

Antibacterial Effectiveness Of Green Shell Nanocitosan (*Perna Viridis*) On The Growth Of The Bacteria *Prevotella Intermedia* (In Vitro Study)

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ABSTRACT

Background: Green mussel shell nanochitosan is a size modification of chitosan obtained from green mussel shells that have been processed with specific standards. Nanochitosan from green mussel shells has not been researched for its anti-bacterial properties against *Prevotella intermedia* bacteria

Purpose: This study aims to determine the antibacterial effect on the growth of *Prevotella intermedia* bacteria. Method: This research is laboratory research using a posttest only control group design.

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Result: Nanochitosan with concentrations of 0.5%, 1%, 1.5% and 2% was able to inhibit the growth of *Prevotella intermedia* bacteria. The test results using the One Way ANOVA test obtained a value of $p = 0.000$ so that $p < 0.5$ and it was stated that there was a significant difference between treatment groups on the growth inhibition value of *Prevotella intermedia* bacteria

Conclusion: There is an anti-bacterial effect and there is an inhibitory zone in the culture of *Prevotella intermedia* bacteria that has been incubated within 24 hours with 37°C. The greater the concentration used, the more effective the antibacterial agent is.

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INTRODUCTION

Endodontic disease is a pathological condition of the dental pulp, especially the soft tissue found in the dental pulp chamber. This condition can be caused by several factors, such as bacterial infection, dental trauma, untreated cavities, or inadequate dental care. Common symptoms of endodontic disease include severe pain in the affected tooth, swelling around the tooth, sensitivity to hot or cold food or drinks, and abscesses in the tissue around the root of the tooth.⁽¹⁾

Root canal treatment is a treatment used to treat dental pulp problems by removing pulp that is still functioning or dead from the root canal and then filling the canal with filling material. The goal of this treatment is to prevent pulp disease from spreading to the periapical tissue⁽²⁾. The medicament commonly used in dental practice is calcium hydroxide which is useful as an antibacterial agent in root canal treatment. Calcium hydroxide is still often used as a root canal treatment, but the use of this material requires attention to the harm that calcium hydroxide can cause. It must be used carefully so as not to cause unwanted side effects⁽³⁾.

Physical modification of chitosan involves changing the particle size to become smaller or what is usually called nanochitosan. This form of modification has advantages in several respects compared to similar normal-sized materials, because this nano-sized material has a larger surface area to volume ratio than similar normal-sized materials, so the nanoparticles are more reactive.⁽⁴⁾

The mechanism of anti-bacterial activity carried out by nanochitosan is by electrostatically sending glucosamine amino groups (positively charged) and cell membranes (negatively charged) to bacteria. This interaction produces general changes on the bacterial cell surface and subsequently causes changes in membrane permeability, which ultimately results in osmotic imbalance and intracellular efflux that causes cell death.⁽⁵⁾. The aim of this research is to prove that nanochitosan from green kernag shells has an anti-bacterial effect against *Prevotella intermedia* bacteria.

RESEARCH METHODS

This research uses true experimental design research with a post test only control group design research design. This research was carried out at the Pharmacy Laboratory of Muhammadiyah University, Surakarta for the manufacture of green mussel shell nanochitosan and the Microbiology Laboratory of the Faculty of Dentistry, Airlangga University for anti- bacterial testing against *Prevotella intermedia*.

Preparation of *Prevotella intermedia* bacterial culture is cultured on Mueller's Hinton agar medium and incubation is carried out first so that the bacteria can live on the medium at a temperature of 37°C in an incubator for 24 hours. Calcium hydroxide paste was prepared as a positive control group, sterile distilled water solution as a negative group, then prepared nanochitosan dispersed solids with concentrations of 0.5%, 1%, 1.5%, 2% with the addition of 100g of acetic acid and 20g of 1% NaTPP. The bacterial media that has been filled with *Prevotella intermedia* colonies is then treated using all the samples on the plate, provided that each plate contains 3 samples, and then incubated again for 24 hours at a temperature of 37°C. The test was repeated 24 times. The inhibition zone that

has been formed will be measured using a sliding caliper and the data obtained will be processed using SPSS version 24.0 software. The data normality test will be tested using the Shapiro - Wilk method, the data homogeneity test will be tested using the Levene's test method, the parametric statistical test will use One Way ANOVA, and the least significant difference test was carried out to determine significant differences between samples.

RESULT

Data of the research results (Diagram 1) obtained show the average inhibition zone formed from all samples that have been tested (Table 1). The research results show that the largest inhibition zone formed is in nanochitosan with a concentration of 2%, which is equal to 13.62 ± 0.40 and the smallest is at a concentration of 0.5%, which is equal to 8.70 ± 0.18 .

