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Antibacterial Effects of Steeped White Tea, Black Tea, and Green Tea against *Streptococcus mutans* and Plaque Accumulation

Damhuji Damhuji[1]*, Budi Suryana[1], M. Ibraar Ayatullah[2]

[1]Department of Dental Health, Poltekkes Kemenkes Pontianak, Pontianak, West Kalimantan, Indonesia; [2]Department of Dental Health, Poltekkes Kemenkes Kupang, Kupang, East Nusa Tenggara, Indonesia

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***Correspondence:** Damhuji Damhuji, Department of Dental Health, Poltekkes Kemenkes Pontianak, Pontianak, West Kalimantan, Indonesia.
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Abstract

BACKGROUND: Tea is the most consumed beverage in the world after water. Tea (*Camellia sinensis*) is native to Southeast Asia and is now available in more than 30 countries. Tea has various health properties. Tea has an active component, namely catechins, which can inhibit the growth of *Streptococcus mutans* as a component of dental plaque formation.

AIM: This study aims to determine the antibacterial effect of steeped white tea, black tea, and green tea on *S. mutans* and plaque accumulation.

METHODS: The research used a quasi-experimental design with pre-test and post-test group design. The population was students of the Department of Dental Nursing. Purposive sampling technique is used with a sample size of 48 students who were divided into three parts of the sample group for the 2.5% concentration of tea mouthwash treatment. The analysis used paired *t*-test to see the antibacterial effect and differences in three-variable test followed by Kruskal–Wallis test.

RESULTS: The results showed that the mouth rinses of steeped white tea, black tea, and green tea were effective in reducing *S. mutans* and plaque accumulation on teeth. There is a significant difference in the inhibition zone of *S. mutans* against the three types of tea, and white has the largest inhibition zone. As for plaque accumulation, there is no difference between the three types of tea.

CONCLUSIONS: It can be concluded that mouth rinsing with steeping white tea, black tea, and green tea with a concentration of 2.5% has antibacterial properties that can inhibit *S. mutans* and plaque accumulation.

Introduction

Dental plaque is a major cause of caries and gum disease. Dental plaque is a soft and thin deposit that adheres tightly to the tooth surface and the gingival margin. Dental plaque is referred to as a very special variable structure formed by colonization of a series of microorganisms on the tooth surface that have the same color as the teeth [1]. The natural physiological forces that clean the oral cavity are unable to remove dental plaque, so controlling plaque is a way to remove plaque and prevent its accumulation. Mechanical plaque control procedures only focus on teeth, while gingivitis and periodontitis can develop from plaque microbes that accumulate in the oral tissue [2]. According to Newman, 2011, 1 g of plaque (wet weight) consists of approximately 10^{11} bacteria [3]. The number of bacteria plaque supragingival on one tooth surface can exceed 10^{9} bacteria. More than 500 microbial species are found in dental plaque.

The composition of dental plaque varies on different surfaces as a result of biological and physical adhesions, which, if balanced by the dominant bacterial population, will develop disease. *Streptococcus mutans* is an important agent causing dental caries. *S. mutans* is a normal oral microflora which has a strong virulence power. These microorganisms are found in 33%–75% in children aged 4 years, 80%–90% in adolescents, and almost all adults. *S. mutans* has various cell surface polymers as antigen materials so that it is able to adhere to and colonize oral tissues. These bacteria are found in dental plaque and saliva with the highest percentage found in plaque [4].

Preventing and controlling the growth of dental plaque can be done by regulating dietary patterns, chemical action against bacteria and extracellular polysaccharides, and mechanical action in the form of cleaning the oral cavity and teeth from all food debris, bacteria, and their metabolic products. However, the minimum level of public knowledge in proper and correct brushing has made mechanical cleaning not optimal, so it is also necessary to clean in a chemical way. It is better if plaque cleaning in the oral cavity is done with a combination of brushing and rinsing. rinsing. Mouthwash is a solution or liquid used to clean the oral cavity for a number of purposes, including ridding off of destructive bacteria, working as a remedy, ridding off of bad breath, having a therapeutic effect, and eliminating infection or preventing dental caries [5].

