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RESEARCH ARTICLE

Antibacterial Effectiveness of Chitosan Solution on Streptococcus mutans

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Abstract Dental caries is a localized destruction on teeth by organic acid produced by carbohydrate fermentation of cariogenic bacteria. Streptococcus mutans is a Gram-positive bacterium, a facultative anaerobe species often found in human oral cavity and is a significant contributor to dental caries. Chitosan is the collective name of a group of partially or fully deacetylated chitin. Because of its unique biological properties, including biodegradability and nontoxicity, a lot of applications have been found for chitosan, on its own or combined with other polymers. Chitosan has a wide spectrum of activity and high killing rate against Gram positive and Gram-negative bacteria, while having low toxicity against mammal cell. In this research a Kirby-Bauer test is done on Streptococcus mutans using 1%, 1.5%, and 2% chitosan solution as treatment group and aquades as control group. Average of each group is calculated and significancy is determined using statistical calculation. The resulting data is analyzed using Kruskal-Wallis and Post-hoc Tukey HSD and is shown there to be a significant difference between each treatment groups. The biggest zone of inhibition is seen on the 2% chitosan solution group. 2% chitosan solution has higher antibacterial effectiveness on Streptococcus mutans than 1% and 1.5% chitosan solution.

Keywords: Streptococcus mutans, chitosan, antibacterial, concentration, infectious disease.

Introduction

Dental caries is a localized damage from dental hard tissue caused by acidic materials produced by bacterial fermentation. Dental caries is a multifactorial disease began with microbiological changes in the biofilm complex and influenced by the flow and composition of saliva, fluoride exposure, sugar consumption, and by preventive act (such as teeth cleansing) [1]. One of the ways to treat dental and oral hygiene effectively is by gargling using mouthwash. The effectiveness of mouthwash use is because its ability to reach difficult places for toothbrush and can damage plaque formation [2]. The use of antiseptics e.g., alcohol in today's mouthwash is thought to have a carcinogenic effect on its users [3]. So that the use of natural biomaterials is used to be able to suppress bacterial growth, because natural ingredients are relatively more acceptable to the body compared to synthetic materials [4].

Chitosan is the collective name of a partially or fully deacetylated chitin compounds group [5,6]. In order of their unique biological properties, including biodegradability and non-toxic, many applications have been found either chitosan alone or mixed with other polymers [7]. Some intrinsic factors of chitosan cause chitosan to have good antibacterial properties [8,9]. One of them is the polycationic structure of chitosan which can bind and disturb the stability of the bacterial cell wall [5]. In addition, the chelating ability of chitosan can also cause damage to bacterial cells. Chitosan has a broad-spectrum activity and a high degree killing of Gram-positive and Gram-negative bacteria, but has low toxicity to mammalian cells [5] This research was conducted with the aim of analyzing the effectiveness of acid soluble chitosan

solution at a higher concentration as an antibacterial agent against Streptococcus mutans bacteria

Materials and Methods

Chitosan Solution Making

Chitosan Solution is made by mixing chitosan powder with 1% acetic acid solvent. Chitosan powder used is an acid soluble chitosan powder obtained from the National Nuclear Energy Agency (BATAN). Preparation of 1%, 1.5% and 2% chitosan solution were carried out at the Ketintang Industrial Research and Consultation Agency (BPKI) Laboratory.

The S. Mutans obtaining Methode

S. Mutans bacteria used were S. Mutans bacteria obtained from the stock of the Faculty of Dental Medicine Research Center, Universitas Airlangga.

Study Setting

This type of research is an experimental laboratory research with a post-test only control group design. In this study the inhibitory zone test was carried out on Streptococcus mutans bacteria using 1%, 1.5%, and 2% chitosan as the treatment group and distilled water as the control group. Testing the inhibition of chitosan solution and measuring the inhibitory zone results were performed at the Faculty of Dentistry Research Center, Universitas Airlangga.

