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THE EFFECT OF SEVERAL TOOTHPAST ON THE GROWTH OF *Streptococcus mutans*

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ABSTRACT

This study aims to determine the effect of several toothpastes on the growth of Streptococcus mutans which is the main cause of tooth decay. The study used 5 types of toothpaste, namely toothpaste A, toothpaste B, toothpaste C, toothpaste D, and toothpaste E. The antibacterial testing method used the diffusion method with the cup-plate technique. The significance level of each type of toothpaste was measured using a completely randomized design followed by a Tukey test to see differences in toothpaste treatment. The results showed that toothpaste A with active ingredients Monofluorophosphate Sodium 1.12%, Triclosan 0.3%, Calcium Glycerophosphate 0.13% had the largest average resistance zone of 27.3 mm. Whereas Toothpaste E with the active ingredient 0.08% Sodium Monofluorophosphate, 0.01% Sodium Fluoride has the smallest average resistance zone, which is 14.25 mm. The diameter of the ANOVA test zone of several types of barrier toothpaste against Streptococcus mutans at 95% confidence intervals showed that there was a significant difference.

Keywords: *influence, toothpaste, streptococcus mutans*

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INTRODUCTION

The oral cavity is the gateway for various kinds of microorganisms to enter the body (Ferdinan, 2007). There are two factors that affect the number and types of bacteria in the oral cavity. The first is microorganisms from air, water, food, and from the environment. The second is the environmental variation caused by the different anatomy of the oral cavity. Climate related to temperature can also affect the number and types of bacteria in the oral cavity (Gerald, 1998). Normal flora in the oral cavity consists of Streptococcus mutans/Streptococcus viridans, Staphylococcus aureus, Lactobacillus sp and Pseudomonas aeroginosa. Even though they are normal flora, under certain conditions these bacteria can turn into pathogens due to predisposing factors, such as poor oral hygiene. Oral cavity bacteria can enter the bloodstream through cavities, dental caries and bleeding gums resulting in bacteremia (Jawetz, 2001). Among the bacteria in the oral cavity, the best known bacteria is Streptococcus mutans. Streptococcus mutans bacteria is one of the normal flora that lives in the oral cavity, but in excessive amounts it is the main causative agent of dental caries (Sharma, 2018).

These bacteria play an important role in the pathogenesis of caries and are capable of forming extracellular polysaccharides and plaque. Plaque is one of the causes of tooth decay. To prevent the formation of plaque, toothpaste is generally used (Anonim, 2009). According to (Nick, 2009), the composition of toothpaste is divided into 2, namely the composition of non-active ingredients and the composition of active ingredients. The non-active ingredients consist of sodium lauryl sulfate, alginate, sodium benzoate, water, sorbitol, glycerine, flavour, sodium saccharine, titanium dioxide and vitamins. While the composition of the active ingredients consists of fluoride, sodium fluoride, sodium monofluorophosphate, calcium phosphate, and potassium nitrate. Some research results show that the addition of certain ingredients to toothpaste can reduce the number of bacteria in the mouth. Ingredients that are generally added are triclosan, alkaloids, certain enzymes (lactoperoxidase, amiloglucoxidase, glucoxidase) which have antibacterial properties (Kidd, 1992). According to *Astuty, 2008) toothpaste does have a very broad market, because most people always brush their teeth using toothpaste. Because of its broad market, currently there are many brands of toothpaste circulating offering various advantages. With the emergence of various new products and improvements to old products, toothpaste manufacturers are increasingly motivated to create products that are able to compete and try to meet consumer desires and tastes. Based on the description above, the authors are interested in examining the effect of several toothpastes on the growth of bacteria *Streptococcus mutans*.

METHODS

The type of research used in this study was laboratory experimental, the research was carried out at the Microbiology Laboratory of FMIPA Unpatti Ambon.

Material

The tools used in this study were stationery, measuring cups, incubators, sterile pipettes, self-modified metal cylinders with a diameter of 6 mm, test tubes, spatulas, test tube racks, sterile petri dishes, autoclaves, ovens, calipers, lamps. bunsen, vortex, circular loop needle, laminar air flow, and water bath. The materials used in this study were 5 types of toothpaste, pure cultures of Streptococcus mutans, Nutrient Agar (NA), Nutrien Broth (NB), distilled water, 75% alcohol, aluminum foil, wrapping paper, label paper, detergent, and petri wrapping paper.

