Antibacterial Effectiveness of Cinnamon Peel Ethanol Extract Toothpaste (*Cinnamomum burmanni*) In Inhibiting the Growth of *Streptococcus* Bacteria

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Abstract

Streptococcus is one of the dominant bacteria in the oral cavity. This bacteria is the bacteria that causes dental caries. Toothpaste containing fluoride is important for preventing tooth decay. Using fluoride toothpaste creates a dilemma because it can cause side effects in the form of fluorosis if used in concentrations that are not recommended. However, there is an alternative way, namely by using toothpaste which contains essential oils and plant extracts. The type of research used is the experimental method. The research was conducted at the pharmaceutical laboratory of the Health Polytechnic of the Ministry of Health Medan and the Microbiology laboratory of the University of North Sumatra Hospital, which was carried out from May to July 2023. The samples in this study were dental plaque taken from 30 respondents pre and post intervention application of 12.5% cinnamon bark ethanol extract toothpaste. The data obtained will be analyzed statistically using the non-parametric Wilcoxon test and the parametric Pair T-Test. The results showed that toothpaste with cinnamon bark ethanol extract with a concentration of 12.5% had significant antibacterial activity in inhibiting the growth of Streptococcus bacteria with a p value <0.05. This toothpaste is able to significantly reduce the number of bacterial colonies found in dental plaque in all bacterial groups. It is hoped that these results will provide information to the public that the ethanol extract of cinnamon bark can be used as an active ingredient in toothpaste.

Keywords: Antibacterial, Cinnamon bark, Streptococcus, Toothpaste

INTRODUCTION

Dental and oral health has an important role for the health and well-being of the body in general. The prevalence of dental and oral diseases in Indonesia continues to increase, especially in children. Based on Basic Health Research data in 2013, dental and oral problems at the age of 5-9 years amounted to 28.9% and aged 10-14 years amounted to 25.2% (Kemenkes RI, 2013). This prevalence continues to increase based on Riskesdas 2018 data, aged 5-9 years by 54% and those who get dental counseling are only 8.3%, then at the age of 10-14 years also increases to 41% and those who get dental counseling are only 5.9% (Kemenkes RI, 2018). The caries rate for the North Sumatra region is 43.1% and people who get dental and oral hygiene care counseling are only 3.1%. When viewed in terms of behavior, the percentage of correct brushing behavior in Indonesian people is only 2.3% (Dinkes Sumut, 2020).

Various ways are done to prevent caries and reduce plaque accumulation in the oral cavity, namely brushing your teeth regularly, rinsing your mouth with antiseptic solutions, cleaning interdental with dental floss, cleaning the tongue, chewing gum, and avoiding sweet

foods (Ladytama et al., 2014). One way to control plaque is to use toothpaste that is effective against bacteria that cause dental caries. Toothpaste also serves as a medium for active substances to remove bacteria and plaque (antiplaque) to be applied to the surface of the teeth (Nurjannah et al., 2018).

Efforts to prevent tooth decay require an antiplaque substance in toothpaste which is currently closely related to fluoride content. Toothpaste with fluoride is not suitable for children under 4 years old. Because the use of toothpaste containing fluoride has certain side effects, it is necessary to find alternative toothpaste formulas from natural ingredients. The addition of herbs to toothpaste is expected to inhibit plaque growth and reduce side effects caused by the addition of fluoride active substances, it is related to the ability of several types of herbs that can inhibit microbial growth (Indrisari et al., 2015).

Research conducted by Ardiansah (2014) There is a significant difference between toothpaste that does not contain herbs and toothpaste with added herbs in reducing plaque accumulation. This shows that toothpaste with added herbs is more effective in reducing plaque accumulation. This suggests that some plants can act as active substances in reducing plaque accumulation on teeth.

One plant that is widely used as an herbal preparation is cinnamon (*Cinnamomum burmanni*). Cinnamon bark and leaves contain essential oils, saponins and flavonoids that have been widely used as herbal medicine (Tan et al., 2018). The largest content of cinnamon bark is essential oil which has the main content of cinamaldehid compounds (60.72%), eugenol (17.62%) and coumarin (13.39%). The content has antibacterial potential (Djarot et al., 2019).

