Denta, *Jurnal Kedokteran Gigi*

Accredited No. 79/E/KPT/2023
p-ISSN: 1907-5987 e-ISSN: 2615-1790

RESEARCH ARTICLE

The Inhibition of *Cyanobacteria spirulina* Extract on S. Mutans Biofilm as an Ingredient in Mouthwash

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Online submission : 17 Maret 2023
Accept Submission : 30 Agustus 2023

ABSTRACT

Background: *The main cause of dental caries is* Streptococcus mutans (S. mutans)*, to suppress the growth of cariogenic bacteria can be done by using mouthwash containing antibacterial ingredients such as chlorhexidine.* Cyanobacteria spirulina *extract contains compounds that have secondary metabolites which act as antibacterial and antioxidant including tannins, flavonoids, saponins and terpenoids.* ***Objective:*** *This study aimed to find out he inhibition of* Cyanobacteria spirulina *against* S. mutans *biofilm.* ***Materials and Methods****: The research sample consisted of 6 groups. Group K(-) was the* S. mutans *biofilm with aquadest, K(+) was Chlorhexidine 0.2% and four treatment groups (P1, P2, P3, P4), which were* Cyanobacteria spirulina *extract with a concentration of 60 , 70, 80 and 90 mg/ml. The sample was applied to a well microtiter plate, then incubated overnight at 37°C for 1 x 24 hours, then painted with 0.1% crystal violet. After that, it was rinsed with sterile distilled water, then given 0.2 mL of Tween 80 concentration of 2% and measured Optical Density with an ELISA Reader with a wavelength of 570 nm, then biofilm inhibition percentage as calculated.* ***Results:*** *The mean value of the percentage of* S. mutans *biofilm inhibition in group K(+): -131,694(SD 10,758) ; P1: -12,234(SD 2,402) ; P2: 11,076(SD 6,387) ; P3: 19.7020(SD 11,670) ; P4: 40,214(12,057) there were significant differences between P2 and P4 also between P3 and P4.* ***Conclusion:*** Cyanobacteria spirulina *at concentrations of 70, 80 and 90 mg/ml had* S. mutans *biofilm inhibition, while Chlorhexidine 0.2% and* Cyanobacteria spirulina *60 mg/ml did not.*

Keywords: *Cyanobacteria spirulina, Chlorhexidine, Mouth Wash, S. Mutans Biofilm*

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Available at http://journal-denta.hangtuah.ac.id/index.php/jurnal/issue/view/23
DOI: 10.30649/denta.v18i2.8

INTRODUCTION

Dental caries is one of the dental health problems experienced by people of all ages. In Indonesia, based on the results of the 2019 Indonesia National Basic Health Research Report, the prevalence of dental caries reached 88.8%. The most common caries-causing micro-organism is *S. mutans*. These microorganisms produce organic acids, especially lactic acid, by fermenting carbohydrates on the tooth surface, resulting in a decrease in salivary pH below 5.5 which results in demineralization of the tooth surface so that dental caries is formed.[1] *S. mutans* is able to produce acids in dental plaque.[2] A colony of microorganisms consisting of several cells of various types of bacteria that adhere to the surface of the teeth is called a biofilm..[3] Bacteria will form a biofilm as a defense to survive in the oral cavity environment so as to make it stronger against immune response attacks from the host and also drugs.[4] The process of biofilm formation can occur through several phases, which are the initiation of bacterial attachment, maturation, colonization and release of biofilm cells.[5] Some conditions that can affect the biofilm formation process include pH levels, nutrition and host defense.[6]

One of the efforts to suppress the high caries rate is to control the number of caries-causing bacteria. This can be done by chemically controlling plaque through the use of mouthwash containing antibacterial ingredients so that it can suppress the growth of cariogenic bacteria.[7] Mouthwash commonly used is chlorhexidine, fluoride and povidone iodine. Chlorhexidine is able to prevent plaque and dental caries because it has a bactericidal and bacteriostatic effect on oral bacteria.[8]

