

Effectiveness of Xylitol Toothpaste on the Growth and Biofilm Formation of *Streptococcus mutans* ATCC 25175

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ARTICLE INFO : Received, December 15, 2022; Revised, February 21, 2023; Accepted, March 5, 2023; Published June 20, 2023
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DOI : [10.24815/jds.v8i1.33375](https://doi.org/10.24815/jds.v8i1.33375)

ABSTRACT

Background: Several toothpaste ingredients have also been proven to prevent dental caries. An alternative material like xylitol can reduce biofilms and caries' formation. **Objective:** This study aims to test the antibacterial xylitol toothpaste against *Streptococcus mutans* biofilm. **Materials and Methods:** The type of this research was true experimental by using two kinds of toothpaste with xylitol active ingredient (samples 1 and 2) and toothpaste with fluoride (sample 3). The toothpaste was diluted using the serial dilution method. Then the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum biofilm eradication concentration (MBEC), and minimum biofilm inhibition concentration (MBIC) were examined. The data were analyzed with ANOVA and Pearson correlation with a significance value of $p < 0.005$. **Results:** The MIC values of samples 1, 2, and 3 were 0.39%, 0.78%, and 0.39%, respectively. The MBC of samples 1, 2, and 3 were 12.5%, 25%, and 6.25%, respectively. The results of MBIC samples 1, 2, and 3 were 1.56%, 1.56%, and 0.78%, respectively. The results of MBEC samples 1, 2, and 3 were 6.25%, 6.25%, and 1.56%, respectively. There was a difference in the decrease of the mass of *Streptococcus mutans* ATCC 25175 biofilm after administration using xylitol and fluoride toothpaste, with a p -value of 0.0295. Additionally, there is a correlation between the time of administration of xylitol toothpaste with the changes in the mass of the biofilm *Streptococcus mutans* ATCC 25175. **Conclusion:** Xylitol toothpaste can inhibit the growth and eradication of *Streptococcus mutans* biofilm ATCC 25175.

Keywords: Biofilm, pertumbuhan, *Streptococcus mutans*, Xylitol toothpaste

ABSTRAK

Latar belakang: Beberapa bahan dasar dari pasta gigi telah terbukti mampu mencegah karies gigi. Bahan alternatif seperti xylitol dapat digunakan untuk mengurangi pembentukan biofilm dan karies. **Tujuan.** Penelitian ini bertujuan untuk menguji antibakteri pasta gigi xylitol terhadap biofilm *Streptococcus mutans*. **Materi dan Metode:** Jenis penelitian ini adalah true eksperimental dengan menggunakan dua jenis pasta gigi berbahan aktif xylitol (sampel 1 dan 2) dan pasta gigi berfluoride (sampel 3). Pasta gigi diencerkan menggunakan metode pengenceran serial, kemudian diperiksa konsentrasi hambat minimum (MIC), konsentrasi bakterisida minimum (MBC), konsentrasi pemberantasan biofilm minimum (MBEC), dan konsentrasi penghambatan biofilm minimum (MBIC). Data dianalisis dengan ANOVA dan Korelasi Pearson dengan nilai signifikansi $p < 0,005$. **Hasil:** Nilai MIC sampel 1, 2, dan 3 masing-masing adalah 0.39% 0.78%, dan 0.39%. MBC sampel 1, 2, dan 3 masing-masing adalah 12.5%, 25%, dan 6.25%. Hasil MBIC sampel 1, 2, dan 3 berturut-turut adalah 1.56%, 1.56%, dan 0.78%. Hasil MBEC sampel 1, 2, dan 3 berturut-turut adalah 6.25%, 6.25%, dan 1.56%. Terdapat perbedaan penurunan massa biofilm *Streptococcus mutans* ATCC 25175 setelah pemberian menggunakan pasta gigi xylitol dan fluoride, dengan p -value 0,0295. Selain itu, terdapat korelasi antara waktu pemberian pasta gigi xylitol dengan perubahan massa biofilm *Streptococcus mutans* ATCC 25175. **Kesimpulan:** Pasta gigi xylitol dapat menghambat pertumbuhan dan merusak biofilm *Streptococcus mutans* ATCC 25175.

