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ORIGINAL RESEARCH

COMPARATIVE ANTIBACTERIAL ACTIVITY OF FLUORIDE TOOTHPASTES WITH HERBAL ADDITIVES (XYLITOL, BETEL LEAF, MISWAK) AGAINST *Streptococcus mutans*: AN IN VITRO STUDY

Santi Chismirina

ABSTRACT

Introduction: *One way to prevent tooth decay is to brush your teeth twice daily for two minutes with fluoride toothpaste. Fluoride is an ingredient in toothpaste that can inhibit the growth of Streptococcus mutans. Currently, many fluoride toothpastes are combined with herbal ingredients for various purposes, such as whitening teeth and preventing discoloration.* ***Aim:*** *This study aimed to evaluate and compare colony forming unit reduction of toothpaste containing fluoride with and without the addition of herbs in the form of xylitol, betel leaves, and miswak on S. mutans in vitro.* ***Methods***: *Suspension of Streptococcus mutans ATCC 25175 with McFarland equivalent turbidity of 0.5 was exposed to each diluted toothpaste (1 g/1 mL of distilled water), then inoculated into Trypticase Soy with Sucrose and Bacitracin (TYS20B) media and incubated anaerobically at 37°C for 48 hours. The number of S. mutans colonies was counted using a colony counter. Data were analyzed using One-Way ANOVA followed by Duncan's test at a significance level of $p<0.05$.* ***Results***: *The results of the ANOVA test showed that there was a significant decrease in the number of S. mutans colony growth compared to fluoride toothpaste without the addition of herbs. The results of further tests showed that the average number of colonies of fluoride toothpaste and miswak was the least, namely 24.33 CFU/ml, followed by fluoride toothpaste with the addition of betel leaves, xylitol, and fluoride toothpaste without the addition of herbs, namely 31 CFU/ml, 32.67 CFU/ml, and 39 CFU/ml.* ***Conclusion***: *Fluoride toothpaste with added herbs had a more significant effect in reducing the number of S. mutans colony compared to fluoride toothpaste without added herbs, with fluoride toothpaste with added miswak having the most significant effect on reducing colony growth compared to betel leaves and xylitol.*

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INTRODUCTION

More than 57% of Indonesians experience dental health problems, often triggered by low awareness of oral hygiene.[1] The most prevalent oral disease in Indonesia is dental caries. Dental caries is defined as localized damage to hard tooth tissue caused by the activity of bacteria that can ferment carbohydrates. This chronic infectious disease is triggered by the acid produced by the

main cariogenic bacteria, *Streptococcus mutans* (*S. mutans*). *Streptococcus mutans* is a Gram-positive, facultative anaerobic, non-motile bacterium capable of producing acid through the fermentation of carbohydrates.[1–3]

Carbohydrate fermentation occurs because the bacteria require a substrate as an energy source for their reproduction, and carbohydrates are one of the food elements that can provide this substrate. Carbohydrates are fermented by *S. mutans* into glucose and fructose as energy sources. *Streptococcus mutans* contributes to dental caries by producing lactic acid that lowers oral pH, leading to enamel demineralization. Consequently, toothpaste with antibacterial and remineralizing properties is essential for reducing cariogenic bacterial activity and preventing the development of dental caries.[4–7]

Regular brushing with fluoride toothpaste is one of the most effective strategies for preventing dental caries. In addition to fluoride, toothpaste formulations have increasingly incorporated natural plant-derived ingredients, such as miswak (*Salvadora persica*) and betel leaf (*Piper betle*), as well as xylitol, a naturally occurring sugar alcohol, to enhance their anticariogenic potential. These ingredients have demonstrated favorable safety profiles for oral use and exhibit antibacterial properties against cariogenic microorganisms, making them promising adjuncts to fluoride in caries prevention.[8,12]

According to the study findings, fluoride can influence the activity of cariogenic *Streptococci* by preventing the synthesis of acid and glucosyltransferase (Gtf), whereas xylitol can lessen *S. mutans* adherence and facilitate its separation from plaque. While research on the effects of herbal plants on caries has shown that betel leaf extract can limit the growth and attachment of *S. mutans* to the tooth surface, other studies have also shown that xylitol can inhibit *S. mutans* by decreasing the quantity of acid produced. This betel leaf extract can also inhibit Gtf activity by affecting the formation of glucan, which will cause the growth environment of *S. mutans* to become less conducive. Some researchers also report the antibacterial effect of miswak against cariogenic bacteria, which can inhibit plaque formation.[7-9,13,14]

