Sys Rev Pharm 2020; 11(6): 836 – 841
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Antibacterial Chitosan of Milkfish Scales (*Chanos chanos*) on Bacteria *Prophyromonas gingivalis* & *Agregatibacter actinomycetemcomitans*

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Article History: *Submitted: 13.04.2020* *Revised: 17.05.2020* *Accepted: 24.06.2020*

ABSTRACT

This research was conducted to appear the effectiveness of milkfish scales chitosan gel *(Chanos Chanos*) against bacterial inhibition of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* which are pathogenic bacteria that cause periodontitis. This research was conducted with five treatments with five repetitions, the five treatments, namely: Positive control (metronidazole), negative control (*aquadest),* chitosan gel in milkfish scales concentration of 1%, 5%, and 10%. Measuring instrument of this research uses calipers with denominations of millimeters (mm). Based on the results of the Mann Whitney test, there was a significant difference in inhibition of chitosan gel on the scale of milkfish 1%, 5%, and 10% against *the Aggregatibacter actinomycetemcomitans & Porphyromonas gingivalis gel* (p <0.005), and based on the Kruskal Wallis test, it was found that the higher concentration of gel milkfish scales, the higher the average inhibition power. It was concluded that milkfish scales gel chitosan (*Chanos chanos*) can inhibit the growth of *Aggregatibacter actinomycetemcomitans & Porphyromonas gingivalis* and the higher the concentration of chitosan gel in milkfish scales, the higher the inhibition zone produced.

Keywords: Chitosan gel, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, Antibacterial.

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DOI: 10.31838/srp.2020.6.121

INTRODUCTION

Tooth and mouth disease is one of the most common diseases affecting Indonesian people today. Dental and oral diseases that are most commonly complained are caries and periodontal disease. Periodontal disease is an inflammatory and destructive disease of periodontal tissue caused by pathogenic bacteria. Periodontal disease causes damage to periodontal tissue and can affect a person's quality of life such as disrupted eating, tooth loss, social and economic conditions.[1]

Periodontitis is a form of periodontal disease. Periodontitis is inflammation of the tooth supporting tissue caused by a specific group of microorganisms, which results in progressive damage to periodontal ligaments and alveolar bone characterized by pockets, recessions, or both.[2] Periodontitis occurs as a result of infection of specific microorganisms from pathogenic bacteria that coexist.[3]

Most of the periodontal pathogens are anaerobes, and others are facultative aerobics, capnophils and microaerophils whose numbers depend on biofilms and periodontal pockets. Many pathogenic bacteria that cause periodontal disease are *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Prevotella intermedia, Campylobacter rectus, Eubacterium nodatum, Treponema sp, S. intermedius, P. micros, P. nigrescens, E. nucleatum, E. cor sp. The bacteria A. actinomycetemcomitans, Tannerella forsythia*, and *Porphyromonas gingivalis* are bacteria that are strongly associated with the initiation of periodontal disease, disease progression, and causes of unsuccessful periodontal therapy.[1,3]

Porphyromonas gingivalis is one of the main etiological agents in the pathogenesis and development of periodontitis. This bacteria is found in 85.75% of plaque samples of patients with chronic periodontitis. This bacteria is an anaerobic gram-negative bacteria and is non-motile which forms a black pigmented colony on blood agar plates.[4]

Aggregatibacter actinomycetemcomitans are non-motile gram-negative coccobacillus with high potential virulence factors. This bacteria is one of the etiologies in the occurrence of local aggressive periodontitis.[5] Colonization of *Aggregatibacter actinomycetemcomitans* is strongly associated with forms of aggressive periodontitis in adolescents and young adults, and this organism is also a systemic pathogen, associated with non-oral infections such as endocarditis.[6]

Treatment of periodontal disease consists of surgical and non-surgical treatments. Surgical treatment can be in the form of Scaling, Root Planning and antimicrobial therapy. Antimicrobial therapy can be used locally such as mouthwash and systemic antibiotic treatment.[7]

The sustainable potential of marine fisheries in Indonesia is very large, this is supported by the vast territorial waters of Indonesia. Milkfish is one type of brackish water aquaculture (ponds) which is also a material for general

public consumption, the average portion of fish meat that can be consumed (edible portion) is 40-50%. The body parts of fish that usually become waste are scales, skin, bones, gills, all internal organs, namely the pancreas, liver, heart, gonads, swimming bubbles, and intestines.[8]

