

**RESEARCH ARTICLE**

**Anti-biofilm Activity of Epigallocatechin gallate (EGCG) against *Streptococcus mutans* bacteria**

**Prawati Nuraini<sup>1\*</sup>, Mega Mocharyono Puteri<sup>1</sup>, EksaArinda Pramesty<sup>2</sup>**

<sup>1</sup>Assistance Professor of Pediatric Dentistry Department, Faculty of Dentistry, Universitas Airlangga, Surabaya, Indonesia.

<sup>2</sup>Student Faculty of Dentistry, Universitas Airlangga, Surabaya, Indonesia.

\*Corresponding Author E-mail: [prawatinuraini@fkg.unair.ac.id](mailto:prawatinuraini@fkg.unair.ac.id)

**ABSTRACT:**

Dental caries is a disease caused by *Streptococcus mutans*. The use of *chlorhexidine* to inhibit bacterial colonization has side effects such as tooth staining and can kill the normal flora when used long term. *Epigallocatechin gallate* (EGCG) is a chemical compound in the form of polyphenols from green tea catechins which have antimicrobial potency to inhibit microorganism growth and biofilm formation. Type Laboratory Experimental Research *In-vitro*. The group that will be studied are the negative control group in the form of *S.mutans* + 5% sucrose, the treatment group in the form of *S. mutans* + 5% sucrose and EGCG concentration of 0.125mg/ml, 0.25mg/ml, 0.375mg/ml and a positive control group is *S.mutans* + 5% sucrose and 0.1% *chlorhexidine*. Data were analyzed using the Kolmogorov-Smirnov test to determine the normality of the data, Levene's test for homogeneity of data, One Way ANOVA Post Hoc Tukey HSD Multiple Comparison to determine differences between treatments. Results: There were significant differences between the treatment groups and the negative control at test results Post Hoc Tukey HSD and the significant differences in the concentration of EGCG 0.375mg/ml with the positive control given *chlorhexidine* 0.1% ( $p < 0.05$ ). *Epigallocatechin gallate* (EGCG) influence on the activity of *S. mutans* biofilm formation and EGCG concentration of 0.375mg/ml are more effective as an antibiofilm of *S. mutans* compared with *chlorhexidine* 0.1%.

**KEYWORDS:** *Epigallocatechin gallate*, antibiofilm, *Streptococcus mutans*.

**INTRODUCTION:**

Oral disease is a part of the list of diseases that most often expressed by the people of Indonesia. Oral disease is often suffered by the people of Indonesia, namely caries. Dental caries is an infectious disease in human teeth, which is characterized by complex interactions that occur between species of oral microorganisms, the products produced by microorganisms, saliva, and the carbohydrates on the tooth surface. This Interaction modulates Biofilm formation (clinically known as plaque) on the surface of the vulnerable teeth and eventually causes demineralization of tooth enamel<sup>1</sup>.

Figures occurrence of oral and dental problems in Indonesia, according to Riskesdas, DMF-T index 5-year-olds at 8.1 DMF-T index of 1.9 at the age of 12, 2.4 at the age of 15 years, and 6.9 in ages 35-44. DMF-T index or DMF-t which is an index for dental caries showed a fairly large number so that this day is one of the carious teeth and oral disease to note<sup>2</sup>.

*Streptococcus mutans* is a gram-positive-bacteria and most commonly found in dental caries. *Streptococcus mutans* has a virulence of the ability to form a biofilm on the tooth surface which is called plaque. Biofilm formation can occur through the action of glucosyltransferase and also through attachment with pelicle<sup>3</sup>. Sixty-five percent of disease caused by a bacterial infection started because of Biofilm formation<sup>4</sup>. The formation of Biofilm can increase the resistance of microorganisms to antimicrobial agents and may worsen the infection<sup>5</sup>. Antibiofilm required inhibiting the growth and development of biofilms.

*Chlorhexidine* (CHX) is known as the "gold standard" for antimicrobial treatment in the oral cavity, but excessive use can cause side effects such as tooth staining or discoloration of the teeth and increases the accumulation of plaque. *Chlorhexidine* is less recommended for use long term<sup>6</sup>. Technological development of natural medicines from herbal plants growing concern by scientists. According to estimates by the World Health Organization (WHO), 80% of the world populations still depend on traditional medicine for their health problems, including the use of drugs derived from plants to minimize the side effects<sup>7</sup>.