Kata Kunci: Pasta gigi, xylitol, biofilm, Pertumbuhan, *Streptococcus mutans*

1. Introduction

Biofilm is a slimy layer formed by microbial colonies that sticks to surfaces and is difficult to remove. Biofilm attachment sites include the tooth and gingival surfaces.¹ *Streptococcus mutans* is the primary bacterium responsible for biofilm formation and caries.² *Streptococcus mutans* is a type of bacteria that adheres to tooth surfaces and is a major pathogen in the development of dental caries.³ According to the World Health Organization (WHO), caries affect 60-90% of children.⁴

According to the 2018 Basic Health Research (Riskesdas) results, 93% of children aged 5-6 years had cavities, and 29.8% of the 12-year-old group had dental caries, according to the 2018 Research and Development Center of the Ministry of Health of the Republic of Indonesia. Children aged 6-14 years are at high risk for caries because they transition from baby to permanent teeth. Mechanical cleaning methods, such as tooth brushing, and chemical methods, such as mouthwash, are two methods for removing biofilm. Several toothpaste ingredients are effective in the prevention of dental caries. The most commonly used toothpaste contains fluoride and is one of the best options for caries control.⁵

Fluoride is a naturally occurring mineral that is highly effective at preventing tooth decay and reducing enamel demineralization.⁶ Fluoride consumption in excess can have toxic and dangerous consequences.⁷ Acute toxicity can occur quickly, resulting in poisoning.⁸ Acute toxicity can result in nausea, vomiting, hypokalemia, cramps, particularly in the hands and feet, hypotension, hypersalivation, metabolic and respiratory acidosis due to renal or respiratory system failure, coma, and convulsions, all of which can be fatal.^{9, 10} Efforts are being made to find alternative caries prevention materials, particularly those that are effective against caries-causing bacteria, have few side effects, and cover a wide range of bacteria. Toothpaste is an essential part of preventive dentistry. Antibacterial agents are used to inhibit biofilm formation or to harm microbial cells.¹¹

Since the mid-1970s, xylitol has been widely used. The American Academy of Pediatric Dentistry (AAPD) recommends xylitol for caries prevention.¹² Over 35 countries have approved using xylitol in food, pharmaceuticals, and oral health products, mainly chewing gum, toothpaste, syrup, and confectionery.¹³ Xylitol has antibacterial properties that can prevent the growth of *Streptococcus mutans*. Using xylitol has been shown to reduce the incidence of dental caries. Xylitol is a non-cariogenic sugar alcohol found in chewing gum, toothpaste, chewable candy tablets, cough mixtures, and mouthwashes.^{14, 15} Xylitol is a type of sugar most plaque-producing bacteria cannot ferment and thus inhibits acid production. Xylitol comprises five-carbon chains and five hydroxyl groups, which bacteria find extremely difficult to ferment into energy. The ability of xylitol to inhibit the growth and metabolism of *Streptococcus mutans* and dental plaque has demonstrated its caries-inhibiting effect.¹⁶

Several methods and materials have been used to reduce *Streptococcus mutans* in the oral cavity to prevent the formation of biofilms, which is the first step in forming caries. Antibacterial xylitol can be used as an alternative to reduce the appearance of biofilms and caries. Splieth et al. discovered that xylitol is more effective than sorbitol at reducing acidogenic plaque.¹⁷ *Streptococcus mutans* in saliva and dental plaque decreases after three weeks of xylitol gum consumption.¹⁸ Wang et al. discovered a significant reduction in plaque accumulation in the group that chewed xylitol gum for two weeks.¹⁵ Caries incidence has also been reduced in children exposed to xylitol for 12 to 40 months. Long-term studies confirm that using xylitol gum can reduce caries risk by 50%, and the optimal time of consumption for caries prevention is at least one year before the eruption of permanent teeth.¹⁹ This study aimed to determine the antibacterial activity of xylitol toothpaste against *Streptococcus mutans* ATCC 25175 biofilm.

2. Material and Methods

The study was carried out at Padjadjaran University's Central Laboratory of Biological Activity and was approved by the Padjadjaran University Research Ethics Commission under reference number 42/UN6.KEP/EC/2021.

