

Catechin Inhibition toward the S. mutans Growth

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ABSTRACT

Streptococcus mutans (*S. mutans*) is one of the components of microorganisms that cause dental plaque. Plaque was the main cause of oral disease. Tea was known has catechin which useful to prevent dental plaque's formation that was caused by *S. mutans*. Catechin is tea polyphenol which helps the formation of leukocyte. The function of leukocyte was to against infection and bacteria growth. The aim of this study was to compare the inhibition of catechin from two type of tea which are green tea and black tea toward *S. mutans* growth. The method of this study was bacteria test with Kirby Bauer's method, and is used culture media Mueller Hinton Blood (MHB). Each extract was diluted to 1%, 2.5% and 5%. The result of this study show that there was significant difference between green tea extract and black tea extract in inhibiting *S. mutans* growth ($\alpha < 0.05$). It can be concluded that resistivity of the green tea extract with a concentration 2.5% is more effective in inhibiting *S. mutans*'s growth than black tea.

Key Words: catechin, *Streptococcus mutans*, green tea, black tea

INTRODUCTION

Dental plaque is the main cause of oral disease. Plaque is a group of bacteria which tied in an organic matrix and stick on the tooth surface. One of the cariogenic-mikroorganisme component in dental plaque is *Streptococcus mutans* (*S. mutans*). *S. mutans* predominate in dental plaque formation.¹ *S. Mutans* is a gram positive bacteria, nonmotil, asidogenic and have an enzyme called glukosiltransferase that is able to synthesize glucose molecule become dextran- $\alpha(1 \rightarrow 6)$ and $\alpha(1 \rightarrow 3)$. Dextran- $\alpha(1 \rightarrow 3)$ is very sticky, insoluble in water and can support other bacteria stick in tooth enamel. The acidogenic character of plaque is able to produce acid to dissolve tooth enamel. That caused colonization of *S. mutans* and plaque formation on the tooth surface.²

Tea is made from young tea leaf that called *Camellia sinensis* L. Kuntze. Based on the way to processing, traditionally tea consist of 3 type, that is green tea, oolong tea, and black tea.³ Some literatures mention that green tea can reduce acid and dental plaque formation of *S. mutans*. Polifenol of green tea so-called katekin, can pursue growth all bacterial type include *S. mutans* standard strain, and has ability as antibacterial and antioxidant. Green tea also contain fluoride to inhibit growth of plaque bacteria.^{4,5} Chemical component in black tea so-called teaflavin, has ability as antibacterial that is preventing bacterial colonization, inhibit plaque formation and reduce acid production. Consuming black tea can assist to prevent dental plaque formation, dental caries and periodontal disease.^{6,7} The aim of this study was to compare resistivity of two type of tea which are green tea and black tea toward *S. mutans*'s growth.

DENTAL PLAQUE

Dental plaque is a semi-transparent coat, stick on tooth surface and consist of a large amount of mikroorganism. Combination of plaque, bacteria and pelicle known as oral-biofilm.^{8,9} According to Besford (1996), plaque consist of *S. mutans*, other type of bacteria and also protein-saliva which forming a microscopic picture at tooth surface. Dental plaque consist of three functional component : (a) cariogenic mikroorganism, especially *S. mutans*, *L. acidophilus*, and *A. viscous*; (b) Periodontopathy mikroorganism, especially *Bacteroides asaccharolyticus* (gingivalis), *Actinobacillus*; (c) adjuvant and supresive materials, the most potential is lipopolysaccharide, dextran, levan, and lipoteichoic acid.⁸ Dental plaque formation started after *S. mutans* c serotype forming extracellular dextran- $\alpha(1 \rightarrow 3)$ which water insoluble, by metabolism of extracellular sucrose, then attachment of bacteria at enamel surface followed by the colonization of bacteria.

