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Research Article

Efficacy and effect of *Salvadora persica* (Miswak), Toothbrush and Toothpaste on colonization of Oral Microbial Flora

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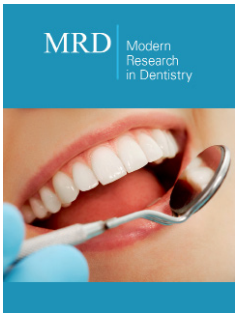

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Abstract

Background: Various oral health measures have been applied throughout the world. The most common way is to use a toothbrush and toothpaste. However, the traditional way of using chewing sticks is found in several parts of the world. Chewing sticks have a mechanical cleansing action similar to a toothbrush in addition to their antimicrobial effect.

Objective: To evaluate the efficacy of *Salvadora persica* (Miswak) products on oral microbial flora (bacteria and fungi) in comparison with toothbrushes and toothpaste.

Method: A cross-sectional study was conducted on 60 randomly selected participants, during the period from March to August 2021. An oral rinses sample was collected at 2-time intervals: Samples were collected before use and after one week of use from two groups (a group used Miswak and group using ordinary toothpaste). The samples were immediately processed for microbiological phenotypic conventional methods and *in vitro* susceptibility testing of the microbial isolates to antimicrobial.

Result: The organisms isolated from Miswak group, toothbrush and toothpaste group were identified. Among the bacterial isolates, *Streptococci mutans* was the most isolates 16 (53.33%) from Miswak group, and in 17 (56.67%) of toothbrush & toothpaste group, followed by *Lactobacillus* 12 (54%) in Miswak group and 10 (33.33%) toothbrush & toothpaste group. *Staphylococci aureus* was found in Miswak group 2 6.67%) and 3 10%) toothbrush & toothpaste groups respectively. *Candida* isolates was manly *C. albicans* 11 (36.67%) from Miswak group, and in 12 (40%) of toothbrush & toothpaste group, the second isolate was *C. dubliniensis* 2 (6.687%) Miswak group, and in 6 (20%) toothbrush & toothpaste followed by *C. glabrata* 1(3.33%) from Miswak group.

Conclusion: Miswak has a significant reduction effect on both bacteria, candida immediately and after a week of use.

Keywords: Oral hygiene; Oral cavity; *Salvadora persica* (Miswak)

Introduction

Oral hygiene is one of the most important daily routine practices, it is necessary for keeping the mouth and teeth clean and preventing many health problems [1]. Different clinical studies have demonstrated the effects of microbial species in the oral cavity. Oral microbial communities are some of the most complex microbial floras in the human body, consisting of thousands of bacterial and hundreds of fungal species [2,3]. Only 54% of these species are cultivable and identified, 14% are cultivable but not identified, the 32% are not even cultivated [4]. The combined activities of microorganisms within the oral and the host responses to them, lead to the progression of the disease and tissue damage [5]. Modern dental care tools are designed to provide both mechanical and chemical means of removing plaque and food residues from the surface and spaces between the teeth. Throughout history, people

have been using different tools and chemicals to maintain their oral health, such as chewing sticks, toothbrushes, gum, mouthwashes, toothpaste, floss, and traditional toothbrush [6,7]. Miswak is known as the "toothbrush tree" *Salvadora persica* L, named chewing sticks, belongs to the family known as Salvadoraceae [8]. Miswak sticks have been used for oral hygiene since ancient times [9]. Fibrous branches of S. persica have been approved by the World Health Organization for use as oral hygiene due to their protective effect on some oral pathogens [1]. The unique complexity of the Miswak phytochemicals and minerals, along with its long fibers, gives it an advantage as a tool for oral and dental health care through providing all of the necessary means of mechanical and chemical cleaning and maintaining healthy teeth and gums. The released chemicals, minerals and some of these biologically active constituents such as Sterol, Phenol, Palmitic Acid, Carvacrol, Eugenol, and Oleic Acids [10-12]. Miswak at the time of usage stimulates saliva production and buffer sits pH [13,14]. These ingredients have the potency to heal the inflamed gums, stimulatory effect on the gingiva, remove tartar, re-mineralize dental hard tissue, whitens teeth, provide enamel barrier, chewing sticks also contains volatile oils, tannic acid, sulphur, and sterols which contribute to anti-septic, astringent and bactericidal properties. It helps in reducing plaque formation, provides anti-carious effects, eliminates bad odor, and improves taste sensation [15], and a strong antimicrobial activity [16]. As well as protecting from pathogens that enters the body through the mouth [17].

Study Objectives

The aim of this research was:

1. To evaluate the efficacy of *Salvadora persica* (Miswak) products on oral bacteria in comparison with toothbrushes and toothpaste.
2. To assess the efficacy of *Salvadora persica* (Miswak) products on oral fungi in comparison with toothbrushes and toothpaste.