2.1. Research procedure

This study used xylitol samples 1 and 2 and fluoride toothpaste as sample 3 (positive control) diluted with aquadest as a solvent. Samples were tested at concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78%, 0.39%, 0.19%, 0.04%, and 0.02%. Furthermore, the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum biofilm inhibitory concentration (MBIC), and minimum biofilm eradication concentration (MBEC) of *Streptococcus mutans* ATCC 25175 were tested.

2.2. Data analysis

The One-Way ANOVA was performed to examine *Streptococcus mutans* biofilm formation and Pearson correlation analysis to see if xylitol toothpaste delivery timing affected biofilm mass.

3. Result and Discussion

Table 1 showed that the MIC value of sample 1 was 0.39%, sample 2 was 0.78%, and sample 3 was 0.39%. The concentration of the xylitol toothpaste (sample 1) has the same MIC value as fluoride toothpaste, which has a sizeable inhibitory power.

Table 1. Value of minimum inhibitory concentration (MIC) of Xylitol toothpaste against *Streptococcus mutans* ATCC 25175

Sample	Concentration (%)	Optical density
Sample 1	0,39	0,29
Sample 2	0,78	0,46
Sample 3	0,39	0,34

The absence of growing bacteria indicates that the sample can kill bacteria. Sample 1 had a MBC value of 12.5%. The MBC value of sample 2 was obtained at a concentration of 25%. The MBC value for sample 3 was 6.25% (Table 2).

Table 2. The minimum bactericidal concentration (MBC) of Xylitol toothpaste against *Streptococcus mutans* ATCC 25175

Sample	Concentration (%)	Bacterial Growth
Sample 1	12,5	0
Sample 2	25	0
Sample 3	6,25	0

Using a microplate reader with an absorbance of 595nm, the MBIC values for samples 1 and 2 were 1.56% and 0.78% for sample 3. (Table 3). This means that xylitol toothpaste has less inhibitory power against *Streptococcus mutans* biofilm than fluoride toothpaste.

Table 3. The minimum biofilm inhibitory concentration (MBIC) of Xylitol toothpaste against *Streptococcus mutans* ATCC 25175 biofilm

Sample	Concentration (%)	Optical density
Sample 1	1,56	0,26
Sample 2	1,56	0,33
Sample 3	0,78	0,31

According to this study, xylitol toothpaste samples 1 and 2 against *Streptococcus mutans* ATCC 25175 had MBEC values of 6.25%, while sample 3 had MBEC values of 1.56%. (Table 4). This means that fluoride toothpaste outperforms xylitol toothpaste in removing *Streptococcus mutans* biofilm.

Table 4. Minimum biofilm eradication concentration (MBEC) of Xylitol toothpaste against *Streptococcus mutans* ATCC 25175 biofilm

Sample	Concentration (%)	Optical density
Sample 1	6,25	0,65
Sample 2	6,25	0,45
Sample 3	1,56	0,44

The biofilm mass of *Streptococcus mutans* was calculated by administering xylitol toothpaste based on the MBEC concentration value. The concentration used in samples 1 and 2 was 6.25%, while the concentration in sample 3 was 1.56%. Each treatment's toothpaste administration time was 1, 30, and 60 minutes. The biofilm mass was measured using a microplate reader with a 595 nm absorbance. Figure 1 shows the change of *Streptococcus mutans* ATCC 25175 biofilm mass induced by three different types of samples for 1, 30, and 60 minutes. Based on these results, it is possible to conclude that the three samples used in this study could reduce *Streptococcus mutans* ATCC 25175 biofilm mass.

