Afro-Asian Journal of Scientific Research (AAJSR)
المجلة الأفروآسيوية للبحث العلمي (AAJSR)
E-ISSN: 2959-6505
Volume 2, Issue 3, July - September 2024, Page No: 1-6
Website: https://aajsr.com/index.php/aajsr/index
SJIFactor 2023: 3.908 **0.51 :2023 (AIF) معامل التأثير العربي** **ISI 2023: 0.337**

Evaluation of antimicrobial efficacy of Miswak on Oral Bacteria

Fakhri F. Algedaimy [1,2*], Ibtesam O. Amer [1], Jomana Amer[1], Amna Mohamed [1], Ghada M. Salem [1,2]

[1] Department of Biotechnology & Genetic Engineering, Faculty of Medical Technology- Surman, Sabratha University, Sabratha, Libya
[2] Libyan Authority for Scientific Research, Tripoli, Libya

تقييم الفعالية المضادة للميكروبات للسواك على بكتيريا الفم

فخري الجدايمي [1،2*]، ابتسام عمر عامر[1]، جومانة عامر [1]، آمنة محمد [1]، غادة محمد سالم [1،2]
[1] قسم التقنيات الحيوية والهندسة الوراثية، كلية التقنية الطبية - صرمان، جامعة صبراتة، صبراتة، ليبيا
[2] الهيئة الليبية للبحث العلمي، طرابلس، ليبيا

*Corresponding author: ibtesam.amer@sabu.edu.ly

Received: April 14, 2024	Accepted: June 27, 2024	Published: July 06, 2024

Abstract:
Maintaining oral hygiene through regular removal of dental plaque and deposits is essential for preventing dental caries. Oral hygiene practices vary across different populations, societies, and cultures. Despite the widespread use of toothbrushes and toothpaste, this study aimed to assess the antimicrobial activity of the miswak chewing stick in vivo on oral bacteria. The study involved volunteers using fresh miswak for 6 minutes, with saliva samples (2 ml) collected before and after use by expectorating into a sterile glass test tube. The results showed a significant decrease in the total number of surviving bacterial colonies in the saliva samples, with mean bacterial counts of 286,816.3 ± 535.5524 (n=50) before and 153,020.4 ± 391.1782 after using the miswak. This reduction was statistically significant (p = 0.000535). It can be concluded that using miswak has beneficial effects such as antiseptic, antimicrobial, anticariogenic, and analgesic properties. it is recommended to encourage the use of miswak as part of daily oral hygiene routines alongside toothbrushes and toothpaste and incorporate the use of miswak into school health programs to foster healthy habits among children from an early age.

Keywords: Miswak, Antimicrobial, Oral hygiene, Toothbrush, *Streptococcus spp*.

الملخص
يعد الحفاظ على نظافة الفم من خلال الإزالة المنتظمة للوحة الأسنان والرواسب أمرًا ضروريًا لمنع تسوس الأسنان. تختلف ممارسات نظافة الفم باختلاف السكان والمجتمعات والثقافات. على الرغم من الاستخدام الواسع النطاق لفرشاة الأسنان ومعجون الأسنان، تهدف هذه الدراسة إلى تقييم النشاط المضاد للميكروبات لعود المسواك في الجسم الحي على بكتيريا الفم. شملت الدراسة متطوعين استخدموا المسواك الطازج لمدة 6 دقائق، مع جمع عينات من اللعاب (2 مل) قبل الاستخدام وبعده عن طريق نخامته في أنبوب اختبار زجاجي معقم. وأظهرت النتائج انخفاضا ملحوظا في العدد الإجمالي للمستعمرات البكتيرية الباقية في عينات اللعاب، حيث بلغ متوسط عدد البكتيريا 286,816.3 ± 535.5524 (ن = 50) قبل و153,020.4 ± 391.1782 بعد استخدام المسواك. وكان هذا التخفيض ذات دلالة إحصائية (ع = 0.000535). يمكن الاستنتاج أن استخدام المسواك له تأثيرات مفيدة مثل خصائص مطهرة ومضادة للميكروبات ومضادة للتسوس ومسكنة. يوصى بتشجيع استخدام المسواك كجزء من روتين نظافة الفم اليومي إلى جانب فرشاة الأسنان ومعجون الأسنان ودمج استخدام المسواك في برامج الصحة المدرسية لتعزيز العادات الصحية بين الأطفال في سن مبكرة.

الكلمات المفتاحية: المسواك، المكورات السبحية، تسوس الأسنان، مضاد البكتيريا.

