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Effect of Green Tea (Camellia Sinensis) Soaking on Reducing the Number of Streptoccocus Mutans on Heat Acrylic Resin Plates

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ABSTRACT:

Background: Plaque is formed by various bacterial microorganisms, one of them is *Streptococcus mutans* which is attached to both tooth surface and acrylic plate through polymers between the bacterial matrix and salivary components, which cancause inflammation of the mucosa, namely denture stomatitis, and angular cheilitiswhich has the potential to cause more serious problems. complex. Green tea(Camellia sinensis) is herbal plant that is reported to have anti-bacterial activity.

Objective : To determine the effect of immersion in green tea with a concentrationof 20%, green tea with a concentration of 50% and the difference in concentration between the two on reducing the number of *Streptococcus mutans* bacteria on heatcured acrylic resin denture plates.

Materials and Methods : This study is an experimental laboratory and uses the pre test and post test method in a control group. The samples were 27 acrylic specimens measuring 10x10x2mm3 which were divided into 3 working groups, namely distilled water, brewed green tea with20% content and brewed green tea with 50% content, each of which was 9specimens. Each sample will be soaked for 15 minutes, the number of colonies is counted and analyzed.

Results: The ANOVA statistical test showed a significant difference between all treatment groups resulting in a p-value smaller than 0.05.

Conclusion: Green tea infusion with a concentration of 50% had a significant effectcompared to a concentration of 20% on the number of Streptococcus mutans bacteria on a heat cured acrylic resin denture base.

KEYWORDS: Heat cured acrylic resin, Green tea, Antibacterial, Streptococcus mutans

INTRODUCTION

A denture plate is a part of a prosthesis that can replace part or all of the missing natural teeth and can also replace the surrounding tissue. Denture plates have components consisting of tooth and plate elements. The part of the denture that is in contact with the soft tissue of the oral mucosa is the plate which has the function of repairing the contours of the tissue so that the results obtained are almost the same as before. In addition, the plate becomes a place for denture elements and receives support from supporting teeth and or remaining alveolar bone tissue[1]. The material of the plate is made of metal or acrylic material. Acrylic resin is a plate material that is often used today[2] . The advantages of acrylic include; has a relatively cheap price, acrylic color can be adjusted to resemble the gingiva, easy manipulation and manufacturing method, does not dissolve easily in saliva, and can be repaired and changes in dimensions are very small[3]. Acrylic resin (heat cured) also has disadvantages, namely porosity, hydrophobicity, and surface roughness which have been reported to affect the attachment of microorganisms to denture plates[4]. Porous nature which is an ideal area for food waste that settles so that it becomes a shelter for the colonization of microorganisms[2].

Bacterial microorganisms attached to both tooth surfaces and acrylic plates form plaque through polymers between the bacterial matrix and salivary components[5]. Therefore, removing denture plaque is very important. There are two methods of cleaning dentures, namely by using a plaque cleaning fluid and cleaning using a brush[6]. Denture cleaning fluid using a lot of solutions containing disinfectants[7]. Recent research using natural ingredients[8]. One ingredient that is often consumed is green tea (Camellia sinensis)[9].

MATERIAL AND METHODS

The type of research used in this study was laboratory experimental and used pre-test and post-test methods in a control group. This research was conducted on May 23, 2022. Located at the Testing and Research Services Laboratory (Qlab) of the Faculty of Pharmacy, Pancasila University on Jalan Serengseng Sawah, Jagakarsa District, South Jakarta City DKI. The material prepared is

green tea (Camellia sinensis). The green tea used in this study is branded Sariwangi, a tea brand originating from Indonesia, owned by PT Unilever Indonesia and its production site is in Cikarang, West Java (figure 1).

Figure 1. Sariwangi green tea sachets

Another material is acrylic resin plate. The acrylic resin plate used as the research sample is 10x10x2 mm3 in size. All samples have the same size and shape after finishing and polishing. The acrylic resin plates were divided into 3 different groups, but were soaked for 15 minutes or 900 seconds, namely: Group I: experienced the immersion process in green tea which had a concentration of 20% (treatment 1). Group II: experienced the immersion process in steeping green tea which has a concentration of 50% (treatment 2). Group III: experiencing the immersion process in distilled water. (control). In this study there were 3 groups with each group consisting of 9 samples, so that the total sample to be used was 27 samples.