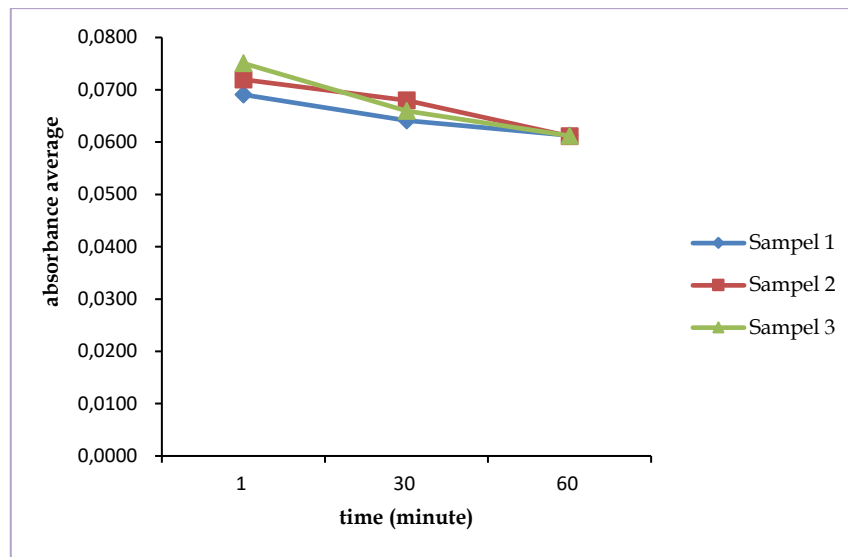


Figure 1. Changes in biofilm mass over time in samples 1, 2, and 3.

The existing data were checked for normality based on ANOVA analysis to determine the data distribution pattern. The research data has a normal distribution pattern because the p-value is more significant than 0.05 (Table 5). The ANOVA test with three times obtained p-value = $0.0295 < 0.05$ for the three treatments (Table 6). This demonstrates the nature of the test, which is statistically significant, indicating that the mass of the biofilm differs between the three samples.

Table 5. Analysis of data distribution

	N	$\bar{x}$	SD	p-value
Sample 1 60'	6	0,0613	0,0100	,4040
Sample 1 30'	6	0,0641	0,0035	,4142
Sample 1 1'	6	0,0691	0,0056	,4142
Sample 2 60'	6	0,0611	0,0051	,4142
Sample 2 30'	6	0,0679	0,0076	,0679
Sample 2 1'	6	0,0719	0,0054	,4142
Sample 3 60'	6	0,0612	0,0049	,4142
Sample 3 30'	6	0,0659	0,0053	,1573
Sample 3 1'	6	0,0751	0,0160	,4142
Total	54	0,0664	0,0087	Distribution Normal *

Table 6. ANOVA test of changes in the mass of *Streptococcus mutans* ATCC 25175 biofilm on induction of three different types of samples over time

N	$\bar{x} \pm SD$	F	p-value
54	0,07 $\pm$ 0,01	2,41	0,0295*

Post-hoc analysis revealed that fluoride toothpaste degraded *Streptococcus mutans* ATCC 25175 biofilm better than xylitol toothpaste.

Table 7. Differences of *Streptococcus mutans* ATCC 25175 biofilm mass after induction of samples 1 and 2

Time (minute)	Sample 1 ($\bar{x}$ dan SD)	Sample 2 ($\bar{x}$ dan SD)	p-value
1'	0,0691 $\pm$ 0,0056	0,0719 $\pm$ 0,0054	,5323
30'	0,0641 $\pm$ 0,0035	0,0679 $\pm$ 0,0076	,4072
60'	0,0613 $\pm$ 0,0100	0,0611 $\pm$ 0,0051	,9740

Table 7 summarizes the differences in *Streptococcus mutans* biofilm mass after induction of samples 1 and 2. The examination results revealed that the average concentration of biofilm mass

in sample 1 was not significantly different from the average mass of biofilm mass in sample 2. Statistical analysis tests at three-time intervals of one, thirty, and sixty minutes revealed a change in the biofilm mass between the toothpaste induction times, but it was insignificant.

Table 8 compares the biofilm mass of *Streptococcus mutans* ATCC 25175 after induction of samples 1 and 3. According to the examination results, the average concentration of biofilm mass between samples 1 and 3 was not significantly different, implying that sample 1 had nearly the same antibacterial activity as sample 3. The results of statistical analysis tests at 1, 30, and 60 minutes revealed no significant difference with a p-value less than 0.05.

Table 8. Differences of *Streptococcus mutans* ATCC 25175 biofilm mass after induction of samples 1 and 3

Time (minute)	Sample 1 ($\bar{x}$ dan SD)	Sample 2 ($\bar{x}$ dan SD)	p-value
1'	0,0691±0,0056	0,0751±0,0160	,1969
30'	0,0641±0,0035	0,0659±0,0053	,6936
60'	0,0613±0,0100	0,0612±0,0049	,9885

The difference in the effect of samples 2 and 3 on *Streptococcus mutans* biofilm mass is shown in Table 9. The average biofilm mass results in samples 2 and 3 did not show a significant difference, implying that samples 2 and 3 have nearly the same antibacterial activity. Statistically, the Post Hoc results at 1, 30, and 60 minutes in samples 2 and 3 did not show a significant difference with a -value> 0.05, according to the ANOVA test.