Over time, many researches have been conducted on fish waste. One part of fish that can be used is scales. In general, fish have scales that contain Chitin. Chitin is then changed into chitosan.[9]

Chitosan is a derivative of chitin which is desethylated. Chitosan is a linear biopolymer consisting of b- (1-4) - related N-acetyl-D-glucosamine which has been highlighted as a potential candidate as an antimicrobial and biocompatibility.[10] Chitosan as a natural carbohydrate biopolymer with unique structure and properties. Chitin and chitosan have been investigated as antimicrobial agents against various target microorganisms such as algae, bacteria, yeast, and fungi in vivo and in vitro experiments involving chitosan in various forms. Linear biopolymers in chitosan show strong activity in reducing dental plaque and proving antimicrobial activity in vitro against various pathogenic bacteria in the oral cavity that are directly involved in plaque formation and periodontal diseases such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Streptococcus mutans*.[11]

Based on the description above, researchers are interested in conducting research on the inhibitory strength of milkfish scales chitosan gel (*Chanos Chanos*) against *Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis bacteria.*

MATERIALS AND METHODS

Type of research used in this study is an experimental laboratory research. The research design used is the post test only control group design. This research was conducted at the Pharmacognosis, Phytochemistry, and Pharmacology Laboratory of the Faculty of Mathematics and Natural Sciences, Pancasakti University and the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University in August 2019.

Making Milkfish Scales Gel Chitosan (Chanos Chanos)

The chitosan of milkfish scales is made by the process of demineralization (removal of minerals), deproteonation and deacetylation of chitin into chitosan. After that chitosan was divided into three groups of gels with concentrations of 1%, 5% and 10%.

Inhibition Test

Tests carried out by the diffusion method using a disk. Prepare pure isolates of *Porphyromonas gingivalis* and *Agregatibacter actinomycetemcomitans* and petri dishes containing MHA medium. Prepare a paper disk for use on the sample to be tested. Prepare positive and negative controls. Prepare chitosan gel with a concentration of 1%, 2% and 3%. Dip the disk paper in the test sample with different concentrations, along with a positive control (gel metronidazole) and a negative control. Pure isolates of *Porphyromonas gingivalis* and *Agregatibacter actinomycetemcomitans* were suspended with 0.9% NaCl and bacterial swabs on petri dishes containing MHA. Insert the paper disk dipped in the test sample in the prepared petri dish. Incubation in an incubator with anaerobic atmosphere at 37ºC for 2x24 hours. Calculate the inhibition zone formed at each concentration with calipers and compare.

RESULTS

Based on the research conducted, obtained the results of the measurement of the inhibition zone diameter of the bacteria *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* are presented in the Table below.

Table 1: Results of measurement of the mean values of inhibition zones of *Porphyromonas gingivalis* bacteria

Group	N	Mean	± SD
Chitosan gel 1%	5	10.94	± 3.92
Chitosan gel 5%	5	11.14	± 0.88
Chitosan gel 10%	5	13.14	± 0.78
Metronidazole control	5	9.24	± 0.88
Aquades control	5	6.20	± 0.00

Table 2: Results of Measurement of the Average Value of *Aggregatebacteri actinomycetemcomitan* Bacteria Inhibition Zones

Group	N	Mean	± SD
Chitosan gel 1%	5	9.34	± 1.28
Chitosan gel 5%	5	10.12	± 1.15
Chitosan gel 10%	5	11.08	± 0.80
Metronidazole control	5	11.34	± 1.66
Aquades control	5	6.20	± 0.00