PROCEDURES

Preparation of culture medium: 1) Mix 3.9 gr of Sabouraud Dextrose Broth (SDB) and 100 ml of water, 2) Set the pH in the range of 5.6 to 0.2, 3) Heat in the microwave until it becomes a homogeneous solution, 4) Pour into 6 ml test tube, 5) Autoclave sterilization at 121°C for 15 minutes, 6) Tilt the test tube and put it in the refrigerator at 4^{o}C, 7) Put Streptococcus mutans bacteria into the test tube by scratching it so that it tilts 1 ose, 8) incubation 36 to 1^{o}C for 24 hours, 9) Put 2 ml of water into a test tube containing agar slant, 10) Put 1 ml of the solution into a solution containing 25 ml of Sabouraud Dextrose Broth (SDB), 11) Incubation 37^{o}C, 150 rpm, 18-24 hours, 12). Perform the calculation of the number of colonization.

Making infusion of green tea (Camellia sinensis): 1) Prepare green tea (Camellia sinensis) weighing 20 gr and 50 gr, 2) Prepare water after that, put it in a measuring cup containing 200 ml of water and boil it to a temperature of 100^{o}C, 4) Pour it into a container containing 20 gr and 50 gr of green tea, 100 ml of boiling water each, 5) Green tea containing 20 gr and brewed with 100 ml of water, then the result of steeping green tea is a solution with a concentration of as much as 20%, 6) Green tea which contains 50 gr and is brewed with 100 ml of water, then the result of steeping green tea is a solution with a concentration of 50%

There were 3 treatment groups of heat cured acrylic plate samples with a total of 9 samples in each group. Group 1 (treatment 1) was soaked in green tea with a concentration of 20% for 15 minutes. Group 2 (treatment 2) was soaked in green tea with a concentration of 50% for 15 minutes. The third group served as a control in an aquadest solution which was soaked for 15 minutes.

The first research data analysis was by testing normality using Shapiro Wilk because the sample used was ≤50, the test was to determine normal or not. The data analysis technique that will be used in this study is One Way Anova. One Way Anova is an analysis of one type of variable. Therefore this type of analysis is carried out for this type
of research by presenting data in the form of tables, charts, and continuum lines.

RESULTS AND DISCUSSION

Analysis was carried out by carrying out a comparative analysis of the results of measuring the number of Streptococcus mutans microbes on 27 acrylic specimens where 9 specimens were given control treatment, namely distilled water, then 9 specimens were soaked with 20% green tea steeping solution and 9 other specimens were soaked with 50% green tea steeping solution. . The research sample used was a heat cured acrylic resin plate measuring 10x10x2 mm3. All samples are of the same size and shape and are polished all over. Next, a test will be conducted to test the effectiveness of steeping green tea on reducing the number of Streptococcus mutans. After the test was carried out, the results were seen from observing the number of bacteria in each group of solutions.

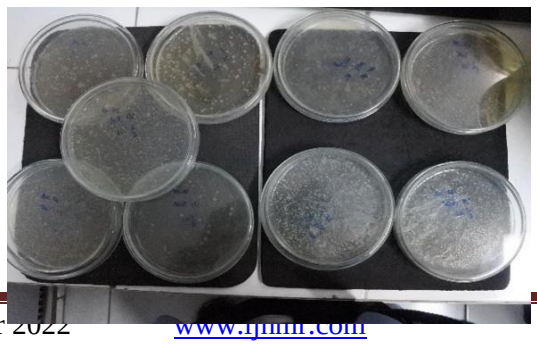

Figure 2. The results of observing the number of Streptococcus mutans bacteria in the control

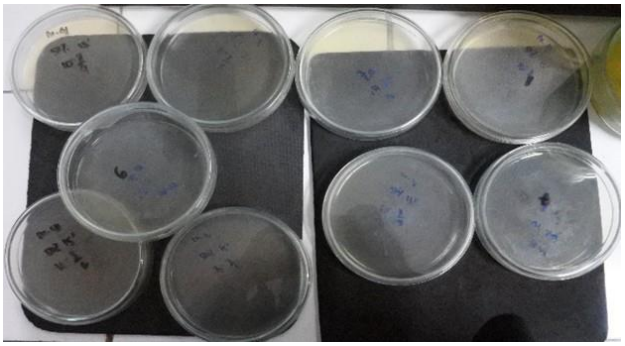

Figure 3. The results of observing the number of Streptococcus mutans bacteria in treatment 1