Mouthwash is generally antibacterial in which it contains active ingredients [6]. Tea contains a rich source of polyphenols (catechins) which are part of flavonoids [7]. The four main catechins are epigallocatechin-3-gallate (EGCG) which is approximately 59% of the total catechins, epigallocatechin (EGC) 19%, epicatechin (EC)-3-gallate 13.6%, EC, and 6, 4% caffeine [8], so that researchers want to use traditional medicine as a medicine to prevent oral disease, namely tea leaves (*Camellia sinensis*).

The animals and humans who were given tea compounds in drinking water showed less dental caries and less plaque formation than those who drank plain water [9]. Types of tea can be divided into tea without fermentation, namely green tea and white tea; semi-fermented tea, namely oolong tea; and fermented tea, namely black tea, although they are produced from the same tea raw material or *C. sinensis*. This study aims to determine the antibacterial effect of steeped white tea, black tea, and green tea on *S. mutans* and plaque accumulation.

Methods

Study design

This research method is a quasi-experimental study with a pre-test and post-test group design, looking at the differences of a particular treatment of the symptoms of the three experimental groups. This study aimed at assessing the antibacterial effect and plaque accumulation by treatment after gargling brewing white tea, black tea, and green tea with a concentration of 2.5%. A concentration of 2.5% indicates the growth inhibition of *Streptococcus mutans*. Tea leaf extract mouthwash is done by the infusion method by means of a 2.5% green tea solution, by brewing 3 g. Green tea with 120-ml distilled water at 70°C–80°C for 3 minutes, which is used as a mouthwash, given twice for 4 days. Rinse gargle for 15 s with a dose of 15 ml. The study was conducted in the Dental Nursing Department of the Polytechnic of the Ministry of Health Pontianak. Laboratory tests were carried out using the Pontianak Provincial Health Office laboratory with the implementation time from June to August 2019. The sampling of germs using a sterile swab and disclosing a solution to see the presence of plaque on the teeth was carried out. Stages of measuring the plaque index, namely:

1. Teeth are divided into 4 parts, namely: mesial, distal, buccal, and lingual/palatal
2. All missing teeth are marked with an "x," and the remaining teeth are noted. For purposes of plaque control, all pontics or bridges should be given the same score as the natural tooth
3. Instruct the patient to rinse his mouth first, its function is to remove food residue or debris that is still attached on the teeth
4. All tooth surfaces are smeared with disclosing solution
5. Patients are instructed to rinse their mouth with water, to checking for plaque at the dentogingival junction can using the tip of the probe. If plaque is found in the dentogingival junction, then the card is colored black or white red.

The plaque index value can be calculated by adding up the total surface of the teeth that are scored and then adding up and dividing by the number of surfaces in the oral cavity patient and multiplying by one hundred.

Study population

The population in this research is the fourth-semester Department of Dental Health students, amounting to 48 students. The sample size was divided into three parts, 16 students were treated with white tea brew mouth rinses, 16 other students with black tea mouth rinses, and 16 students with mouth rinse with green tea brewing. The sampling procedure used is purposive sampling technique, where only respondents who have certain criteria are selected as samples.

Inclusion criteria

a. Respondents are in good health
b. Willing to be a respondent
c. Respondents are not currently undergoing dental care and are not currently undergoing taking antibiotics
d. Respondents do not smoke.

Exclusion criteria

a. Respondents are taking antibiotics
b. Respondents do not have index teeth
c. Respondents are unable or unwilling to be respondents.

Ethical approval

This research was authorized by the Health Research Ethics Committee at Health Polytechnic of Pontianak, and it complied with the guidelines associated with the "Research Ethics on Living Organisms"' issued with no. 376/KEPK-PK.PKP/VII/2019.