The description shows that fluoride toothpaste with the addition of herbs, namely xylitol, betel leaf, and miswak, not only whitens and prevents tooth discoloration, but also has antibacterial properties that can prevent caries. Nevertheless, no research has used equivalent fluoride bases to directly assess these three herbal additions under normal in vitro circumstances. Thus, the purpose of this study was to scientifically demonstrate how fluoride toothpaste, both with and without the addition of these herbal substances, affected *S. mutans* in vitro.

METHODS

This experimental laboratory study was conducted at the Research Laboratory, Faculty of Dentistry (FKG) USK. The sample used in this study was *S. mutans* ATCC 25175, with test materials consisting of 3 toothpastes containing herbs and 1 non-herbal toothpaste as a control. The toothpastes were labeled as follows: 1) toothpaste A contains herbal ingredients xylitol and sodium monofluorophosphate (Pepsodent

Sensitive Expert®); 2) toothpaste B contains herbal ingredients betel leaf and sodium monofluorophosphate (Pepsodent Complete 8 Herbal®) ; 3) toothpaste C contains herbal ingredients miswak and sodium monofluorophosphate (Miswak-F®); 4) toothpaste D, which was used as a control, contains the active ingredient sodium monofluorophosphate (Pepsodent Action 123 Pencegah Gigi Berlubang®).

At the beginning of this study, *S. mutans* was grown in Trypticase Soy with Sucrose and Bacitracin (TYS20B) which consists of sucrose, yeast extract, trypticase soy agar, bacto agar, and bacitracin. This media is a selective media for the growth of *Streptococcus mutans*. For 48 hours, the cultivation process was carried out in an anaerobic jar in an incubator set at 37^0C. Suspensions were then made using the resultant *S. mutans* colonies. After adding one loop of pure culture to five milliliters of Trypticase Soy Broth (TSB), the combination was incubated for a full day at 37^0C. After incubation, a standard Mc Farland 0.5 ($1,5 \times 10^8$ CFU/mL) solution was used to balance the turbidity of the *S. mutans* culture.[5,15]

To find out how toothpaste affected *S. mutans* growth, the colonies were then counted. The first step in this process was creating the toothpaste. To manufacture the toothpaste, one milliliter of distilled water was mixed with one gram of each toothpaste. Twelve test tubes were then made, divided into four groups, and labeled with the kind of toothpaste used, such that each group contained three tubes. 0.5 ml of liquid TSB medium and 1 ml of *S. mutans* suspension were added to each tube. After that, each tube was filled with 1 milliliter of toothpaste in accordance with the label. Each tube was initially shaken to homogenize the fluid therein. The tube and the petri dish were then labeled, and 50 µl of each tube was placed into a petri dish to be grown on TYS20B medium. After the inoculation, the results were cultivated for 48 hours at 37°C in an anaerobic jar. A colony counter was used to count how many *S. mutans* colonies formed on each medium. The study's data were assessed using the one-way ANOVA technique, and any significant differences were further examined using the Duncan test.

RESULTS

Streptococcus mutans colonies grown on TYS20B media from each group can be seen in Figure 1, characterized by milky white, convex colonies, rough edges, and a yeast-like odor. Meanwhile, Gram staining results show that the colonies are round and appear to form pairs or chains, as seen in Figure 2.

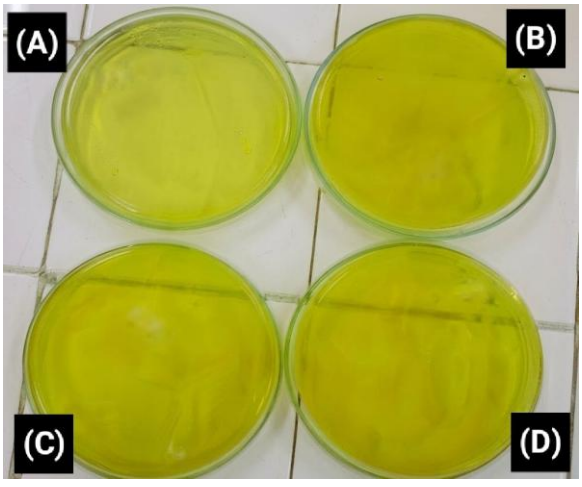

Figure 1. *Streptococcus mutans* colony growing on TYS20B media after exposure to toothpastes - A.fluoride and xylitol; B. fluoride and betel leaf; C. fluoride and miswak, and D. control toothpaste