Procedures

a. Rejuvenation of Streptococcus mutans Isolate. Streptococcus mutans from the stock was taken with a sterile loop needle and then put into a test tube containing solid NA media and incubated at 37 °C for 24 hours [10].
b. Toothpaste Preparation

Table 1. Composition and Active Ingredients of Toothpaste Tested

Toothpaste	Composition as stated in Packaging	Active compound
Toothpaste A	Calcium Carbonate, Air, Sorbitol, Hidrated Silica, Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Flavour, Cellulose Gum, Potassium Citrate, Sodium Silicate, Triclosan, Sodium Saccharin, Calcium Glycerofosfat, DMDM Hydantoin Cl 73360, Cl 74260, Cl 77891	1,12% Sodium Monofluorophosphate, 0,3% Triclosan, 0,13% Calcium Glycerofosfat
Toothpaste B	Calcium Carbonate, Air, Sorbitol, Hidrated Silica, Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Flavour, Cellulose Gum, Potassium Citrate, Sodium Silicate, Sodium Saccharin, Calcium Glycerofosfat, DMDM Hydantoin, Fluoride , Cl 77891	1,12% Sodium Monofluorophosphate, 0,13% Calcium Glycerofosfat
Toothpaste C	Sorbitol, Air, Hydrated Silicone Dioxide Precipitaed, Abrasive Silica, Polyethylene Glycol, Sodium Lauryl Sulfate, Flavour, Sodium Carboxy Methyl Cellulosa, Sodium Monofluorophosphate, Saccharin, Trisodium Phosphate, Vitamin E Acetate, Milk Protein, Titanium Dioxide, Fluoride, Cl 74160	1,18% Sodium Monofluorophosphate

Toothpaste D	Precipitate Calcium Carbonate, Sorbitol, Peg 600, Hydrated, Silocone Dioxide Precepitated, Air, Sodium Lauryl Sulphate, Sodium Carboxy Methyl Cellulosa, Sodium Saccharin, CaGP, Triclosan, Formaldehyde, Flavor Sodium Monofluorophosphate, MonosodiumPhosphate,	0,1% Triclosan, 0,8% Sodium Monofluorophosphate, 0,13% Calcium Glycerophosphate
Toothpaste E	Silicon Dioxide, Sorbitol, Xanthan Gum, Sodium Lauryl Sulfate, Sacharine Natrium, Sodium Monofluorophosphate, Sodium Fluoride, Buthyl	0,08% Sodium Monofluorophosphate, 0,01% Sodium Fluoride

The samples taken for the manufacture of toothpaste preparations in this study had the criteria of containing detergent (Surfactant), phosphate, and being the type of toothpaste that is generally used by the public. The way to make toothpaste preparations according to (Pratiwi, 2005) is that each toothpaste is weighed 1 gram and then put in a test tube containing 1 mL of sterile distilled water and then diluted using a sterile spatula.

c. Preparation of Bacterial Suspension

The bacterial suspension was prepared for stock multiplication by inoculating 1 ose of pure culture into 5 mL of NB media, then incubating at 37°C for 24 hours in an incubator (Pratama, 2005).

d. Tested

Diffusion method using well technique, namely 15 mL of NA medium at 50 °C is poured into a petri dish aseptically in laminar air flow and allowed to solidify as a base layer. 2 mL of the bacterial suspension from NB media was put into 10 mL of NA media in a test tube then vortexed and poured over the NA media which had solidified earlier. The second layer, which had started to semi-solid, was placed in a metal cylinder with a diameter of 6 mm and a height of 10 mm. This treatment was repeated 3 times. The negative control was sterile distilled water and the positive control was Tetracycline with a concentration of 30 µL/g. After that, it was incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured for each tested toothpaste using calipers.

Experimental design

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

Y_{ij} = treatmen response

μ = Mean

τ_i = Effect of toothpaste treatment factor on – i

ε_{ij} = Error in experiment

i = 1, 2……., p (p = treatment)

j = 1, 2……., k (k = repeated)

If the ANOVA results show a significant effect, it will be continued with the Tukey test to find out the differences in each treatment using SPSS 17 software.

DISCUSSION RESULT

The results obtained from this study can be seen in Table 1.