Cyanobacteria are known as "blue green algae" because the bluish color of this species is due to the formation of phycobilin and phycocyanin pigments in certain concentrations.[9] This *Cyanobacteria spirulina* extract contains compounds that have the potential as antitumor, anti-inflammatory, antimicrobial, and antioxidant.[10] The presence of effective antimicrobial properties in Cyanobacteria spirulina extract is due to the content of linolenic acid, synergistic effect of lauric acid.[11] *Cyanobacteria spirulina* contains secondary metabolites that act as antibacterials including tannins, flavonoids, saponins and terpenoids.[12]

MATERIALS AND METHODS

Preparation of solutions by weighing *Cyanobacteria spirulina* powder with sterile distilled water to produce solutions with concentrations of 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml. To conduct a biofilm formation detection test on *Streptococcus mutans* bacteria, it is necessary to inoculate Streptococcus mutans bacteria on Congo red agar in a petri dish, incubated at a temperature of 37°C, for 48 hours, under anaerobic conditions. Then checked after 48 hours, if a black colony were formed, it means that the bacterial strain forms a biofilm.

The biofilm of *Streptococcus mutans* which in a well microtiter plate and then it needs to be incubated at 37°C for 24 hours. Then into each well was added a solution of green microalgae *Cyanobacteria spirulina*, then incubated overnight for 24 hours, at 37°C. The contents of each well were aspirated and washed with 0.2 mL of Phosphate buffered saline, 3 times. The biofilm formed and attached to the well was stained using 0.1% crystal violet. After that, it was rinsed using sterile distilled water, then dried. The analysis stage of biofilm formation was carried out by adding 0.2 mL of 2% Tween 80 in each well. Measurement of Optical Density (OD) using ELISA Reader 570nm wavelength.

RESULTS

The average value along with the standard deviation of the Optical Density measurement using an ELISA Reader tool using 570nm wavelength in all groups.

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Table 1. The mean value and standard deviation of *Streptococcus mutans* biofilm inhibition

Group	Replication	Mean	SD
K (+)	5	-131,6940	10,75857
K (-)	5	0	0
P1	5	-12,2340	2,40246
P2	5	11,0760	6,38713
P3	5	19,7020	11,67030
P4	5	40,2140	12,05749

Note:
K (+) : Chlorhexidine 0.2% + the biofilm of Streptococcus mutans
P1 : Cyanobacteria spirulina solutions with concentrations of 60 mg/ml + the biofilm of Streptococcus Mutans
P2 : Cyanobacteria spirulina solutions with concentrations of 70 mg/ml + the biofilm of Streptococcus Mutans
P3 : Cyanobacteria spirulina solutions with concentrations of 80 mg/ml + the biofilm of Streptococcus Mutans
P4 : Cyanobacteria spirulina solutions with concentrations of 90 mg/ml + the biofilm of Streptococcus Mutans

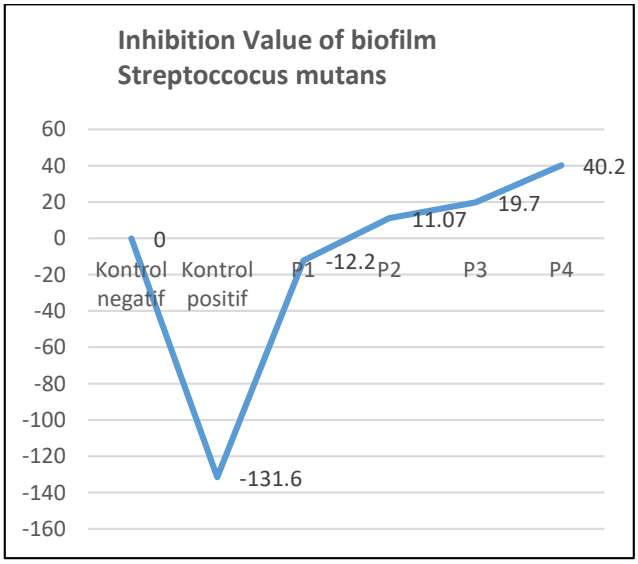

Figure 1. Graph of the inhibition value *Streptococcus mutans* biofilm

Note: K - (Aquades); K+ (Chlorhexidine 0,2%); P1 (solution Cyanobacteria spirulina concentration 60mg/ml); P2 (solution Cyanobacteria spirulina concentration 70mg/ml); P3 (solution Cyanobacteria spirulina concentration 80mg/ml); P4 (solution Cyanobacteria spirulina concentration 90mg/ml).

