# Periodontal Health and Microbiological Characteristics of Siwak (Chewing Stick) and Toothbrush Users: A Cross-Sectional Study

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## Abstract

**Objective** Siwak is a chewing stick used to clean the teeth and oral structures. Many studies have been conducted to assess the potential use of siwak in dentistry and concluded that it can be an alternative to a toothbrush in reducing plaque and gingivitis. However, some observations have reported more periodontal attachment loss and gingival recession among siwak users. This study aimed to compare the periodontal health and oral microbial characteristics between siwak and toothbrush users.

**Materials and Methods** This was a cross-sectional study, and participants were recruited from the public who attended community engagement programs. They were assigned to two groups based on whether they used siwak or a toothbrush. Participants who consented were examined for periodontal health. Supragingival and subgingival plaque samples were collected for bacterial identification and quantification.

**Statistical Analysis** The SPSS package version 21.0 was used for data entry. Data normality was statistically tested using Kolmogorov–Smirnov and Shapiro–Wilk tests, while data comparison used either *t*-test or Mann–Whitney *U* test.

**Results** A total of 36 participants were included in this study. The findings revealed that the plaque scores, bleeding scores, and periodontal pocket depths between siwak and toothbrush users were comparable ($p > 0.05$). Both groups had no evidence of gingival recession. Most participants had bacteria with characteristics of *Streptococcus* spp., which were present in 12 toothbrush users and 6 siwak users.

**Conclusions** The periodontal health status of siwak users was comparable to that of toothbrush users. Despite this, siwak users had fewer types of bacteria than toothbrush users, suggesting that siwak may serve as an alternative device to conventional toothbrushes for oral hygiene when properly used.

**Keywords**
- bacterial morphology
- chewing stick
- gingivitis
- periodontal pocket
- *Salvadora persica*

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## Introduction

Siwak is mainly derived from the roots or young twigs of trees, particularly *Salvadora persica*.[1] Typically, it is prepared as a 15- to 20-cm-long pencil-sized stick with a diameter ranging of 1.0 to 1.5 cm. When soaked in water for 1 to 2 minutes, the natural fibers of the stick soften, making it easier to separate. It is then tapered or chewed at one end until it becomes frayed in a brushlike form, which is then used for cleaning the teeth.[2] Siwak has an elastic fiber trunk that will not damage the teeth even under hard pressure. Physically, it is available in small-diameter rods and has high flexibility, allowing it to be bent in all directions, which can assist in removing dental plaque. Furthermore, it is known to give a pleasant fragrance to the mouth, eliminate bad breath, enhance the sense of taste, and give the teeth a radiant and shiny appearance.[3] Among the global Muslim community, it is one of the most commonly used medicinal plants for oral hygiene.[4]

Siwak use is widespread and is a commonly observed practice in numerous countries across the globe, spanning Asia, the Middle East, and Africa.[5–8] Due to its relative accessibility, popularity, and cost-effectiveness, siwak is an excellent and useful tool for controlling dental plaque and improving oral health.[8,9] The siwak stick serves mechanical functions similar to a toothbrush, allowing it to reach all parts of the mouth. Based on observational, analytical, and cross-sectional studies, it was found that the effect of siwak on plaque removal and improvement of gingival health was comparable to that of using a toothbrush, whether its use was exclusive or in combination with a toothbrush.[10] Furthermore, siwak users exhibited lower mean probing pocket depths (PPD) and gingivitis scores compared to toothbrush users.[11] However, significantly greater reductions of plaque and gingivitis scores were observed in clinical trials when siwak was used as an adjunct to the toothbrush.[12,13] Many other studies were conducted to assess the potential use of the siwak in dentistry, and they concluded that siwak could provide an alternative to a toothbrush for reducing plaque and gingivitis.[14,15]