Material and Methods

Study design

This is a study with a cross-sectional design. It was conducted on 60 volunteers, divided into two groups. Group Miswak uses, while the other group uses toothbrush & toothpaste (3 times a day for a week). This study was conducted between the Department of Medical Laboratory Sciences, Faculty of Medical Technology, and the Department of Medical Microbiology and Immunology, College of Human Medicine, from March to August 2021.

Study population

Sixty subjects aged 10- 65 y (39 females and 21males) consented to participate and were enrolled in the study. The selected subjects had not used any antibiotics or antiseptic mouthwash during the week. Smokers were not included in the study; none of the participants had used Miswak previously. The volunteers were divided into two groups with 30 in each group. Data were collected by questionnaire, were including age, sex, oral condition health.

Sample collection culture and identifications

Sampling was carried out two times:

1) *Salvadora persica* (Miswak) group: In two stages: The first sample was before use Miswak, and the second sample was after a week of use Miswak. Subjects were instructed to Miswak using, 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.
2) Toothbrush & toothpaste group: The first sample was before use toothbrush & toothpaste, and the second sample was after a week of use toothbrush & toothpaste, instructed to use ordinary toothpaste three times daily for a week.

Samples were obtained by an oral concentrated rinse in which the mouth was washed for 30 seconds with 10ml of sterile water. The mouthwash liquid is then deposited in tubes until microbiological processing, 100µl aliquots were inoculated by a spiral plating system onto the surface nutrient agar, blood agar, and mannitol salt agar, and Sabouraud's dextrose agar with antibiotics. Culture plates were incubated for up to 24 h at 37 °C and at 30 ºC for 24-72h for yeast The identification of these isolates was performed using convention and standard biochemical tests including Gram stain, catalase, coagulase, and oxidase tests as described [18]. Growth assessed by enumeration of colonies and expressed as candida colony forming units per mL (cfu/mL^{-1}) of rinse [19]. Yeasts species identified by germ-tube production, and chlamydospores production on cornmeal agar plus 1% Tween 80. All isolates show germ tube test and chlamydospores test positive on cornmeal agar and Tween 80 was identified as C*. albicans* or *C. dubliniensis*. Sunflower seed agar and xylose hypertonic media were used to differentiate both species [20].

Statistical analysis

The raw data were entered into excel spreadsheets and later imported to SPSS software version 26 (IBM Corp., Armonk, N.Y., USA). 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 statistically significant difference was set at P<0001.

Result

Of the 60 study volunteers, 39 (65%) were female, and 21 (35%) were male. The mean age of the participants was 34.23 years ± 17.428 SD (range: 10-65). On two groups, 30 were Miswak users, and 30 were toothbrush & toothpaste users. This study also indicated biochemical tests adopted for identification of isolated strains and their results were tabulated in Table 1 & 2. The results show that there was a statistically significant difference from the collected samples after a week using Miswak, compere toothbrush with toothpaste, according to the microbial growth on a nutrient agar plate, and Sabouraud's dextrose agar, found that Miswak more effective than toothbrush & toothpaste, showed a significant decrease in the total number of colonies for each sample with a

correlation coefficient. These indicate that a positive relationship between the Miswak and the total number of the bacteria, and candida with a P < 0.0001. Tables 3 & 4 as show the effect of Miswak on the bacterial strains, and candida, were total bacterial count (Mean±SD 22.633±17.88948, IQR=20, P-value=0.000) from Miswak group, and in [Mean±SD 149.767±26.27006, IQR =39, P-value=0.237] toothbrush & toothpaste group. Total candida count [Mean ± SD 27.5±18.48422, IQR=30, P-value=0.000] from Miswak group, and in [Mean±SD 247.0±90.13976, IQR =110, P-value=0.979] toothbrush & toothpaste group. Table 5 Shows the pattern of all the organisms isolated from Miswak group & toothbrush & toothpaste group, amongst the bacterial isolates *Streptococci mutans* had the highest occurrence with 16 (53.33%) from Miswak group, and in 17 (56.67%) of toothbrush & toothpaste, followed by *Lactobacillus* 12 (54%), and in Miswak group, 10 (33.33%) toothbrush & toothpaste group. *Staphylococci aureus* was found in Miswak group 2 (6.67%) and 3 (10%) toothbrush & toothpaste groups. The most frequent Candida isolates organisms were *C. albicans* 11 (36.67%) from Miswak group, and in 12(40%) of toothbrush & toothpaste group, the second isolate was *C. dubliniensis* 2 (6.687%) in Miswak group, and in 6 (20%) toothbrush & toothpaste group, followed by *C. glabrata* 1 (3.33%) from Miswak group. However, the isolated organisms can become pathogenic if the conditions become congenial.

Table 1: Tests used for the diagnosis of bacterial isolates.