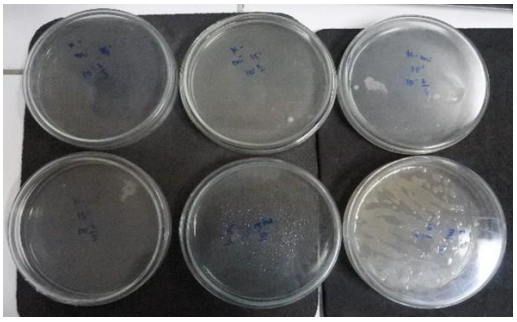

Figure 4. The results of observing the number of Streptococcus mutans bacteria in treatment 2

The first analysis to be carried out is to carry out descriptive statistical analysis to determine the characteristics of the data from the results of the research that has been carried out. The descriptive statistics that are calculated are the average and standard deviation. The results of the analysis can be seen in Table 1.

Table 1. Mean and Standard Deviation of Streptococcus Mutans Microbial Data Streptococcus Mutans (CFU/ml)

	Specimen	**Number Of Microbial**	
	Control	Treatment 1	Treatment 2
1	220000	110000	8000
2	220000	140000	11000
3	220000	110000	10000
4	200000	56000	18000
5	220000	96000	1000
6	200000	120000	8000
7	250000	110000	23000
8	260000	20000	17000
9	260000	140000	14000
Mean	227777,78	100222,22	12222,22
Standart Deviation	**228641**,98	99135,80	12691,36

The average number of Streptococcus Mutans microbes in the control (aquades) was 227,777.78; in treatment 1 was 100,222.22; then in treatment 2 it was 50% which was 12,222.22.

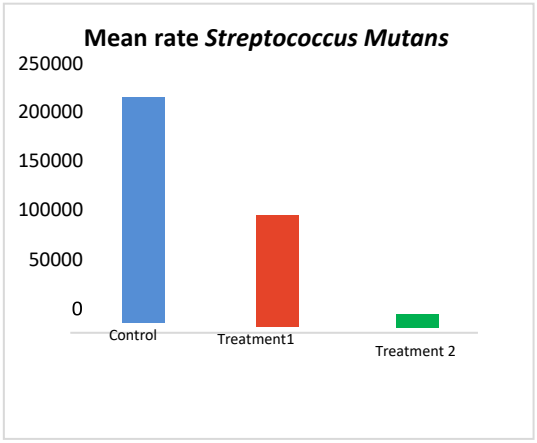

Figure 5. Streptococcus Mutans Microbial Data (CFU/ml) for Each Specimen in Each Treatment

Based on Figure 2, it can be seen that the highest number of Streptococcus Mutans microbes in acrylic specimens was found in specimens with control treatment. When soaked with 20% green tea steeping solution (treatment 1), the number of microbes decreased compared to the control treatment. Then when the specimens were soaked with 50% green tea steeping solution (treatment 2), it was seen that the number of microbes decreased more when compared to the control and treatment 1.

Before carrying out a comparative test on the data on the number of Streptococcus Mutans microbes, a normality test was previously carried out. The normality test was carried out using the Shapiro-Wilk normality test method because the sample in each group to be tested was less than 50 data. The normality test was carried out to find out which parametric statistical test method or non-parametric statistical test method is more suitable for analyzing research data. Decision making is seen based on the p-value or sig. value on the SPPS output, thus

• the p-value < error level (α) is 5%, then H0 is rejected
• p-value > error rate (α) which is 5% then H0 is accepted.

The results of the normality test on the data on the number of Streptococcus Mutans microbes in each group are shown in Table 2.

Table 2. Test for Normality of Data on the Number of Streptococcus Mutans Microbes

		Shapiro-Wilk		
	control	*Statistic*	*df*	*P-value*
	Treatment 1	0,847	9	0,068
	Treatment 2	0,860	9	0,095
		0,981	9	0,969

Based on Table 2, namely the results of the normality test on the data on the number of Streptococcus Mutans microbes in each treatment, it is known that all measurement data in each treatment group are normally distributed because all data groups produce a p-value greater than 0.05. So that the comparison test can be carried out with the parametric statistical method, namely the ANOVA test.