To find out whether all data were normally distributed, data analysis was carried out by conducted a normality test used Shapiro-Wilk.

Table 2. Result of normality test with Shapiro-Wilk

Group	Sig.
K (+)	0,959*
P1	0,228*
P2	0,376*
P3	0,318*
P4	0,154*

Note: ***** (p>0,05)

In the normality test, the obtained is p > 0.05, this indicates that the data is normally distributed. Because all data are normally distributed, it can be continued with a homogeneity test using the Levene Test.

Table 3. Test result for the variance homogeneity with Levene's test

Levene test	Sig.
2,501	0,075*

Note: ***** (p >0,05)

The results of the variance homogeneity test with Levene's test obtained is p > 0.05, so it can be said that the data in this study are homogeneous. The results of data testing on all percentage values of *Streptococcus mutans* biofilm inhibition obtained that all data obtained is normally distributed and homogeneous, then it can be continued with data analysis using the one-way ANOVA test. This is to find out whether

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there are differences in all research sample groups.

Table 4. The result of *Oneway* ANOVA test

	Sig.
Between Groups	0,000*****

Note: ***** (p < 0,05)

The p < 0,05 was obtained from the results of data analysis using the one-way ANOVA test, this indicates that there are differences in all groups, so to find out in more detail about the differences between each group, for this reason, data analysis is required by conducting an LSD test.

Table 5. The result of LSD test

	K+	P1	P2	P3	P4
K (+)		0,000*	0,000*	0,000*	0,000*
P1			0,001*	0,000*	0,000*
P2				0,163	0,000*
P3					0,003*
P4					

Note: * (p < 0,05)

The results of the LSD test conducted, obtained data p value <0.05 between group K (+) with groups P1, P2 and P3; group P1 with groups P2, P3 and P4; group P4 with groups P2 and P3. From these data it can be said that there are significant differences between: group K (+) with groups P1, P2 and P3; group P1 with groups P2, P3 and P4; group P4 with groups P2 and P3.

DISCUSSION

Cyanobacteria spirulina is a prokaryotic blue-green algae or single-celled organism, is a type of phytoplankton organism that comes from the Cyanophyta group (blue-green algae), where the Cyanobacteria spirulina species is often used for various needs, including as a food additive, industrial raw material, natural feed, pharmaceuticals and also for cosmetics. With a protein composition of 60-70% of the total mass. *Cyanobacteria spirulina* is known to have antimicrobial activity, one of which is against *Staphylococcus aureus (S. aureus)*.[10] In research on the evaluation of microalgae and cyanobacteria as potential sources of antimicrobial compounds, it was found that the dominant compounds identified and purified in the extract were docosahexanoic acid (DHA), oleic acid, linoleic acid and eicosapentaenoic acid (EPA). The presence of these compounds as ingredients in Cyanobacteria spirulina is a potential candidate that inhibits the growth of gram-positive bacteria.[13] Pure antimicrobial compounds produced by Spirulina platensis appear to be more active in inhibiting gram-positive, gram-negative bacteria and unicellular fungi as well as candida albicans.[14]

Cyanobacteria spirulina also has the potential as a strong natural antioxidant, this is because this species contains chlorophyll, phycobiliprotein, phenolic and carotenoid compounds which are able to donate hydrogen atoms to free radical compounds.[10] Dental caries is caused by host interactions which include teeth and saliva, food, time, oral hygiene and microorganisms. *Streptococcus mutans* bacteria are the main microorganisms that cause dental caries.[15] *Streptococcus mutans* has the ability to form biofilms on teeth so that it can cause dental caries which can cause abnormalities in tissues in the oral cavity and body systems. Biofilm is a colony of microbes consisting of both organic and inorganic substrates which are coated with extracellular microbial products to form an intermicrobial matrix. The ability of *Streptococcus mutans* to synthesize glucan homopolymers extracellularly from sucrose is something that plays an important role in the process of initial attachment, colonization and biofilm accumulation on the tooth surface.[16]

In this research wanted to know about the inhibition of *Streptococcus mutans* biofilm against 0.2% Chlorhexidine mouthwash as a