The test results was found that nanochitosan with a concentration of 2% with the highest amount of nanochitosan showed the strongest anti-bacterial effect compared to the others, followed by concentrations of 1.5%, 1% and 0.5%.

Test results Shapiro-Wilk normality in this study shows that the data is normally distributed ($p > 0.05$) (Table 2). The homogeneity test using the Levene's test method shows that this research data has homogeneous results ($p > 0.05$) (Table 3). The One Way ANOVA test on

$p = 0.000$ ($p < 0.05$) (Table 4). The post hoc test using least significant difference displays data regarding the difference in mean differences between one test group and another (Table 5).

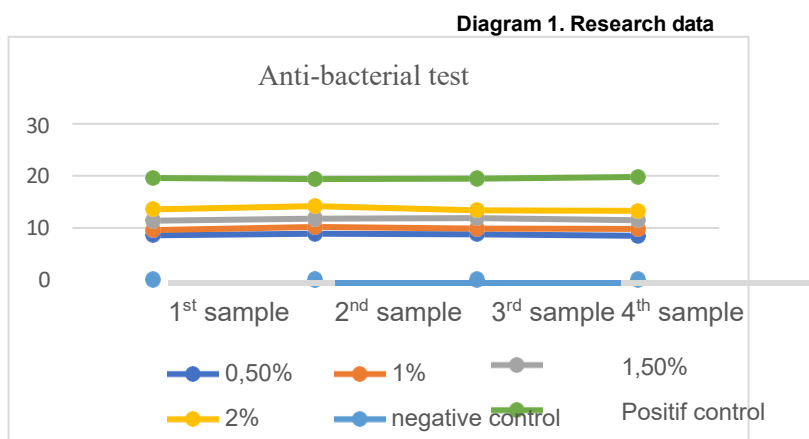


Table 1. Mean value of nanochitosan antibacterial test results on the growth of *Prevotella intermedia* bacteria

Test group	Mean±SD
0.5%	8.70 ± 0.18
1%	9.87 ± 0.25
1.5%	11.65 ± 0.23
2%	13.62 ± 0.40
Negative control	0.1±0
Positive control	19.57 ± 0.17

Table 2. Shapiro-Wilk normality test

Test group	Sig.
0.5%	0.714
1%	0.911
1.5%	0.488
2%	0.304
Negative control	0,000
Positive control	0.850

Table 3. Levene's test of homogeneity

Test	
Levene Statistics	Sig.
2,424	0.076

Table 4. One Way ANOVA Test

Test	
One Way ANOVA	Sig.
Results	0,000

Table 5. Least significant difference test

	1	2	3	4	5	6
1		-1.17*	-2.95*	-4.92*	8.70*	-10.87*
2	2.95*		-1.77*	-3.75*	9.87*	-9.70
4	4.92*	1.77*		-1.97*	11.65*	-7.92*
5	-8.70*	3.75*	1.97*		13.62*	-5.95*
6	-9.87*	-11.65*	-13.62*	19.57*		-19.57*
	10.87*	9.70*	7.92*	5.95*	19.57*	

*Information:

1: Nanochitosan 0.5%

2: Nanochitosan 1%

3: Nanochitosan 1.5%

4: Nanochitosan 2%

5: Negative control

6: Positive control

*:Mean difference

DISCUSSION

This test aims to determine the anti-bacterial properties of green mussel shell nanochitosan against *Prevotella intermedia* bacteria. Green mussel shell nanochitosan showed an anti-bacterial effect on the growth of *Prevotella intermedia* bacteria as indicated by the differences in inhibition zones that appeared on petri dishes or the growth medium for *Prevotella intermedia* bacteria. Nanochitosan has this ability because green mussel shell nanochitosan is a natural ingredient that has anti-bacterial effects⁽⁶⁾.

The results of The One Way ANOVA test shows that $p=0.000$ ($p<0.05$), with this result proving that there is a significant difference between one test group and another. The mechanism that occurs

between nanochitosan and *Prevotella intermedia* bacteria is to electrostatically send glucosamine amino groups (positively charged) to the cell membrane (negatively charged) of the bacteria. This interaction creates general fluctuations on the cell surface and then causes changes in membrane permeability and causes osmotic imbalance and intracellular leakage that causes bacterial cell death.⁽⁵⁾.

Previous research conducted by Divya et al explained that the anti-bacterial mechanism of chitosan which is most often used as a reference is that chitosan binds to the negative charge of bacterial cell walls, causing cell lysis, then causing changes in membrane permeability, then binding to DNA, blocking DNA replication and then causing cell death. Another possible mechanism is that chitosan, as a chelating agent, can bind other metal elements nearby, selectively binding these metal elements, causing the production of toxic factors and inhibiting the growth of microorganisms.⁽⁷⁾.