Analytic approach

Data analysis was performed by SPSS v20 software with paired t-test to see the difference between *S. mutans* and plaque scores before and after mouth rinse with steeped white tea, black tea,

and green tea. Meanwhile, to see the differences of the three treatment variables, we are using the analysis of variance test. If it does not meet the normality and homogeneity requirements of the data, then we use the Kruskal–Wallis test.

Results

A comparison of the number of *S. mutans* bacteria and dental plaque on the index tooth surface before and after using mouthwash using steeped white tea, black tea, and green tea was carried out on 48 respondents, with each treatment being 16 respondents.

Table 1: Bacteria *Streptococcus mutans* before and after gargling of steeping white tea

Treatment	*S. mutans* minimum	*S. mutans* maximum	Mean ± SD	t	p
Before gargling steeped white tea	145	210	175.50 ± 21.18	17.09	0.000
After steeped white tea	50	80	66.88 ± 8.80		

S. mutans: Streptococcus mutans, SD: Standard deviation.

Descriptive analysis showed the number of *S. mutans bacteria* before gargling with white tea, the lowest number of bacteria was 145 and the highest was 210, with an average of 175.50 *S. mutans* (Table 1). Meanwhile, after gargling, the lowest bacterial count was 50 and the highest was 80, with an average of 66.88. The mean difference was 108.62, namely the difference between the average before and after gargling with white tea. It can be seen that the t-count is 17.09 with a probability value of 0.000, therefore, the probability of 0.000 < 0.05 then H0 is rejected, which means that the number of S. mutans before gargling white tea and after gargling white tea is not the same or significantly different. Hence, it is found that white tea gargle is effective in reducing *S. mutans* on teeth.

Table 2: Plaque index before and after rinse mouthwash white tea

Treatment	Plaque score minimum	Plaque score maximum	Mean ± SD	t	p
Before mouthwash white tea steeped	0.8	1.6	1.31 ± 0.28	15.23	0.000
Then rinse steeped white tea	0	0.3	0.15 ± 0.12		

SD: Standard deviation.

The results showed plaque scores before gargling with steeped white tea, the lowest score was 0.8 and the highest was 1.6, with an average plaque score of 1.31 (Table 2). Meanwhile, after gargling with white tea, the lowest plaque was 0.0 and the highest was 0.3, with an average plaque score of 0.15. Gargle with steeped white tea showed a mean difference of 1.16, namely the average difference before and after gargling tea. White t-count shows 15.23 with a probability value of 0.000, therefore, the probability of 0.000 < 0.05 then H0 is rejected, which means that the plaque scores before rinsing and after gargling steeped white tea are not the same or significantly different. Hence, it is concluded that the gargle of steeped white tea is effective in reducing the plaque score.

Table 3: Bacteria *Streptococcus mutans* before and after gargling black tea steeped

Treatment	*S. mutans* minimal	*S. mutans* maximum	Mean ± SD	t	p
Before steeped black tea gargle	156	206	183.38 ± 14.59	24.89	0.000
After gargle steeped black tea	74	98	84.38 ± 8.22		

S. mutans: Streptococcus mutans, SD: Standard deviation.

Descriptive analysis showed the number of *S. mutans* bacteria before being carried out by gargling with steeped black tea, the lowest number of bacteria was 156 and the highest was 206, with an average of 183.38 bacteria (Table 3). Meanwhile, after gargling, the lowest bacterial count was 74 and the highest was 98, with an average of 84.38. The average difference was 99.00, namely the difference between the average before and after gargling steeped black tea. It can be seen that the t-count is 24.89 with a probability value of 0.000 , therefore, the probability of 0.000 < 0.05 then H0 is rejected, which means that the number of bacteria before gargling and after gargling steeped black tea is not the same or significantly different. Hence, it can be concluded that gargling with steeped black tea is effective in reducing the *S. mutans* bacteria on the teeth.

Table 4: Plaque index before and after rinse mouthwash black tea by steeping

Treatment	Plaque score minimum	Plaque score maximum	Mean ± SD	t	p
Before steeped black tea mouthwash	0.8	2.3	1.23 ± 0.44	15.48	0.000
After gargle steeped black tea	0	0.8	0.20 ± 0.26		

SD: Standard deviation.