Sample Size Calculation

This research used 28 samples, with 7 samples divided into each group. The group was divided into the control group, the group with 1% chitosan concentration, 1.5% concentration and 2% concentration. Anti-bacterial potential test was done by inhibitory test method. A total of 7 petridishes containing 15ml MHA solid media were added 0.5 McFarland active bacteria flattened with spreader until it dried. Petridish is divided into 4 zones for each treatment. Each group treatment repeated 7 times. All petridish were incubated at 37 ° C for 24 hours.

Determination of Chitosan Solution Against *S. Mutans*

Antibacterial potential was measured by measuring transparent zones using calipers. Measurements were made three times and calculated the average diameter of the inhibition zone measurement.

Statistical Analysis

The average score of each group is calculated and determined its significance by using the Kolmogorov-Smirnov Test statistical calculation to find out the data distribution, Levene's Test to find out the variance of data from each sample tested is the same (homogeneous), Kruskal Wallis to see the significance of the whole group, and Honestly Significant Difference (Tukey HSD) test to find out which treatment group has meaningful difference in mean

Results and Discussion

The results showed that the use of 1%, 1.5% and 2% chitosan solution produced inhibitory zones of Streptococcus mutans and in controls using aquades there was no inhibitory zone.

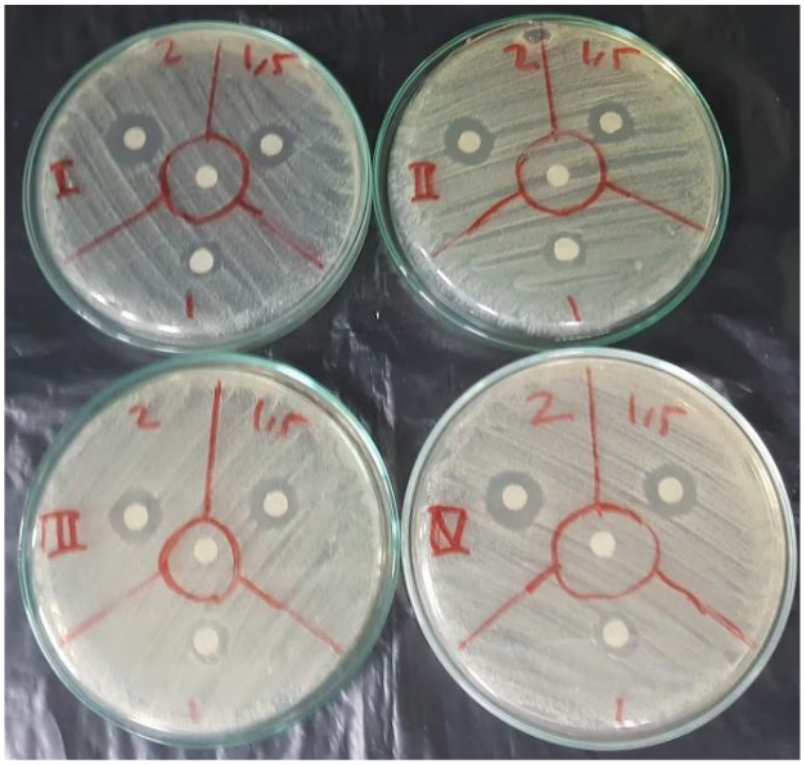

Figure 1: The antibacterial effect of Chitosan Solution against Streptococcus mutans

Figure 1 shows that chitosan solution inhibits the growth of Streptococcus mutans bacteria. Where the antibacterial potential at 2% concentration is greater than other concentrations.The measurement results of the inhibition zone in each treatment group are as follows.

Table 1. Mean and Standard Deviation Inhibitory Zones in Each Treatment Group.