Base on data, the results of this test show that the lowest nanochitosan capability is nanochitosan with a concentration of 0.5%, followed by 1%, 1.5%, and the highest result is nanochitosan with a concentration of 2%. The cause of the difference in anti-bacterial effect is due to the comparison of the concentration of nanochitosan with the solvent, namely acetic acid (10gr) and NaTPP 1% (20gr), which is different for each concentration. Nanochitosan which has the highest concentration will have a stronger anti-bacterial effect compared to nanochitosan with a low concentration⁽⁸⁾.

The impact exerted by nanochitosan through ionic and/or hydrophobic action is responsible for damage to cell membranes. This reaction causes the hydrophilicity effect of gram-negative bacteria to be much higher than that of gram-positive bacteria, so they are more sensitive to the anti-bacterial effect provided by nanochitosan. The difference in sensitivity of the two bacteria is caused by chitosan activity, especially due to differences in the cell wall structure of the two bacteria. Gram-negative bacteria have three membranes, namely the outer membrane (hydrophobic), a thin peptidoglycan layer and a cell membrane, while gram-positive bacteria only have a thick peptidoglycan layer.⁽⁹⁾.

The anti-bacterial ability of nanochitosan is obtained from its content which has the chemical structure NH₂ which can affect negatively charged cell walls, thereby disrupting the stability of the cell walls. This event ends with damage to the cell wall which results in leakage of the cell's essential components and leads to cell death. Nanochitosan also has the ability to chelate bacterial cells which can inhibit bacterial growth⁽¹⁰⁾.

Calcium hydroxide can maintain its antibacterial effect as long as the pH of the calcium hydroxide remains alkaline. The antibacterial activity of calcium hydroxide will depend on the release of hydroxyl ions in an aqueous environment. The lethal effect of hydroxyl ions on bacteria is thought to be because these ions are capable of damaging the cytoplasmic membrane, denaturing it. proteins, and damage the DNA of the bacteria. Damage to the cytoplasmic membrane in these bacteria is due to bacterial enzymatic processes taking place in the cytoplasm. The pH gradient in the cytoplasmic membrane is damaged by the high concentration of hydroxyl ions from calcium

hydroxide which acts on membrane proteins (protein denaturation). The high pH of calcium hydroxide alters the integrity of the cytoplasmic membrane through chemical damage to organic components and nutrient transport or through the destruction of phospholipids or unsaturated fatty acids in the cytoplasmic membrane during the peroxidation process, which is a saponification reaction. Drastic changes in pH will cause the cessation of bacterial enzymatic processes for long periods of time and cannot be changed⁽¹¹⁾.

Nanochitosan is difficult to dissolve at pH > 6.5. Nanochitosan has different properties from chitosan because the higher the pH it contains, the more the effect will increase (4.0 increased to 6.0) and shows high antibacterial results at pH 6.0 on gram-negative bacteria, namely *Escherichia coli* and gram-positive bacteria *Streptococcus aureus*. *Pseudomonas aeruginosa* bacteria were treated with nanochitosan at a pH level of 5.0 and showed significant results in terms of anti-microbial effects. Other research shows that the antibacterial ability of nanochitosan does not appear at pH > 7.0. Other research proves the comparison of the antibacterial effect of nanochitosan at pH 4.0; 7.0; 10.0 against the bacteria *Escherichia coli* and *Staphylococcus aureus* and the results show that the antibacterial effect increases at a comparison of pH 4.0 to 7.0 but decreases at pH 10.0⁽⁵⁾.

Tooth demineralization occurs due to a reaction of hydroxyapatite against hydrogen ions in the oral cavity with a pH ≤ 5.5. H⁺ and phosphate ions will react on the surface of tooth enamel and will change PO₄³⁻ to HPO₄²⁻ and will dissolve hydroxyapatite⁽¹²⁾. The nanochitosan that was made in this research will not affect demineralization because in the manufacturing process before being converted into nanochitosan, the acidity level of the chitosan was measured and a pH of 6.7 was obtained, which is close to neutral pH (7.0).

CONCLUSION

Based on the results of research that has been carried out regarding the anti-bacterial effect of green mussel shell nanochitosan on the growth of *Prevotella intermedia* bacteria, it can be concluded that nanochitosan can inhibit the growth of *Prevotella intermedia* bacteria. The most effective concentration of green mussel shell nanochitosan to inhibit the growth of *Prevotella intermedia* bacteria is a concentration of 2%.

SUGGESTION

Further research is needed to test nanochitosan on other bacteria with higher concentrations of nanochitosan. Adding other anti-bacterial ingredients to nanochitosan to find out whether the addition of these ingredients can increase the anti-bacterial effect. Increases the degree of deacetylation of nanochitosan to achieve maximum anti-bacterial effects. Increasing the pH level of nanochitosan so that it is alkaline and not acidic, which is safe for tooth roots.

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