The results of the treatment showed a plaque score before gargling black tea steeped, the lowest score was 0.8 and the highest was 2.3, with an average plaque score of 1.23 (Table 4). Meanwhile, after gargling the plaque, the lowest was 0.0 and the highest was 0.8, with an average plaque score of 0.20. Gargle with steeped black tea showed a mean difference of 1.03, namely the average difference before and after steeped tea black. It can be seen that the *t*-count is 15.48 with a probability value of 0.000, therefore, the probability of 0.000 < 0.05 is that H0 is rejected, which means that the plaque scores before and after gargling black tea are not the same or significantly different. Hence, it is concluded that gargling with steeped black tea is effective in reducing the plaque score.

Table 5: *Streptococcus mutans* bacteria before and after

Treatment	*S. mutans* minimum	*S. mutans* maximum	Mean ± SD	t	p
Before steeped gargling green tea	134	184	161.25 ± 14.17	26.71	0.000
After gargling with green tea	55	70	63.37 ± 5.31		

S. mutans: Streptococcus mutans, SD: Standard deviation.

Descriptive analysis showed the number of *S. mutans* bacteria before gargling with steeped green tea, the lowest number of bacteria was 134 and the highest was 184, with an average of 161.25 bacteria (Table 5). Meanwhile, after gargling, the lowest number of *S. mutans* was 50 and the highest was 70, with an

average of 63.37. The average difference was 57.87, namely the average difference before and after gargling steeped green tea. It can be seen that the t-count is 26.71 with a probability value of 0.000, therefore, the probability of 0.000 < 0.05 then H0 is rejected, which means that the number of bacteria before and after gargling steeped green tea is not the same or significantly different. Hence, it is concluded that gargling with steeped green tea is effective in reducing the *S. mutans* bacteria on the teeth.

Table 6: Plaque index before and after gargling with green tea

Treatment	Plaque minimum	Score plaque maximum score	Mean ± SD	t	p
Before gargling steeped green tea	0.8	1.8	1.23 ± 0.36	11.29	0.000
After gargling steeped green tea	0	1.0	0.23 ± 0.35		

SD: Standard deviation.

The results showed the plaque score before gargling green tea, the lowest plaque score was 0.8 and the highest was 1.8, with an average plaque score of 1.23 (Table 6). Meanwhile, after gargling, the lowest plaque score was 0.0 and the highest was 1.0, with an average plaque score of 0.23. Gargle with the steeped green tea showed a mean difference of 1.00, namely the average difference before and after steeped tea rinse green. It can be seen that the t-count is 11.29 with a probability value of 0.000, therefore, the probability of 0.000 < 0.05 then H0 is rejected, which means that the plaque scores before and after gargling green tea are not the same or significantly different. Hence, it is concluded that gargling with steeped green tea is effective in reducing the plaque score.

Table 7: Distribution of white, black, and green tea mouthwash frequency by plaque

Category	White		Tea black		Tea green tea	
	Before, n (%)	After, n (%)	Before, n (%)	After, n (%)	Before, n (%)	After, n (%)
Good very	-	4 (25)	-	6 (37.5)	-	8 (50)
Good	2 (12.5)	12 (75)	2 (12.5)	10 (62.5)	2 (12.5)	6 (37.5)
Medium	14 (87.5)	-	12 (75)	-	14 (87.5)	2 (12.5)
Bad	-	-	2 (12.5)	-	-	-
Total	16 (100)	16 (100)	16 (100)	16 (100)	16 (100)	16 (100)

Based on the plaque index after gargling, steeped white tea was very good for category 4 (25%) of respondents, followed by good category 12 (75%) respondents. Steep black tea for very good category 6 (37.5%) and good 10 (62.5) respondents. Meanwhile, green tea shows a very good category with 8 (50%) and the lowest is in the medium category with 2 (12.5) respondents (Table 7).