Dextran- $\alpha(1\rightarrow3)$ which water insoluble is the most important in mechanism of plaque formation. Improvement of bacterial colonization through three base cell interaction, that is (a) germ attachment at tooth surface; (b) attachment of homotypic cell; (c) attachment of heterotypic cell. Glukosiltransferase (GTF) is a katalisator to forming dextran- $\alpha(1\rightarrow3)$ that acting as mediator among *S.mutans*, *S.sanguis* and *A.viscosus*, which represent expression of *S.mutans* essential virulensi.^{8,10}

STREPTOCOCCUS MUTANS

Streptococcus mutans found first time by JK Clark in 1924 after the bacteria is isolated from the wound. This bacteria found again when researcher start learn dental caries. *Streptococcus mutans* represent one of bacterial type in group of *Streptococcus* α -haemolyticus which consist of 7 subspecies that is serotype-a, until serotype-g. *S.mutans* serotype-c represent one of the most spread over the human population and around 80% plaque isolate contain serotype-c.^{1,11,12} *Streptococcus mutans* is an acidogenic bacterial (producing acid which can dissolve tooth enamel), aciduric (can maintain acid environment), and producing dextran to support other bacteria stick on tooth enamel so that increasing bacterial colonization on tooth surface.^{2,13} According to Keyes and Fitzgerald (1960), *S.mutans* is a predominant cause in dental plaque formation.

TEA

Tea generally grown in plantation both in height 200-2.300 m above sea level. There are two groups of famous tea varieties, that is *Camellia Sinensis* var. *assamica* coming from Assam and *Camellia sinensis* var. *sinensis* coming from Chinese. *Camellia sinensis* var. *assamica* have rather large leaves with a pointed tip, whereas *C. sinensis* var. *sinensis* leaves are smaller and rather blunt ends.¹⁴ According to its processing, traditionally tea consist of 3 type, that is green tea, oolong tea, and black tea. Syah (2006) state that manufacturing of the three type of tea is different each other. Green tea is tea that does not undergo the process of fermentation, oolong tea is experiencing semi-fermentation processes, and black tea is tea that undergo full-fermentation process.¹⁵ Recent years, tea gets a lot of attention related to the physiological potential as antimutagenic and antitumorigenik

1. CONTENT OF TEA

A cup of tea contains: (a) water (97%) that help move food elements into the process of digestion, absorption, and disposal; (b) 5-6% minerals, namely: Potassium to maintain the electrolyte balance of the body, Fluor plays an important role in maintaining and strengthening the teeth to avoid plaque and caries, Magnesium acts as a regulator of the body's electrolytes and Vitamin C, K, A, B1, B2; (c) Chlorophyll and Carotene support around 0.0019% of the dry weight of the leaves; (d) phenolics, namely polyphenols and flavanols; (e) caffeine (5-10%) that can stimulate the nerve center, improving blood circulation, aid kidney function, and improve concentration.^{5,16}

Tea polyphenols called catechins (25-30%) is unique because of the different substances with catechins found in other plants. Catechins that give a bitter taste to the tea, consisting of various kinds of epicatechin (EC), epicatechin Gallic (ECG), epigallocatechin (EGC), epigallocatechin Gallic (EGCG) and gallocatechin (GC). The most dominant bioactive component² and the most beneficial to the health is EGCG. Catechins have antimicrobial properties (bacteria and viruses), antioxidants, strengthens blood vessels, and launched secretion of urine. In black tea, catechins is converted into teaflavin and tearubigins due to the fermentation process by the enzyme polyphenol oxidase. Teaflavin have the ability to free-radical inhibitors smaller than the catechins. Tea flavonoids are antioxidants that have anticarcinogenic, hypocholesterolemic and cariostatic properties. Tea flavanols is a compound

that is very similar to the chemical composition of catechins. tea flavanols include quersetin, kaempferol, and mirisetin

2. GREEN TEA

Asian green tea is green tea that has the best quality, namely: Dragonwell Green Tea and Matcha Green Tea.⁵ The process of making green tea can not be done arbitrarily, because the objectives to be achieved in producing green tea is maintaining its health benefits, its purity, and the active compound of fresh tea leaves so that all of it can be felt when the tea is served. The composition of green tea compounds can be seen in table 1.¹⁷

Table 1. Composition of green tea compounds

component	% (w / w) dry weight
caffeine	7.43
total polyphenol	35.89
(-) Epicatechin	1.98
(-) Epicatechin gallic	5.20
(-) Epigallocatechin	8.42
(-) Epigallocatechin gallic	20.29
flavonol	2.23
theanine	4.70
glutamic acid	0.50
aspartic acid	0.74
arginine	0.50
Other amino acids	0.74
sugar	0.74
Materials that can precipitate	
alcohol	6.68
Potassium	3.96

Source (Tuminah, 2004)

