

The Inhibition Effect of Green Microalga Ethanol Extract (Nannochloropsis Oculata) on The Number of Bacteria Streptococcus Mutans (In Vitro)

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ABSTRACT

Background: Antibacterial is a substance produced by a microorganism, which has the ability to inhibit growth or kill other microorganisms. Antibacterial activity is measured in vitro to determine the potency of an antibacterial agent in a solution, its concentration in body fluids or tissues, and the susceptibility of certain microorganisms to certain concentrations of drugs. **Purpose:** The purpose of this research was to find the power of extract ethanol Nannochloropsis oculata as an alternative antibacterial causes of the growth of the caries which is Streptococcus mutans. **Material and methods:** extract ethanol. Nannochloropsis oculata divided into three parts of concentration 1,5%, 2% and 2,5%, Chlorhexidine 0,12% as a control, culture bacteri Streptococcus mutans with McFarland 0.5 standart. The metode used in this study was One way analysis of varians (ANOVA), but first it had to be a homogenius test and a normalitas test. **Result:** The result of this study showed that no barrier power at concentration 1,5%, 2% and 2,5%. **Conclusion:** The result showed no inhibition in the three observed groups of 1.5%, 2%, and 2.5% of extract ethanol Nannochloropsis oculata. This meant that this article cannot prove the inhibition characteristic of Nannochloropsis oculata. This may be because the concentration of the extract Nannochloropsis oculata used in this study was too small. Henceforth, it is necessary to conduct research with a higher concentration of extract. On the other hand, the positive control group, using 0.12% Chlorhexidine solution, showed an inhibition characteristic while the negative control group, using 1 % of DMSO showed no inhibition.

Keywords: Antibacterial, nannochloropsis oculata, streptococcus mutans

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INTRODUCTION

Dental caries is an infectious disease that involves four main factors, namely host, substrate, microorganisms, and time. This disease occurs due to the continuous demineralization process of hard tooth tissue by organic acids that come from foods containing sugar and fermented by bacteria. The demineralization process takes place when the pH of saliva is below 5.5 or acidic. In this state, bacteria acidogenic causes of dental caries, such as *Streptococcus mutans* and *Lactobacillus* reproduce rapidly.¹

The pH value of saliva is inversely proportional, where the lower the pH value the more acid in the solution, conversely, the higher the pH value means the increase in base in the solution. At pH 7, there is no acidity or basicity of the solution, and this is called neutral. Bacterial growth occurs at the optimum salivary pH ranging from 6.5-7.5 and if the oral cavity pH of saliva is low (4.5-5.5) it will facilitate the growth of acidogenic germs such as *Streptococcus mutans* and *Lactobacillus*.²

The bacteria that can cause dental caries can be gram positive and gram negative both in the form of cocci and rods. Bacteria that play an important role in the formation of dental plaque and dental caries are bacteria from the genus *Streptococcus*, namely *Streptococcus mutans* bacteria.³

Streptococcus mutans is a cariogenic gram-positive bacterium because it is able to immediately form acids from distributed carbohydrates. These bacteria can thrive in an acidic environment and can stick to the tooth surface because of their ability to form extra cell polysaccharides. These extra cell polysaccharides mainly consist of glucose polymers which cause the plaque matrix to have a gelatin-like consistency, as a result of which the bacteria adhere to the teeth and stick to each other. Plaque gets thicker over time, so it will inhibit the function of saliva to carry out antibacterial activity.⁴

One of the efforts that can be done to prevent caries is a plaque control, especially in children, this is intended to minimize the incidence of dental caries in children. Plaque control can be done in two ways, namely by mechanical and chemical means. Plaque control by mechanical means, one example is by brushing teeth properly regularly every day. Meanwhile, chemical plaque control can be done by using mouthwash or toothpaste with antibacterial agents, because the occurrence of caries is related to the presence of bacteria.⁵