Table 8: Test results of *streptococcus mutans* inhibition zone differences with gargling treatment steeped white tea, black tea, and green tea

Category	Mean	χ^2	Significant
White tea inhibition	39.63	30.99	0.000
Zone black tea inhibition	12.75		
Zone green tea inhibition zone	21.13		

The significance probability value is 0.000, therefore, the significance probability value is 0.000 < 0.05, then the H0 hypothesis is rejected, which means that there is a significant difference in the inhibition zone of S. mutans which is done with the mouth rinses of steeped white tea, black tea, and green tea as an antibacterial effect of S. mutans on teeth (Table 8).

Table 9: Test results of differences in plaque accumulation with gargling treatment steeped white, black tea, and green tea

Category	Mean	χ^2	Significant
White	25.63	0.28	0.86
Tea black	24.75		
Tea green tea	23.13		

The obtained significance probability value of 0.86 > 0.05 (Table 9). The H0 hypothesis is accepted, which means that there is no significant difference in mouth rinse by steeped white tea, black tea, and green tea against the index plaque.

Discussion

The results showed the effective use of steeped white tea, black tea, and green tea as a mouthwash to reduce the number of *Streptococcus mutans* and dental plaque; this was because the three types of tea had an inhibitory effect on the growth of *S. mutans*. However, steeped white tea showed a greater inhibitory effect on *S. mutans* growth than green and black teas. This is due to the presence of a greater amount of phytochemicals (terpenoids, steroids, alkaloids, phenolics, flavonoids, and saponins) in white tea compared to black and green teas [10].

White tea is a type of tea that does not undergo a fermentation process at all, where the drying and evaporation processes are very short. The tea leaves that are picked are young leaves that have not yet bloomed; then, they are dried by the method of evaporation (steam dried) or allowed to dry by air (air-dried). White tea leaves are the least processed tea leaves, while other types of tea generally undergo four to five processing steps. With this shorter process, white tea has a high catechin content which serves to reduce the formation of dental plaque by killing the bacteria that cause so that it has higher properties than other types of tea [11].

Black tea is obtained through a fermentation process, which is carried out by the polyphenol oxidase enzyme found in the tea leaves itself. In this process, most of the catechins are oxidized to theaflavins and thearubigins. Black tea is the tea leaf that experiences the most fermentation, so it can be said that black tea processing is carried out with full fermentation [11]. In black tea, the sinensis leaves are first allowed to wither to remove moisture and then rolled and allowed to ferment. This process results in leaf oxidation by enzymes present in tea leaves and conversion of catechins to polyphenol compounds with higher molecular weight such as theaflavins and thearubigins that give black tea its distinctive color and taste. Non-catechin components, such as tannins and thearubigins, are the main components that support the antioxidant activity of black tea [12].

Green tea is obtained by drying the leaves. Sinensis to remove moisture followed by a steam

treatment to deactivate the enzymes in the tea leaves. Green tea is obtained without a fermentation process (enzymatic oxidation), which is made by activating the polyphenol oxidase enzyme in the shoots of fresh tea leaves, by heating so that oxidation of catechins (antioxidants) can be prevented [13]. Heating can be done in two ways, namely by dry air (roasting/ roasting) and wet heating with hot steam [11]. Green tea is characterized by the presence of non-oxidized phenolic compounds called catechins, which are mainly responsible for green tea's antioxidant capacity.

According to Subramaniam and Eswara, 2012; Moghbel, *et al*., 2013; and Ferrara, *et al*., 2001, black tea and green tea contain the same number of flavonoids, however, green tea contains more catechins (simple flavonoids), while most black teas contain polymerized catechins (theaflavins and thearubigins), so black tea showed less inhibition of *S. mutans* due to the fermentation process compared to processing for white and green teas [10], [14], [15]. This research is in line with previous research, where the methanol extract of white tea can inhibit bacterial growth [16]. Another study also mentions that the antibacterial effect of green tea is stronger than black tea; this is because the polyphenol content of green tea is higher than black tea. This study also strengthens the statement that fermented tea leaves will reduce antibacterial activity, proven to be antibacterial of white tea ethanol extract which is not fermented stronger than black tea [11], [17].