Based on the normality test, it is known that all data meet the normal distribution assumption, so that the comparison test is carried out using the parametric statistical method, namely the ANOVA test. This comparison test was carried out to find out whether there was a significant difference or whether there was a significant difference in the data on the number of Streptococcus Mutans microbes in each treatment group, namely control, treatment 1 and treatment 2. The results of the ANOVA test can be seen in Table 3.

Table 3. ANOVA Test on Streptococcus Mutans Microbial Amount Data

	Sum of Squares	*df*	*Mean Square*	*F*	*P-value*
Between Groups	211435851851,852	2	105717925925,926	149,683	0,000
Within Groups	16950666666,667	24	706277777,778		
Total	228386518518,519	26			

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It can be seen from Table 3 that the results of the ANOVA test on data on the number of Streptococcus Mutans microbes in acrylic specimens yield a p-value that is less than 0.05. So it can be concluded that the treatment gave a significant difference in the number of Streptococcus Mutans microbes. Because based on the ANOVA test it is known that there are significant differences in the number of Streptococcus Mutans microbes, so to find out more precisely which treatment has the difference, it is necessary to proceed with a post-hoc test using the LSD (Least Significant Difference) test. The results of the LSD test can be seen in Table 4.

Table 4. P-value of LSD Post Hoc Test

Treatment		*P-value*	Keterangan
Control	Treatment 1	0,000*	significantly different
	Treatment 2	0,000*	significantly different
Treatment 1	Control	0,000*	significantly different
	Treatment 2	0,000*	significantly different
Treatment 2	Control	0,000*	significantly different
	Treatment 1	0,000*	significantly different

The p-value followed by a sign (*) is a pair of groups that differ significantly from each other because the p-value is less than 0.05. It can be seen that based on the results of the LSD (Least Significant Difference) test, it is known that the average number of Streptococcus Mutans microbes in the control, treatment 1 and treatment 2 were all significantly different from each other. It can be seen previously in Table 1 and Figure 2 that there is a decrease in the number of microbes when soaked with 20% green tea steeping solution, based on the post hoc test results in Table 4 it is proven that this decrease is significant. Then, it can be seen that the number of microbes decreased even more when the specimen was immersed in 50% green tea steeping solution which based on the post hoc test this decrease also proved significant. Based on the results of this study, it can be concluded that the green tea steeping solution proved effective in killing Streptococcus Mutans bacteria both at concentrations of 20% and 50%.

This study was conducted to determine the effect of soaking green tea (Camellia sinensis) with a concentration of 20% and 50% on reducing the number of Streptococcus mutans on heat cured acrylic plates. This research was conducted on May 23 2022 at the Testing and Research Services Laboratory (Qlab), Faculty of Pharmacy, Pancasila University, Jagakarsa, South Jakarta. The researcher used a sample of 27 acrylic specimens measuring 10x10x2 mm3 which were divided into 3 working groups namely distilled water, green tea steeping with a content of 20% and green tea steeping with a content of 50% where each of the 9 specimens was then tested for the effectiveness of green tea steeping on a decrease in the amount Streptococcus mutans.

Streptococcus mutans bacteria was chosen as the research sample because it is a microorganism that exists in the mouth and can be found on the surface of teeth and dentures and will then colonize and proliferate into biofilms on the denture plate if not cleaned [10].

In this study, heat cured acrylic denture denture bases were soaked for 15 minutes in a green tea steeping solution with a concentration of 20% and 50% and in a control solution in the form of distilled water. The cup count method used is the pour plate method. This method is often used to quantify the number of microorganisms in mixed samples, which are added to a liquid agar medium prior to solidification. This process results in colonies that are evenly distributed throughout the solid medium when dilution of the sample is appropriate. This method aims to determine the number of living bacteria in a liquid only on the surface of the solid media. However, the calculation results do not always show the actual number of cells, because adjacent cells may form colonies. In order to avoid microbial density that can cause inaccuracy, dilution must be done first. Multilevel dilutions were carried out to produce suspension concentrations. The diluted sample is counted in a new cup and then poured into the medium (pouring method). Then after incubation at 32.5oC for 24 hours, observe the growth of the colonies [11,12].