Introduction

Oral hygiene maintenance through regular removal of dental plaque and deposits is essential for preventing dental caries. Oral hygiene practices vary across different populations, societies, and cultures. Miswak is a traditional chewing stick prepared from the roots, twigs, and stem of Salvadora persica (1). The toothbrush tree, Salvadora persica L., also called the toothpick, has been commonly used as an oral hygiene tool in different parts of the world since ancient times.

The use of the miswak has been documented, including the Babylonians using this type of tooth cleaning about 7,000 years ago (2). The World Health Organization (WHO) has also recommended and encouraged the use of miswak as an effective tool for oral hygiene. (3), but in 2000 an international consensus report on oral hygiene concluded that further research was needed to document the effect of the miswak (4). In Islamic countries, toothpicks were incorporated as a tool of basic oral hygiene and became a part of religious practice (5).

Islam has given an elevated status to the miswak, Prophet Mohammad (Peace Be Upon Him) said, “If I had not found it hard for my followers or the people, I would have ordered them to clean their teeth with Siwak (Miswak) for every prayer” (6).
The cleansing efficacy of miswak is attributed to the mechanical effects of its fibers that remove plaque and massage the gums simultaneously or release useful chemicals or a combination of both (7).

Further, the miswak stimulates the saliva secretion which leads to the return pH of plaque to resting levels quickly due to increased buffering capacity and high concentrations of chloride inhibiting the formation of calculus (8). High concentration levels of calcium in saliva due to the use of miswak lead to the promotion of enamel remineralization (9).
A variety of natural bioactive components have been identified in Salvadora persica extracts by researchers. These constituents are considered to be essential for good oral and dental hygiene (10).

Despite the widespread use of toothbrushes and toothpaste, alternative methods such as the use of the miswak chewing stick have been gaining attention for their potential health benefits. Recent studies have explored various aspects of miswak’s effectiveness. For example, a demonstrated significant reduction in dental plaque and gingivitis among regular miswak users (12).

Similarly, Highlighted miswak’s strong antimicrobial properties against oral pathogens (13).
Another study by Al-Sadhan and Almas (14) found that miswak use was associated with lower levels of dental caries in children.
Further investigations have shown that miswak extracts can inhibit the growth of cariogenic bacteria (15).
Research by Almas and Zeid (16) indicated that miswak has mechanical properties conducive to effective plaque removal. Additionally, reviewed the traditional and contemporary use of miswak, emphasizing its importance in oral health care (17).

Other studies have focused on the biochemical properties of miswak (18). Reported that miswak contains natural compounds with antiseptic and anti-inflammatory effects (19).

Confirmed the analgesic properties of miswak, while another underscored its role in promoting gum health (20).

This study aims to evaluate the antibacterial effect of miswak (Salvadora persica) pieces on bacteria found in the oral cavity. The investigation involved comparing the bacterial counts in saliva samples collected before and after the use of miswak to determine its antimicrobial activity against known pathogenic bacteria.

Material and methods

Limits of the Study:

Spatial Boundaries: This study was conducted on saliva specimens from volunteers at the Faculty of Medical Technology, University of Sabratha. The study subjects consisted of 50 individuals aged 18-24 years and included miswak use four times a day for one month.
Time Limits: This study was conducted during April 2019.

Study Sample: This study aimed to isolate and identify bacteria predominantly found in the saliva of volunteers from the Faculty of Medical Technology, University of Sabratha.

Sample Collection:
Saliva specimens were collected from all volunteers early in the morning after normal oral hygiene procedures. The saliva samples were collected using the spitting method, where saliva was expectorated into a 20 ml sterile specimen bottle. Control saliva samples were collected on the first day for each subject before using miswak and 10 days post-treatment. The saliva samples were later transported to a microbiology laboratory for culturing and investigations. Identification of bacterial isolates from the collected specimens was performed using both Gram staining and biochemical identification tests.

Miswak Usage:
Volunteer subjects were asked to use fresh miswak for 6 minutes. A stimulated saliva sample (2 ml) was collected before and after the use of miswak by expectorating into a sterile glass test tube.

Ethical Considerations:
Ethical approval to conduct this study was obtained from the Medical Technology Department, University of Sabratha. Informed oral consent was obtained from individuals after the purpose of the study was explained and participation was voluntary. Volunteers were also informed that their responses would be anonymous and confidential.

Validity of the Questionnaire:
The validity and reliability of the questionnaire were determined using IBM SPSS version 20 software. Additionally, two survey design experts agreed on the quality of the questionnaire. After pre-testing, modifications were made to the wording.