Polyphenols are an important part of the active secondary metabolites of plants which exhibit activity against several pathogens. This antibacterial capacity, against both Gram-positive and Gram-negative bacteria, can be explained by different mechanisms such as interactions with bacterial protein and cell wall structure, damage to the cytoplasmic membrane, reduction of membrane fluid, inhibition of nucleic acid synthesis, cell wall synthesis, or energy metabolism [12].

In the oral cavity, there are many microorganisms that can cause disease and one of the diseases that can arise is caries. Caries is an infectious disease that affects the hard tissues of the teeth and causes hard tissue damage. The main bacteria that causes dental caries and is dominant in the oral cavity is *S. mutans* which is abundant in dental plaque. *S. mutans* have a role in the occurrence of carbohydrate fermentation which produces acid and causes demineralization of tooth enamel. *S. mutans* is one of the most important cariogenic bacteria because it ferments sugars which support the creation of an acidic environment. Bacterial growth and metabolism promote changes in the oral environment, allowing other bacteria to take part in the formation of dental biofilms. Hence, it is important to prevent bacterial growth and colonization [12].

Plaque is a soft deposit that is grayish-white or yellow in color that forms a collection of bacteria and their products that stick closely to the surface of the teeth. This accumulation of bacteria cannot happen by chance but is formed through a series of stages. The process of plaque formation occurs in three stages, namely pellicle formation, bacterial colonization, and plaque maturation. Plaque is formed when the pellicle leftovers and bacteria combine. Many chemical anti-plaque agents in various formulations have been tried as adjuncts for mechanical action to improve oral health. Numerous reviews have supported the feasibility of a chemical approach in controlling plaque formation, thereby assisting individuals in achieving acceptable gingival status. This anti-plaque ingredient can be given in the form of mouthwash, toothpaste, gum, and gel. Mouthwash, a safe and effective delivery system for antimicrobials, can play an important role in plaque reduction [18].

Green tea extracts have been reported to show antibacterial effects against bacterial species from dental plaque [2]. Although there was no statistically significant difference between the three types of tea because of the similar anti-plaque effect. Steeped black tea is able to prevent the formation of dental plaque because of the high polyphenol content. Compounds from polyphenols play an active role in inhibiting the formation of dental plaque. In addition, polyphenols also function to kill bacteria that cause dental plaque are catechin compounds, especially EGC, and EGC gallate (EGCg) compounds. This compound is able to inhibit the activity of the glucosyltransferase (Gtf) enzyme produced by *S. mutans*. The enzyme glucosyltransferase plays a very important role in converting sucrose to glucose for the attachment of these bacteria, catechincompounds cause bacterial growth to be inhibited and due to a decrease in acid production by bacteria [19].

Tea has diverse pharmacological properties which include antibacterial, antiviral, anti-oxidative, anti-inflammatory, anticariogenic, and antiaging properties. Several animal studies, such as studies conducted by Shinta *et al.* [20], show that frequent consumption of green tea can significantly reduce caries formation, even in the presence of sugar in the diet. Thomas, *et al*., 2017, and Okamoto, *et al.*, 2003, suggested that green tea catechins may have the potential to reduce periodontal damage due to the strong protease activity of *Porphyromonas gingivalis* [21], [22]. For decades, the determination of bacterial count has been an accepted test by the scientific community to investigate the antibacterial effects of mouthwash [23].

Tea leaves contain three main components that act on human health: ordinary xanthic (theophylline), essential oils, and polyphenolic compounds. Tea has been considered a medicine and a healthy drink for headaches, body aches and pains, digestion, depression, detoxification, as an energizer. Herbal mouthwash has received special attention because it is non-chemical and non-synthetic, and has long been used in traditional medicine [23].

Conclusions

It can be concluded that mouth rinsing with steeping white tea, black tea, and green tea with a concentration of 2.5% has antibacterial properties that can inhibit *S. mutans* and plaque accumulation.

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