In addition to its mechanical properties, siwak contains biochemical components, such as antibacterial and antiplaque properties.[1] These substances can help prevent dental issues including dental plaque accumulation, dental caries, and periodontal diseases.[3,16] Benzyl isothiocyanate (BITC) is a significant antibacterial component detected in the essential oils of siwak (*S. persica*) extract. The BITC component demonstrates a rapid bactericidal effect against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*, which are oral pathogens associated with periodontal disease and along with other gram-negative bacteria.[17] It is also capable of inhibiting the growth of *Streptococcus mutans*.[18] This enzymatic release of BITC occurs when individuals chew on a siwak stick before brushing their teeth. The mechanical action is believed to facilitate the penetration of freshly released BITC into the deeper structures of the plaque biofilm, thus inhibiting bacterial plaque formation.

Notably, it was discovered that practicing siwak five times per day resulted in a reduction in *A. actinomycetemcomitans* count in subgingival plaque.[19] However, when used at least once daily, facultative species, including *A. actinomycetemcomitans* and anaerobic bacteria, were found to be highly prevalent.[20] It is important to acknowledge that these inconsistencies in the results may be attributed to the varied methods of siwak practice employed in each study. Moreover, some studies reported more sites with gingival recession and clinical attachment loss among siwak users.[6,14,21] To overcome these inconsistencies, our study aimed to compare the periodontal status and dental plaque microflora between siwak and toothbrush users.

## Materials and Methods

The study was initiated after obtaining approval from the university ethics committee (CODE MEC: USIM/FPg-MEC/2016/No. 15). This was a cross-sectional study with a nonprobability convenience sampling design.

### Sample Size

The sample size was estimated using a mean plaque score difference of 0.19, a pooled standard deviation of 0.23, a significance level of 5%, and accepts power of study by 80% with possible type II error of 20% ($\beta = 0.2$).[19] Assuming that the data are normally distributed, the sample size was analyzed using PS software (Power and Sample Size Calculation) and estimating that 24 participants are required in each group.

### Recruitment of Participants

The public attending the community service program in three locations in Selangor, Malaysia, were screened according to the inclusion and exclusion criteria. Participants were selected among Malay, Muslim, adult individuals of both genders who were generally healthy and who were either using siwak (use siwak only and siwak and toothbrush with or without toothpaste) or toothbrush (those who use a toothbrush with or without toothpaste). Participants wearing orthodontic appliances, those with severe teeth crowding, and individuals using electrical toothbrushes were excluded from the study. Participants who fulfilled the criteria were invited to participate in this study, and written informed consent was obtained from them prior to data collection.

### Data Collection

The three examiners were blinded at all times during the procedures, except for the main examiner who was responsible for the allocation of participants. The examiners were calibrated, and an interobserver error test using the kappa statistics was conducted, obtaining a score of 0.605, indicating "substantial agreement." The periodontal health status of the participants was assessed using a plaque score index (O'Leary), PPD measurements, bleeding on probing (BOP) assessment, and gingival recession measured as the distance

between the cementoenamel junction and the gingival margin.

Supragingival plaque samples were collected using sterile cotton pellets from six sites: the buccal surface of the most anterior molar in each quadrant and the labial surface of tooth nos. 11 and 31. Subgingival plaque samples were then collected using sterile periodontal curettes, which were then stored in different labeled tubes.[22,23] Both supragingival and subgingival plaque samples were suspended in thioglycolate broth separately and stored at –70°C until processing.[24]

### Plaque Sample Analysis

In the anaerobic chamber, the supragingival and subgingival dental plaque was diluted serially in 10-fold steps. Each sample (10 µL) was cultured on blood agar medium. Every single colony was then subcultured to get pure samples and then proceeded with the determination of morphology identification and colony forming unit (CFU). In determining its morphological identification, four characteristics of a bacterium were considered: (1) form (circular, irregular, filamentous, rhizoid, etc.); (2) elevation (flat, raised, umbonate [has protruding protrusion], crateriform, convex, pulvinate [pillow shaped]); (3) size (pinpoints, small, medium, large); and (4) pigmentation (white, buff, green, red, etc.). This procedure was conducted by a single examiner using a reference, but lacking calibration.[25]

### Data Analysis

SPSS package version 21.0 was used for data entry. The normality of the periodontal health data was determined by performing the normality test using Kolmogorov–Smirnov and Shapiro–Wilk tests. When the data were normally distributed, a *t*-test was used; otherwise, the Mann–Whitney *U* test was employed for non-normally distributed data. A *p*-value of less than 0.05 with a 95% confidence interval was considered statistically significant.