The results showed that soaking heat cured acrylic resin plates with steeping green tea significantly reduced the number of Streptococcus mutans colonies growing on the plates. This can happen because according to Denny et al (2018) steeping green tea contains many compounds such as antimicrobial activity such as polyphenolic compounds which work by damaging bacterial cell membranes, inhibiting fatty acid synthesis and enzyme activity so that bacterial growth and development can be inhibited. In addition, the content of green tea contains many catechin compounds which play a role in causing damage to bacterial cell membranes, inhibiting fatty acid synthesis, and inhibiting enzyme activity[13].

A decrease in the number of Streptococcus mutans colonies can be seen when the specimens are soaked with 50% green tea steeping solution, the number of microbes has decreased more when compared to the control and 20% green tea steeping solution. [Figure 2] This is supported by research conducted by Wijaya et al (2022) which used green tea leaf extract at concentrations of 3.125%, 6.25%, 12.5%, 25% and 50%. This is in line with Rilaksana et al (2016) which stated that infusion of green tea has antibacterial activity at a concentration of 25% and 50% where at a concentration of 50% it has a better antibacterial effect. The

greater the concentration of green tea extract, the larger the diameter of the inhibition zone formed. There is a tendency that the higher the concentration of green tea steeping, the lower the growth of Streptococcus mutans on the acrylic resin plate after soaking [14].

Green tea leaf steeping solution contains polyphenols, amino acids, caffeine, organic acids. In addition, the components of green tea also have anti-bacterial activity, namely catechins, tannins, saponins that can inhibit bacteria, one of which is Streptococcus mutans. One cup of Japanese green tea contains 37-56% polyphenols, 30-42% catechins and 10-13% epigallocatechin gallate or 67.5 mg of catechins in 100 ml. 36 Catechin compounds in green tea can also work to inhibit the activity of glycotransferase enzymes from bacteria so that The attachment of bacteria to the pellicle is inhibited and the process of plaque formation is also inhibited. Epigallocatechin-3-gallate (EGCG) is a catechin derivative. It is proven that the most powerful bioactive ingredient in green tea is EGCG, referred to as the main polyphenol of green tea. A mixture of green tea catechins, especially EGCG, was observed to reduce Streptococcus mutans on the plate surface[15]. It has been observed that the higher the EGCG content, the better the antibacterial effect on the oral microflora and showed significant antibacterial activity against Streptococcus mutans and human dental bacterial samples[16].

This study used steeping green tea but did not rule out the possibility that brewing green tea could very well be used as an antibacterial solution. This is supported by the research of Anita et al. 2018 states that green tea leaf extract can inhibit the growth of Streptococcus mutans[17]. This statement is in line with the research by Armidin et al. who stated that gargling with green tea solution was more effective in reducing total Streptococcus mutans compared to black tea solution[18].

Based on the results of research and statistical testing, namely the effect of steeping green tea with a concentration of 20% and 50% on the number of Streptococcus mutans bacteria on acrylic plate resin, it can be said that the hypothesis in this study can be accepted. Utilization of the results of this study in the field of dentistry, namely that it can be used as an alternative for people who use acrylic plaque resin to reduce the number of Streptococcus mutans bacteria in acrylic plaque resin herbally if chemical use is inconvenient to use. In addition, based on several references, soaking acrylic resin plates in green tea has the advantage of reducing Streptococcus mutans bacteria, the price is cheaper, the materials are easy to find, easy to practice and can prevent denture stomatitis.

CONCLUSION

There is an effect of the content of green tea brewing on reducing the number of Streptococcus mutans bacteria on heat cured acrylic resin denture plates. Green tea steeping solution with a concentration of 50% was more effective in reducing the number of Streptococcus mutans bacteria in heat cured acrylic resin denture plate immersion compared to green tea steeping solution with a concentration of 20%. This is because brewing green tea contains a lot of antimicrobial compounds, one of which is polyphenolic compounds which work by damaging bacterial cell membranes, inhibiting fatty acid synthesis and enzyme activity so that the growth and development of Streptococcus mutans bacteria can be inhibited.

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