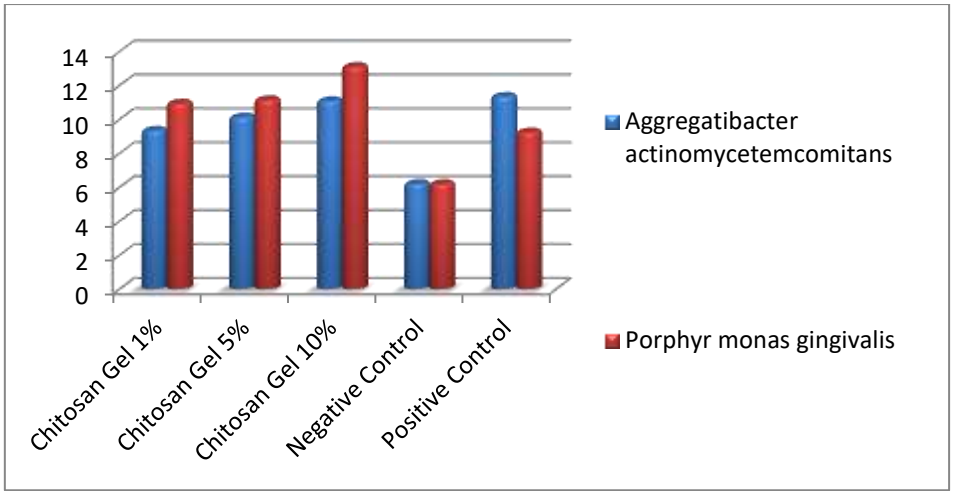

Chart 1: Comparison of effectiveness of milkfish chitosan gel *(Chanos chanos)* against *Agregatibacter actinomycetemcomitan bacteria* and *Porphyromonas gingivalis*

Table 3: Results of Statistical Test for *Porphyromonas gingivalis* Bacteria Inhibition Zones

Group	N	*Normality test*	*Comparison test*
Chitosan gel 1%	5	0.090	
Chitosan gel 5%	5	0.802	
Chitosan gel 10%	5	0.090	0,000
Metronidazole control	5	0.802	
Aquades control	5	-	

Shapiro Wilk test: p>0.05; data distribution normal
**One-Way Anova: p<0.05; significant

Table 4: Post hoc LSD statistical test results of zone of inhibition of Porphyromonas gingivalis bacteria

Treatment group (i)	Comparison (j)	Mean difference (i-j)	p-value
Chitosan gel 1%	Chitosan gel 5%	-0.20	0.868
	Chitosan gel 10%	-2.20	0.078
	Metronidazole control	1.70	0.167
	Aquades control	4.74*	0.001
Chitosan gel 5%	Chitosan gel 10%	-2.00	0.107
	Metronidazole control	1.90	0.125
	Aquades control	4.94*	0.000
Chitosan gel 10%	Metronidazole control	3.90*	0.004
	Aquades control	6.94*	0.000
Metronidazole control	Aquades control	-3.04*	0.019

*. The mean difference is significant at the 0.05 level.

Table 5: Results of statistical tests for Agregatibacter actinomycetemcomitan bacterial inhibition zones

Group	N	Normality test	Comparison test
Chitosan gel 1%	5	0.696	
Chitosan gel 5%	5	0.721	
Chitosan gel 10%	5	0.708	0,000
Metronidazole control	5	0.569	
Aquades control	5	-	

*Shapiro Wilk test: p>0.05; data distribution normal
**One-Way Anova: p<0.05; significant

Table 6: Results of Post hoc statistical tests for LSD zone for inhibition of Agregatibacter actinomycetemcomitan bacteria

Treatment group (i)	Comparison (j)	Mean difference (i-j)	p-value
Chitosan gel 1%	Chitosan gel 5%	-0.78	0.289
	Chitosan gel 10%	-1.74*	0.025
	Metronidazole control	-2.00*	0.011
	Aquades control	3.14*	0.000
Chitosan gel 5%	Chitosan gel 10%	-0.96	0.195
	Metronidazole control	-1.22	0.104
	Aquades control	3.92*	0.000
Chitosan gel 10%	Metronidazole control	-0.26	0.720
	Aquades control	4.88*	0.000
Metronidazole control	Aquades control	-5.14*	0.000

*. The mean difference is significant at the 0.05 level.