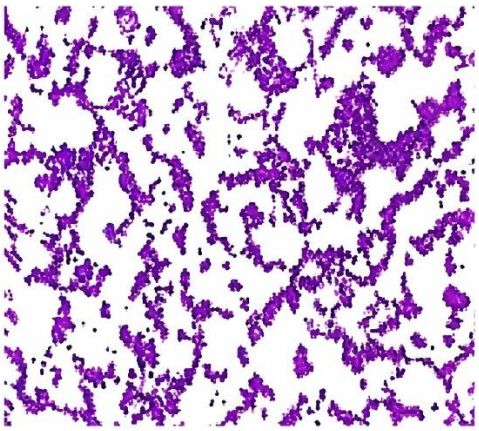

Figure 2. Gram staining results of *S. mutans* from colonies growing on TYS20B media

The result of mixing toothpaste that has been dissolved in distilled water with 1 ml of S. mutans suspension in TSB and 0.5 ml of TSB media into a test tube (Figure 3).

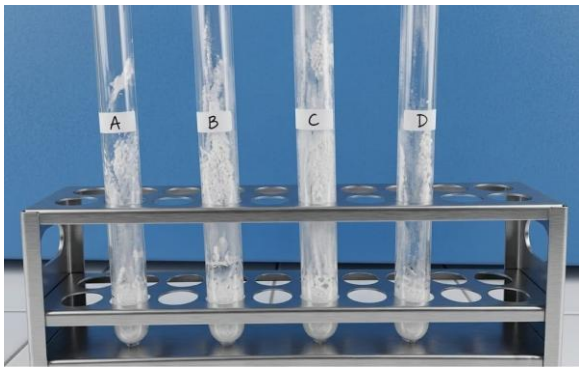

Figure 3. *Streptococcus mutans* suspension and toothpaste solution in TSB media – A. fluoride and xylitol; B. fluoride and betel leaf; C. fluoride and miswak, and D. control toothpaste.

The method used in this study involved counting the number of *S. mutans* colonies that were developing in each petri dish three times. Figure 4 below shows the results of counting the number of colonies that grew on TYS20B media from each repetition. The results of the tests carried out showed that the number of *S. mutans* colonies exposed to toothpaste containing fluoride and xylitol in the first treatment was 35 CFU/ml. This value is higher than the number of *S. mutans* colonies exposed to toothpaste containing fluoride and betel leaves and fluoride and miswak, which were 28 and 26 CFU/ml, respectively, but still lower than the number of *S. mutans* colonies exposed to control toothpaste, which was 38 CFU/ml. In the second and third repetitions, the number of *S. mutans* colonies exposed to toothpaste containing fluoride and betel leaves was higher, namely 31 and 34 CFU/ml, when compared to fluoride and xylitol toothpaste, which amounted to 30 and 33 CFU/ml, and fluoride and miswak toothpaste, which were 24 and 23 CFU/ml. This value is also still lower compared to the number of *S. mutans* colonies exposed to control toothpaste, namely 40 and 37 CFU/ml.

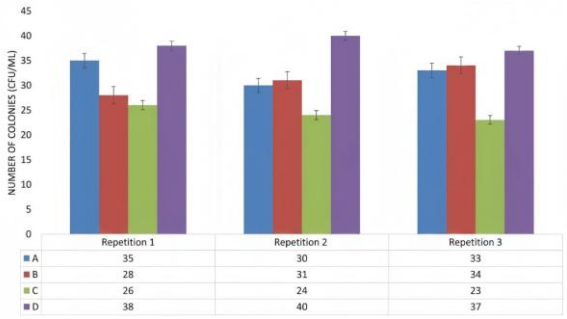

Figure 4. Effect of toothpaste on *S. mutans* growth in three replications – A. fluoride and xylitol; B. fluoride and betel leaf; C. fluoride and miswak; and D. control toothpaste