Treatment Group	N	Mean (mm)	Standard Deviation
Control	7	0	0
Chitosan solution 1%	7	2.885	0.166
Chitosan solution 1.5%	7	3.821	0.312
Chitosan solution 2%	7	5.571	0.473

Table 1 shows that in the treatment group the inhibition zone occurs while it does not show in the control group. The treatment group with a concentration of 2% chitosan solution had the largest inhibition zone area compared to the treatment group with a concentration of 1% and 1.5% chitosan solution. This shows the inhibition of chitosan solution with a concentration of 2% greater than chitosan solution with a concentration of 1% and 1.5%.

Table 2. Normality, Homogeneity, Comparative Test Results.

Data Analysis Type	P value	Information	Significances
Kolmogorov-Smirnov Test	0.020	$p < 0.05$	Abnormal
Levene's Test	0.132	$p > 0.05$	Homogen
Kruskal-Wallis Test	0.000	$p < 0.05$	Significant

Table 2 shows Kolmogorov-Smirnov normality test results show a p value of 0.20, which means that the data are not normally distributed because p <0.05. The results of the Levene homogeneity test showed a p value of 0.132 which means the variance between treatments was homogeneous because p> 0.05. Significance test uses non parametric test, Kruskal-Wallis, because data is not normally distributed. The Kruskal-Wallis test results show a p value of 0.00, which means that the treatment shows a significant effect on the results of the study.

Table 3. Post Hoc Tukey Test Result.

	K	1%	1.5%	2%
K	-	0.000 (p < 0.05)	0.000 (p < 0.05)	0.000 (p < 0.05)
1%	0.000 (p < 0.05)	-	0.000 (p < 0.05)	0.000 (p < 0.05)
1.5%	0.000 (p < 0.05)	0.000 (p < 0.05)	-	0.000 (p < 0.05)
2%	0.000 (p < 0.05)	0.000 (p < 0.05)	0.000 (p < 0.05)	-

The Kruskal-Wallis test result showed significant results (p<0.05), the analysis was continued using the Tukey Post-hoc test to determine the significance of the mean differences between groups (Table 3). The results of the Tukey Post-hoc test showed significant differences in the comparison of all treatment groups (p <0.05).

The results showed that the largest inhibition zone radius was in the treatment group of chitosan solution with 2% concentration. In the control group, the study used a treatment variable in the form of aquades that were neutral, so it does not affect the bacterial growth. In the treatment group with 1% concentration of chitosan solution, the mean radius of the inhibitory zone was 2.885mm. In the treatment group with a 1.5% concentration of chitosan solution, the average radius of the inhibitory zone was 3,821mm. In the treatment group with a 2% concentration of chitosan solution, showed the mean radius of the inhibitory zone radius of 5,571mm. Based on the data analysis found a significant difference between each treatment group.

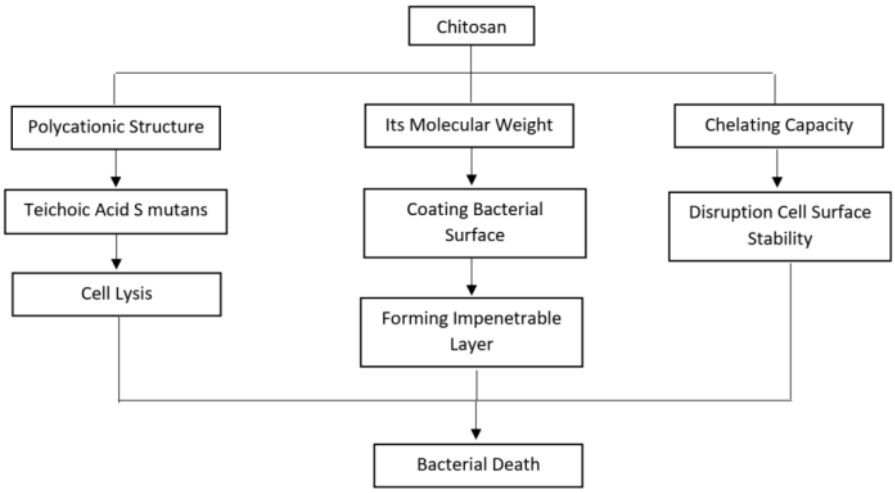

Figure 2. Graphical abstract of the chitosan.