The content of polyphenols in tea or often called catechins may help in increasing the number of white blood cells responsible for fighting infection and even helped reduce plaque formation by affecting employment in oral bacteria.⁵ The results showed that the catechins contained in green tea is beneficial for health and oral health. The result of Sakanaka, et al (1991) found that green tea containing catechins can inhibit the growth of *S. mutans* as the bacteria that cause dental plaque formation which will cause dental caries and prevent the inflammation of the gums.^{15,16} Prof. Dr. Masao Onishi from Tokyo University, Japan said that the fluoride content in tea helps maintain healthy tooth enamel and can inhibit bacteria that cause bad breath and dental plaque. Antibacterial content in tea is also active in preventing *S. mutans* strains that can lead to tooth loss. Research on mouthwash showed that gargling with green tea to inhibit the growth of dental plaque with a concentration of 0.2% was more effective than rinsing with Chlorhexidine with the same concentration.⁵

3. BLACK TEA

Black tea or red tea is referred to as the most powerful tea, in which contains tannins highest among other types of tea.¹⁷ Black tea has antioxidant and antimicrobial properties. The content of caffeine in black tea have a detrimental effect on fluid balance and status of iron in the body, according to some studies show that caffeine intake associated with dehydration (loss of body fluids) and iron deficiency anemia.

Table 2. Composition of black tea compounds

Component	% (w / w) dry weight
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caffeine	7.56
theobromine	0.69
theophylline	0.25
flavonol glycosides	slightly
Bisflavanol	slightly
Teaflavic acid	slightly
total polyphenol	33.52
Teaflavin	2.62
Tearubigin	35.90
gallic acid	1.15
chlorogenic acid	0.21
sugar	6.85
pectin	0.16
polysaccharides	4.17
Oxalic acid	1.50
malonic acid	0.02
succinic acid	0.09
malic acid	0.31
acid akonitat	0.01
citric acid	0.84
lipids	4.79
Potassium	4.83
other minerals	4.70
Peptide	5.99
tannins	3.57
Other amino acids	3.03
aroma	0.01

Source (Tuminah, 2004)

While several other large studies concluded that drinking **three cups of black tea a day** can nourish the **heart**, but to avoid potential dehydration and iron deficiency anemia, black tea should not be consumed more than eight cups per day.¹⁸ Black tea processing through stages: leaf harvested, cleaned, withered, cut and fermented, after the tea leaves are colored dark red or black, placed in a hot wok to stop the fermentation process and add flavo**s**.⁹ Chemical components in black tea called teaflavin have antimicrobial properties to inhibit **the growth of bacteria that cause** dental plaque **and** reduce **the** rate of acid production. This is supported by the research of Dr. Hittori and other research, shows that polyphenols (teaflavin) contained in black tea extract of *Camellia sinensis* group has antimicrobial properties against *S. mutans* activity.^{6,7}

MATERIAL AND METHODS

This study is divided into three stages, ie:

1. Phase I: Preparation of green tea extract and black tea **g** extracts respectively 1%, 2.5%, 5%. Procedures total length of maceration 6 days, so we get a **green tea extract** and **black tea extract**.
2. Phase II: Cultivation of *S. mutans* in media Mueller Hinton Blood. Make 5 pieces petri dish of Mueller Hinton Blood media and incubation for media sterility test, hereinafter *S. mutans* which has been cultured, planted in MHB, subsequent incubated at 37 degrees C for 18-24 hours.
3. Phase III : testing the effectiveness of green tea and black tea against streptococcus mutans using Kirby Bauer method or the disc diffusion method, that is germ sensitivity tests against a particular substance by means of a disc containing a standardized amount of material placed on plates that were **g** ultivated by the bacteria tested later the clear zone was measured. Absorbent paper soaked in **green tea extract and black tea with** each concentration of 1%, 2.5%, 5%, for 1 hour. After 1 hour, lifted absorbent paper, and stick it on Blood agar, which has been cultivated bacteria. Put back into the incubator with a temperature **1** of 37° C for 18-24 hours, will form a zone of inhibition. To determine the inhibitory effects **of green tea and black tea extract, the** inhibition zone was measured using calipers. The inhibition zone is characterized by the presence of a clear zone around the absorbent paper. How to calculate the inhibition zones, as follows :

1. Diameter of clear zone: $\geq 20\text{mm}$, means: very strong inhibition (bacteria susceptible/sensitive)
2. Diameter of clear zone: 10 - 20mm, means: strong inhibition (bacteria moderately susceptible)
3. Diameter of clear zone: 5 - 10mm, means: moderate inhibition (bacteria intermediate)
4. Diameter of clear zone: 2 - 5mm, means: weak inhibition (bacteria resistant)

Data were analyzed by one-way ANOVA using SPSS to determine the difference between green tea and black tea in inhibiting growth of *S. mutans*. Next step is LSD test, to determine the difference in each concentration of green tea and black tea.