Table 1. Diameter of Inhibition Zones of Several Types of Toothpaste Tested

Types of Toothpaste	Inhibition Diameter (mm)			mean (mm)	Barrier Response
	1	2	3		
Toothpaste A	27,5	28	26,5	27,3	Strong
Toothpaste B	22,5	16	12	16,83	Currently
Toothpaste C	19	15,25	16	16,75	Currently
Toothpaste D	16,5	19,25	26	20,58	Strong
Toothpaste E	15,5	13,25	14	14,25	Weak

Each toothpaste has an average diameter of the inhibition zone that varies against Streptococcus mutans (Figure 1 – Figure 5). The average diameters of the inhibition zones of toothpaste A, toothpaste B, toothpaste C, toothpaste D, and toothpaste E against Streptococcus mutans were 27.3 mm, 16.83 mm, 16.75 mm, respectively. 20.58mm, and 14.35 mm.

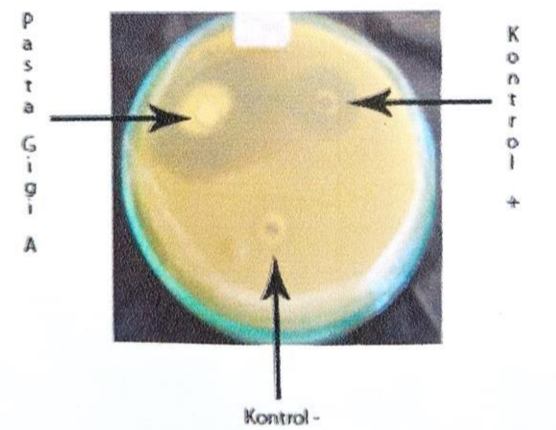

Figure 1 : Zone of Inhibition of Toothpaste A Against *Streptococcus mutans*

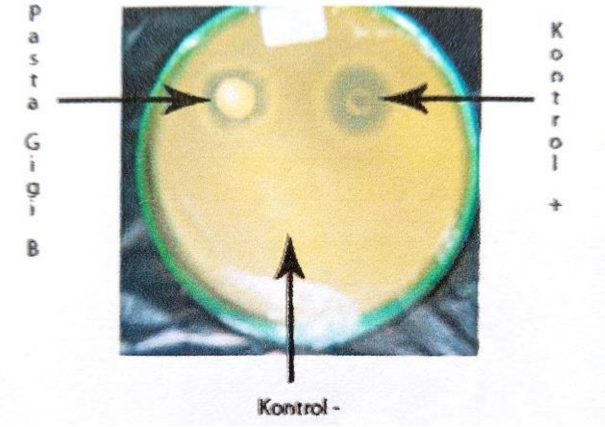

Figure 2 : Zone of Inhibition of Toothpaste B to *Streptococcus mutans*

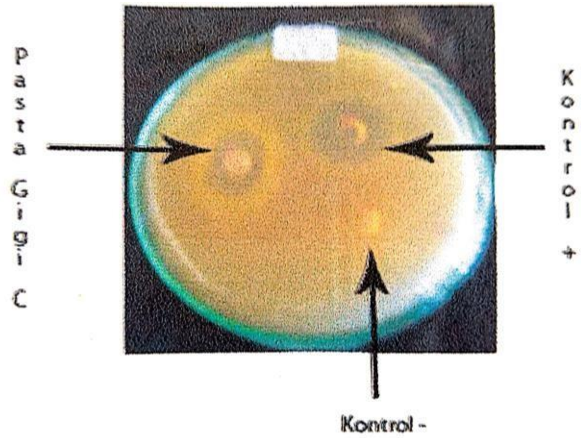

Figure 3 : Zone of Inhibition of Toothpaste C to *Streptococcus mutans*

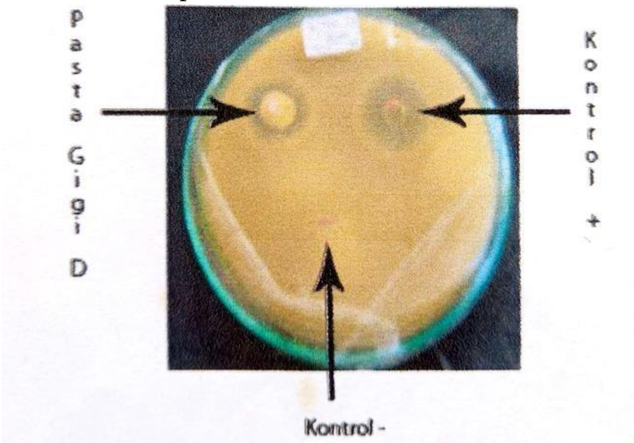

Figure 4 : Zone of Inhibition of Toothpaste D to *Streptococcus mutans*

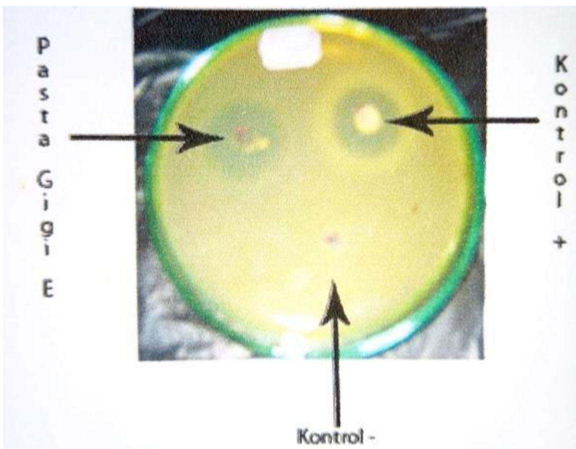

Figure 5 : Zone of Inhibition of Toothpaste

Analysis of variance shows that the calculated F value is 7.101. The degree of freedom factor (df between group) is 4 and the degree of freedom of error (df within group) is 10. Meanwhile, the resulting significant value is 0.006, meaning $p < 0.05$. So that it was stated that there was a significant difference, then a further test was carried out with the Tukey test.

Table 2. Tukey test results. Inhibition zone diameter
of several types of toothpaste tested against Streptococcus mutans

Types	**Toothpaste A**	**Toothpaste B**	**Toothpaste C**	**Toothpaste D**	**Toothpaste E**
A	-	0.021*	0.017*	0.173	0.005*
B	0.021*	-	1.000	0.656	0.872
C	0.017*	1.000	-	0.570	0.927
D	0.173	0.656	0.570	-	0.215
E	0.005*	0.872	0.927	0.215	-

*= significan

Regular maintenance of oral health through mechanical plaque control with a toothbrush using toothpaste will ensure dental and oral health (Pratiwi, 2005). According to (Aguilar et al, 2008) plaque is a collection of bacteria, especially Streptococcus sp., one way to kill or inhibit its growth is by adding certain chemicals in toothpaste. Toothpastes tested used materials containing Sodium