Green tea is one of the herbs that are widely consumed by the people in Indonesia because it contains ingredients that are beneficial to health. *Epigallocatechin gallate* (EGCG) is a chemical compound in the form of polyphenols from green tea catechins have an anti-infective potential site to inhibit the growth of various microorganisms and formation biofilm<sup>8</sup>. EGCG is an effective antimicrobial that can bind to the bacterial cell membrane and then damage the cell, especially in gram-positive bacteria<sup>9</sup>.

Previous research suggests that EGCG may inhibit the growth of *Streptococcus mutans* by lowering the activity of glucosyltransferase<sup>10</sup>. Decreased activity of glucosyltransferase on GTFB and GTFC can reduce the ability of bacteria to attach on teeth surfaces<sup>3</sup>. EGCG also can inhibit biofilm formation by destroying the bacterial cell wall through peptidoglycan<sup>11</sup>. Based on the research of green tea catechins against *Streptococcus mutans* bacterial virulence factor<sup>10,11</sup>, so this research of the activity of *Epigallocatechin gallate* (EGCG) as the material for the bacteria *Streptococcus mutans* antibiofilm will be done.

## MATERIAL AND METHODS:

The research will be carried out an analytical study with the laboratory experimental research with Post Test Only Control Group Design. The research sample in the form of cultured *Streptococcus mutans* in the medium Brain Heart Infusion Broth (BHIB) at 37°C for 24 hours. The sample that will be used is calculated using the Lemeshow formula (1999) and obtained the minimum number of samples per group is as much as 3.33. To overcome the possibility of failure were calculated correction factor of 20%, using Higgins formula and found the number of samples per group is 4.

The bacteria used in this study is *Streptococcus mutans* bacteria obtained from the stock in the Research Center, Faculty of Dentistry, Airlangga University. The materials used are *Epigallocatechin gallate* (EGCG) in a powder form preparation with brands named Chem

Faces No. CFN99569 and dissolved in distilled water corresponding concentration required. In this experiment, sucrose 5% is used to trigger the formation of biofilms. A positive control using *chlorhexidine* 0.1%. This study has received approval from the Ethics Committee of the Faculty of Dentistry, Airlangga University (Number: 401 / HRECC.FODM / VI / 2019).

Before being used in the study, all the tools are sterilized by autoclaving for 15 minutes at a temperature of 121° and a pressure of 1.5 atm. Culturing bacteria were performed to reproduce *Streptococcus mutans* by inoculating 1 ose pure cultures of *Streptococcus mutans* in the medium Brain Heart Infusion Broth (BHIB) and incubated at 37°C for 24 hours in an incubator. EGCG in the formation of powder dilution using distilled water following the concentration required.

The minimum inhibitory concentration test and minimum bactericidal concentration test performed by the microdilution method. Preparations EGCG at concentrations 100% to do serial dilution and put BHIB media in test tubes with a final volume of 1ml using McFarland standard (1.5x10<sup>8</sup> CFU ml<sup>-1</sup>). Created a positive control containing medium and bacteria as well as the negative control containing media without bacterial suspension was added. 1ml of *Streptococcus mutans* suspension incorporated into the test tube and implanted with scratched at the surface, then incubated at 37°C for 24 hours. The lowest concentration that produces an inhibitory effect (bacteriostatic) was recorded as the Minimum Inhibitory Concentration (MIC) and the lowest concentration that produces a bactericidal effect on bacteria levels is recorded as the Minimum Bactericidal Concentration (MBC). The tests performed three replication.

*Streptococcus mutans* (10<sup>8</sup>-10<sup>9</sup> CFU mL<sup>-1</sup>) that have been cultured in media BHIB with sucrose 5%, BHIB + 5% sucrose and EGCG, and BHIB + sucrose 5% and *chlorhexidine* for one night at 37°C, taken respectively 100µL and put in a microtiter plate. Incubate the bacteria for 48 hours at 37°C, then the media and cells that do not stick to the microtiter plate removed. Planktonic cells rinsed with 200µL PBS (Phosphate Buffer Saline). Each well was colored using Crystal Violet 200µL of 0.1% for 15 minutes at room temperature. Eliminating dye using 95% ethanol 100µL after rinsed two times with PBS and sterile water. The microtiter plate is placed in a shaker and then vibrated for 10 minutes. The formation of biofilm formed was measured by measuring the optical density of the suspension formed using ELISA Reader (BioTek) with a wavelength of 540 nm.

The data analysis was conducted to determine significant differences in each treatment. Data were analyzed using the Kolmogorov-Smirnov Test to see the normality of the data distribution, Levene's test for homogeneity of data, One-Way ANOVA, Post Hoc Tukey HSD to determine differences between treatments. Calculations using SPSS software version 21.