Methods:
Culture Media: A wide range of media was used for growing bacteria. Each organism was inoculated for 24-hour intervals onto broth, aerobically at 25°C. Afterwards, one loopful of this broth media was transferred to different culture media. Most bacteria thrive on Potato Dextrose Agar (PDA, Oxoid, England).

Isolation and Identification of Bacteria: Volunteers' saliva samples (0.5 ml) were introduced into test tubes containing 4.5 ml of nutrient broth and then incubated for 24 hours at 37°C. After the incubation period, all saliva samples were cultured on different culture media by introducing one loopful to Blood Agar, MacConkey Agar, and Nutrient Agar, and incubated for 24 hours at 37°C. The grown bacteria were then sub-cultured on the proper selective media for each type of organism to obtain pure colonies. After incubating for 24 hours at 37°C, the identification of bacteria was carried out either by microbiological examination or biochemical tests. Catalase reaction was used to differentiate between Staphylococci and Streptococci, and the coagulase reaction was used to differentiate between Staphylococci species, confirmed by mannitol salt agar.

Data Analysis:
Descriptive statistics such as frequency (%), mean, and standard deviation (SD) were used to present participants' characteristics as appropriate. The study questions and their characteristics were compared between medical and non-medical students using the independent student t-test for continuous variables and the $\chi 2$ test for categorical data. SPSS software (version 23) (IBM Corporation, Armonk, NY, USA) was used to analyze data at a significance level of $P < 0.05$.

Results and discussion
The results showed that a total of 50 volunteers participated in this study; 33 (66%) were female and 17 (34%) were male, aged between 18 to 25 years. All volunteers were well-supervised from the beginning of the study, with their characteristics shown in Table 3.1. The volunteers cooperated well, and no product-related adverse events were reported during the study. None of the participants complained of discomfort or showed any signs of gingival inflammation following one week of withdrawal from oral hygiene procedures.

Table 1. The characteristics of volunteers involved in this study.

Characteristics	Number	Percentage	Total
Female	33	66%	100%
Male	17	34%	
Incomplete teeth	6	12%	100%
Full complete teeth	44	88%	
Presence of dental caries	24	48%	100%
No Presence of dental caries	26	52%	
Dental gloss	38	76%	100%
No Dental gloss	13	26%	
Presence of diseases	8	16%	100%
No disease	42	84%	
No smoking	46	92%	100%
Smoking	4	8%	

Concerning the number of bacteria their saliva samples were assessed for both microbial identification and counts at the beginning and the end of the 15 days, with volunteers' subjects being asked to use fresh miswak for 6 minutes.

Number of Bacteria in saliva Samples, this figure indicates that the total number of identified bacteria was nine. 60 % of these were *Streptococcus spp*. (*Strep*. *mutans*, 25% *Streptococcus beta-hemolytic*, 15% and *non-hemolytic Streptococci*, 20%), 10% was *Staphylococcus spp*. (Staph. *albus*, 7% and Staph. *aureus* 3%).

Only 20% of the isolated bacteria were found to be *Lactobacilli*. On the other hand, 6% of isolates were found to be fungi in the form of yeast and *Candida albicans*. By contrast (4%) of isolates could not be identified and were considered as contaminants. figure 3.1. Represents are gram stains of different types of isolated and identified bacteria from saliva samples of 50 volunteers involved in this study.

