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Research Article

Root Canal Irrigation NaOCl plus Nano Chitosan as an Antibacterial *Pseudomonas Aeruginosa*

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KEYWORDS

Root canal irrigation NaOCl, nano chitosan, antibacterial

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ABSTRACT

Introduction: Chitosan tilapia scales is one of the chitosans which has benefits as an antibacterial in dental root canal, the physical size of chitosan can be converted into smaller particle sizes in the form of nanoparticles. For the material of root canal irrigation as a *gold standard* is sodium hypochlorite 0.5% -5.25%. One of the bacteria found in the root canal is *Pseudomonas aeruginosa*. **Objective:** The purpose of this study was to determine the effectiveness of nano chitosan tilapia scales plus 1.5% and 2.5% NaOCl in inhibiting *Pseudomonas aeruginosa*. **Methods:** This research is a laboratory experimental study with the research design used is the *Post Test Only Control Group Design* antibacterial test *Kirby Bauer*. The sample used was the bacterium *Pseudomonas aeruginosa* which was divided into 4 groups: group 1 was the treatment group given nano chitosan of tilapia scales plus 1.5% NaOCl, group 2 was the treatment group given nano chitosan of tilapia scales plus 2.5% NaOCl, group 3 of the treatment group was given a positive control with *Chlorhexidine* and group 4 of the treatment group was given a negative control of aquadest with 6 repetitions for each. **Results:** The results of the Least Significant Difference (LSD) test showed significant differences in the inhibition zone between the nano chitosan groups with 1.5% and 2.5% NaOCl (<0.05). The average inhibition power of nano chitosan of tilapia scales plus 1.5% NaOCl was 21.33 mm and nano chitosan of tilapia scales plus 2.5% NaOCl was 24.83. **Conclusion:** Both nano chitosan groups showed strong antimicrobial activity against *Pseudomonas aeruginosa* with the 2.5% NaOCl group being more effective.

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INTRODUCTION

Dental and oral health is an important aspect of a person's overall health. Based on the 2023 Indonesian Health Survey (SKI), the national prevalence of dental and oral problems was 33,19% among individuals aged 3 years and older[1]. One of the most common dental and oral diseases found in the community is a disease that affects the pulp and periapical tissue[2]. One of the treatments for pulp and periapical diseases is root canal treatment which is done by removing the infected pulp tissue from the pulp chamber and root canal, then filled with root canal filling[3] material to prevent re-infection[4]. Root canal irrigation is one of the important stages in supporting the success of root canal treatment which can facilitate the removal of necrotic tissue, microorganisms and dentin fragments from infected root canals with the rinsing action of irrigation solutions. NaOCl is one of the ideal irrigation materials to use [5].

In recent dental studies, the focus of the use of natural materials such as chitosan is one of the materials that has received attention. Chitosan, which is now being utilized as an irrigation solution in root canal treatment, is a derivative of linear polysaccharide chitin that can be synthesized. It is generally derived from crustaceans animals [6]. Nano chitosan is a type of chitosan that has better adsorption capacity because it has a specific surface and smaller size [7,8]. Chitosan can also be found in cells under the dermis layer of tilapia fish scales, Nano chitosan from tilapia fish scales is a material that is biocompatible, non-toxic and biodegradable. Nano chitosan is a chelating agent that can remove the smear layer on the walls of the t root canal so that the chelating agent cleans the entire surface of the root canal wall and opens blockages in the dentin tubules without causing increased dentin erosion.

This study aims to determine the effectiveness of nano chitosan from Tambak Danau Batur tilapia scales combined with NaOCl in inhibiting *Pseudomonas aeruginosa* bacteria in root canals, Root canal irrigation is a stage that determines the success of root canal treatment, where 1,5% and 2,5% NaOcl are the most frequently used materials that do not cause periapical tissue irritation.[9] *Pseudomonas Aeruginosa* bacteria are anaerobic bacteria that are very difficult to destroy on the surface of proteins, so smart materials are needed, namely nano particles in the form of nano chitosan to destroy Pseudomonas Aeruginosa bacteria found in the root canals of teeth that have experienced pulp necrosis. The use of Nano chitosan plus 1,5% and 2,5% NaOCL has not been widely carried out by researchers so there are still shortcomings related to the references used, so this research needs to be carried out considering that nano chitosan is a smart material.

