

## Microbial Contamination of *Salvadora persica* (Miswak Chewing Stick): Comparison with Commercially Available Toothbrush

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**Received:** September 04, 2022; **Published:** September 23, 2022

### Abstract

**Background:** Miswak is widely used and multicultural. It is mostly used as an oral hygiene aid. However, its bacterial contamination was not addressed in the literature.

**Aim:** To investigate microbial contamination different kinds of preserved Arak Miswak and compare it with commercially available toothbrush in bacterial retention.

**Materials and Methods:** A sample of freshly harvested unpacked/packed *Salvadora persica* (Arak Miswak) and toothbrushes were incubated in laboratory before use. Then distributed to 40 volunteered female dental students aged 21 - 24 years. They were divided into four groups. First group used the toothbrush, the second used Packed Miswak, the third used unpacked and the fourth used Freshly Harvested Miswak. All used Miswaks and toothbrushes were incubated and cultured after use.

**Results:** This study shows that toothbrushes are free from bacteria before use. On the other hand, all Miswaks sample contained *Bacillus cereus*. After use toothbrushes were found to contain the higher percentage of *Streptococcus viridans* (40%), *Staphylococcus epidermidis* (26.6%), *Staphylococcus aureus* (20%). Packed/unpacked Miswak sample contained higher *Bacillus cereus* (33.3%) and less retention of *Streptococcus viridans* (33.3%), *Staphylococcus epidermidis* (16.6%), *Staphylococcus aureus* (8.3%) and *Streptococcus salivarius* (8.3%). Freshly Harvested Miswak contained the highest *Bacillus cereus* (66.6%) and retained the least *Streptococcus viridans* (25%) and *Pseudomonas aeruginosa* (8.3%).

**Conclusion:** In general, Miswak retains the lowest number of bacteria when compared to toothbrushes. The Freshly Harvested Miswak retains the least but had the highest number of *Bacillus cereus*.

**Keywords:** Microbial Contamination; *Salvadora persica*; Miswak; Toothbrush

### Introduction

Miswak (Tooth Stick, *siwak*, *arak*) is a natural toothbrush made from the twigs of the *Salvadora persica* tree [1]. It is commonly used as a tooth cleaning aid particularly among educated people [2]. Miswak does not only provide spiritual benefits, but is also useful for the

achievement and preservation of healthy oral environment [3]. Its wide use, as an oral hygiene aid, is possibly due to the low cost and availability [4].

Miswak was found to be better than tooth brushing in reducing both periodontal and gingival diseases if preceded by professional instructions [5].

In a crossover randomized trial, they found that combining toothbrush and miswak had a significant reduction in plaque and bleeding when compared to using toothbrush only [6].

Recent systematic review and meta-analysis concluded miswak to be equivalent in decreasing plaque accumulation in comparison to regular toothbrushes [7].

Rinsing with Miswak extract increases plaque PH, thus, may prevent caries with an effect extending up to 30 minutes [8].

In addition, Miswak has shown similar effectiveness against development of new caries lesions when compared to regular toothbrushes and toothpastes [9].

Hala A Khoriba, *et al.* *in vitro* study found that miswak increase enamel and dentin resistant to acids [10].

It also enhances fibroblasts growth and inhibits cariogenic bacteria growth [11].

Miswak has antifungal, anti-parasitic, antiviral, and antibacterial properties [12-17]. As an irrigant, 10% of *Salvadora persica* was found to be effective antimicrobial in the endodontic treatment of necrotic pulps [18] and in improving the post-operative complications' severity following lower third molar extraction [19].

When compared to commonly used mouth washes, fifty percent Miswak extract has a low anti-bacterial effect against *Streptococcus faecalis*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [20].

The World Health Organization (WHO) recommended the use of the Miswak in 1986 and in 2000, an International Consensus report on oral hygiene concluded that further research was needed to document the effect of the Miswak [21]. However, a review of the literature has shown that no previous investigation has compared the antibacterial effects against bacterial retention of Freshly Harvested Miswak, Packed Miswak, unpacked Miswak and toothbrush.