The average number of *S. mutans* colonies that developed following exposure to toothpaste containing fluoride and xylitol was 32.67 CFU/ml; toothpaste containing fluoride and betel leaves was 31 CFU/ml; toothpaste containing fluoride and miswak was 24.33 CFU/ml; and the control toothpaste was 39 CFU/ml. This demonstrates that after being exposed to toothpaste containing fluoride and miswak, the average number of *S. mutans* colonies was lower than that of toothpaste

containing fluoride plus additional herbal compounds such as xylitol and betel leaves, as well as control toothpaste. The average number of *S. mutans* colonies following exposure to toothpaste containing fluoride and betel leaves was lower than that of toothpaste containing fluoride and xylitol and control toothpaste, whereas the average number of colonies following exposure to control toothpaste was the highest. The average value is shown in Figure 5 below.

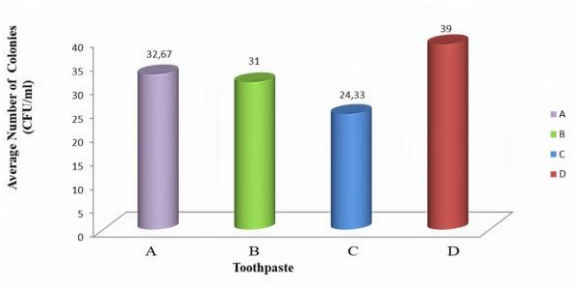

Figure 5. Average number of *S. mutans* colonies after exposure to each toothpaste – A. fluoride and xylitol; B. fluoride and betel leaf; C. fluoride and siwak; and D. control toothpaste

The One-Way ANOVA test showed a significant difference in the mean number of *S. mutans* colonies among treatment groups (F = 23,351; p < 0.00026 (<0,05)). As a result, the null hypothesis is rejected, and it is possible to conclude that the kind of toothpaste influences the amount of *S. mutans* colonies significantly. Next, a post hoc test was used to discover which groups differed significantly.

Table 1. Duncan test for the mean number of *Streptococcus mutans* colonies following exposure to different toothpaste formulations

Treatment Group	Mean ± SD (CFU/mL)	Duncan Grouping
Control	39.00 ± 1.00	A
Fluoride + Xylitol	32.67 ± 2.52	B
Fluoride + Betel Leaf	31.00 ± 3.00	B
Fluoride + Miswak	24.33 ± 1.53	C

Note: Different letters indicate significant differences (p<0.05).

Based on the results of the Duncan presented in Table 1, the control toothpaste group had the highest average number of *S. mutans* colonies (39.00 CFU/ml) and was significantly different from all treatment groups (group a). The toothpaste groups containing fluoride and xylitol (32.67 CFU/ml) and fluoride and betel leaf (31.00 CFU/ml) were in the same letter group (group b), indicating that they were not significantly different (p>0.05). Meanwhile, the toothpaste group containing fluoride and miswak had the lowest average number of *S. mutans* colonies (24.33 CFU/ml) and formed a separate group (group c), which was significantly different from all other groups (p<0.05). These results indicate that toothpaste containing fluoride and miswak has the highest effectiveness in inhibiting the growth of *S. mutans* compared to toothpaste containing fluoride and xylitol, fluoride and betel leaf, and control toothpaste.

DISCUSSION

Compared with the control fluoride toothpaste, all fluoride toothpastes supplemented with herbal ingredients (xylitol, betel leaf, or miswak) produced lower *S. mutans* colony counts. The fluoride–miswak toothpaste showed the greatest reduction, followed by fluoride–betel leaf and fluoride–xylitol, indicating the strongest inhibitory effect under the present experimental conditions. However, because this study did not quantify the antibacterial concentrations or phytochemical contents of the herbal ingredients, these findings should not be interpreted as evidence that miswak is inherently more potent than xylitol or betel leaf. The observed differences may instead reflect variations in

toothpaste formulation, active compound concentration, or interactions between fluoride and the herbal extract, which require further investigation. These findings are consistent with previous studies reporting the antimicrobial activity of miswak against cariogenic bacteria, although direct comparison is limited because earlier studies evaluated natural miswak sticks rather than miswak-containing toothpaste.[7,10,16,17]