Cinnamon bark extract affects the growth of *Streptococcus mutans* which is the main bacteria that causes dental caries (Puspita, 2014). Ekstrak kulit kayu manis dalam bentuk obat kumur juga dapat menghambat pertumbuhan *Streptococcus* di dalam mulut (Waty et al., 2018). Penelitian Waty (2022) also stated that toothpaste preparations of cinnamon bark extract (*Cinnamomum burmannii*) concentrations of 6.25% and 12.5% had antibacterial activity in inhibiting the growth of *Streptococcus mutans* (ATCC) bacteria with an average inhibitory zone diameter of 10.0 mm and 15.72 mm. This study aimed to see the effectiveness of antibacterial activity of cinnamon bark ethanol extract toothpaste with a concentration of 12.5% in inhibiting the growth of *Streptococcus bacteria*.

METHODS

This type of research is a pure experiment or true experiment, which is to make cinnamon bark ethanol extract and formulate it into toothpaste then test its antibacterial activity with a quantitative descriptive approach.

The research was conducted at the pharmaceutical laboratory of the Health Polytechnic of the Ministry of Health Medan and the Microbiology laboratory of the University of North Sumatra Hospital, which was carried out from May to July 2023.

The sampling technique in this study was purposive sampling. The sample in this study was 30 respondents dental plates.

Statistical testing on 30 samples using the *Saphiro Wilk normality test* with a confidence level of 95%, the Wilcoxon non-parametric *test and* the Pair T-Test *parametric test*. Based on the normality test, the data is not normally distributed, so it is continued with the Wilcoxon non-parametric test.

Making toothpaste from cinnamon bark ethanol extract with a concentration of 12.5% begins with the first stage, namely making mucilago by putting 2.75 grams into a beaker containing 500C aqueous and stirring until completely dispersed (mixture a). Put cinnamon bark ethanol extract mixture a, stirred until homogeneous (mixture b). Put 0.22 grams of menthol into the mortar then drip with 2 drops of ethanol and grind until smooth (mixture c). Dissolved sodium cyclamate 0.16 grams using a small amount of water (mixture d). Put the mixture c and d into the same mortar and grind until homogeneous. 8.25 ml glycerin is added to the mortar and crushed until homogeneous. Added 11 grams of CaCO3 to the mortar while continuing to grind. Mixture b is added to the mortar and crushed until homogeneous. Added 0.55 grams of Sodium Lauryl Sulfate and 0.16 grams of Methyl paraben to the mortar and then crushed to form toothpaste. Put toothpaste preparations in pots of ointment (Nurjannah et al., 2018).

Isolation of dental plaque from respondents who experienced dental caries was carried out on the buccal part of teeth 16, 26, 36 and 46 and labials of teeth 11 and 31. Application of toothpaste formula of 12.5% cinnamon bark ethanol extract was carried out on 30 respondents. Each respondent was given cinnamon bark extract toothpaste. Respondents' dental plaque before brushing was isolated and 30 minutes after brushing was re-isolated. Dental plaque samples are stored in a closed container and then taken to the laboratory for Streptococcus isolation on TYCSB media that has been made.

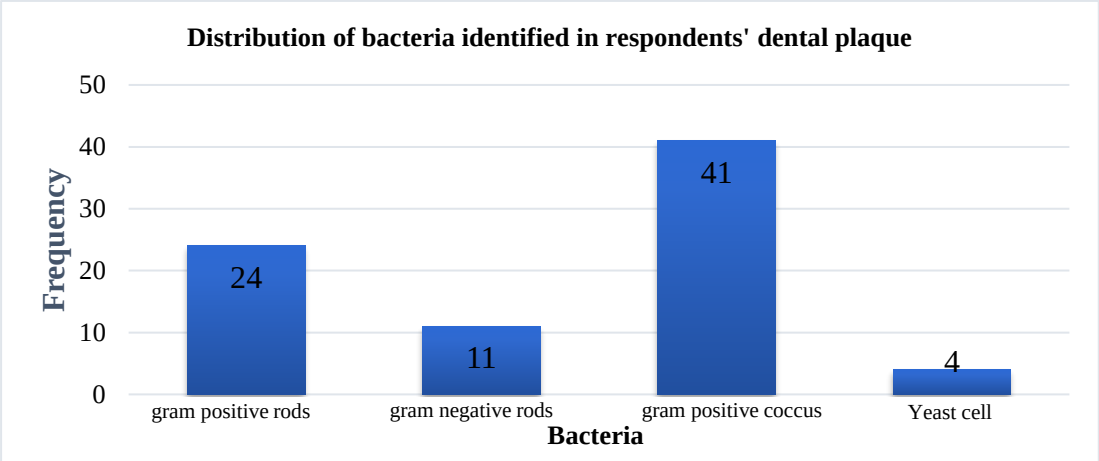
Dental plaque isolate was suspended with sterile aqueous as much as 1 ml then divortex then scratched on TYCSB media and incubated at 37°C for 24 hours. After that, it was

