Antibacterial Chitosan of Milkfish Scales (Chanos Chanos) Onbacteria Prophyromonas Gingivalis & Agregatibacter Actinomycetemcomitans

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Abstract

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

Keywords: *Chitosan gel, Fish scales, Aggregatibacter actinomycetemcomitans, Antibacterial.*

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 coexisting bacteria.

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. [1,3,20,21]

Aggregatibacter actinomycetemcomitans are gram-negative coccobacillus anaerobes, measuring around 0.4x1.0 μm, dominated by bacilli with several forms of coccal. These bacteria are non-sporulation, non-motile, unbranched, capnophilic and facultative bacteria.[4,5]

A. actinomycetemcomitans which is one of the main causes of periodontal disease is local aggressive periodontitis. This bacteria is described as five serotypes (a-e), with more than one serotype found in the human mouth. Serotype B of A. actinomycetemcomitans is more common in aggressive periodontitis. The natural habitat of this organism is the oral cavity and can be isolated from various non-oral infections such as bacteremia, septicemia, endocarditis, atherosclerotics, pneumonia, skin infections, osteomyelitis, inflammation of the urinary tract, and various types of abscesses.[5]

This bacterium has a complex life cycle, obtained through transmission from the saliva of an infected individual and may initially colonize the oral mucosa as a facultative intracellular pathogen. These bacteria move from the colonies in the oral cavity to the gingival fissures and compete with other bacteria. Successful formation of persistent colonization in subgingival fissures by A. actinomycetemcomitans can cause periodontal damage and the development of periodontitis in susceptible individuals.[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 studies 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.

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 ability of milkfish scales gel chitosan (Chanos Chanos) against Aggregatibacter actinomycetemcomitans

Materials and Method

This 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, Phytochemical 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 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. The pure isolate of Agregatibacter actinomycetemcomitans was suspended with 0.9% NaCl and did a bacterial swab 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 Aggregatibacter actinomycetemcomitans bacteria are presented in the Table below.

Table 1: Results of Measurement of the Average Value of Aggregate bacteria 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

Table 2: Results of statistical tests for Agregatibacter actinomycetemcomitan bacterial inhibition zones

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

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

Table 3. Results of Post hoc statistical tests of LSD zone for inhibition of Agregatibacter actinomycetemcomitan bacteria

Group treatment (i)	Comparisom (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 that is Oneway Anova (Table 5.5). Based on the One-way Anova statistical test it was found that the significance value was 0,000 (p <0.05) which meant that there were significant differences between treatment groups. The results of the Post Hoc LSD test for zone of inhibition between treatment groups on Aggregacter bacteria 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 negative control of aquades has 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. Meanwhile, when compared with negative controls aquades have a p value <0.05 which means there are significant differences or have different effects. The 10% chitosan gel when compared with the positive control Metronidazole has a p value> 0.05 with a negative mean difference which means there is no significant difference or has the same effect, but the

Metronidazole control has a better effect than the 10% chitosan gel.

Discussion

The result of this research is that milkfish scales chitosan gel with concentration of 1%, 5%, and 10% shows the presence of clear zone in Aggregatibacter actinomycetemcomitans isolates. with research conducted by Ummah (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 Aggregatibacter actinomycetemcomitans.[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 (≤ 5 mm), Medium (5-10 mm), Strong (10-20 mm), and Very strong (≥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 ≤ 5 mm, the growth inhibition is weak. Thus, the conclusion of the inhibition response of the growth of milkfish chitosan gel scales has moderate to strong resistance to Aggregatibacter actinomycetemcomitans. 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] 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.[15,19]

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 which is one of the pathogens that cause periodontal disease. The results obtained indicate that the greater the concentration of chitosan gel, the inhibitory power of Agregatibacter actinomycetemcomitans will also be greater. 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

Conflict of Interest: There is no conflict of interest in this study

Source Of Funding: Domestic Government

Ethical Clearance: This study has obtained information on ethical qualifications number: 0263/ PL.09/KEPK FKG-RSGM UNHAS/2019 and registration number UH 17120269 dated21November 2019.

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