**RESULT:**

The results from the dilution of *Epigallocatechin gallate* (EGCG) 100% to a concentration of 50%; 37.5%; 25%; 12.5%; 6.25% showed minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *Streptococcus mutans*. bacteria are at a concentration of 0.375mg/ml and 0.5 mg/ml.

Results assay minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) obtained in the control group (-) treatment of substance tested without any bacteria, did not obtain the growth of bacteria, which indicate that the medium is sterile and does not contaminate with bacteria. In the control group (+) bacteria is given without test substance, it was found that the bacteria can grow on the medium used. The treatment group *Epigallocatechin gallate* (EGCG) 100% and 50% is obtained a minimum bactericidal concentration of bacterial growth. The treatment group was 37.5%, 25%, 12.5%, and 6.25% obtained some bacteria still grow because the concentration is the minimum inhibitory concentration of *Epigallocatechin gallate* (EGCG).

The concentration of *Epigallocatechin gallate* (EGCG) were used in this study is 0.125mg/ml, 0.25mg/ml, and 0.375mg/ml. The use of these concentrations is intended to see the ability of anti-biofilm in *Streptococcus mutans* bacteria. According to the calculation of the number of samples obtained the number of samples for each treatment was 4. Antibiotic activity of *Epigallocatechin gallate* (EGCG) against *Streptococcus mutans* was tested and read using an ELISA reader with a wavelength of 540 nm expressed in Optical Density (OD) units. The results of the Optical Density (OD) readings of the study can be seen in (Table 1):

**Table 1: Optical Density (OD) results of the experiment**

Group	N	Mean OD ± Standard Deviation
Negative control	4	2.617 ± 0.199
0.125mg / ml	4	2.412 ± 0.232
0.25 mg / ml	4	2.087 ± 0.009
0.375mg / ml	4	1.259 ± 0.102
Positive control (CHX)	4	1.734 ± 0.162

Based on the results of Optical Density (OD) reading by looking at the turbidity level of the bacterial suspension using the ELISA reader, it was found that the three concentrations tested showed OD values lower than negative control OD values, which can be interpreted that Epigallocatechin gallate (EGCG) starting from a concentration of 0.125mg/ml is antibiofilm against *Streptococcus mutans*.

The results of data analysis using the Kolmogorov Smirnov Test obtained  $p = 0.833$  ( $p > 0.05$ ) which means the data is normally distributed. After a normality test was carried out, a homogeneity test was performed with the Levene Test and  $p = 0.056$  ( $p > 0.05$ ) was obtained, which means homogeneous data. One-Way ANOVA test was used to see the significance of differences between treatment groups. In this test,  $p = 0.000$  ( $p < 0,005$ ) showed that there were significant differences between the concentration groups and showed differences in effectiveness. To find out the treatment group that had significant OD differences, a Post-Hoc Multiple Comparison Test was conducted. The method used is Tukey HSD. A value is considered to have a significant difference if the significant value is less than equal to 0.05.

Based on the results of the Post-Hoc Multiple Comparison Test, the results show that the OD value of the negative control is higher than the positive control OD value and has a significant difference. Higher negative control OD indicates that no biofilms were inhibited because no treatment was given, while positive controls showed lower OD because it was given *chlorhexidine* 0.1% treatment.

EGCG with a concentration of 0.375mg/ml has a lower OD value than a concentration of 0.25mg/ml and 0.125 mg/ml and has a significant difference which means that at a concentration of 0.375mg/ml, biofilms are inhibited more than other concentrations, however at concentrations of 0.25mg/ml and 0.125mg/ml with OD values of 0.25mg/ml lower than the concentration of 0.125mg/ml had no significant difference which

could be interpreted that the number of biofilms inhibited was not much different. OD values at concentrations of 0.375mg/ml indicate lower values than positive control OD values given *chlorhexidine* 0.1% and have significant differences so that it can be interpreted that *Epigallocatechin gallate* (EGCG) provides a more biofilm inhibitory effect than *chlorhexidine* 0.1%.

## DISCUSSION:

This laboratory experimental study of *Streptococcus mutans* was carried out in vitro using the microtiter plate assay method. This study aims to determine the activity of *Epigallocatechin gallate* antibiofilm (EGCG) against *Streptococcus mutans* bacterial biofilms based on the theory that the active ingredients in green tea catechins have antibacterial and antibiofilm properties.