### Aim of the Study

The aim of the study was to investigate contamination of Miswak and compare Miswak (Chewing Stick) with commercially available toothbrush in term of bacterial contamination and retention before and after use.

### Materials and Methods

Samples of the most commonly used chewing sticks in Saudi Arabia (*Arak*, *Salvadora persica*) and toothbrushes were obtained as follows:

- a. Freshly harvested miswak: Freshly harvested Miswak, was obtained and cleaned from dust with water and stored in the fridge to preserve its natural constituents.

- b. Packed miswak: Sample from the open market of packed Miswak was obtained from different companies (Al-Muslem, Taybah and Safa Sewak).
- c. Unpacked miswak: Sample from different street vendors of unpacked Miswak was obtained.
- d. Toothbrush (Oral-B) were obtained from open market and used in this investigation.

A sample of each of the four groups was obtained using a sterilized blade and scissor and cultured. All were incubated for 24 hours in 37°C in Thioglycolate Media. They were later subcultured in Nutrient Agar Media and incubated for 24 hours in 37°C.

Samples of toothbrushes and Miswaks (ten from each group) were stored in sterilized bags to be used in this investigation.

Forty female Dental students (King Saud University, Riyadh, Saudi Arabia) volunteered in this study. They were divided into three groups, ten in each group. All subjects received written brushing instructions 24 hours before the sample collection. They were asked to practice brushing prior to the sample collection. On the experimentation day, the samples of Miswak and toothbrushes were distributed, and subjects were asked to use it under supervision of the main author for 6 minutes. Miswak and Toothbrushes were then collected in sterilized bags and transferred to the laboratory.

Samples were obtained from the used areas of Miswaks and toothbrushes. They were cultured using Cooked Meat Media and incubated for 24 hours in 37°C. Subculture has been taken by using Blood Agar Media incubated for 24 hours in 37°C. The bacteria were identified before and after subjects use.

## Results

Pre-use cultures were negative for all toothbrushes. All samples of Miswak (unpacked, Packed and Fresh) had *Bacillus cereus* growth. After use culture results are summarized in table 1 and figure 1. Post usage contamination is presented in table 1. *Streptococcus viridans* was cultured in all specimens ranging from 40% in toothbrush to 25% in fresh Miswak. *Bacillus cereus* was found mostly in fresh Miswak accounting for 66%. *Staphylococcus aureus* was cultured in both toothbrush (20%) and unpacked and packed Miswak (8.3%).

Bacterial Growth	Toothbrush	Unpacked and Packed Miswak	Fresh Miswak
<i>Streptococcus viridans</i>	40%	33.3%	25%
<i>Staphylococcus epidermidis</i>	26.6%	16.6%	0%
<i>Staphylococcus aureus</i>	20%	8.3%	0%
<i>Enterobacter cloacae</i>	6.6%	0%	0%
<i>Streptococcus milleri</i>	6.6%	0%	0%
<i>Bacillus cereus</i>	0%	33.3%	66.6%
<i>Streptococcus salivarius</i>	0%	8.3%	0%
<i>Pseudomonas aeruginosa</i>	0%	0%	8.3%
<i>Clostridium difficile</i>	0%	16.6	0%

Table 1: Frequency and distribution of bacteria post-usage in the three groups.

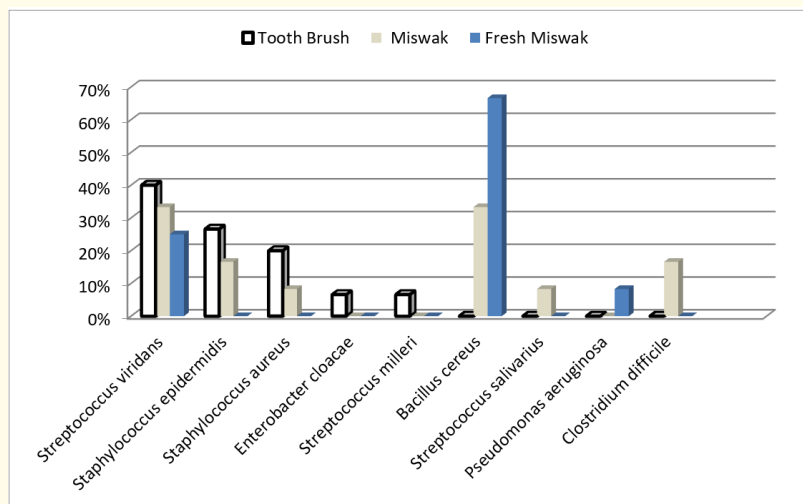


Figure 1: Post-usage bacterial culture results.