## Results

A total of 179 people underwent oral examination at the community service program in three locations. Of the 36 participants who met the inclusion criteria, 25 were toothbrush users, while 11 were siwak users. The periodontal health data were normally distributed and analyzed using a *t*-test, except for PPD and gingival recession, which were assessed using the Man–Whitney *U* test. Even though the mean plaque scores of siwak users was lower (42.55) than that of toothbrush users (44.56), the difference was not significant ($p > 0.05$; ►**Fig. 1**). Similarly, the bleeding scores and PPD at the posterior and anterior teeth showed no significant differences between siwak and toothbrush users (►**Fig. 2** and ►**Table 1**). Furthermore, none of the participants presented with gingival recession.

►**Tables 2** and **3** display the characteristics of the bacteria found in this study. About 140 bacteria samples were successfully isolated from both supragingival and subgingival sites. In total, 93 samples exhibited circular colonies (67%), 23 samples were filamentous colonies (16%), 23 samples had irregular colonies (16%), and 1 was a rhizoid colony (1%). The most frequently isolated bacteria were circular, convex, and small white colonies (13%). Additionally, 7.9% of samples exhibited circular, convex, punctiform, and white colonies, while 7% of samples displayed circular, moderate, convex, and white colonies. These features were observed in 12 toothbrush users and 6 siwak users. These tables also indicate that the types of bacteria found in toothbrush users outnumbered those in *siwak* users.

►**Table 2** showed that among siwak users, the predominant characteristics of supragingival bacterial plaque are circular, convex, punctiform, and white, accounting for 4 of 19 samples (23%). In contrast, toothbrush users predominantly exhibited circular, convex, small, and white, constituting 9 out of 45 samples (20%). These findings suggest a greater diversity of bacteria in toothbrush users compared to siwak users. Of the 29 bacterial characteristics listed in the table, 10 were found in both supra- and subgingival surfaces.

►**Table 3** shows that there are no bacteria present in the supra- and subgingival surfaces of siwak users that are absent in toothbrush users. The majority of subgingival bacterial plaque in siwak users exhibited characteristics such as circular, convex, small, and white, accounting for 4 out of 21 samples (19%). In contrast, toothbrush users predominantly

