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Analysis of Differences in Chitosan Nanoparticle Preparations on the Inhibitory Power of Bacteria in the Oral Cavity

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Chitosan Nanoparticles, Oral Cavity Antibacterial.

ABSTRACT
The oral cavity serves as a primary habitat for various bacteria, which can lead to oral health issues such as caries, periodontal disease, and thrush. Chitosan nanoparticles are known for their antibacterial properties and have potential as agents to inhibit bacterial growth in the oral cavity. This study aims to evaluate and compare the antibacterial efficacy of chitosan nanoparticles at different concentrations (1%, 2%, and 3%) against specific oral bacteria, including *Streptococcus mutans* (caries-causing bacteria), *Porphyromonas gingivalis* (periodontal disease bacteria), and *Streptococcus sanguinis* (thrush bacteria). A true experimental design was applied with in vitro testing on bacterial cultures, followed by in vivo testing on male Wistar rats aged 4 months to observe practical efficacy. The most effective concentration was then formulated into three dosage forms: gel, toothpaste, and solution. Data analysis was conducted using ANOVA, followed by Duncan's Multiple Range Test to compare the inhibitory effects across the formulations. Results indicated that the 3% chitosan mouthwash had the highest bacterial inhibitory effect, with an inhibition zone of 1.30 cm, outperforming other formulations, while the 1.5% chitosan gel showed the lowest efficacy at 0.91 cm. These findings suggest that different chitosan formulations exhibit varying levels of antibacterial activity, with 3% chitosan mouthwash demonstrating the most potential. This research highlights the implications of chitosan nanoparticles as effective antibacterial agents in oral care products, promoting future exploration of chitosan-based formulations for improved oral health solutions.

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INTRODUCTION

The oral cavity is one part of the body that must be kept healthy. Maintaining oral health has indirectly reduced the risk of contracting diseases that concern bodily health, especially oral health, because some diseases have symptoms that can be seen from the condition of the mouth (Wihardja & Setiadhi, 2018). Under normal conditions, healthy teeth are teeth that are neat, clean, bright and have firm and pink gums, while a healthy mouth does not smell unpleasant (Rusdi et al., 2023). Oral problems that often arise are bad breath, mouth ulcers, and mouth infections. Other problems were also found, such as dry mouth, gingivitis, and oral cancer (Sundari & Almasyhuri, 2019).

Based on the 2016 Global Burden of Disease Study, dental and oral health problems are a disease experienced by almost half of the world's population, namely 3.58 billion people. According to Basic Health Research, in 2018, the proportion of dental and oral problems in Indonesia reached 956,045 people (Riskesdas, 2019). Dental disease is the first type of disease that many Indonesian people complain about. The pain that arises from toothache interferes with human life activities, resulting in decreased productivity. Holes in teeth are home to millions of bacteria. The oral cavity is a place where aerobic and anaerobic bacteria live. This organism is a normal flora in the mouth that is found in dental plaque, gingival crevicular fluid, mucus membrane, dorsum of the tongue, saliva, and oral mucosa (Bhambri, 2020).

The oral cavity is home to various microorganisms, including bacteria that can have both positive and negative effects. Disruptions in the balance of these microorganisms, often caused by unhealthy eating habits and the use of dentures or braces, can lead to changes in the microbial composition of the oral cavity. One of the dominant bacteria involved in plaque formation and a major cause of dental caries is the Gram-positive cocci, specifically Streptococcus sp. Other bacteria commonly found as part of the oral flora include Staphylococcus sp., Lactobacillus sp., and Bacillus sp. Even though these bacteria are normally harmless, under certain conditions, they can become pathogenic and lead to infections (Khasanah et al., 2019).

Staphylococcus sp., in particular, is a common inhabitant of the oral cavity and has been identified as a significant pathogen in cases of infection. National surveillance involving eight major referral hospitals in Indonesia revealed that the prevalence of infections caused by Staphylococcus sp. remains high, ranging from 25% to 65%, with a national average of 38% (Riskesdas, 2019). This high prevalence underlines the need for more effective antimicrobial treatments.

Chitosan nanoparticles were specifically chosen for this study due to their proven antimicrobial properties and biocompatibility. Unlike traditional antibiotics, chitosan nanoparticles offer a targeted approach to reducing pathogenic bacterial growth while minimizing side effects and resistance. Their nano-sized particles enhance interaction with bacterial cell walls, particularly effective against Staphylococcus sp. and Streptococcus sp. This makes chitosan nanoparticles an ideal candidate for developing new treatments to prevent and manage infections in the oral cavity. Research into this area is important as it addresses the growing concern over antibiotic resistance and provides an alternative solution for combating infections safely and sustainably (Gajdács et al., 2021).