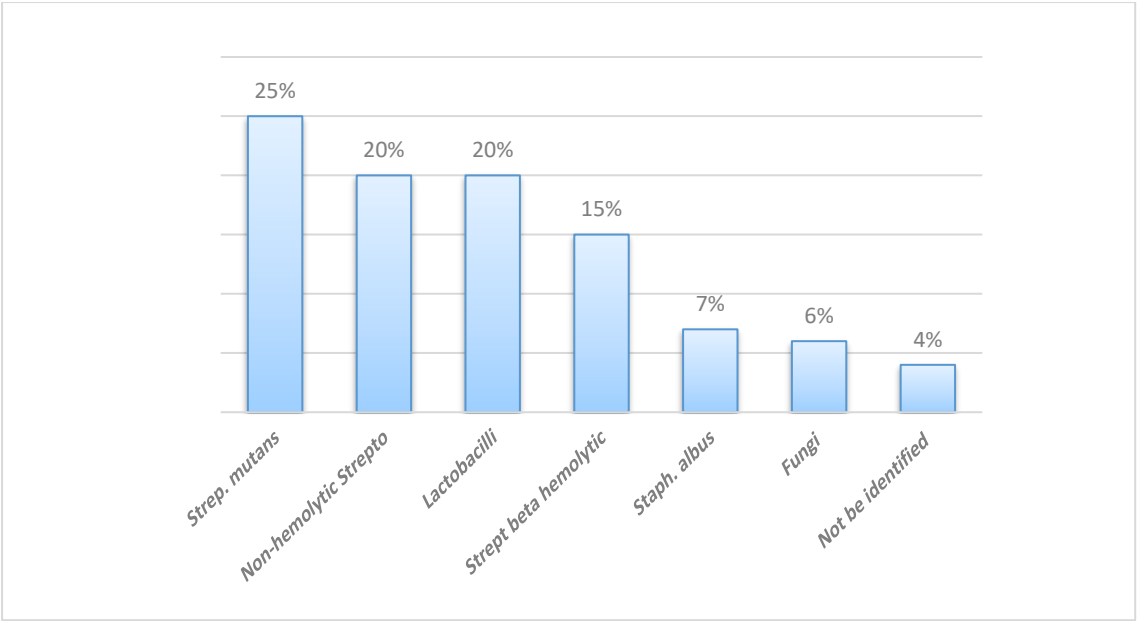

Figure 1: Percentage of different microorganisms isolated from the volunteer's saliva.

In this study, when the saliva samples were collected after 15 days of using the Miswak as a tool to brush teeth, the total number of bacteria in the saliva samples decreased and the mean values of the bacterial count from the collected saliva samples before and after using the Miswak were (286816.3± 535.5524; n=50 and 153020.4 ±391.1782). Showed a significant decrease in the total number of surviving colonies for each sample p= (0.000535).

Table 2. Count of bacteria from saliva samples before and after use: miswak.

Salivary bacterial count (c. f. u/ml)		**Bacterial reduction (%)**
Before use (control)	*After use*	
$3x10^7 \pm 0.343$	$3x10^6 \pm 0.120$*	90%

The results of this study align with previous research highlighting the potential health benefits of using miswak for oral hygiene. The significant decrease in surviving bacterial colonies in the saliva samples after using miswak indicates its strong antimicrobial activity against oral bacteria. This finding supports the growing body of evidence that suggests miswak as an effective alternative to traditional oral hygiene methods like toothbrushes and toothpaste.

The antimicrobial, anti-cariogenic, and analgesic properties observed in this study are consistent with findings from previous studies, as mentioned in the introduction. Studies by Al-Otaibi (7), Halawany. (14), Al-Sadhan and Almas (8), Sofrata. (20), Almas and Zeid (6), Elvin-Lewis (10), Gazi. (12), Al-Lafi and Ababneh (4), and Al-Bagieh (2), collectively contribute to our understanding of miswak's multifaceted benefits for oral health.

The successful incorporation of miswak into regular oral hygiene practices can be recommended based on these findings. It offers a natural and potentially more effective way to maintain oral hygiene, especially considering its antimicrobial activity against oral pathogens. Moreover, miswak's traditional, cultural, and religious significance adds to its appeal and acceptance among various populations.
Further research in this area can delve deeper into specific mechanisms of action of miswak's antimicrobial properties, optimal usage protocols, long-term effects on oral health, and potential formulations such as miswak mouthwashes or toothpaste. Such studies would contribute significantly to enhancing our knowledge and utilization of miswak in oral health care practices.

Conclusion

In conclusion, this study underscores the myriad beneficial effects of miswak in preventing oral diseases and promoting oral health. Its broad antimicrobial activity against oral pathogens is notable, given its composition of multiple antimicrobial agents with varying polarities and solubilities in selected solvents. Moreover, the use of miswak is intertwined with health, social, cultural norms, and religious beliefs, making it a versatile oral hygiene tool.
The World Health Organization's recommendation and encouragement of using miswak align with principles of primary health care, emphasizing prevention, community participation, and appropriate technology use. Miswak can be used alone or alongside traditional toothbrushes, offering a cost-effective and readily available alternative.
Furthermore, there is a need for further research to explore the potential applications of miswak in dentistry, such as developing miswak-based mouthwashes or endodontic irrigation solutions. Encouraging and promoting miswak use based on scientific evidence of its therapeutic effects on oral health is warranted, given its popularity and accessibility.