subcultured back to TYCSB media to compare the number of Streptococcus colonies contained in the media. Counting the number of bacterial colonies using Total Plate Count (TPC) (Nurjannah et al., 2018). Antibacterial activity of cinnamon bark ethanol extract seen from the calculation of Total Plate Count of bacterial colonies.

The gram staining procedure performed to determine the bacteria growing on TYCSB selective media is *Streptococcus* gram (+). Gram staining using gentien violet, lugol, acetone alcohol and water fuxin. The results of gram staining are viewed on a microscope with a magnification of 1000X ocular lens. Isolates that have been obtained are identified using a vitec device to determine the type of bacteria on the media.

RESULTS

This study was conducted on 30 respondents. Each respondent was taken pre-intervention dental plaque samples (before using toothpaste) and post-intervention (after using toothpaste). The results of this study are presented in the form of graphs, figures and tables below :



Graph 1. Distribution of bacteria identified in respondents dental plaque

Graph 1. The above shows the distribution of bacteria identified from respondents' dental plaque. Various bacteria found in dental plaque include gram-positive coccus bacteria most commonly found at 51.25%, gram-positive rod bacteria at 30% and gram-negative rod bacteria at 13.75%. In addition to bacteria, yeast (fungi) was also found by 5%.

Furthermore, the following figure below is a picture of dental plaque bacterial colonies on TYCSB *(Tryptone Yeast Cystein) Agar Base* pre and post intervention media.

B11 (Pre-interventions) A11 (Post interventions)

Figure 1. Test results of antibacterial activity of cinnamon bark ethanol extract toothpaste against bacteria in dental plaque before and after intervention

Figure 1. The above showed that the bacterial colony before the intervention (B11) reduced the number of bacterial colonies in the A11 sample after the use of cinnamon bark ethanol extract toothpaste. This illustrates the activity of cinnamon bark ethanol extract toothpaste in inhibiting bacterial growth in dental plaque. The bacteria found in dental plaque can be viewed microscopically as follows:

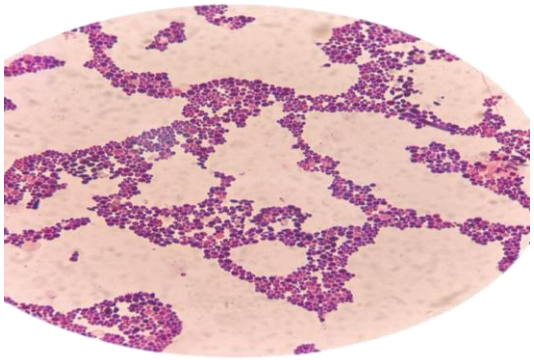


Figure 2. Microscopic description of *Streptococcus* batteries found in toothpaste samples

Figure 3. Cinnamon bark ethanol extract toothpaste 12.5%

From Figure 2. it can be seen that *Streptococcus* bacteria are *coccus* (round) like grapes and purple in color indicating as gram-positive bacteria, and Figure 3. shows a toothpaste used as antibacterial against dental plaque bacteria containing 12.5% cinnamon bark ethanol extract.

The following table below illustrates the antibacterial activity of cinnamon bark ethanol extract against bacteria in dental plaque.

