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ORIGINAL RESEARCH article

Evaluation of the antifungal activity of Miswak (*Salvadora persica*) and toothpaste against oral cavity Candida species

Hamida S. El Magrahi [1 *], Abir M. Ben Ashur [1], Shada M. Agha [1], Shahed A. Khaleel [1], Arij M. Mousa [2], Ahmed E. Atia [3], Manal O. Abuagela [2], Eman A. Abdulwahed [1], Rehab S. Jerbi [4], Najia N. Alwaseea [2], Fawzia A. Mohamed [2] and **Eman A. Alaqeli [5]**

[1] Department of Medical Laboratories Sciences, Faculty of Medical Technology, University of Tripoli, Tripoli,
[2] Department of Public Health, Faculty of Medical Technology, University of Tripoli, Tripoli,
[3] Department of Anesthesia and Intensive Care, Faculty of Medical Technology, University of Tripoli, Tripoli,
[4] Department of Community Medicine, Faculty of Medicine, University of Tripoli, Tripoli,
[5] Department of Health Services Administration, Faculty of Public Health, University of Benghazi, Benghazi, Libya
[*]Author to whom correspondence should be addressed

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Abstract: Around the world, several oral health measures have been implemented. The most popular method is to use a toothpaste. However, chewing sticks are still used in many cultures around the world in a conventional manner. Chewing sticks have a mechanical cleansing action similar to a toothbrush in addition to their antimicrobial effect. The purpose of this study is to evaluate the effect of Miswak on the growth of Candida species and to compare it to that of toothpaste. A cross-sectional study was carried out on 120 selected randomly volunteers from January to April 2022. Two groups of participants were used for two weeks. The participants were separated into a group using Miswak and a group using ordinary toothpaste. Samples were collected before and after two weeks in these two groups. Samples were immediately processed for microbiological phenotypic conventional methods and in vitro susceptibility testing of the microbial isolates to antimicrobial. The findings show a significant effect of Miswak against an oral cavity candida species. Total candida count was 27.5±18.48, P=0.001 from Miswak group and 247.0±90.14, P=0.979 in toothpaste group. The most frequent Candida isolates organisms were *C. albicans*: 22 (36.67%) in the Miswak group and 24 (40.0%) in the toothpaste group, the second isolate was *C. dubliniensis*: 04 (6.69%) in the Miswak group and 12 (20.0%) in toothpaste group followed by *C. glabrata:* 02 (3.33%) in Miswak group. In conclusion, Miswak has a considerable antifungal impact immediately and after two weeks of use.

Introduction

The oral cavity is a habitat for a large number of microorganisms species that coexist with one another as a normal microbiota [1]. There are more than 20 species of candida, the most common opportunistic oral fungus associated with oral candidal infection, which colonize from 20.0% to 80.0% of adults without evidence of infection [2, 3].

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Candida albicans are the predominant oral cavity species in medically compromised and otherwise healthy individuals [4]. Oral candidiasis is a major condition that arises due to some predisposing factors such as poor oral hygiene, immune-suppression, nutritional deficiencies, long-term of use antibiotic/radiation therapy, dental prostheses, diabetes mellitus and a high carbohydrate diet or cigarette smoking [5]. Oral health has been linked to a number of chronic and systemic disorders and good oral hygiene is vital for disease prevention [6]. Modern dental care tools are designed to provide mechanical and chemical means through the regular removal of plaque and food residues from the surface and spaces among the teeth. One of the most popular ways to maintain oral hygiene is by using a toothbrush and dentifrices. Other notable methods include chewing gum, mouthwash and dental floss [7]. In many countries, chewing sticks made from the twigs, stems or roots of various plant species are chosen and prepared as natural methods of brushing teeth [8].

Salvadora persica is the most commonly used plant for this practice [9]. This plant is commonly known as the Miswak tree. Due to its broad association with the practice, the Arabic word Miswak, which means tooth cleaning stick, has become a common name for *S. persica* [10]. Due to their ability to defend against some oral infections and their potential to advance oral health, chewing sticks of *S. persica* have been recommended for use in oral hygiene by the World Health Organization [11]. *S. persica* has been comprising a wide variety of organic and inorganic compounds within its extract. Among the organic compounds were glycoside, saponins, flavonoids, alkaloids, tannins, benzyl derivatives, phenol compounds and organic acids [12]. For inorganic compounds, i.e. anionic compounds such as fluoride, chloride, sulphate, thiocyanate and nitrate have been identified [13]. Long fibers and the specific complexity of phytochemicals and minerals offer it an advantage as a tool for oral and dental health care by supplying all of the necessary means of mechanical and chemical cleaning [14].