Streptococcus mutans bacteria as Gram positive bacteria have cell walls formed from peptidoglycan and TA (11). Figure 2 describes of the polyanionic structure of TA causes an electrostatic interaction between TA and chitosan with polycationic structure. Because TA has a vital role in maintaining the structure of cell walls, this electrostatic bond between TA and chitosan causes disruption of the cell wall stability which ultimately causes cell lysis [5,12].

Chitosan has antibacterial properties that are influenced by the intrinsic characteristics of chitosan. Some

characteristics of chitosan that influence antibacterial properties include polycationic properties of chitosan, chitosan molecular weight, and chelating capacity of chitosan. In this study, a high molecular weight was used. The chitosan molecular weight plays a role in determining the antibacterial mechanism. In high molecular weight chitosan, chitosan will coat the surface of bacterial cells, forming an impenetrable layer. This layer causes no transport of essential ingredients for the bacterial cells' life. This will cause death from bacterial cells [5,10].

Chitosan has a high chelating capacity for various metal ions under acidic conditions. The bacterial cell wall stability joint with metal ions from chitosan were very important. The loss of these metal ions causes disruption of cell surface stability. Chitosan chelating activities play a role in the antibacterial ability of chitosan at high pH (alkaline). In this study, the chitosan solution used has a low pH (acidic). Therefore, the main antibacterial mechanism has electrostatic interaction between the cell surface and chitosan, but chelating activity still occurs and still influential in antibacterial activity [5,10].

In this research, increasing the efficiency of chitosan testing correlated with an increase in antibacterial potential against Streptococcus mutans bacteria. An increase of chitosan solution concentration causes an increase the amount of chitosan contacted with bacterial cells. Contact between chitosan and bacterial cells is very important in the antibacterial relationship of chitosan. Increasing the amount of chitosan will cause more chitosan to interact with bacterial cells, thereby increasing the antibacterial potential in chitosan solution.

In the treatment using a 2% concentration of chitosan solution obtained the largest inhibition zone results. A higher concentration means that there is a greater amount of chitosan in the solution. This has an effect on the antibacterial potential of chitosan solution. Along with the increasing amount of available chitosan, the electrostatic bond that occurs between chitosan and TA on the cell wall will also increase. The increase in the number of electrostatic bonds between chitosan and TA cause more unstable cell walls. In addition, the number of electrostatic bonds that can occur is also limited by the number of NH3 + groups available. Increasing the amount of chitosan means increasing the number of NH3 + groups available to bind to TA, allowing more bacteria to be affected by chitosan solution [12].

The same applies to the chelating process [5]. The amount of metal ions that could be bind by chitosan is very dependent on the amount of available chitosan, because chitosan could only bind a limited number of metal ions. 2% concentration of chitosan solution were contained more chitosan so that more metal ions that can be bound by chitosan in the chelating process than the chitosan solution with a concentration of 1% and 1.5%. Metal ions are an important part in maintaining the stability of the cell wall, therefore if more metal ions are taken, the cell wall will become increasingly unstable, which in turn will facilitate the occurrence of lysis [10].

To form an impenetrable layer that lines the entire surface of the bacterial cell, a number of chitosan is needed to bind to the surface of each bacterial cell. Without enough chitosan to coat the entire cell surface, this process could not occur perfectly, and the entire cells surface could not be blocked. With a higher concentration of chitosan solution, an increase in the amount of chitosan will increase the possibility of forming a perfect blocking layer on more bacterial cells, which causes the death of bacteria [10].

Conclusions

In conclusion, Chitosan solution with a 2% concentration has a greater antibacterial effect on the Streptococcus mutans bacteria than chitosan solution with 1% and 1.5% concentration.

Data Availability

The data used to support this research are available from the corresponding author upon the request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding Statement

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