*Streptococcus mutans* is a gram-positive bacterium identified as a risk factor for caries development and is also a major pathogenic species in dental caries. Increasing the value of dental caries in the population, the research related to efforts to decrease the value of caries also increases. Currently, the most widely used therapy to inhibit bacterial activity is to use antibiotics. The use of mouthwash is also commonly done to inhibit bacterial growth and prevent the formation of plaque on teeth<sup>6</sup>.

The use of antibiotics and mouthwash cannot be used in the long term because it causes side effects, one of which can kill normal flora in the oral cavity<sup>11</sup>, so research is conducted to examine herbal ingredients that can be used as an alternative to bacterial antibiofilm inhibitory ingredients which are expected to have minimum side effects. Some herbal ingredients that have been researched to inhibit bacterial antibiofilm include *Ellagic acid* from green tea leaves, *Quercetin* from wind wood, *Eugenol* from basil plants, *Curcumin* from turmeric, *Epigallocatechin gallate* (EGCG) from green tea leaves and others<sup>12</sup>. The material used in this study was *Epigallocatechin gallate* (EGCG) which is an active ingredient of green tea catechins.

EGCG has various functions such as antioxidant, anti-microbial, antibiofilm, anti-inflammatory, anti-diabetic, anti-atherosclerosis, and anti-cardiac hypertrophy<sup>13</sup>. In previous studies, EGCG is an effective antimicrobial that can fight gram-negative and gram-positive bacteria, EGCG has antimicrobial effects on the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus* which is highly pathogenic bacteria, besides that EGCG can inhibit the formation of biofilms from the bacteria *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*<sup>12</sup>.

*Streptococcus mutans* produce glucosyltransferase enzyme which can synthesize sucrose into glucan adhesive. This glucan is a strong intermediate for attaching bacterial cells to the tooth surface and also attachment between the bacteria themselves which will later form biofilms. The presence of glucans can also increase plaque permeability by increasing the number

of acidic products on the tooth surface and acting as an energy source for bacteria.

In this study, *Streptococcus mutans* was induced using 5% sucrose. The addition of sucrose is intended to trigger the activity of the glucosyltransferase enzyme in *Streptococcus mutans* to synthesize sucrose into glucan adhesive in the sucrose-dependent adhesion process. *Streptococcus mutans* can interact with glucans through the Glucan Binding Protein (Gbps) process. Gbps acts as a mediator of the binding of glucan synthesis derived from sucrose which is synthesized by the enzyme glucosyltransferase<sup>3</sup>.

Previous studies have concluded that EGCG can inhibit the activity of glucosyltransferase, which serves to assist the formation of biofilms in the oral cavity. Inhibited glucosyltransferase activity can cause decreased glucan synthesis. Decreased amount of glucans causes the attachment of bacteria to the dental pellicle to be inhibited thereby inhibiting the process of colonization. As a result, the process of forming bacterial biofilms is also inhibited<sup>10</sup>.

Another study by Roy *et al* explained that EGCG can inhibit bacterial growth by damaging bacterial cell wall proteins and their teichoic acid. Damaged teichoic acid causes the breakdown of peptidoglycan which is a component of the bacterial wall so that the cell membrane is damaged and lysis. The lysis of bacterial cell membranes causes bacterial metabolism disrupted so that the process of colonization decreases and inhibits bacteria to form biofilms<sup>11</sup>.

*Epigallocatechin gallate* (EGCG) starting from a concentration of 0.125mg/ml to a concentration of 0.375mg/ml showed a decrease in OD values, which means that antibiofilm activity has increased. Based on the working mechanism of the optical density measurement tool that is the light scattering technique, the light is passed through a microorganism suspension which will then be captured by the detector. When visible light passes through the cell suspension, the light will be diffused<sup>14</sup>. The greater spread of light indicates that there are more bacteria or other substances present in the suspension, which means that if the value of OD measurements is higher, it indicates that there is still a bacterial biofilm and vice versa if the OD value is lower then the formation of bacterial biofilms decreases.

In this study, EGCG was antibiofilm as evidenced by the difference between the treatment group and the non-treatment group. The treatment group with a concentration of 0.125mg/ml has no difference with the group without treatment, this can occur because of the

possibility of glucosyltransferase activity in *Streptococcus mutans* bacteria that has not been completely inhibited by EGCG material so that there are still biofilms formed and the adhesin properties of the bacteria according to the theory that adhesin can also play a role in attachment between bacteria through sucrose-dependent or sucrose-independent<sup>15</sup> mechanisms or because of the presence of I/II antigens. External factors such as the possibility of contamination of the test material during the dissolution or suspension of bacteria-contaminated by the surrounding air.