RESULT

The results showed that green tea with a concentration of 2.5% has the most extensive clear zone compared with green tea and black tea in the other concentrations (Table 3). In the measurement of the concentration 2.5% green tea showed inhibition zone diameter 21mm-22mm, meaning that green tea extract concentration 2.5% has a very strong inhibition. Whereas for the concentration of green tea extract 1%, 5%, black tea extract 1%, 2.5%, 5% and 96% alcohol does not have a zone of inhibition. Means that the inhibition against bacteria does not occur at these concentrations.

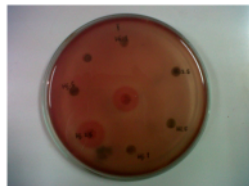


Figure 1. Kirby Bauer method



Figure 2. Measurement clear zone at the Kirby Bauer method

In this study, repetitions performed 7 times, which are presented in Table 3. Table 4 shows the results of the data analysis by one-way ANOVA.

Table 3. Diameter of clear zone

Repetition	Green tea			Black tea			(-)
	1%	2,5%	5%	1%	2,5%	5%	
I	0	22mm	0	0	0	0	0
II	0	21mm	0	0	0	0	0
III	0	21mm	0	0	0	0	0
IV	0	21mm	0	0	0	0	0
V	0	22mm	0	0	0	0	0
VI	0	21mm	0	0	0	0	0
VII	0	22mm	0	0	0	0	0

Table 4. One way Anova

	N	Mean	SD	df	F	Sig.
Black tea 1%	7	.00	.000	6	11250.000	.001
Black tea 2,5%	7	.00	.000			
Black tea 5%	7	.00	.000			
Green tea 1%	7	.00	.000			
Green tea 2,5%	7	21.43	.535			
Green tea 5%	7	.00	.000			
alcohol 96%-control (-)	7	.00	.000	49		
Total	49	3.06	7.579			

Based on one-way ANOVA, significance level was 0.001, less than 0.05, indicating that all groups had a mean value significantly different (Table 4). In Table 5 it can be seen that there are significant differences ($\alpha < 0.05$), between 2.5% green tea with 1% black tea, black tea 2.5%, 5% black tea, green tea 1%, 5% green tea, and alcohol 96%.

Table 5. LSD test

(I) extract	(J) extract	Mean Difference (I-J)	Sig.
4 Black tea 1%	Black tea 2,5%	.000	1.000
	Black tea 5%	.000	1.000
	Green tea 1%	.000	1.000
	Green tea 2,5%	-21.429*	.001
	Green tea 5%	.000	1.000
	Alcohol 96%-control (-)	.000	1.000
Black tea 2,5%	Black tea 1%	.000	1.000
	Black tea 5%	.000	1.000
	Green tea 1%	.000	1.000
	Green tea 2,5%	-21.429*	.001
	Green tea 5%	.000	1.000
	Alcohol 96%-control (-)	.000	1.000
Black tea 5%	Black tea 1%	.000	1.000
	Black tea 2,5%	.000	1.000
	Green tea 1%	.000	1.000
	Green tea 2,5%	-21.429*	.001
	Green tea 5%	.000	1.000
	Alcohol 96%-control (-)	.000	1.000
7 Green tea 1%	Black tea 1%	.000	1.000
	Black tea 2,5%	.000	1.000
	Black tea 5%	.000	1.000
	Green tea 2,5%	-21.429*	.001
	Green tea 5%	.000	1.000
	Alcohol 96%-control (-)	.000	1.000
7 Green tea 2,5%	Black tea 1%	21.429*	.001
	Black tea 2,5%	21.429*	.001
	Black tea 5%	21.429*	.001
	Green tea 1%	21.429*	.001
	Green tea 5%	21.429*	.001
	Alcohol 96%-control (-)	21.429*	.001
Green tea 5%	Black tea 1%	.000	1.000
	Black tea 2,5%	.000	1.000
	Black tea 5%	.000	1.000
	Green tea 1%	.000	1.000
	Green tea 2,5%	-21.429*	.001
	Alcohol 96%-control (-)	.000	1.000
	Black tea 1%	.000	1.000
	Black tea 2,5%	.000	1.000
	Black tea 5%	.000	1.000
	Green tea 1%	.000	1.000
	Green tea 2,5%	-21.429*	.001
	Green tea 5%	.000	1.000

*. The mean difference is significant at the 0.05 level.