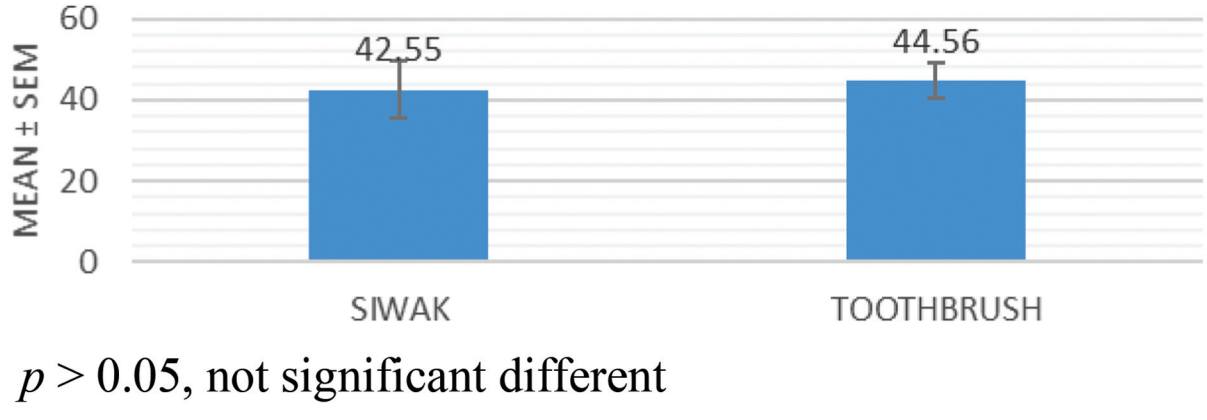


**Fig. 1** Plaque score between siwak and toothbrush users. SEM, standard error of the mean.

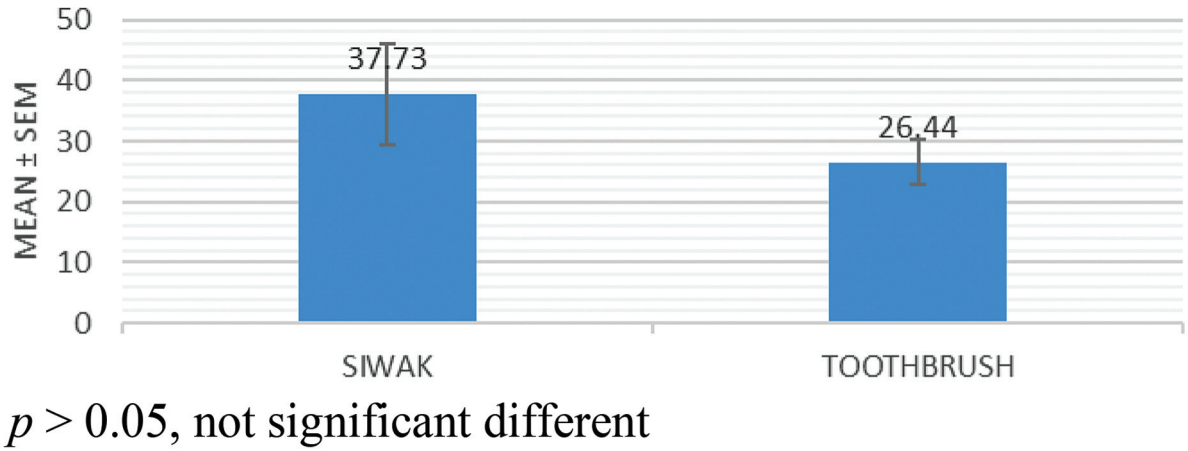


**Fig. 2** Bleeding score between siwak and toothbrush users. SEM, standard error of the mean.

**Table 1** Probing pocket depth between siwak and toothbrush users

| Parameter | Median (IQR) | | *p*-value |
|---|---|---|---|
| | Siwak | Toothbrush | |
| Probing pocket depth Upper right posterior | 3 (2) | 4 (2) | 0.957 |
| Probing pocket depth Upper anterior | 3 (2) | 3 (2) | 0.256 |
| Probing pocket depth Upper left posterior | 4 (2) | 4 (2) | 0.593 |
| Probing pocket depth Lower left posterior | 3 (2) | 3 (2) | 0.732 |
| Probing pocket depth Lower anterior | 3 (2) | 3 (1) | 0.372 |
| Probing pocket depth Lower right posterior | 3 (1) | 4 (2) | 0.291 |

Abbreviation: IQR, interquartile range.
Note: $p > 0.05$; no significant difference.

displayed circular, convex, moderate, and white plaque, constituting 6 of 53 samples (11.3%). Once again, these findings suggest a greater diversity of bacteria in toothbrush users compared to siwak users.