## Discussion

Miswak is one of the most commonly used oral hygiene aid in the Middle East Region [22]. The predominance of the use was reported to be due to its good taste, low-cost and availability [4,22,23]. It also has anti-plaque and many pharmacological properties [22].

In this study, *Bacillus cereus* has been found in all Miswaks' samples properly from soil. *Bacillus cereus* is Gram-positive spore forming bacteria. They are ubiquitous in nature and widely distributed in the food, air, water, soil, feces or even as a part of the normal flora [24,25]. Previous studies showed that it has been isolated from many foods, including cereal and cereal derivatives, spices, milk and dairy products, vanilla sauce, chicken soup, Mashed potatoes, vegetables, rice dishes and dried foods [24,25]. It is known to cause food poisoning and many infections especially in immunosuppressed patients. Its pathogenicity and virulence are related to toxins and enzymes produced. The spores require quite a severe heat process to destroy. They are hydrophobic and have the ability to adhere to surfaces including epithelial cells [24,25]. In the oral cavity, *B. cereus* was isolated from the dental plaque, root canal infection and from patient with gingivitis and a patient with juvenile periodontitis [24].

Studies have found that Miswak has natural minerals that kills microbes and germs and remove plaque [26]. It has beneficial ingredients in it one of them is antibacterial acidic inhibitors [27]. It has been suggested that it may have a selective inhibitory effect on the level of certain bacteria in the saliva, particularly several oral *Streptococcus species* [28]. Thus, Miswak users harbored significantly higher *Streptococcus intermedius*, *Actinobacillus actinomycetemcomitans*, *Veillonella parvula*, *Actinomyces israelii*, and *Capnocytophaga gingivalis*, and significantly lower *Selenomonas sputigena*, *Streptococcus salivarius*, *Actinomyces naeslundii* and *Streptococcus oralis* than did Tooth Brush users [29].

Al-Otaibi 2004 concluded that Miswak use was at least as effective as tooth brushing in reducing plaque and gingivitis and that the antimicrobial effect of *Salvadora persica* is beneficial for prevention and treatment of periodontal disease [30]. Similarly, Miswak has been reported to be more effective than tooth brushing for reducing plaque and gingivitis, when preceded by professional instruction in its

correct application especially from the embrasures [5]. Because the wicks of the Siwak clean between the teeth and do not break under any amount of pressure rather, they are flexible and strong [27]. In line with the previous studies, the results of this study demonstrated the superiority of Miswak, over the toothbrushes, in term of its antibacterial effects.

Other than *B cereus*, Sewak was less in harboring bacteria than toothbrushes particularly if fresh. Toothbrushes were found to retain cariogenic bacteria depending on the arrangement of the filaments and time duration [31-33]. Both unpacked and packed Miswak showed similar results. Anti-bacterial properties, thus, depends on time elapsed since harvesting.

### Conclusion

In conclusion, the present study had confirmed that toothbrushes are sterilized before packaging. Yet, the amount of retained bacteria was higher after use. This could be attributed to antimicrobial constituents of Miswak. It also demonstrated the contaminations of Miswak from the soil by *Bacillus cereus*. Freshly Harvested Miswaks were the least in term of contamination. Careful decontamination of Miswak prior to the use. Toothbrush and Miswak should undergo cleaning process and dryness after use. Careful use of both in immunocompromised individuals is also recommended.

Further research is needed with a larger sample size to assess the effect of Miswak on the salivary bacteria and to determine the contents of Miswak which combine the mechanical and chemical effect of tooth cleaning aids.

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**Volume 21 Issue 10 October 2022**

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