Antibacterial activity is measured in vitro to determine the potency of the antibacterial agent in solution, its concentration in body fluids or tissues, and the susceptibility of certain microorganisms to certain concentrations of drugs. There are several factors that influence in vitro antimicrobial activity, namely environmental pH, medium components, drug stability, inoculum size, incubation time, and metabolic activity of microorganisms.⁶

Nannochloropsis oculata is a green single-celled (unicellular) microalgae that has a very simple morphology.⁷ It is a non motile, non flagellated, and spherical, with a diameter of 2-4 μm .⁸ It has the potential due to high growth rates and ease of cultivation even in unfavorable environmental conditions.⁹

The results showed that the *Nannochloropsis oculata* extract contained a compound derived from fat oxidation called oxylipin. Through these compounds various types of secondary metabolite compounds are produced including terpenoids, alkaloids, and flavonoids. These various compounds are antibacterial.¹⁰ In the research of Yanuhar et al in 2011, it was stated that *Nannochloropsis oculata* extract was able to inhibit the growth of *Vibrio alginolyticus* bacteria at concentrations of 20%, 25%, 30% and 35% and had the ability to kill bacteria at a concentration of 40% of the colony.¹¹ In a study by Kafaie et al (2012), it was shown that *Nannochloropsis oculata* did not have acute and subchronic toxicity effects on plasma cells in mice at low to high doses.¹²



MATERIALS AND METHODS

The material used in this study was *Nannochloropsis oculata*. *N. oculata* was obtained in the form of dry powder from Balai Perikanan Budidaya Air Payau, Situbondo, Jawa Timur, Indonesia.¹³

Nannochloropsis oculata extracted by maceration method, then made into several concentrations, namely 1.5%, 2%, 2.5%. Chlorhexidine 0.12% as positive control.

In this study, samples of *Streptococcus mutans* bacteria were made from stock derived from culture by making *Streptococcus mutans* colony suspensions using Brain Heart Infusion Broth (BHIB) in a test tube, incubated at 37 °C for 24 hours in an anaerobic atmosphere and standardized equally with a standard of 0.5 McFarland.

This type of research is true experimental laboratories. Because in this study, control was carried out on all variables that could affect the research process and results. In addition, the sampling in this study was carried out randomly.¹⁴

The research design in this study was the post test only control group design. The research object was divided into 4 groups. Group 1 was the positive control group, group 2,3, and 4 were the treatment groups that were given extracts of green microalgae (*Nannochloropsis oculata*) with concentrations of 1.5%, 2% and 2.5%.

RESULTS

Observation data in the clear zone against *Streptococcus mutans* bacteria colonies using calipers. Analysis of the data obtained was then tested using statistical tests with a significance level of 95% ($p = 0.05$) and then processed by the SPSS version 23 program. The data from the research results were analyzed descriptively.

Table 1. Table of Inhibitory Power Examination Results of the inhibition zone diameter of *Nannochloropsis Oculata* extract against the growth of *Streptococcus mutans* bacteria.

Sam ple	K+	K-	P1 (1,5%)	P2 (2%)	P3 (2,5%)
1	22,45	6,00	6,00	6,00	6,01
2	30,43	6,00	6,01	6,00	6,00
3	31,35	6,01	6,00	6,00	6,00
4	31,01	6,00	6,00	6,00	6,00

Table 2. The mean diameter of the inhibition zone of *Nannochloropsis Oculata* extract against the growth of *Streptococcus mutans* bacteria.

Group	N	Mean	Std. Deviation
K-	4	6.0025	0.005
K+	4	28.81	4.25698
P1	4	6.0025	0.005
P2	4	6	0
P3	4	6.0025	0.005
Total	20	10.5635	9.51188

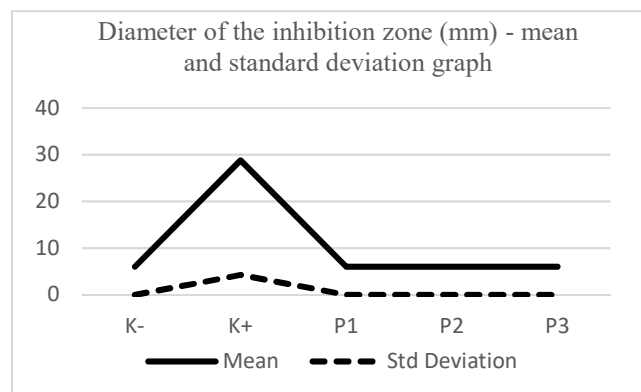


Figure 1. Diameter of the inhibition zone (mm) - mean and standard deviation graph.