## Discussion

### Periodontal Health between Siwak and Toothbrush Users

The statistics in this study showed no significant differences between the users of toothbrush and siwak in terms of plaque scores, gingival bleeding, gingival recession, and PPD on all sextants. While all the users of toothbrushes use toothpaste, it does not enhance plaque removal. This finding is consistent with a systematic review that investigated the efficacy of brushing with and without dentifrice for dental plaque removal.[26]

In this study, the periodontal health of participants who primarily used siwak in combination with a toothbrush,

**Table 2** Bacterial characteristic in supragingival between siwak and toothbrush users

| Supragingival | Siwak | Toothbrush |
|---|---|---|
| Circular, umbonate, punctiform, yellow | 0 | 1 |
| Circular, umbonate, punctiform, white | 1 | 0 |
| Circular, umbonate, small, white | 1 | 1 |
| Circular, umbonate, moderate, cream | 1 | 1 |
| Circular, pulvinate, moderate, black | 0 | 1 |
| Circular, convex, punctiform, yellow | 1 | 0 |
| Circular, convex, punctiform, white | 4 | 2 |
| Circular, convex, punctiform, black | 1 | 2 |
| Circular, convex, punctiform, cream | 1 | 2 |
| Circular, convex, small, yellow | 1 | 1 |
| Circular, convex, small, white | 2 | 9 |
| Circular, convex, small, black | 0 | 1 |
| Circular, convex, small, cream | 0 | 2 |
| Circular, convex, moderate, white | 0 | 1 |
| Circular, convex, moderate, black | 0 | 1 |
| Circular, raised, punctiform, black | 0 | 1 |
| Circular, raised, moderate, white | 0 | 1 |
| Circular, flat, punctiform, yellow | 2 | 2 |
| Circular, flat, punctiform, white | 0 | 1 |
| Circular, flat, punctiform, cream | 0 | 1 |
| Filamentous, umbonate, moderate, white | 0 | 1 |
| Filamentous, umbonate, moderate, cream | 0 | 1 |

(*Continued*)

**Table 2** (*Continued*)

| Supragingival | Siwak | Toothbrush |
|---|---|---|
| Filamentous, umbonate, large, yellow | 0 | 1 |
| Filamentous, umbonate, large, white | 0 | 1 |
| Filamentous, umbonate, large, black | 1 | 0 |
| Filamentous, pulvinate, moderate, black | 0 | 1 |
| Filamentous, convex, moderate, black | 0 | 1 |
| Filamentous, convex, large, white | 0 | 1 |
| Filamentous, convex, large, cream | 0 | 1 |
| Irregular, umbonate, moderate, white | 1 | 2 |
| Irregular, convex, moderate, white | 1 | 0 |
| Irregular, flat, punctiform, yellow | 0 | 1 |
| Irregular, flat, small, yellow | 0 | 2 |
| Irregular, flat, large, white | 1 | 2 |
| Rhizoid, convex, moderate, black | 0 | 1 |
| Total | 19 | 47 |

**Table 3** Bacterial characteristic in subgingival plaque between siwak and toothbrush user

| Subgingival | Siwak | Toothbrush |
|---|---|---|
| Circular, umbonate, moderate, yellow | 1 | 0 |
| Circular, umbonate, moderate, white | 1 | 0 |
| Circular, umbonate, moderate, cream | 0 | 2 |
| Circular, umbonate, large, white | 1 | 0 |
| Circular, pulvinate, moderate, white | 0 | 1 |
| Circular, pulvinate, moderate, black | 0 | 1 |
| Circular, convex, punctiform, yellow | 1 | 1 |
| Circular, convex, punctiform, white | 1 | 4 |
| Circular, convex, punctiform, black | 1 | 2 |
| Circular, convex, punctiform, cream | 0 | 1 |
| Circular, convex, small, yellow | 0 | 2 |
| Circular, convex, small, white | 4 | 3 |