Organisms	Biochemical Tests				Other Test	
	Catalase	Coagulase	Oxidase	Mannitol	Gram stain	Hemolytic activity
Streptococci mutans	-	-	-	+	G +ve cocci	∝
Lactobacillus spp	-	-	-	-	G +ve coccobacilli	Y
Staphylococci aureus	+	+	-	+	G +ve cocci	β

Table 2: 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 3a: Bacterial count in the different brushing groups.

Group	Time	Mean±SD	Median	IQR	P value
Miswak group, n=30	Before	151.433±65.46159	150	96	0
	After	22.633±17.88948**	19	20	
Toothbrush & toothpaste group, n=30	Before	219.067±32.80447	200	50	0.237
	After	149.767±26.27006	152.5	39	

Table 3b: Candida count in the different brushing groups.

Group	Time	Mean±SD	Median	IQR	P Value
Miswak group, n=30	Before	253±97.12323	249.5	100	0
	After	27.5±18.48422**	30	30	
Toothbrush & toothpaste group, n=30	Before	247.778±89.89834	240.5	111	0.979
	After	247.0±90.13976	238	110	

Table 4: Comparison between Miswak and toothbrush & toothpaste through cultural growth before and after use.

Demographic Data	Oral Microbial Flora Colonization(CFU/ml)						
Miswak group, n=30	Count	No		10-90 CFU/ml		>105 CFU/ml	
		Candida	Bacteria	Candida	Bacteria	Candida	Bacteria
Before using the Miswak	<200	4	12	4	9	0	3
	200-500	9	15	5	7	4	8
	>500	1	3	0	2	1	1

Total number		14	30				
After a week of using the Miswak	<200	4	30	4	29	0	1
	200-500	0	0	0	0	0	0
	>500	0	0	0	0	0	0
Total number		4	30				
Toothbrush & toothpaste group, n=30	<200	6	8	1	6	2	2
	200-500	11	17	1	8	7	9
Before &after using the tooth brush & toothpaste	>500	1	5	0	3	1	2
Total number		18	30				

Table 5: Microorganisms isolated from the mouth of test group, n=30.

Type of Microorganisms	Isolated Species	Miswak Group, No (%)	Toothbrush & Toothpaste Group, No (%)
Bacteria	*S. mutans*	16(53.33%)	17(56.67%)
	Lactobacillus	12(40%)	10(33.33%)
	S. aureus	2(6.67%)	3(10%)
Total		**30(100%)**	**30(100%)**
Candida	*C. albicans*	11(36.67%)	12(40%)
	C. dubliniensis	2(6.67%)	6(20%)
	C. glabrata	1(3.33%)	0
Total		**14(46.67%)**	**18(60%)**

Discussion

Several studies have shown, Miswak has been reported to impart an essential antibacterial role, and antifungal [21,22]. where explained that Miswak has a more effective antimicrobial effect against *S. aureus* and *C. Albicans* compared to ordinary toothpaste, thereby preventing oral candidiasis [21,23,24]. The Miswak has been contain several medicinal properties; it has been scientifically proved to be very helpful in preventing tooth decay, even when used alone and without other methods of cleaning teeth. Several studies have assessed Miswak and its effect on oral health [25,26]. The potential of Miswak chemical components releasing during chewing may reduce the potential of contamination of Miswak [27]. As a consequence, the antimicrobial activity of the released phytochemicals reduces the total number of bacteria [28]. In this study, an assay after week was conducted to illustrate the difference between Miswak use, and toothbrush with toothpaste. The results showed that Miswak had an effect on all bacteria and candida in all. Miswak is effective against various types of oral bacteria which are involved in caries or periodontal diseases [29]. Miswak was significantly more effective in reducing *S. mutans* than ordinary toothpaste both immediately and after two weeks of use. This is also attributed to the antibacterial effect of Miswak. The importance of this study is based on being *in vivo* assessment of Miswak on oral flora followed up for the week. Despite that, it is highly recommended to extend the period of follow-up in future studies to measure the long-term effects on Miswak.

Recommendations

A. For the first use, the person should soak the tip of the Miswak for several hours, likely about 24hrs.

B. Before the use of Miswak, the tip meant for brushing must be washed with water.

C. The Miswak, which will be used, should be immersed in water for a few minutes (between 2 and 5 min) before using.

D. It is necessary to cut the tip of the Miswak every time.

E. When Miswak not used, it should be advisable to be stored in a humid place.

F. Must be dried after use in order not to cause mold due to the remaining water inside the used part.

G. It is recommended that further research should be carried out to study the role of Miswak on oral infections including, oral ulcers and other lesions in the oral cavity.

Conclusion

Our study results indicated that the total oral bacterial and oral candida carriage, on Miswak use was significantly reduced as compared with a toothbrush with toothpaste, thus the use of Miswak can limit the risk for oral bacterial contamination and translocation.

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