Miswak at the time of usage stimulates saliva production and buffer sits pH [15]. These ingredients have the ability to remineralize dental hard tissue, stimulate the gingiva and treat inflammatory gums. Chewing sticks also contain volatile oils, tannic acid, sulphur and sterols which contribute to anti-septic, astringent and bactericidal properties. It reduces plaque formation, has anti-carious properties, eliminates bad odors and improves taste sensation [16]. It contains a strong antimicrobial activity [17] as well as protecting from pathogens that enter the body through the mouth [18]. Thus. The present study aimed to assess the effect of Miswak on the growth of Candida species and to compare it with toothpaste use.

Materials and methods

Study design: This study was conducted at the University of Tripoli, Faculty of Medical Technology, Department of Medical Laboratory Sciences, from January to April 2022, and was approved by the Research Ethics Committee of the University (2021). It was performed on 120 volunteers who were divided into two main groups. Group Miswak uses, whereas, the other group uses toothpaste for two weeks, three times daily.

Study population: 120 subjects aged 15-65 years (78 females and 42 males) consented to participate and were enrolled in the study. During the two weeks, neither antibiotic nor antiseptic mouthwash had been utilized by the chosen participants. The study excluded smokers and none of the individuals had ever utilized Miswak. There were 60 volunteers split between the two groups. Data were collected by questionnaire including age, sex and oral condition health.

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Sample collection culture and identifications

Sampling was carried out two times

Miswak group: The first sample was before using Miswak and after utilizing Miswak for two weeks, the second sample was taken. Volunteers were instructed about the Miswak use, advised to use it three times daily and shown how to keep it fresh by cutting off the edge of the Miswak every day and storing it in the refrigerator at night.
Toothpaste group: The first sample was taken before using toothpaste and the second sample was taken after using toothpaste for two weeks as directed. By using an oral concentrated rinse, samples were acquired by washing the mouth for 30 seconds with 10 ml of sterile water. The mouthwash liquid is then placed in conical tubes and placed in an insulated container until it is microbiologically processed. The sample suspension was washed three times in sterilized 0.10 M phosphate-buffered saline pH 7.4 (PBS) by centrifugation at 3,500 rpm. The pellet was then resuspended in 01.0 mL of PBS and 100 µl aliquots were inoculated by a spiral plating system onto the surface of Sabouraud's dextrose agar with an antibiotic. The growth of the colonies on the plates was counted and expressed as the number of Candida colony forming units per milliliter (CFU per ml) of rinse after 24-72 hours of incubation at 30^{o}C [19]. Yeast species identified by germ-tube production, micromorphology and chlamydospores production on corn meal agar plus 01.0% Tween 80. On corn meal agar, all isolates tested positive for germ tube test and chlamydospores and Tween 80 was identified as *C. albicans* or *C. dubliniensis*, Sunflower seed agar. Candida species were identified by chromogenic media [20].

Statistical analysis: The raw data were entered into spreadsheets in Excel and then imported into SPSS software version 26 (IBM Corp, Armonk, N.Y, USA). All data were expressed as Mean and standard deviation (Mean±SD). Descriptive statistics were used to calculate the frequency distribution, mean, standard deviation and median. For the total number of microorganisms in the two groups, a *t*-test was used. The level of significant difference was set at $^{*}p<0.05$, $^{**}p<0.01$ as highly significant and $^{***}<0.05$ as very highly significant.

Results

In this study, of the 120 adult participants, 78 were females (65.0%) and 42 were males (35.0%). The mean age of the participants was 34.2±17.42 years old (range: 15-65 years). In the two groups, 60 participants were Miswak users and 60 participants were toothpaste users. The present findings of the biochemical tests used in this study to identify isolated strains are given in **Table 1**. The results show that there is a statistically significant difference between the collected samples after two weeks of using Miswak compared to toothpaste according to the microbial growth on Sabouraud's dextrose agar. Where Miswak is more effective than toothpaste, showed a significant decrease in the total number of colonies for each sample with a correlation coefficient. This shows that there is a positive correlation between the Miswak and the overall number of candida species with $p<0.001$. **Tables 2** and **3** show the effect of Miswak on the candida. Thus, the total candida count was 27.50±18.48, IQR=30, p=0.001 from Miswak group and 247.0±90.14, IQR=110, p=0.979 in toothpaste group. **Table 4** depicts the distribution of all the organisms from the Miswak group and toothpaste group that have been isolated. The most frequent Candida isolated organisms were *C. albicans* 22 (36.67%) in the Miswak group and 24 (40.00%) in the toothpaste group, the second isolate was C. *dubliniensis* 04 (6.687%) Miswak group and 12 (20.0%) in toothpaste group followed by *C. glabrata* 02 (03.33%) in Miswak group.