**Table 3** (*Continued*)

| Subgingival | Siwak | Toothbrush |
|---|---|---|
| Circular, convex, small, black | 0 | 1 |
| Circular, convex, small, cream | 0 | 1 |
| Circular, convex, moderate, yellow | 0 | 1 |
| Circular, convex, moderate, white | 3 | 6 |
| Circular, convex, moderate, black | 0 | 1 |
| Circular, convex, moderate, cream | 0 | 2 |
| Circular, convex, large, yellow | 0 | 1 |
| Circular, convex, large, white | 0 | 1 |
| Circular, flat, punctiform, yellow | 1 | 0 |
| Circular, flat, punctiform, white | 0 | 1 |
| Circular flat, small, cream | 0 | 1 |
| Filamentous, umbonate, moderate, yellow | 1 | 1 |
| Filamentous, umbonate, moderate, black | 0 | 1 |
| Filamentous, umbonate, large, yellow | 1 | 1 |
| Filamentous, umbonate, large, white | 0 | 2 |
| Filamentous, umbonate, large, cream | 0 | 1 |
| Filamentous, convex, moderate, black | 0 | 1 |
| Filamentous, convex, large, white | 0 | 3 |
| Filamentous, convex, large, cream | 0 | 1 |
| Filamentous, raised, moderate, white | 0 | 1 |
| Irregular, umbonate, small, yellow | 0 | 2 |
| Irregular, umbonate, moderate, white | 0 | 1 |
| Irregular, umbonate, large, white | 1 | 0 |
| Irregular, convex, moderate, cream | 0 | 1 |
| Irregular, flat, punctiform, yellow | 1 | 1 |
| Irregular, flat, small, yellow | 0 | 1 |
| Irregular, flat, moderate, yellow | 2 | 0 |
| Irregular, flat, moderate, white | 1 | 0 |
| Irregular, flat, large, white | 0 | 2 |
| Total | 21 | 53 |

including one exclusive siwak user, was comparable to that of those using only a toothbrush with toothpaste. These findings align with previous clinical studies among adjunctive siwak users of lower plaque scores, bleeding scores, and PPDs.[14] Furthermore, a meta-analysis of grouped randomized clinical trials revealed a significant reduction in plaque and gingivitis scores when siwak was used adjunctively.[27]

Although it significantly reduced gingivitis scores and PPDs, a study reported noticeable gingival recession in siwak users.[11] Gingival recession discovered in some users was generally reported to be due to excessive siwak use and improper technique.[6] In contrast, our study showed comparable PPD between groups and had no evidence of gingival recession. Additionally, a study by Ramadan and Alshenqiti discovered greater clinical attachment loss in toothbrush users than in siwak users.[15] Thus, our findings indicate that siwak may serve as a viable alternative to conventional toothbrushes for oral hygiene, provided that it is used properly.

### Microbiological Characteristic

There were 53 characteristics of the bacteria identified in this study. Among these 53 bacterial features, there was a possibility that the same bacteria could exhibit different aspects. For instance, bacteria with circular, convex, small, white characteristics were likely to be the same bacteria that have circular, convex, small, cream characteristics. This could occur when distinguishing between the colors white and cream exhibited by these bacteria was occasionally challenging. Similarly, bacteria characterized as circular, convex, punctiform, black, and circular and those described as convex, small, black, with sizes fallen between punctiform and small were sometimes difficult to differentiate. Consequently, it was crucial to conduct specific tests, such as using 16S ribosomal ribonucleic acid (rRNA) to determine each bacterial type.[28]

In our study, it became evident that the most commonly found bacteria in both siwak and toothbrush users shared similar characteristics. They appeared circular, convex, and white, with the primary difference being size, either punctiform, small, or medium. There was a possibility that these bacteria originated from a single bacteria type, as distinguishing features between punctiform, small, and medium sizes was somewhat challenging. These bacteria exhibit characteristics typical of Streptococcus, with features including small, circular colonies that are semi-transparent and convex, with a small clear zone of hemolysis on blood agar.[29] This finding is aligned with the observations of Sofrata et al, who noted that the inhibitory effect of siwak on *S. mutans* was less pronounced compared to *Actinobacillus actinomycetemcomitans* and *Lactobacillus acidophilus*.[30] Similarly, as reported by Zaenab et al, the antibacterial activity of siwak extract was better against *Bacteroides melaninogenicus* than against *S. mutans*.[31]

In addition to *Streptococcus* spp., the primary colonizer in plaque biofilm formation that accumulates on the tooth surface are mostly gram-positive bacteria, such as *Actinomyces* sp.[32] The morphological characteristics of *Actinomyces* spp. include being either white or off-white and size ranging from 3 to 4.5 mm. Their colonies on starch casein agar exhibit irregularities with filamentous margins rarely appearing wavy. The elevation is typically raised, although in a few cases, it can be convex and flat.[33] In our study, filamentous bacteria that have similar characteristics as *Actinomyces* spp. exhibited greater diversity in toothbrush users compared to siwak users.