MATERIAL AND METHODS

Tools and Materials

The materials used include tilapia fish scales, *Pseudomonas aeruginosa* bacteria, 2,5% sodium hypochlorite, 1,5% sodium hypochlorite, 1 M NaOH, 1,15 M NaOH, 1,5 M HCl, 2% *chlorhexidine* 2%, acetic acid, distilled water, and *Mueller Hinton Agar* media.

Research Procedures

1. Each gram of *Oreochromis niloticus* Nano Chitosan powder is processed in a size of 100 nm which forms a nano chitosan suspension.Preparation of a 0,5% nano-chitosan solution from tilapia fish scales combined with 1,5% sodium hypoclorite. *Oreochromis niloticus* nano chitosan solution of 0,5% was made by dissolving 0,5 grams of *Oreochromis niloticus* nano chitosan powder (measured using a digital scale) in 100 ml of 2% acetic acid and stirred until homogeneous using a vortex for 2 hours. Then, 10 ml of *Oreochromis niloticus* nano chitosan that had been mixed with acetic acid was added with 10 ml of 1,5% sodium hypochlorite and stirred until homogeneous using an ultrasonic cleaner for 1 hour.

   Preparation of a 0,5% *Oreochromis niloticus* nano chitosan solution combined with 2,5% sodium hypoclorite. *Oreochromis niloticus* nano chitosan solution of 0,5% was made by dissolving 0,5 grams of *Oreochromis niloticus* nano chitosan powder (measured using a digital scale) in 100 ml of 2% acetic acid and stirred until homogeneous with a vortex for 2 hours. Then, 10 ml of the nano chitosan solution in acetic acid was mixed with 10 ml of 2,5% sodium hypoclorite and the mixture was stirred until homogeneous using an ultrasonic cleaner for 1 hour.

2. In-Vitro Antibacterial Activity Test

   The inhibition test of nano chitosan *Oreochromis niloticus* plus 1,5% and 2,5% NaOCl against *Pseudomonas aeruginosa* bacteria was carried out using the diffusion method. Cultivation of *Pseudomonas aeruginosa* bacteria as many as 6 colonies were taken using a sterile ose, then spread evenly on *Mueller Hinton Agar*. Prepare 24 blank discs, each soaked with 20 µl of the following solutions: 6 discs with a 0,5% *Oreochromis niloticus* nano chitosan solution combined with 2,5% sodium hypoclorite, 6 discs with 0,5% *Oreochromis niloticus* nano chitosan solution combined with 1,5% sodium hypoclorite, 6 discs with aquadest as a negative control, and 6 discs

with 2% *chlorhexidine* as a positive control. The discs were then placed onto the *Mueller Hinton Agar* that had been inoculated with *Pseudomonas aeruginosa*, resulting in four types of solutions, which were repeated six times. The plates were then incubated in an incubator at 37ºC for 24 hours.

Ethical approval was obtained from the Dentistry Research Ethics Committee, Faculty of Dentistry, University of Mahasaraswati Nomor: 03.0042/KEP-Unmas/IX/2025.

RESULTS

Based on the research conducted, the inhibition zone diameter of nano chitosan *Oreochromis niloticus* 0,5% plus sodium hypochlorite 1,5% and nano chitosan *Oreochromis niloticus* 0,5% plus sodium hypochlorite 2,5% against *Pseudomonas aeruginosa* bacteria were obtained. The inhibition zone with test method Agar Diffusion (*Kirby Bauer*), results of measurements are presented in the following table 1. The results of descriptive analysis of *Pseudomonas aeruginosa* bacteria are presented in Table 2.

