilm is due to the interaction of the positively charged NH^+ amino group of chitosan with the negatively charged OH^- carboxylate group of the bacterial cell membrane forming an electrostatic force which will make the permeability of the bacterial cell membrane unstable and increase, resulting in leakage and lysis of bacterial intracellular constituents such as K^+ ions, proteins, nucleic acids, and glucose which will eventually cause bacterial cell death and reduce biofilm integrity [10].

This is in line with Tan's 2013 research which states that chitosan has a free amino group that is protonated to form a polycationic acid in an acidic environment, so that the chitosan polysaccharide is positively charged. This polycationic will interact with anionic groups on the cell wall, this interaction will form an impermeable layer around the bacterial cell, then this layer will block the transport of molecules needed by bacterial cells.

Chitosan can also penetrate into the bacterial nucleus by permeation which then enters the nucleus and binds to DNA to then inhibit the process of DNA transcription, RNA and protein synthesis [10]. Tania 2020 in her research proved that chitosan Haruan fish scale has a specific shape that contains an amino group in its carbon chain and is positively charged, so that in a liquid state it will be sensitive to high ionic strength. Chitosan has an amine group (-NH2) which is positively charged and highly reactive, so that it is able to bind to the negatively charged *P.gingivalis* bacterial cell wall [12].

The results of this study showed that 0.2% chlorhexidine gluconate was more effective than the other treatment groups, this was because 0.2% chlorhexidine was able to precipitate cytoplasmic acid proteins

resulting in cell wall permeability and cell leakage which could interfere with the growth of *P gingivalis* biofilms [12]. Sinaredi 2014 in his research also stated that chlorhexidine gluconate was effective for destroying gram-positive and gram-negative bacterial biofilms, depending on the concentration used. The chlorhexidine gluconate molecule has a positive charge and most of the bacterial molecular charge is negative, this causes a strong attachment between chlorhexidine gluconate and the bacterial cell membrane. Chlorhexidine will cause an increase in the permeability of the bacterial cell membrane, causing the release of the cell cytoplasm and low molecular weight components capable of penetrating the cell membrane, causing bacterial death and followed by a decrease in the integrity of the biofilm. This is in line with the research conducted by Mareta 2014 which stated that 0.2% chlorhexidine gluconate is bacteriostatic and bactericidal and has a broad spectrum and can inhibit microbial activity. Based on the result of this study, it can be concluded that Chitosan of Haruan Fish Scales at concentrations of 2,5%, 10%, 20%, dan 40% proed capable of damaging the biofilm, resulting in a decrese in the untegrity of the *Porphyromonas gingivalis* biofilm.

Acknowledgments

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