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Table 1: Phenotypic and microscopic characteristics of isolated candida species

Types	Microscopic characteristics	Chlamydospores	Colonies on chromogenic media
C. albicans	*G* +ve, spherical or semi-spherical and germ tube in human serum	+	Light green colonies
C. dubliniensis	G+ve, spherical or semi-spherical and germ tube in human serum	+	Bluish green colonies
C. glabrata	G +ve, oval.	-	Smooth creamy

Table 2: Candida count in the different brushing groups

Group, each n=60	Time	Mean±SD	Median	IQR	P value
Miswak group	Before	253±97.12	249.5	100	
	After	27.5±18.48**	30	30	0.001
Toothpaste group	Before	247.78±89.89	240.5	111	
	After	247.0±90.14	238	110	0.979

IQR = Interquartile Range

Table 3: Comparison between Miswak and toothpaste through cultural growth before and after use

		Candida colonization (CFU per ml)		
Demographic Data	**Count**	**No**	**10-90 CFU/ml**	**> 105 CFU/ml**
Miswak group, n=60				
Before using Miswak	<200	08	08	00
	200-500	18	10	08
	>500	02	00	02
Total number		**28**	**18**	**10**
After 2 weeks of using Miswak	<200	04	04	00
	200-500	00	00	00
	>500	00	00	00
Total number		**04**	**04**	**00**
Toothpaste group, n=60				
Before using toothpaste	<200	12	08	04
	200-500	22	14	04
	>500	02	04	02
Total number		**36**	**26**	**10**
After 2 weeks of using toothpaste	<200	12	07	000
	200-500	21	14	04
	>500	02	04	02
Total number		**35**	**25**	**10**

Table 4: Microorganisms isolated from the mouth of the test group

Type of microorganisms	Isolated species	Miswak group no = 60	Toothpaste group no = 60
Candida	*C. albicans*	22 (36.67%)	24 (40.0%)
	C. dubliniensis	04 (06.67%)	12 (20.0%)
	C. glabrata	02 (03.33%)	00
Total		**28 (46.67%)**	**36 (60.0%)**

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Discussion

Good oral hygiene maintenance is a key to preventing oral diseases [21]. Miswak is used in the Arab world and is represented by flexible and strong sticks [8, 9]. This study was conducted for the first time in Libya to investigate the anti-candida activities. The findings showed that Miswak displays certain antifungal activity compared to toothpaste particularly against *C. albicans*, *C. dubliniensis* and *C. glabrata* strains. The highest candida count in colony forming units was recorded in the toothpaste group indicating the least antifungal activity while the least candida count was recorded in Miswak indicating the highest antifungal activity. A significant difference between Miswak before and after use while there was no significant difference between the toothpaste group before and after use. Several studies assessed Miswak and its effect on oral health. Miswak contains several medicinal properties and scientifically proven to be helpful even when used alone and without other methods of cleaning teeth [22-27]. It has also been indicated that Miswak released a variety of beneficial chemicals such as fluoride, saponins and sterol which possess antimicrobial activity. Chewing minimizes Miswak contamination [28, 29]. Miswak Chewing stick stimulates salivary flow. Saliva plays a vital role in maintaining homeostasis by balancing the pH in the oral cavity [30, 31]. As a result, using Miswak can reduce the risk of oral microbial contamination and translocation [32, 33]. The antimicrobial activity of released phytochemicals reduces the number of candida [27, 34]. Studies have indicated that Miswak possesses antifungal properties compared to ordinary toothpaste, thus, preventing oral candidiasis [35, 36]. Where Naeini et al. [37] reported from an *in vitro* study that an alcoholic extract of Miswak has strong to moderate activity, on oral Candida species, including *C. albicans*, *C. dubliniensis* and *C. glabrata*. More, hexane components in the roots of Miswak were found robust against *C. albicans* [37]. In the current study, an assay after two weeks was conducted to illustrate the difference between Miswak use and toothbrushes with toothpaste. Where the results showed that Miswak affected candida in all the investigated strains. Miswak was much more effective than toothpaste in eliminating candida immediately and after two weeks of use. These results indicate that the extracts of Miswak contain compounds with therapeutic potential against different Candida strains, and hence, they can potentially be utilized as therapeutic agents. The significance of this study came from an *in vivo* assessment of Miswak on oral candida that was followed up on for two weeks. In case both treatments are combined or used, Miswak has antibacterial and antifungal activities as well as preventing plaque. Toothpaste contains fluoride which prevents tooth decay and thus, the use of both for one month may reach an ideal level of oral hygiene and dental health. On the other hand, more studies are needed to verify the use of Miswak as an effective means to improve oral hygiene tools and fungicidal therapeutic agent for *C. albicans*.

Conclusion: Miswak reduces substantially the total oral fungus more than toothpaste, thus, Miswak use can limit the risk of oral candida species contamination and translocation.

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Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Ethical issues: Including plagiarism, informed consent, data fabrication or falsification and double publication or submission were completely observed by the authors.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

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