K + (positive group / Chlorhexidin 0.12% solution); K- (negative group / DMSO 1% solution); P1 (1.5% extract group); P2 (2% extract group); P3 (2.5% extract group).

Based on Figure 1 and Table 2, the research data showed an inhibition zone of green microalgae extract *Nannochloropsis Oculata* against the growth of *Streptococcus mutans* bacteria in the K + group, while the K-, P1, P2 and P3 groups did not show any inhibition zones. The treatment was then tested for significance with an error rate of 5% ($p = 0.05$).



Furthermore, the results of the study were tested for the normality of the distribution using Shapiro-Wilk, because the number of research subjects was less than 50. This test aims to determine whether the data obtained is normally distributed or not with a significance level of 0.05 ($p = 0.05$). If the data is normally distributed ($p > 0.05$) it can be continued with a homogeneity test and if the data is not normally distributed ($p < 0.05$), then the data transformation is carried out.

Table 3. Shapiro-Wilk test results

	Shapiro-Wilk		
	Statistic	Df	Sig.
VAR00001	.710	4	.015
VAR00002	.632	4	.001
VAR00003	.630	4	.001
VAR00004	.	4	.
VAR00005	.630	4	.001

After the normality test was carried out and it was found that the data was not homogeneous, then the Kruskal-Wallis non-parametric hypothesis test was carried out. The results of the Kruskal-Wallis test were used to determine whether there was a significant difference in inhibition of the inhibition zone extract of the green microalgae extract of *Nannochloropsis Oculata* on the growth of *Streptococcus mutans* bacteria.

Table 4. Kruskal-Wallis test for data variables

Independent-Samples Kruskal-Wallis Test Summary	
Total N	20
Test Statistic	13.114 ^a
Degree Of Freedom	4
Asymptotic Sig.(2-sided test)	.011

Remarks * there is a significant difference

Table 4 shows a significance value of 0.011 ($p < 0.05$). This indicates that there is a difference in meaning between the positive

control and each treatment group that has different concentrations. Based on this, the Mann-Whitney test was continued.

Table 5. Mann-Whitney test results

	K+	K-	P1	P2	P3
K+		0.18*	1.00*	0.317*	1.00*
K-			0.18	0.18	0.18
P1				0.317	1.00
P2					0.317
P3					

Based on the results of the Mann-Whitney test, there was no significant difference in the inhibition of *Streptococcus mutans* bacteria ($p < 0.05$) in the K + group with the K-, P1, P2 and P3 groups, P1 groups with K- groups, P2 groups with K- groups. and P1, as well as the P3 group with the K-, P1 and P2 groups because the significant value is greater than 0.05 ($p > 0.05$).

DISCUSSION

In this study, *Nannochloropsis Oculata* extract was used because this species is known to have fast and easy growth in culture. The selection of concentrations of 1.5%, 2%, and 2.5% is based on research by Revianti and Parihsini (2013), proving that *Nannochloropsis oculata* extract is not toxic in fibroblast stem cell culture with a concentration threshold of 2.5%, whereas above that concentration is toxic to fibroblast stem cells.¹⁵

In making the extract of *Nannochloropsis Oculata*, researchers used ethanol solvent with a concentration of 96% because it can produce easier separation of the content. Ethanol is a universal polar compound that can dissolve polar compounds and few non-polar compounds. Ethanol can generally extract components from the glycoside and essential oil groups. The test results of the content *triterpene glycoside* in the ethanol extract were greater than the content *triterpene glycoside* in the hexane extract.¹⁶ *Triterpene glycoside* is the for *termholothurin* which is a saponin compound in



sea cucumbers and functions as an antibacterial.¹⁷ The concentration of ethanol used as the solvent in this study was 96% because it resulted in an easier separation of the content.¹⁸