Table 1 The measurement results of the inhibition zone diameter (mm) against *Pseudomonas aeruginosa*

Repetition	Inhibition Zone (mm)			
	NK+OMN+ NaOCl 1,5%	NK+OMN+ NaOCl 2,5%	Control (+)	Control (-)
Average	21.33	24.83	19.16	0

(+) Positive control: *Chlorhexidine 2%*; (-) Negative control:Aquades

Table 2 Results of descriptive analysis of the effectiveness of *Oreochromis niloticus* nano chitosan plus 1,5% sodium hypochlorite and *Oreochromis niloticus* nano chitosan plus 2,5% sodium hypochlorite in inhibiting the growth of *Pseudomonas aeruginosa* bacteria.

Group	N	Average	SD (Standard Deviation)
Nano chitosan *Oreochromis niloticus* plus 1.5% sodium hypochlorite	6	21.33	1.21
Nano chitosan *Oreochromis niloticus* plus 2.5% sodium hypochlorite	6	24.83	1.47
Positive control	6	19.17	0.75
Negative control	6	0	0

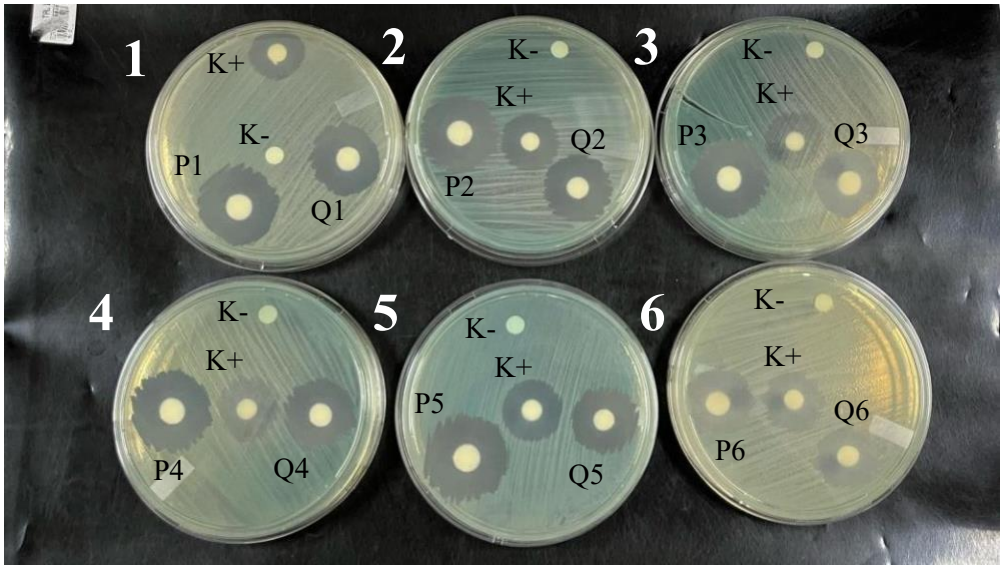

Figura no 1,2,3 results of bacterial culture 1,5% NaOCl solution group, no 4,5,6 the 2,5% NaOCl solution group and the control group (+). While the control group (-) with Kirby Bauer test results

Based on the descriptive test result in Table 5.2, the measurement of the inhibition zone of *Oreochromis niloticus* nano chitosan against *Pseudomonas aeruginosa* bacteria was repeated 6 times in this study.

In the treatment group of *Oreochromis niloticus* nano chitosan 0,5% plus 1,5% sodium hypochlorite showed an average value of 21.33 with a standard deviation of 1.21. In the treatment group of *Oreochromis niloticus* nano chitosan 0,5% plus 2,5% sodium hypochlorite showed an average value of 24.83 with a standard deviation of 1.47. In the positive control treatment group, namely *chlorhexidine* 2%, the average value was 19.17 with a standard deviation of 0.75, and in the negative control group, namely distilled water, the average value was 0 with a standard deviation of 0.

Table 3 Results of the normality test of the effectiveness of *Oreochromis niloticus* nano chitosan plus 1,5% sodium hypochlorite and *Oreochromis niloticus* nano chitosan plus 2,5% sodium hypochlorite in inhibiting the growth of *Pseudomonas aeruginosa* bacteria.