During the acute phases of subgingival biofilm development, periodontopathic bacteria often experience a significant increase. These bacteria include *A. actinomycetemcomitans*, *P. gingivalis*, *Tannerella forsythia*, and *Spirochetes*.[33] On a blood agar substrate *A. actinomycetemcomitans* forms small, convex, translucent, circular colonies with diameters of approximately 1 mm after 2 to 3 days. These colonies have irregular edges and are firmly attached to the agar surface.[34] *Porphyromonas*, named after the Greek word "porphyrin" for its cell, produces black pigmented colonies on blood agar plates after 6 to 10 days due to heme accumulation.[35] The morphology of *P. gingivalis*, which displays as circular, convex, and having black characteristics, was also observed in this study. Among subgingival bacteria, these characteristics were more prevalent in toothbrush users compared to siwak users. In contrast, they were only found in supragingival plaque of toothbrush users. After 2 weeks of incubation, *Spirochetes* sp. produced pinpoint colonies that were circular with raised, convex centers and irregular edges, flat, and spreading. After 1 month of anaerobic incubation, the colonies reached a diameter of approximately 2.0 mm.[36] In this study, the same characteristic as *Spirochetes* sp. were found in both subgingival and supragingival plaques.

Even though the periodontal health comparison between siwak users and toothbrush users in this study did not yield significant difference, it is noteworthy that toothbrush users exhibited a greater diversity of bacteria compared to siwak users. This discrepancy could be attributed to the presence of antibacterial properties in the essential oil extracted from siwak, as reported by previous studies. Specifically, BITC was identified as the primary antibacterial compound in the essential oil of *S. persica* (siwak) and demonstrated effectiveness against *P. gingivalis*,[37] while octadecenoic acid, n-hexadecanoic acid, pentadecanoic acid, 2,6,10,14-tetramethyl-, methyl ester, and 9-hexadecanoic acid were the volatile compounds identified in the alcoholic extract of *S. persica*, conferring antibacterial effects, and inhibiting both *P. gingivalis* and *A. actinomycetemcomitans*.[38] Furthermore, gram-positive bacteria, including *S. mutans* and *Peptostreptococcus* sp., were significantly inhibited by the alcoholic extract of *S. persica*.[39] In another study, BITC demonstrated bactericidal effect and antibiofilm activity against *S. mutans*.[18]

## Limitations

Despite the inclusion of a small number of participants from the public and siwak users, monetary constraints limited the procedures for a specific test of bacterial identification. Morphology identification procedures were conducted by two examiners without calibration, which may introduce information bias. Therefore, the results should be interpreted with caution. Additionally, the technique and routine of

toothbrushing, which were not included in this study, could have influenced oral hygiene.

## Conclusion

Within the limitations of this study, the periodontal health status of siwak users was comparable to that of toothbrush users. In total, 53 types of bacteria were discovered, with most of them exhibiting morphological characteristics as circular, convex, small, and white, and these were similar to *Streptococcus* sp. Despite the comparable periodontal health status between these groups, toothbrush users had more types of bacteria (44 types) than siwak users (24 types). This suggests that individuals with a higher diversity of oral bacteria may elevate the risk of oral infections, including periodontal disease. Therefore, siwak exhibits the potential to function as both a mechanical and a chemical plaque remover, contributing to the maintenance of periodontal health.

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Conflict of Interest
None declared.

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