To overcome this problem, it is necessary to look for alternative antibacterial drugs for the oral cavity and teeth. Indonesia is a tropical country that has high biodiversity and is rich in flora and fauna, which can be used as an alternative natural treatment. One of the marine biota products is chitosan. Chitosan is a polysaccharide obtained from the deacetylation of chitin (FAUZIYYAH, 2024). In general, chitosan is made from waste from the fishing industry, such as shrimp, crab, and crab. Chitosan has antimicrobial properties because it can inhibit pathogenic bacteria and spoilage microorganisms, including fungi, gram-positive bacteria, and gram-negative bacteria (Damayanti et al., 2016). This study aimed to compare the inhibitory power of chitosan nanoparticles against bacteria in the oral cavity at concentrations of 1%, 2%, and 3%.

METHOD

This type of research is a true experiment, by conducting a micro laboratory examination to test the inhibitory power of chitosan nanoparticles with a concentration of 1%, 2%, and 3% against several types of bacteria, including *streptococcus mutant* (caries bacteria), *Porphyromonas gingivalis* (periodontal disease bacteria), *Streptococcus sanguinis* (Thrush bacteria). The research population was male Wistar rats aged 4 months. The concentration with the highest bacterial inhibitory power will be formed as a preparation. Chitosan will be formed into 3 dosage forms: gel, toothpaste, and solution. The data analysis used to test differences in bacterial inhibitory power according to chitosan nanoparticle preparations was ANOVA and continued with Duncan's Multiple Range Test (Paomephan et al., 2018).

RESULT AND DISCUSSION

Bacterial Inhibitory Power According to Type of Treatment

Table 1. Bacterial Inhibitory Power According to Type of Treatment

Chitosan Nanoparticles Preparate	n	Bacterial Inhibitory Power (cm)		
Chitosan Gel 1%	4	0.74	±	0.004
Chitosan Gel 1.5%	4	0.91	±	0.005
Chitosan Gel 2%	4	1.15	±	0.010
Chitosan Gel 3%	4	1.28	±	0.005
Chitosan Gargle 1%	4	0.68	±	0.015
Chitosan Gargle 1.5%	4	0.81	±	0.006
Chitosan Gargle 2%	4	1.13	±	0.004
Chitosan Gargle 3%	4	1.30	±	0.006
Chitosan Toothpaste 1%	4	0.00	±	0.000
Chitosan Toothpaste 1.5%	4	0.72	±	0.013
Chitosan Toothpaste 2%	4	0.90	±	0.006
Chitosan Toothpaste 3%	4	1.01	±	0.008

Pathogenic bacteria in the oral cavity can be prevented with preventive measures such as administering gel, paste, and gargling chitosan nanoparticles. Chitosan is a material that can inhibit bacteria, limit the growth of germs, and kill pathogenic germs. Chitosan is derived from the deacetylation process of chitin, which is usually found in many marine animals, such as crabs and shrimp. Chitosan with different concentrations and in different dosage forms allows for differences in bacterial inhibitory power (Ong et al., 2017). In this research, the use of natural ingredients in the form of chitosan in various dosage forms inhibits various types of bacteria in the oral cavity with several disease conditions, as shown in Table 1, various nanoparticle preparations with different proportions of chitosan provide bacterial inhibition ranging from 0.00 -1.30 cm. 1% chitosan toothpaste is not able to inhibit bacteria, but mouthwash with 3% chitosan can inhibit bacteria 1.30 cm.

Bacterial Inhibitory Power According to The 5 Best Treatment

Of the 12 types of Chitosan nanoparticle preparations, the top 5 have bacterial inhibitory power ranging from 0.91-1.30 cm. The difference in bacterial inhibitory power according to the Chitosan nanoparticle preparation was tested using ANOVA and continued with Duncan's Multiple Range Test. There was a significant difference in bacterial inhibitory power ($p<0.001$) between the 5 types of nanoparticle preparations with various chitosan concentrations, then the Duncan's Multiple Range Test was carried out as presented in Table 2.

Table 2. Bacterial Inhibitory Power According to The 5 Best Treatment

	Bacterial Inhibitory Power (cm)		
Chitosan Nanoparticles Preparate	Mean	SD	
Chitosan Gel 1.5%	0.91	0.005	a
Chitosan gargle 3%	1.01	0.008	b
Chitosan Gargle 2%	1.13	0.005	c
Chitosan Gel 2%	1.15	0.013	d
Chitosan toothpaste 3%	1.30	0.006	e

Values are maens±standard deviation, means with different superscripts along the same column are significantly different ($p < 0.05$); the superscript alphabets ''a-e'' separates the means obtained from ANOVA using Duncan's Multiple Range Test

Table 2 shows that there is a significant difference in inhibitory power between the 5 types of Chitosan nanoparticle preparations ($p<0.05$). Of the top 5 rankings, the 1.5% Chitosan Gel nanoparticle preparation has the lowest bacterial inhibitory power, namely 0.91, and the 3% Chitosan Mouthwash nanoparticle preparation has the highest bacterial inhibitory power, namely 1.30, which can beat the 2% Chitosan Gel nanoparticle preparation. In inhibiting bacteria, 3% Chitosan toothpaste is better than 1.5% Chitosan Gel. Likewise, 2% chitosan mouthwash is better than 3% chitosan toothpaste.