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positive control group and with *Cyanobacteria spirulina* solution with concentrations of 60, 70, 80 and 90 mg/ml respectively as the treatment group. The results showed that a solution of *Cyanobacteria spirulina* with a concentration of 70, 80 and 90 mg/ml had the ability to inhibit the formation of biofilm Streptococcus mutans, this was supported by the research of Winahyu et al (2020) it was found that the *Cyanobacteria spirulina* extract with concentrations of 25%, 50%, 75% and 100%, could be used as an antibacterial against gram-positive bacteria *Streptococcus aureus* in which *Streptococcus aureus* bacteria had the same characteristics. similar to that of Streptococcus mutans.[17] In microalgae lipid compounds there are Fatty Acid Methyl Ester (FAME) which are known as suitable supercurators and superior antipathogens. FAME extract obtained from Scenedesmus intermedius microalgae has antimicrobial properties against Gram-positive bacteria (Bacillus cereus, Sterptococcus mutans and Streptococcus aureus); on Gram-negative bacteria (P. aeruginosa and E. coli); and fungi (Candida albicans and Aspergillus parasiticus).[18] The antimicrobial effectiveness of the *Cyanobacteria spirulina* extract is related to the secondary metabolite compounds contained therein, namely tannins, flavonoids, saponins and triterpenoids.[12] Tannin compounds as antimicrobials can occur by inactivating essential enzymes. After that, tannin molecules interact hydrophobically with proteins to form complexes. Hydrophobic bonds will cause denaturation and disrupt cell metabolism.[19] Flavonoid compounds can form complexes with proteins from bacteria through hydrogen bonds so that they can cause changes in the structure of cell walls and cytoplasmic membranes so that bacterial cells become lysed.[20] Protein denaturation is how saponins exert antimicrobial effects. Saponin is an antibacterial compound, because it can reduce the tension on the surface of the bacterial cell wall, so that the permeability of the bacterial membrane will be disrupted, the content of the active substance is similar to detergent. Bacterial life will be disrupted if the cell membrane is damaged. The stability of the cytoplasmic membrane will then be disturbed due to the diffusion of saponin across the membrane, causing cytoplasm leakage and cell death.[21] The antibacterial effect of triterpenoids is mediated by lipid damage to the cytoplasmic membrane, which disrupts the production of cell walls or membranes. As a result, the cell membrane or wall fails to develop or develops imperfectly.[22]

The antibacterial potential of algae extracts against *Streptococcus mutans* and Streptococcus aureus is higher than standard antibiotics. The antimicrobial activity of this microalgae is due to the presence of lipopolysaccharides, cyclic peptides and alkaloids.[23] Spirulina platensis can stimulate cellular antioxidant enzymes, reduce DNA damage, reduce lipid peroxidation and scavenge free radicals, and can also enhance the effects of superoxide catalase and dismutase.[24] Other studies that support the antimicrobial activity of Spirulina platensis extract at concentrations of 1, 10 and 100 mg/ml against gram-negative bacteria *Pseudomonas aureginosa (P. aureginosa)*, gram-positive bacteria *Streptococcus aureus* and pathogenic fungi.[25] The results of the inhibition percentage in 0.2% Chlorhexidine and a solution of *Cyanobacteria spirulina* with a concentration of 60 mg/ml showed a negative value, this indicates that there is no *Streptococcus mutans* antibiofilm power. The higher the concentration of Cyanobacteria spirulina solution, the higher the amount of active antibacterial compounds, this is evidenced by the large inhibitory power of Streptococcus mutans biofilm. The largest inhibition value of Streptococcus mutans biofilm was found in group P4, namely Cyanobacteria spirulina solution with a concentration of 90 mg/ml. There was a significant difference in *Streptococcus mutans* biofilm inhibition between groups P4 and group P2 and P3.

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CONCLUSION

Cyanobacteria spirulina solutions with concentrations of 70, 80 and 90 mg/ml had the ability to inhibit the formation of *Streptococcus mutans* biofilm. The highest value of *Streptococcus mutans* biofilm inhibition was found in *Cyanobacteria spirulina* solution with a concentration of 90 mg/ml.

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