Group	Shapiro-Wilk	
	N	Sig.
1.5% solution	6	0.415
2.5% solution	6	0.804
Control (+)	6	0.212

Normality test was performed using the Shapiro-Wilk test because the sample is less than 50. In addition, the data used are data from the 1,5% NaOCl solution group, the 2,5% NaOCl solution group and the control group (+). While the control group data (-) cannot be tested for normality because all data is equal to zero. The basis for decision making in the test can be done through a probability approach, the significance used is $\alpha = 0.05$. The basis for decision making Sig.> 0.05 normality is met and Sig. <0.05 isnot met.

Based on the results of the Shapiro-Wilk normality test in the table above, it is known that the significance value for the 1,5% solution group, the 2,5% solution group, and the control group (+) are 0.415, 0.804 and 0.212 respectively. This significance value is greater than 0.05, normality is met using the *Levene's Test* statistical technique.

Based on the results of the Shapiro-Wilk test in the table above, it is known that the probability value is 0.000, this probability value is smaller than the significance level of 0.05. Referring to the basis for decision making, analysis results is rejected or there is a difference in antibacterial effectiveness between nano chitosan *Oreochromis niloticus* added with 2,5% sodium hypochlorite and nano chitosan *Oreochromis niloticus* added with 1,5% sodium hypochlorite in inhibiting the growth of *Pseudomonas aeruginosa* bacteria.This study still requires further research on experimental animals, because in experimental animals various types of bacteria will be found in necrotic root canals. In necrotic root canals there are anaerobic and aerobic bacteria so further research is needed in vivo.

Table 4. Results of the antibacterial effectiveness difference test between *Oreochromis niloticus* nano chitosan with 2,5% NaOCl and *Oreochromis niloticus* nano chitosan with 1,5% NaOCl

	$\bar{x}$	F	*p*
Between Groups	744,111	708,677	0,000
Within Groups	1,050		
Total			

DISCUSSIONS

Nano chitosan *Oreochromis niloticus* with lower concentrations has higher bacterial inhibition compared to higher concentrations, this is due to the lower viscosity of the solution so that the diffusion process is better and the ability to suppress bacterial growth is faster [10]. In general, chitosan cannot dissolve in water, chitosan is also insoluble in alkaline solvents due to the presence of amine groups. Chitosan will dissolve in acidic solvents with a pH below 6 such as acetic acid, formic acid and lactic acid [11,12]. Among the various irrigation materials, sodium hypochlorite solution with a concentration of 0,5% -5,25% is still the gold standard because effectively dissolves tissue and is antiseptic. The use of 2,5% sodium hypochlorite concentration has the potential to reduce toxicity without reducing tissue dissolving ability and antimicrobial activity [12,13]. Root canal irrigation using 0,5% nano chitosan able to dissolve the smear layer in the coronal, middle and apical third root canals. Irrigation material of 0,5% nano chitosan solution can clean the smear layer more effectively compared to irrigation material of 2,5% sodium hypochlorite and 17% EDTA [14,15,16]

Judging from the difference in the average diameter in each group, the 2,5% solution group has a positive difference in the average diameter. This means that the average diameter of the 2,5% solution group is the highest compared to other groups. Based on the results of the study, the antibacterial inhibitory power of nano chitosan *Oreochromis niloticus* added with 1,5% sodium hypochlorite and 2,5% sodium hypochlorite against *Pseudomonas aeruginosa* bacteria is included in the very strong category (≥21 mm).

CONCLUSIONS

Based on the research results, Nano chitosan *Oreochromis niloticus* plus 1,5% NaOCl and nano chitosan *Oreochromis niloticus* plus 2,5% NaOCl have strong inhibitory power against *Pseudomonas aeruginosa* bacteria. In inhibiting the growth of *Pseudomonas aeruginosa* bacteria, nano chitosan *Oreochromis niloticus* plus 2,5% NaOCl is more effective than nano chitosan *Oreochromis niloticus* plus 1,5% NaOCl.

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