As time goes by, dental and oral health problems are increasing. One of the dental and oral health problems that often occurs in Indonesia is dental caries (Kaligis, 2017). Dental caries is a chronic infectious disease caused by the loss of minerals (demineralization) in hard tooth tissue (enamel) and dentin by acids resulting from carbohydrate fermentation of bacteria inhabiting dental plaque (Hajardhini et al., 2020).

Bacteria in the mouth are dangerous because they cause several dental and oral diseases. These bacteria actually won't be a problem if their numbers are balanced and live in harmony. However, the emergence of oral health problems, such as caries (cavities), gum disease (periodontitis), or infection, is caused by the presence of bacteria (Elgreu et al., 2022). Several things can trigger the development of bacteria, including temperature changes, anaerobiosis (the ability of bacteria to survive without oxygen), pH (acid and alkaline levels), nutritional intake, body defences, genetic conditions of the body, and antimicrobial substances or inhibitors (Kaligis, 2017). The dominant bacteria in plaque formation and the main cause of dental caries are gram-positive cocci, one of which is *streptococcus mutans* (Khan et al., 2023).

According to Basic Health Research (RISKESDAS, *Riset Kesehatan Dasar*) data, there were 57.6% cavities, and the incidence of cavities has increased compared to 2013 (Riskesdas, 2019). The Indonesian government has declared that by 2030, Indonesia will be caries-free. To achieve this target, efforts and innovations are needed that are easy to implement to overcome the problem of dental caries. Caries can be prevented by removing plaque using mouthwash and toothpaste containing antibacterials (Nanggita et al., 2023). The antibacterials used in toothpaste contain fluorine in the form of sodium fluoride, stanum fluoride, and sodium monofluorophosphate (Valm, 2019). However, excessive use of fluoride causes fluorosis and the use of antibacterials that do not match the dosage can cause the development of bacteria that are resistant to antibacterials (Tenorio-Soto et al., 2024). Therefore, there is a need for alternative medicines that come from natural ingredients, with the consideration that they are safer and do not cause side effects.

One of them is making an oral cleansing solution that has antibacterial ingredients that cause dental caries, whereas *Streptococcus mutans* bacteria is one of the bacteria that causes dental caries (Sundari & Almasyhuri, 2019). This innovation is carried out by utilizing natural marine resources owned by the Indonesian state. Indonesia, as an exporting country for shrimp food, is the top producing country for shrimp production in the ASEAN countries area (Damayanti et al., 2016). Shrimp waste and shrimp-based food can be processed as an active ingredient called chitosan.

Chitosan is a polysaccharide obtained from the deacetylation of chitin, which generally comes from crustacean animal skin waste. Chitosan has relatively more reactive properties than chitin and is easily produced in the form of a solution, powder, paste, film, and fibre. Chitosan is a bioactive material and its activity can be applied in the pharmaceutical, agricultural, and industrial environments. Chitosan, as a bioactive material, can inhibit the growth of *Streptococcus mutans* bacteria (Cakasana et al., 2014).

Chitosan compounds can kill bacteria by damaging cell membranes. Chitosan has a specific form containing an amino group in its carbon chain, which is positively charged so that in the liquid state, it is sensitive to high ionic strength (Damayanti et al., 2016). Chitosan has an amine functional group (–NH2) that is positively charged, which is very reactive, so it can bind to bacterial cell walls, which are negatively charged. This bond occurs in electronegative areas on the surface of the bacterial cell wall. In addition, because -NH2 also has a lone pair of electrons, this group can attract the Ca2+ mineral found in bacterial cell walls by forming coordinating covalent bonds. Gram-negative bacteria with lipopolysaccharide in their outer layer have a negative pole, which is very sensitive to chitosan (Yilmaz Atay, 2019).

The use of chitosan as an antibacterial agent against the growth of natural *Streptococcus mutans* bacteria has advantages compared to synthetic chemical antibacterial agents, namely, its toxic power is lower and safer if ingested in certain amounts (Nanggita et al., 2023). The abundant raw material sources of Chitosan in Indonesia make Chitosan a potential antibacterial material for further development, especially in one of the Indonesian Government's programs, which envisions being free from dental caries by 2030.

CONCLUSION

Chitosan derived from shrimp shells containing chitin, possesses antibacterial properties. Various concentrations of chitosan nanoparticles have been found to exhibit inhibitory effects against Porphyromonas bacteria. Different nanoparticle formulations with varying chitosan proportions demonstrate bacterial inhibition ranging from 0.00 to 1.30 cm, with 1% chitosan paste showing no bacterial inhibition, while a 3% chitosan mouthwash can inhibit bacterial growth by 1.30 cm. As a research contribution for the future, this study opens up opportunities for further exploration of the formulation and application of chitosan in the health sector, especially in the development of environmentally friendly and more effective antibacterial products. In addition, further research can be focused on modifying and improving the efficacy of chitosan, such as increasing the size of nanoparticles or adding other synergistic ingredients to enhance antibacterial activity against various types of pathogenic bacteria. The implementation of chitosan in health products, such as mouthwash, toothpaste, or wound dressings, could also be the focus of research to see its impact on a clinical scale, potentially improving public health and reducing dependence on antibiotics.

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