DISCUSSION

S. mutans is a bacterium that has an important role in the formation of dental plaque. Dental plaque formed after *S. mutans* serotype c forming extracellular dextran, then going on adhesion and aggregation bacteria on the enamel surface followed by an increase in colonization. Presence of dextran receptors on the cell surface causes aggregation of bacteria, so there are interactions between cells during the formation of dental plaque.

This study showed that green tea extract concentration 2.5% has a wider inhibition zone compared with green tea and black tea with other concentration. This is probably because green tea contains catechins that can be inhibit the growth of *S. mutans*. Mechanism of action of catechins in inhibiting the growth of bacteria, as follows: initially catechins inhibit bacterial cell wall synthesis,

hereinafter undermines the integrity of the bacterial cell wall, by way interfere the function of the cytoplasmic membrane, which functions as the controller so that the permeability of the cell structure and cell wall changed or even be damaged , then the critical components such as proteins, nucleic acids, nucleotides, and others out of the cell, and then the cell gradually die, so that bacterial growth be inhibited. This is supported by Dyayadi, which states that the catechins in green tea inhibits the enzyme amylase in the fermentation of sucrose by *S. mutans*, so that acid formation is inhibited, and the growth of bacteria becomes disturbed. Syah said that the catechins in green tea can inhibit the formation of glucans by glucosyltransferase enzymes which can reduce bacterial colonization. According Setiawati and Nasikun, the amount of catechins in green tea are 15.65%, so the green tea extract with a concentration 2.5%, has a 0.37% catechins. Research conducted by Karlina et al. revealed minimal concentration of catechins that can be inhibit the growth of *S. mutans* was at 0.05%. This study is consistent with research by Cau jin who proves that tea concentration above 2.5% causes catechins in tea lost its ability to inhibit the formation of glucan extracellular, ability to inhibit plaque formation and bacterial growth. This means that the function of caries prevention by catechin limited to a certain concentration, and this mechanism needs furthermore research.

In this research, the inhibition zones on black tea extract did not appear. All three concentration black tea : 1%, 2.5%, and 5%, not able to inhibit the growth of *S. mutans*. This is not in accordance with the theory of dr. Wu, who claimed that the consumption of black tea can reduce the formation of dental plaque and prevent periodontal disease.⁶ Black tea in the manufacturing process is different from green tea. In the manufacture of green tea is not fermented, whereas in the manufacture of black tea, black tea is fully fermented. It is likely that the cause of the amount catechins in black tea are smaller than green tea, because the fermentation process of black tea can lower the natural content of tea, and one of them is polyphenols, or catechins. So black tea is less effective in inhibiting the growth of *S. mutans*. According to Soraya, black tea is made through a fermentation process by the enzyme polyphenol oxidase, can oxidize catechins in the fresh leaves into theaflavins and thearubigin. Theaflavins have many hydroxyl groups (OH) that functions as an antioxidant. The ability of theaflavins in inhibiting free radical is smaller than the catechins. No inhibition zone formation in black tea, presumably because black tea used in this study is a packaging tea, in which the manufacturing process can not be controlled by the researcher. Maybe in the process of drying or fermentation, causing a natural ingredient in tea is not maximal, so do not fit with the existing theories. The results of this study reinforced by research conducted by Wijaya et al. , which examines three types of tea ie green tea, black tea, and oolong tea against *S. mutans*. The study showed that green tea is more effective in inhibiting the growth of *S. mutans* among the three types of this tea.²⁰

CONCLUSION

Based on the research that has been done, it can be concluded that it significantly ($\alpha < 0.05$), green tea extract with a concentration 2.5% was more effective in inhibiting the growth of *S. mutans* than black tea extracts.

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