Table 9. Differences of *Streptococcus mutans* ATCC 25175 biofilm mass after induction of samples 2 and 3

Time (minute)	$\bar{x}$ dan SD Sample 2	$\bar{x}$ dan SD Sample 3	p-value
1'	0,0719±0,0054	0,0751±0,0160	,4998
30'	0,0679±0,0076	0,0659±0,0053	,6619
60'	0,0611±0,0051	0,0612±0,0049	,9856

Table 10 shows the correlation between the time of administration of xylitol toothpaste and changes in the mass of *Streptococcus mutans* ATCC 25175 biofilm. The correlation between the time of administration and changes in the mass of biofilm *Streptococcus mutans* ATCC 25175 in sample 1 is -0.45, which is significant (p-value≤0, 05). The correlation between induction time and changes in *Streptococcus mutans* ATCC 25175 biofilm mass in sample 2 was -0.62 and significant (p-value≤0.05). The correlation between induction time and changes in *Streptococcus mutans* ATCC 25175 biofilm mass in sample 3 was -0.52 and significant (p-value≤0.05).

Table 10. Correlation between the time of administration of Xylitol toothpaste and changes in *Streptococcus mutans* ATCC 25175 biofilm mass

Perlakuan	R	p-value
Sample 1	-,45	0,0310*
Sample 2	-,62	0,0032*
Sample 3	-,52	0,0138*

Based on the previously described data, it was discovered that xylitol toothpaste could inhibit the growth of *Streptococcus mutans* at a concentration of 0.39% in sample 1 and 0.78% in sample 2. The findings of this study are consistent with the results of Soderling et al.,¹⁶ Zou et al.,¹⁷ and Misra et al.,¹⁸, who discovered that xylitol toothpaste could inhibit the growth of *Streptococcus mutans*. In a study conducted by Soderling et al., the MIC value of xylitol was 0.1%.¹⁶ According to Zou et al., the MIC value of xylitol against *Streptococcus mutans* was 20%.¹⁷ According to Misra et al.,¹⁸ the MIC value of xylitol against *Streptococcus mutans* was 10%. The difference in MIC values is due to the different types of preparation and sample concentration used in the research. Low optical density (OD) values result from high sample concentrations. Rachmawati et al.¹⁹ agreed with the findings of this study, concluding that the higher the concentration, the lower the average OD or absorbance value of biofilm turbidity. This condition is expected because the concentration of antibacterial compounds in the sample increases with concentration.

Differences in the outcomes of teaching and learning activities in this study could be attributed to sample composition differences. According to Zou et al.,¹⁷ xylitol has an MBC value of 20% against *Streptococcus mutans*. According to research by Resti et al.,²⁰ and Agustina et al.,²¹ toothpaste containing xylitol has an MBC value of 10%. The findings of this study are consistent with previous research findings that xylitol can kill *Streptococcus mutans*.¹⁰ The results were caused by differences in the toothpaste brands studied and their active ingredients or ingredients. The toothpaste in Sample 1 contains eucalyptus and cloves. According to Lee and Kim's research, the minimum concentration of eucalyptus required to kill *Streptococcus mutans* is 1.25%, and the MBC value of cloves is 25%.²² COQ10, which effectively suppresses periodontal inflammation caused by plaque accumulation, is present in sample 2.²³

Gargouri et al. discovered that MBIC xylitol had a 50% inhibitory effect on *Streptococcus mutans*.²⁴ In this study, the MBIC value of xylitol toothpaste in samples 1 and 2 was 1.56%. Brown et al., Edwardsson et al., Loesche et al., Makinen et al., and Scheinen et al. discovered that xylitol can alter *Streptococcus mutans* in plaque and saliva. Makinen et al. discovered that xylitol cannot be fermented by bacteria present in the biofilm mass.²⁵ When xylitol is administered orally through mouthwash, toothpaste, or chewing gum, the mass of the biofilm can change. Direct contact between teeth and xylitol is required to reduce biofilm and caries. *Streptococcus mutans* xylitol toxicity is caused by intracellular accumulation of xylitol phosphate, which interferes with *Streptococcus mutans* and reduces its cariogenicity.