Bioactive substances found in miswak, including isothiocyanate, fluoride, tannin, and flavonoids, are crucial in preventing *S. mutans* from growing. An antibiotic called isothiocyanate damages the cell wall and cytoplasmic membrane of bacteria to prevent their growth. In order to regulate the structure of the cell, the cytoplasm is confined by a membrane that acts as a barrier with active permeability and performs active transport functions. Isothiocyanate damages or kills cells by interfering with the integrity of the cytoplasmic membrane. Flavonoids and tannins are additional antibacterial substances that have the chemical characteristics of phenol compounds since they are members of the polyphenol group. Tannins and flavonoids function as antibacterials by disrupting the permeability and structure of the bacterial cell wall. Lysis results from the bacterial cell's internal osmotic pressure being higher than its external osmotic pressure.[13,18,19]

The lower *S. mutans* colony counts observed in the fluoride–miswak group may reflect the combined antimicrobial effects of fluoride and miswak-derived bioactive compounds. However, because the fluoride content and phytochemical composition were not quantified, the contribution of each component cannot be determined. Fluoride inhibits bacterial metabolism while enhancing enamel remineralization, whereas miswak contains antibacterial phytochemicals, including salvadorine, flavonoids, tannins, saponins, and alkaloids. Therefore, the greater reduction in *S. mutans* may result from their combined effects, although further studies are needed to clarify the role of each component and any potential synergism.[12,16,20]

In the present study, the fluoride–betel leaf toothpaste produced lower *S. mutans* colony counts than the fluoride–xylitol and control fluoride toothpastes, indicating greater antibacterial activity under the experimental conditions. This finding is consistent with previous in vitro studies reporting the antimicrobial effect of *Piper betle* against *S. mutans*. However, direct quantitative comparison should be interpreted cautiously because differences in toothpaste formulation, extract concentration, exposure time, and experimental methods may affect bacterial inhibition. The antibacterial activity of betel leaf is mainly attributed to phenolic compounds, such as hydroxychavicol, which disrupt bacterial cell membranes and denature proteins by altering their secondary and tertiary structures. In combination with fluoride, these compounds may provide complementary antimicrobial effects, although the contribution of each component and any potential synergism could not be determined in the present study and requires further investigation.[7,19,20]

In addition to miswak and betel leaf, xylitol is a naturally occurring sugar alcohol (polyol) commonly added to fluoride toothpaste

because of its anticariogenic properties. In the present study, the fluoride–xylitol toothpaste reduced *S. mutans* colony counts compared with the control fluoride toothpaste but was less effective than the fluoride–miswak and fluoride–betel leaf formulations. These findings are consistent with previous studies reporting that xylitol inhibits *S. mutans* by disrupting carbohydrate metabolism through the futile xylitol cycle, thereby reducing acid production and bacterial growth, while also enhancing the cariostatic effect of fluoride. However, because the concentrations of xylitol and fluoride were not quantified, their individual contributions and potential synergistic effects could not be determined and require further investigation.[8,9,21]

Although the control fluoride toothpaste reduced *S. mutans* colony counts, its inhibitory effect was lower than that of fluoride toothpastes supplemented with miswak, betel leaf, or xylitol. These findings should be interpreted with caution because this study has several limitations. First, the *in vitro* model does not fully replicate the complex oral environment, including saliva, acquired pellicle, pH fluctuations, temperature changes, and mechanical toothbrushing. Second, only a single *S. mutans* ATCC reference strain was evaluated, which may not represent the behavior of clinical isolates. Third, only one exposure concentration was tested. Finally, because the fluoride concentration, phytochemical content, and other formulation components were not quantified, the observed differences cannot be attributed solely to the added ingredients. In addition, the small sample size (n = 3) limits the statistical reliability and generalizability of the findings.

CONCLUSION

Fluoride toothpaste containing herbs, namely xylitol, betel leaf, and miswak, was able to reduce the growth of *S. mutans* better than toothpaste containing only fluoride. Fluoride toothpaste with the addition of herbs, namely miswak, had a higher effect in reducing the number of *S. mutans* colonies compared to fluoride toothpaste with the addition of herbs, namely betel leaf and xylitol.

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