Researchers used *Streptococcus mutans* bacteria because these bacteria are the most dominant cause of human dental caries.¹⁹ The metabolic acid produced by *Streptococcus mutans* can cause demineralization of the tooth surface and play a role in dental caries. The glucosyltransferase enzymes produced by these bacteria are the key to this process. These bacteria use sucrose as a substrate to synthesize soluble and insoluble glucans. Kinetic studies with divalent metal ions have shown the strength of the affinity bond for glucosyltransferase. The metal ions also exhibit the inhibitory power of the enzyme.²⁰

The positive control used 0.12% Chlorhexidine solution because the components consisted of two parts of para-chlorophenol and three parts of camphor. Its disinfectant irritant properties are less than formocresol, has a broad antibacterial spectrum and is effective against fungi. Chlorhexidine 0.12% is able to destroy various microorganisms in the root canal. Kamfer as a means of diluting and reducing the irritation properties of pure para-chlorophenol. Apart from that it extends the effect of the antimicrobial properties and has also been compared with the antimicrobial effect of other root canal sterilizers. Menthol reduces the irritating properties of chlorophenol and relieves pain. This material can be used in the treatment of root canals that have apical disorders.²¹

This study has divided bacteria into five groups. The K + group as a positive control was given 0.12% Chlorhexidine solution. The administration of DMSO 1% in the K- group (negative control), 1.5% *Nannochloropsis Oculata* extract as the first treatment group (P1), 2% *Nannochloropsis Oculata* extract as the second treatment group (P2), and 2.5% *Nannochloropsis Oculata* extract as the third treatment group (P3). The results showed that

the inhibition zone of *Nannochloropsis oculata* extract against the growth of *Streptococcus mutans* bacteria in the K + group, while the K-, P1, P2 and P3 groups did not show any inhibition zones.

The negative control used is DMSO 1% because it does not have antibacterial properties that will affect the inhibition of bacteria and the extract tested is a natural material. In addition, DMSO solution functions as a solvent that quickly penetrates into epithelial extracts without damaging cells and is often used in medicine and health.²²

Based on the results of the Kruskal-Wallis test, it was obtained $p = 0.011$ ($p < 0.05$) which indicated a significant difference in all groups, followed by the Mann-Whitney test to see the significance of the two groups' data. There was no significant difference in the inhibition of *Streptococcus mutans* bacteria ($p < 0.05$) in the K + group with the K-, P1, P2 and P3 groups, P1 groups with K- groups, P2 groups with K- and P1 groups, and P3 groups with groups K-, P1 and P2 because the significance value is greater than 0.05 ($p > 0.05$). The mean of the antimicrobial zone at a concentration of 1.5% (6.01 mm), 2% (6.01mm), 2.5% (6.00mm), DMSO negative control 1% (6.00 mm), and control Chlorhexidine positive 0.12% (28.81 mm).

This research is still qualitative in nature, which is to show differences in the inhibition of *Nannochloropsis oculata* extract against the growth of *Streptococcus mutans* bacteria with concentrations of 1.5%, 2%, and 2.5%, and is a preliminary research.

CONCLUSION

The results obtained showed that there was no inhibition at concentrations of 1.5%, 2%, and 2.5%. This may be because the concentration of the *Nannochloropsis oculata* extract used in this study was too small, whereas based on previous research by Fadhilah (2013), showed the effectiveness of the inhibition of *Nannochloropsis oculata* extract on bacterial



growth in root canals at a concentration of 80% .¹⁰ Because it is suggested for further research, research can be carried out with a higher concentration of *Nannochloropsis oculata* extract.

Henceforth, it is necessary to conduct quantitative research to determine the decrease in the number of bacterial colonies.

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