Table 1. Results of Antibacterial Activity of Cinnamon Bark Ethanol Extract Toothpaste against Dental Plaque Bacteria

Types of Bacteria	Average Amount		Difference in Number of Colonies
	Pre-Intervention Colonies (CFU/ml)	Post-Intervention Colonies (CFU/ml)	Pra-Post
Gram Negative Rod Bacteria			
Aeromonas salmonicida	171,60	50,00	121,60
Oligella ureolytica	127,75	43,50	84,25
Spingomonas paucimobilis	216,50	128,50	88,00
Gram Positive Rod Bacteria			
Rothia dentocariosa	204,37	83,26	121,11
Kocuria rosea	213,00	122,20	90,80
Gram Positive Coccus			
Granulicatella elegans	217,75	96,50	121,25
Staphylococcus aureus	250,67	129,00	121,67
Staphylococcus lentus	185,33	55,00	130,33
Streptococcus mitis	185,08	74,23	110,85
Streptococcus oralis	180,33	102,00	78,33
Streptococcus pluranimalium	165,33	100,67	64,67
Streptococcus pneumoniae	282,00	157,00	125,00
Streptococcus pseudoporcinus	300,00	254,00	46,00
Streptococcus salivarius	214,00	78,50	135,50
Streptococcus sanguinis	240,00	97,00	143,00
Streptococcus thermophilus	128,00	39,00	89,00
Yeast			
Candida famata	119,00	17,00	102,00
Candida albicans	171,00	58,00	113,00

From table 1. above it can be seen that the bacteria found in dental plaque samples are not only the genus *Streptococcus* but also gram-positive rod bacteria, gram-negative rod bacteria and *yeast* or fungi. The table above shows the antibacterial activity of cinnamon bark ethanol extract toothpaste against bacterial colonies found in dental plaque. The number of bacterial colonies pre and post application of cinnamon bark ethanol extract toothpaste was clearly decreased.

Table 2. Gram Negative Rod Bacteria
Wilcoxon Signed Ranks Test
Ranks

		N	Mean Rank	Sum of Ranks
Number of Gram Negative Rod Colonies Post Intervention - Number of Gram Negative Rod Colonies Pre Intervention	Negative Ranks	11[a]	6.00	66.00
	Positive Ranks	0[b]	.00	.00
	Ties	0[c]		
	Total	11		

	Number of Gram Negative Rod Colonies Post Intervention - Number of Gram Negative Rod Colonies Pre Intervention
Z	-2.936[b]
Asymp. Sig. (2-tailed)	**.003**

In Table 2, the p value <0.03 shows that there is a significant difference in the number of gram negative rod colonies pre and post intervention.

Table 3. Gram Positive Rod Bacteria
Wilcoxon Signed Ranks Test
Ranks

		N	Mean Rank	Sum of Ranks
Number of Gram Positive Rod Colonies Post Intervention - Number of Gram Positive Rod Colonies Pre Intervention	Negative Ranks	24[a]	12.50	300.00
	Positive Ranks	0[b]	.00	.00
	Ties	0[c]		
	Total	24		

Test Statistics[a]

	Number of Gram Positive Rod Colonies Post Intervention - Number of Gram Positive Rod Colonies Pre Intervention
Z	-4.288[b]
Asymp. Sig. (2-tailed)	**.000**

In Table 3. The p value <0.05 shows that there is a significant difference in the number of gram-positive rod colonies between before or pre and post intervention.

Table 4. Gram-positive cocci
Wilcoxon Signed Ranks Test
Ranks

		N	Mean Rank	Sum of Ranks
Number of Gram Positive Coccus Colonies Post Intervention - Number of Gram Positive Coccus Colonies Pre Intervention	Negative Ranks	41[a]	21.00	861.00
	Positive Ranks	0[b]	.00	.00
	Ties	0[c]		
	Total	41		

Test Statistics[a]

	Number of Gram Positive Coccus Colonies Post Intervention - Number of Gram Positive Coccus Colonies Pre Intervention
Z	-5.582[b]
Asymp. Sig. (2-tailed)	**.000**

In Table 4. The p value <0.05 shows that there is a significant difference in the number of gram positive coccus colonies between before or pre and post intervention.

Tabel 5. YEAST
Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Number of Yeast Colonies Pre-Intervention - Number of Yeast Colonies Post-Intervention	110.250	50.308	25.154	30.198	190.302	4.383	3	**.022**

In Table 5. The p value <0.05 shows that there is a significant difference in the number of yeast colonies between before or pre and post intervention.