EGCG with a concentration of 0.375mg/ml showed a difference with the *chlorhexidine* 0.1% treatment group which meant that the antibiofilm activity of EGCG material against the *Streptococcus mutans* bacterial biofilm was higher because inhibition of glucosyltransferase activity was more maximal and cell wall protein damage was more than *chlorhexidine* 0.1%. Based on research<sup>6</sup>, *chlorhexidine* 0.1% can reduce the number of living bacterial cells and can inhibit the growth and formation of *Streptococcus mutans* biofilms. The results of this study are following research<sup>10,11</sup> which explains that EGCG can inhibit the activity of the glucosyltransferase enzyme and damage the cell wall protein which results in disturbed bacterial metabolism so that the process of attachment between bacteria and biofilm formation is inhibited.

Measurement with other methods to determine the shape, characteristics and attachment of *Streptococcus mutans* bacterial biofilms can be done with the confocal scanning laser microscopy method to determine the three-dimensional picture of the biofilm formed. The infrared spectroscopy method can also be performed to determine the amount of protein and polysaccharides that makeup biofilms and the scanning electron microscopy (SEM) method to find out the details of the attachment of bacteria on biofilms and the attachment of biofilms to the surface of the substrate<sup>11</sup>.

## CONCLUSION:

From this study we can conclude that *Epigallocatechin gallate* (EGCG) with a concentration of 0.375 mg/ml is more effective as an antibiofilm against the *Streptococcus mutans* bacteria compared with *chlorhexidine* 0.1%.

## REFERENCES:

1. Lamont, RJ., Hajishengallis, GN., Jekinson, HF. Oral Microbiology and Immunology third Edition. ASM Press. Washington DC. 2019; 229-230. [internet] <http://asmscience.org>
2. Basic Health Research in the Ministry of Health (MoH). Jakarta: Agency for Health Research and Development MOH. 2018; 197-207.

3. Nakano, M. Role of *Streptococcus mutans* Surface Proteins for Biofilm Formation. Japanese Dental Science Review. 2018; 54(1): 22-29.
4. Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M. Nawaz, MA, Kamil, MA Bacterial Biofilms and Associated Infections. Journal of the Chinese Medical Association. 2018; 81(1): 7-11.
5. Singh, S., Singh, SK, Chowdhury, I., and Singh, R. Understanding the Mechanism of Bacterial Resistance to Antimicrobial Agents biofilms. The Open Microbiology Journal. 2017; 11(1): 53-62.
6. Wang, H., and Ren, D. Controlling *Streptococcus mutans* and *Staphylococcus aureus* biofilms with direct current and chlorhexidine. AMB Express. 2015; 7(1): 204.
7. World Health Organization. WHO Global Report on Traditional and Complementary Medicine. World Health Organization. 2019
8. Guo, YJ, Zhang, B., Feng, XS, Ren, HX, and Xu, JR Human cathelicidin LL-37 Enhance the antibiofilm effect of EGCG on *Streptococcus mutans*. BMC oral health. 2016; 16(1): 101.
9. Reygaert, WC The antimicrobial possibilities of green tea. Frontiers in Microbiology. 2018; 5(AUG): 1-8
10. Xu, X., Zhou, XD, and Wu, CD The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. Antimicrobial Agents and Chemotherapy. 2011; 55(3): 1229-1236.
11. Roy, R., Tiwari, M., Donelli, G., and Tiwari, V. Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. Virulence. 2018; 9(1): 522-554.
12. Vidigal, PG, Musken, M., Becker, KA, Haussler, S., Wengender, J., Steinmann, E., Steinmann, J. Effects of green tea compound epigallocatechin-3-gallate against *Stenotrophomonas maltophilia* infection and biofilm. PLoS ONE. 2014; 9(4): 1-8.
13. Eng, Q., Thanikachalam, P. and Ramamurthy, S. Molecular understanding of epigallocatechin gallate (EGCG) in cardiovascular and metabolic disease. Journal of Ethnopharmacology. 2018; 210: 296-310.
14. Sutton, S. Measurement of microbial cells by optical density. Validation Journal Techn. 2011; 17(1): 46-49.
15. Yadav, K., and Prakash, S. Dental caries: A Microbiological Approach. Journal of Clinical Infectious Diseases and Practice. 2017; 02(01): 1-15.