At a concentration of 6.25%, MBEC values of biofilm *Streptococcus mutans* samples 1 and 2 were present (Table 4). Gargouri et al. investigated the antibacterial activity of xylitol on oral biofilms containing *Streptococcus mutans* B509, *Streptococcus mutans* B782, *Streptococcus mutans* B234, *Streptococcus mutans* B200, *Streptococcus salivarius* B468, *Streptococcus consrellatus* B629, and *Streptococcus oralis* B634.²⁴ The accumulation of intracellular xylitol phosphate causes xylitol toxicity in *Streptococcus mutans*. Changes in the formation of extracellular polysaccharides or changes in the relative amount of biofilm polysaccharides with different solubility are expected to be metabolic consequences of intracellular accumulation of xylitol phosphate reactions.²⁵

This study found a correlation between the time of administration of xylitol toothpaste and changes in the mass of *Streptococcus mutans* ATCC 25175 biofilm. The growth of *Streptococcus mutans* biofilm increased gradually within 12 hours. Still, it decreased after 24 hours in the study by Zou et al.¹⁷ and Loimaranta et al.²⁶ concluded that inhibition of *Streptococcus mutans* growth could be seen in the early stages of biofilm formation but was less significant later on. When the biofilm of *Streptococcus mutans* is 8 hours old can be inhibited by 5% xylitol, but not when it is 24 hours old.²⁶ Because *Streptococcus mutans* cannot ferment xylitol, changes in the mass of this biofilm can occur. *Streptococcus mutans* transports xylitol in an energy-consuming cycle. The phosphoenolpyruvate system then converts xylitol to xylitol-5-phosphate. Phosphoenolpyruvate is a system that promotes intracellular vacuole formation and cell membrane degradation. *Streptococcus mutans* dephosphorylate Xylitol-5-phosphate. Because xylitol dephosphorylation wastes a lot of energy and *Streptococcus mutans* do not get energy from xylitol, xylitol has the potential to kill by starving *Streptococcus mutans*.

This study's results differ from those of several other studies because of the sample differences. The *Streptococcus mutans* used by Soderling et al.¹⁶ were obtained from the saliva of mothers and children who had consumed xylitol gum since the 1990s. They had the lowest inhibition value at a concentration of 0.1%. The *Streptococcus mutans* culture was transferred to growth media containing an additional 0.001%-1% xylitol concentration.¹⁶ Misra and colleagues fermented *C. tropicalis* to produce xylitol.¹⁸ His findings revealed that 5% and 10% xylitol concentrations could inhibit the growth of *Streptococcus mutans* by 50.30% and 96.04%, respectively. Resti et al.²⁰ discovered a minimum inhibition value formed at a concentration of 10% using a sample derived from Pepsodent brand toothpaste produced by Unilever Finland Oy, Helsinki. The study by Zou et al.¹⁷ used pure xylitol as a sample and discovered that 10% did not inhibit *Streptococcus mutans* biofilm growth.

4. Conclusion

Xylitol toothpaste can inhibit the growth and eradication of *Streptococcus mutans* ATCC 25175 biofilm. An incubation time of 1 minute improved inhibiting biofilm and *S. mutans* growth compared to 20 and 60 minutes.

5. Acknowledgments

The authors would like to thank Meita Kartika Sapitri, a Laboratory technician at the Central Laboratory of Biological Activity at Padjadjaran University, Indonesia.

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Authors Contribution

Contribution	Liyana	Ahmad I	Gunawan, K L	Gartika, M
Concepts or ideas	√	√	√	√
Design		√		√
Definition of intellectual content	√			√
Literature search		√		
Experimental studies	√			√
Data acquisition			√	√
Data analysis	√			√
Statistical analysis			√	√
Manuscript preparation	√	√	√	√
Manuscript editing	√	√	√	√
Manuscript review	√	√	√	√



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Citation Format: Liyana L, Ahmad I, Gunawan KL Gartika M. Effectiveness of Xylitol Toothpaste on the Growth and Biofilm Formation of *Streptococcus mutans* ATCC 25175. *J Syiah Kuala Dent Soc.* 2023; 8(1): 84-91.

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