DISCUSSION

There are several bacteria found in dental plaque, the majority of which are gram-positive coccus bacteria at 51.25% **Graph 1.** There are 8 types of *Streptococcus bacteria found such as* Streptococcus mitis, Streptococcus oralis and *Streptococcus sanguinis* (Figure 1). Bacteria of this genus are the cause of dental caries. This is also in accordance with the results of research by Waty, *et.al* 2008 which also found 6 types of *Streptococcus* bacteria in dental plaque including *S. mitis, S. oralis, S. sanguinis* and *S. salivarius. The mythic Streptococcus is a member of the group* Streptococcus mutans. This group is known as *the oral group of Streptococci* which is karyogenic in nature. Another type that is also included in the *Streptococcus mutans* group is *S. sobrinus.* This group of bacteria is a group of bacteria that are first involved in plaque formation and initiate dental caries (Chandrabhan et al., 2012). A microscopic picture of *Streptococcus* bacteria can be seen in **Figure 1.** which indicates gram-positive bacteria are purple and coccus-shaped or round like grapes.

The antibacterial activity test of cinnamon bark ethanol extract toothpaste against *Streptococcus bacteria* and other bacteria in dental plaque was carried out using an extract concentration of 12.5%. The results of reducing bacterial colonies are shown in **Figure 2.** which showed antibacterial activity contained in toothpaste was able to reduce the number of bacterial colonies between before and after the intervention on the culture media used.

The results of statistical analysis showed a significant difference in the number of battery colonies between pre and post intervention with a p<0.05 value. A significant difference was also found in the number of colonies of gram-negative rod bacteria between pre and post intervention with a value of p<0.03. Furthermore, a significant difference of p<0.05 was found in the number of gram-positive rod bacterial colonies between pre and post intervention.

Significant results were also found in colonies of gram-positive coccus bacteria between pre and post intervention with $p<0.05$. In dental plaque samples, yeast or fungus was also found. Furthermore, statistical analysis was carried out on the sample with the Shapiro Wilk normality test, but because the data were not normally distributed, there was a significant difference in the number of yeast colonies before and after the intervention with a value of $p<0.05$.

Previous research has obtained the results of toothpaste preparations of cinnamon bark extract *(Cinnamomum burmannii*) concentrations of 6.25% and 12.5% have antibacterial activity in inhibiting the growth of *Streptococcus mutans (ATCC) bacteria* with an average inhibitory zone diameter of 10.0 mm and 15.72 mm. The results of Anova's statistical analysis of the diameter of the inhibitory zone of cinnamon bark ethanol extract toothpaste preparations showed a significant difference or significant difference for the $p<0.05$ test group (Waty, 2022). Other researchers who also use green tea catechins stated that green tea catechins toothpaste proved very effective for inhibiting the activity of karyogenic bacteria, namely *Streptoccocus mutans (17.21 mm) and* Lactobaccilus achidophilus *(19.57 mm)* (Fajriani & Djide, 2015).

Research Waty et al., (2018) wrote that *Cinamomum burmannii* plants contain alkaloids, saponins, flavonoids and glycosids. Puspita (2014) also wrote cinnamon bark extract contains transinamaldehid, polyphenols, flavonoids, saponins and tannins.

According to Cowan (1999) alkaloid plant products, flavonoids, tannins, and phenolics are antimicrobial agents. Alkaloids can inhibit microbial growth due to their ability to intercalate cell walls and DNA. Flavonoids have antibacterial activity by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes. Tannins have antibacterial activity based on their ability to damage cell membranes. Phenolics have hydroxyl functional groups which will later form complexes with bacterial cells so that the permeability of the bacterial cell membrane becomes disrupted. With the content of antibacterial compounds in cinnamon bark plants (*Cinnamomum burmannii*) causes these plants to be able to inhibit *Streptococcus mutans (ATCC)* bacteria which are bacteria that cause plaque on teeth.

CONCLUSIONS

Cinnamon bark ethanol extract toothpaste with a concentration of 12.5% was effective in inhibiting the growth of *Streptococcus* bacteria and other bacteria on dental plaque significantly with a $p<0.05$ value. This toothpaste showed antibacterial activity that significantly reduced the number of bacterial colonies found in dental plaque in all bacterial groups.

This toothpaste is able to significantly reduce the number of bacterial colonies found in dental plaque in all bacterial groups. It is hoped that these results will provide information to the public that the ethanol extract of cinnamon bark can be used as an active ingredient in toothpaste.

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