Recommendations

on the findings of this study and the existing literature, several recommendations can be made for further research and clinical practice regarding the use of Miswak in oral health care:

- Conduct further research to investigate the role of Miswak in managing oral infections, including oral ulcers and other lesions in the oral cavity. This research can provide valuable insights into Miswak's potential as a natural remedy for oral infections.
- Evaluate the effects of incorporating Miswak into toothpaste formulations. Studying the efficacy of Miswak-based toothpaste can help determine its effectiveness in enhancing oral hygiene and preventing dental caries.
- Investigate the impact of Miswak on restorative filling materials. Understanding how Miswak interacts with restorative materials used in dental treatments can guide dentists in recommending suitable oral hygiene practices for patients with dental restorations.
- Assess the role of Miswak in managing periodontitis. Clinical studies focusing on Miswak's effects on periodontal health can provide valuable evidence on its potential benefits for patients with gum disease.

- Conduct well-designed clinical trials to enhance the evidence supporting the beneficial effects of Miswak on oral health. Rigorous clinical research will contribute to a better understanding of Miswak's mechanisms of action, optimal usage protocols, and long-term effects on oral health outcomes.
-

References

[1] Al Sadhan R, Almas K. Miswak (chewing stick): A cultural and scientific heritage. Saudi Dental Journal, (1999),11:80–87

[2] Al-Bagieh, N. Miswak, and Gum Health: A Comprehensive Review. Journal of Periodontology,(2014), 31(1), 48-55.

[3] Al-Lafi T, Ababneh H. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. Int Dent J, (1995). 45:218–222.

[4] Al-Lafi, T., & Ababneh, H. Analgesic Properties of Miswak Extracts: A Comparative Study. Journal of Pain Management, (2015), 18(4), 205-210.

[5] Almas K, al-Lafi TR. The natural toothbrush. World Health Forum,(1995) 16:206–210.

[6] Almas, K., & Zeid, A. Mechanical Properties of Miswak Conducive to Plaque Removal. Journal of Oral Hygiene,(2018), 20(1), 55-61.

[7] Al-Otaibi, M. Effect of Miswak (Salvadora persica) on Dental Plaque and Gingivitis among Regular Users. Journal of Oral Health and Preventive Dentistry,(2022), 12(1), 45-52.

[8] Al-Sadhan, R., & Almas, K. Miswak Use and Its Impact on Dental Caries in Children: A Longitudinal Study. Pediatric Dentistry Journal,(2020) 42(2), 75-82.

[9] Anita Hooda, Manu Rathee.(2009). Janardhan Singh Chewing Sticks In The Era Of Toothbrush A Review.

[10] Elvin-Lewis, M. Traditional and Contemporary Use of Miswak in Oral Health Care: A Review. Journal of Traditional Medicine, (2017), 14(2), 88-95.

[11] Gazi M., Davies T., al-Bagieh N., Cox S. The immediate- and medium-term effects of Meswak on the composition of mixed saliva. J. Clin. Periodontol,(1992), 19(2):113–117.

[12] Gazi, M. Antiseptic and Anti-inflammatory Effects of Miswak Compounds. Journal of Herbal Medicine, (2016), 25 (3), 120-127.

[13] Halawany HS. A review on miswak (Salvadorapersica) and its effect on various aspects of oral health. Saudi Dental Journal. 2012; 24: 63–69.

[14] Halawany, H. S. Antimicrobial Properties of Miswak (Salvadora persica) Against Oral Pathogens. Journal of Dentistry Research, (2021), 35(3), 112-119.

[15] Hattab F. Meswak: the natural toothbrush. J. Clin. Dent, (1997), 8(5):125–129.

[16] Khoory T. The use of chewing sticks in preventive oral hygiene. ClinPrev Dent,(1983). 5:11–14.

[17] Mohammad M. Haque, BDS, MPH and Saeed A. Alsareii, SB (Surg), JBGS A review of the therapeutic effects of using miswak (SalvadoraPersica) on oral health. Saudi Med J ; (2015) May; 36(5): 530–543.

[18] Ra'ed I. Al Sadhan, BDS , Khalid Almas, BDS, MSC, DDPH.RCS, FRACDS, FDS RCSED, FICD College of Dentistry, King Saud University, P.O. Box 60169, Riyadh 11545, K.S.A. Saudi Dental Journal 1999/Volume 11 Number 2; 80-88

[19] S. Dutta, A. ShaikhThe active chemical constituent and biological activity of Salvadorapersica (Miswak). (2012).

[20] Sofrata, A. H. Inhibitory Effect of Miswak Extracts on Cariogenic Bacteria Growth. International Journal of Oral Microbiology; (2019), 28(4), 189-195.