Monofluorophosphate, although the concentrations were different for each type of toothpaste. Another active ingredient that is most widely used in toothpaste is fluoride which is effective in preventing caries. One source of fluoride in toothpaste is sodium monofluorophosphate. Sodium monofluorophosphate is an inorganic compound with the formula Na_2PO_3F. These compounds restore lost minerals to the teeth (remineralization) caused by acids formed by bacteria in dental plaque. Thus, caries can be prevented and produce good oral hygiene so as to prevent gingivitis. In Table 1, all tested toothpastes were shown to have inhibition against Streptococcus mutans (Aguilar et al, 2008; Ministry, 2009).

The results of research conducted by (Klimek, 1997) also proved that Sodium Monofluorophosphate will produce a degradation enzyme commonly known as Monofluorophosphatase (MFPase). MFPase has high activity when the amount of plaque is large and the presence of Streptococcus mutans and Lactobacillus sp is abundant in saliva. This is because the activity of Sodium Monofluorophosphate will consistently increase the amount of fluoride ions within a few minutes after Sodium Monofluorophosphate is in the mouth after brushing your teeth. With increasing fluoride ions, plaque can be hydrolyzed (Jackson, 1982). According to research conducted by (Restina, 2008) stated that toothpaste containing Sodium Monofluorophosphate and Xylitol can reduce the number of Streptococcus sp in plaque for the toothpaste group containing the least Sodium Monofluorophosphate compared to the toothpaste group containing Xylitol. Toothpaste A has the same amount and type of active ingredient as toothpaste D, but the concentration of the active ingredient triclosan in the two toothpastes is different. This is thought to cause the average diameter of the inhibition zone produced by Toothpaste A to be larger. Whereas in toothpaste B the absence of triclosan is thought to cause the average inhibition zone produced to decrease by almost half of the average diameter of the inhibition zone of toothpaste A. Because bacterial metabolism contained in dental plaque can also be inhibited by the presence of triclosan, this is stated by (Van et al, 2000) showed that toothpaste containing triclosan, calcium carbonate, silica and fluoride can inhibit Streptococcus mutans and Lactobacillus acidophilus bacteria.