Based on the Shaphiro-Wilk statistical test to determine the normality value obtained p value> 0.05 which means the data is normally distributed so the test is continued with the parametric test namely Oneway Anova. Based on the One-way Anova statistical test it was found that the significance value was 0,000 (p <0.05) which mean that there were significant differences between treatment groups. The results of the Post Hoc LSD test for zone of inhibition between treatment groups on the bacteria *Porphyromonas gingivalis* and *Agregatibacter actinomycetemcomitan* have shown significant values (p <0.05). Chitosan gel 1% when compared with a concentration of 5%, has a p value> 0.05 which means there is no significant difference or have the same effect. Meanwhile, when compared with a concentration of 10%, and positive control Metronidazole and negative control aquades have a p value <0.05 which means there are significant differences or have different effects. In chitosan gel 5% when compared with a concentration of 10%, and positive control Metronidazole has a p value> 0.05 which means there are no significant differences or have the same effect. Whereas when compared with negative controls aquades have a p value <0.05 which means there are significant differences or have different effects. On chitosan gel 10% when compared with positive controls Metronidazole has a p value> 0.05 with a negative mean difference which means there were no significant differences or had the same effect, but the Metronidazole control had a better effect than the 10% chitosan gel. Likewise, if the positive control of Metronidazole compared with negative control of aquades has a p value <0.05 which means there are significant differences or have different effects.

DISCUSSION

The result of this research is that the milkfish scales chitosan gel with a concentration of 1%, 5%, and 10% reveals the presence of clear zones in *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* isolates which show that the milk chitosan chitosan gel has an anti-bacterial power against the bacteria *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* which shows that the chitosan gel in the milkfish scales has anti-bacterial power against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. The results of this study are in line with research conducted by Ummah in 2017 which states that milkfish scales (*Chanos chanos*) contain chitosan which can be used as an antibacterial, besides that according to Loekito in 2018 and Adha in 2017 chitosan has antibacterial power against *Aggregatibacteracteric actinomycetemcomitans* and *Porphyromonas gingivalis*.[12-14]

By finding several concentrations that have been tested, it can be seen that the higher the concentration given by the milkfish scales chitosan gel, the higher the antibacterial inhibition. Concentration of 10% milkfish scales gel chitosan has the biggest inhibition zone. This is in line with research conducted by Hosseinnejad in 2016 which states that the higher the concentration of chitosan, the higher the inhibitory power of bacteria. At lower concentrations, chitosan binds to cell surfaces that are negatively charged, disrupts cell membranes, and causes cell death by inducing leakage of intracellular components. Meanwhile, at higher concentrations, protonated chitosan can coat the cell surface and prevent intracellular component leakage. In addition, positively charged bacterial cells repel each other and prevent agglutination.[15]

Categories of bacterial inhibition are divided into 4, namely: Weak ($\leq$ 5 mm), Medium (5-10 mm), Strong (10-20 mm), and Very strong ($\geq$20 mm).[16] Based on the category of inhibition, can be seen that if the diameter of the inhibition zone is greater than 20 mm, then the growth inhibition response is very strong. If it is 10-20 mm, the growth inhibition is strong and if it is 5-10 mm, the growth inhibition is moderate, while if it is $\leq$ 5 mm, the growth inhibition is weak. Thus, the conclusion of the response of growth inhibition of milkfish scales gel chitosan bacteria has a moderate to strong inhibition response to the bacteria *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. It was seen that each concentration of chitosan gel in milkfish scales had inhibition zone diameters ranging from 9-12 mm.

The main factors influencing the antibacterial activity of chitosan are molecular weight and concentration. The minimum inhibitory concentration (MIC) of chitosans ranges from 0.005 to 0.1% depending on the bacterial species and molecular weight of Chitosan and varies

depending on the pH of the chitosan preparation.[17,18,19,20] The chitosan antimicrobial activity is higher at low pH, this is due to the fact that the chitosan amino group become ionized at a pH below 6.[15,21,22,23]

Based on the results and the previous discussion, it was concluded that chitosan gel from milkfish scales (*Chanos chanos*) has inhibitory properties against *Agregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* which is one of the pathogens that cause periodontal disease. The results obtained showed that the greater the concentration of chitosan gel, the inhibitory power of the bacteria *Agregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* would also be large. This can be seen in the 10% chitosan gel which has the greatest inhibition compared to other control groups. For these results, it is recommended that further research be conducted on the toxicity test of chitosan milkfish scales gel, so that it can be developed as an alternative antimicrobial agent that causes periodontitis and for further research on milkfish scales chitosan gel on experimental animals that have been induced by bacteria that cause periodontal disease.

CONCLUSION

The milkfish scales gel chitosan (*Chanos chanos*) can inhibit the growth of *Aggregatibacter actinomycetemcomitans & Porphyromonas gingivalis* and the higher the concentration of chitosan gel in milkfish scales, the higher the inhibition zone produced.

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