The effect of triclosan was also investigated by (Jannesson et al, 2002) adding 10% xylitol to toothpaste containing triclosan could reduce the number of Streptococcus mutans in savila and dental plaque. In addition, the formation of dental plaque in vitro by Streptococcus mutans can also be inhibited even though the concentration of triclosan given is lower than the minimum concentration for bacteria. In addition, triclosan can also inhibit the growth and attachment of bacteria, this was stated by (Imasatto, 1995). Because triclosan is a multitarget inhibitor for Streptococcus mutans, especially in the process of glycolysis in dental plaque (Phan et al, 2006). Another function of triclosan stated by (Van, 2000) is to protect the tooth enamel surface from low acid. It can be seen in Table 1 that the average diameter of the inhibition zone of toothpaste B is not much different from the average diameter of the inhibition zone of toothpaste C, although the average diameter of the inhibition zone of toothpaste B is larger than that of toothpaste C. This is presumably because toothpaste C only has one active ingredient, namely Sodium Monofluorophosphate, while toothpaste B has two active ingredients, namely Sodium Monofluorophosphate and Calcium Glycerophosphate. Because according to (Lynch, 2004) that Calcium Glycerophosphate has an anticariatory ability and the effect will be seen if it is applied continuously and in high concentrations in vivo. The potency of calcium glycerophosphate in vivo will increase if it is used before the entry of caries-causing compounds to the teeth (Lynch et al, 2006). In addition, the presence of calcium glycerophosphate and fluoride in the toothpaste used can help tooth enamel survive the demineralization process. This was concluded by (Tenuta, 2006) after testing three types of toothpaste, namely (A) a paste containing fluoride and calcium glycerophosphate; (B) pastes containing fluoride; (C) paste that does not contain fluoride, against dental plaque derived from Streptococcus mutans. The results showed that Toothpaste A was very significant and efficient in increasing enamel resistance to the demineralization process when compared to Toothpaste C of all dental plaques tested.

Toothpaste E has the smallest average diameter of the inhibition zone, which is 14.35 mm. This is thought to be due to the very low concentration of the two active ingredients in E toothpaste. Although only Toothpaste E has the active ingredient sodium fluoride when compared to the other four toothpastes tested. Because according to research conducted by (Goncalves et al, 2006) a 0.05% sodium fluoride solution containing 2.5% or 1.25% xylitol can cause a significant reduction in the number of Streptococcus mutans. However (Caufild et al, 1982) found that sodium fluoride had a lower ability to fight Streptococcus mutans when compared to iodine. A study conducted by (Lobo et al, 2008) showed that sodium fluoride did not significantly reduce the amount of Streptococcus mutans in saliva when compared to chlorhexidine. A study conducted by (Sari, 2008) who tested the effect of toothpaste containing cetylpyridinium chloride and sodium fluoride on the growth of Streptococcus mutans in dental plaque concluded that cetylpyridinium chloride was more effective than sodium fluoride in suppressing and inhibiting the growth of Streptococcus mutans in dental plaque. Although it has a low ability to inhibit Streptococcus mutans, according to (Anonim, 2009) sodium fluoride is used to increase tooth strength by forming fluorapatite which is a natural component of tooth enamel formation. Apart from the active ingredients previously mentioned, there are also ingredients contained in toothpaste such as hydrated silica which is an abrasive material; surfactants are antibacterial and can destroy dental plaque; sodium bicarbonate (baking soda) can also reduce the number of bacteria that live in an acidic environment in the mouth (Anonim, 2009).

Five toothpastes tested had a significant effect on the growth of Streptococcus mutans. And the results of the Tukey test in Table 3B show that the treatment with toothpaste A had a significantly different effect from the treatment of toothpaste B, toothpaste C, and toothpaste E on the growth of Streptococcus mutans, while the comparison of the effects of the other treatments was not significantly different. This is presumably due to the presence of other active ingredients such as triclosan and calcium glycerophosphate contained in the tested toothpaste. According to (Van, 2000) triclosan added to toothpaste can increase the effect of inhibiting the metabolic results of bacteria found in dental plaque. Meanwhile, according to (Tenuta et al, 2006) calcium glycerophosphate added to toothpaste containing fluoride can increase the anticaries effect. And in Table 1 it can be seen that toothpaste containing the active ingredients fluoride, triclosan and calcium glycerophosphate has the largest diameter of the inhibition zone when compared to toothpaste containing only the active ingredient fluoride or a combination of active ingredients containing fluoride and calcium glycerophosphate.

CONCLUTION

Toothpaste A has a better effect than toothpaste B, toothpaste C, and toothpaste E in inhibiting the growth of Streptococcus mutans with an average effect value of 0.021, 0.017, and 0.005, respectively. Meanwhile, the average effect between other toothpastes was not significantly different.

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