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RESEARCH ARTICLE

EFFECT OF AN EXPERIMENTAL MOUTHWASH EXTRACTED FROM MISWAK (SALVADORAPERSICA) ON CARIOGENIC BACTERIA AND DENTAL PLAQUE FORMATION

[1]Bshayer N.Wali, [2]Ibtesam K. Afifi [3]Jihan F. Balata and Nadia A. Badr

[1]Dental Intern, Faculty of Dentistry (UQU Dent), UMM AL-Qura University, KSA
[2]Professor of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University affiliated to UQU Dent, UMM AL-Qura University, KSA
[3]Associate Professor of Pharmaceutics, Faculty of pharmacy, Zagazig Universityaffiliated to Faculty of Pharmacy, UMM AL-Qura University, KSA
[4]Professor of Dental Biomaterials, Faculty of Oral & Dental Medicine, Cairo University affiliated to UQU Dent, UMM AL-Qura University, KSA

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ABSTRACT

This study aimed atpreparation of an experimental mouthwash from Siwak sticks, and compare it with the gold standard mouthwash; chlorohexidine (CHX), regarding the effectiveness on some bacteria responsible for dental plaque formation qualitatively and quantitatively.

Materials and Methods: This study was conducted in Makkah, Faculty of dentistry, Umm Al-Qura University. Twenty female patients with gingivitis and/or periodontitis, aged 20-40 years, systemically free were selected. An aqueous extract of Miswak was prepared by grinding Siwak sticks. The ground powder was soaked in distilled water for 48 hrs, filtered and dried. The filtrate was added in concentration of 10% to preservative, sweetener, freshness, surfactant, Co-surfactant and purified water. CHX was used as control. Plaque index (PI) was determined using modified *Quigley-Hein plaque index* and plaque samples were collected using sterile wooden toothpicks for microbiologic analysis using Caries Risk Test (CRT) for counting *Streptococcus mutans* and *Lactobacilli*. Then, the investigated mouthwashes were used twice daily for two weeks during which patients were instructed to perform regular oral hygiene measures. At the end of investigation period, PI was redetermined and bacterial plaque samples were collected again. For qualitative assessment of the mouthwashes, a subjective questionnaire was answered by the selected patients.

Results: There is always substantial improvement of PI and significant reduction in bacterial counts after the use of both mouthwashes.

Conclusion: An experimental Siwak mouthwash proved comparable effect in diminishing (PI) and reduction in counts of bacteria aid in dental plaque formation. The experimental mouthwash; Siwak, proved better patient's tolerance than CHX.

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INTRODUCTION

Oral health has been believed to be the main portal for the health of our bodies, as there are many diseases reflect their symptoms firstly in the mouth as for gastrointestinal diseases e.g. ulcerative colitis (Daley, 2007). On the other hand, oral health might affect certain diseases such as infective endocarditis where some oral bacteria increase the possibility of its occurrence. One of the oral hygiene measurements is the mouthwashes which have been recommended for treatment of infection, decreasing of inflammation, relieve of pain, lessening of halitosis (Oral and Dental Expert Group, 2007). Moreover, Fluoride containing mouthwashes are recommended for patients at high risk of dental caries including those with xerostomia after irradiation and chemotherapy to reduce dental caries (Marinho, 2016). Another well-known approach for oral hygiene is Miswak (*Salvadora Persica*, Siwak). It is made from the roots, branches, twigs, or stems of a plant called Salvadora Persica which is a member of the Salvadoraceae family, and derived mainly from Arak tree that grows in Saudi Arabia and in other parts of the Middle East (Almas, 1992). The efficiency of Miswak has been proven a long time ago. From a scientific aspect, many studies provided evidence that aqueous extracts of Salvadora Persica plant ha**ve** an inhibitory

Corresponding author: **Bshayer N.Wali,**
Dental Intern, Faculty of Dentistry (UQU Dent), UMM AL-Qura University, KSA.

effects on several oral microorganisms (Al-Bagieh, 1994). reducing *candida albicans* (Nyman, 1998), and an analgesic effect (Ali, 2002). From a religious aspect, our prophet Mohammed, peace be upon him recommended us to use Miswak saying "Were it not that I might over-burden the Believers I would have ordered them to use Siwak at the time of every prayer" (http://www.ummah.net/islam/taqwapalace). A recent research endorsed the use of Miswak on regular basis as one of strong efficient oral hygiene aid (Halawany, 2012). Further therapeutic benefits of Miswak are represented by anti-inflammatory and hypoglycemic effect and it might improve appetite and regulate movements of gastro-intestinal tract (Monforte, 2001). It has also protective action against stress induced ulcers (Sofrata, 2007). Nowadays the most effective mouthwash for reducing plaque and gingivitis is clorhexidine gluconate. It is a cationic bis-guanide with broad-spectrum antimicrobial activity. The recommended use is for twice-daily at only a short-term adjunct as long-term use has numerous adverse effects, which include tooth and restoration staining, soft tissue staining, increased calculus deposition, unpleasant taste, taste alteration, and burning sensation, desquamation and mucosal irritation (Farah, 2009). Despite Salvadora Persica had proven an antiplaque activity,it was reported that using its extract as a mouth rinse was less effective than CHX in preventing plaque accumulation (Al-Bayaty, 2010). Rinsing with Miswak extract resulted in protracted raise in plaque pH (Safrata, 2010). Thus, this study aimed to throw the light on an experimental mouthwash prepared from Siwak extracts regarding the qualitative and quantitative effectiveness on two main cariogenic bacteria; *Streptococcus mutans* and *Lactobacilli;* that aid in dental plaque formation. The gold standard mouthwash; chlorohexidine (CHX), was used as control.

MATERIALS AND METHODS

Preparation of Miswak (*Salvadora Persica)* extracts

Two kilograms of *Salvadora Persica*, Miswak chewing sticks were cut into small pieces, and then ground into powder form. The fine powder was soaked in five liters of distilled water for 48 hrs at 4°C. The immersion solution was filtered through Whatman No.1 filter paper, and the filtrate was then dried using a freeze drier and stored in air-tight closed bottles.

Preparations of Miswak (Salvadora Persica) mouthwash

Miswak extract was added in concentration of 10% guided by Al-Bayaty et al., 2010, toother ingredients listed in (Table *1).*

Table 1. Compositions of Salvadora Persica mouthwash

Ingredient	Description	(W/W) %
Salvadora Persica extract	Main active component	10
Sodium benzoate	Preservative	0.1
Sodium saccharin	Sweetener	0.1
Menthol	Freshness	0.2
Polysorbate 80	Surfactant	4
Glycerol	Co-surfactant	4
Purified water	Vehicle	To 100

Preparation of Tris-EDTA buffer

Tris-EDTA buffer solution was used for preservation of plaque samples as recommended by*Mannaa et al, 2013* [15]. A stock of 100 ml of Tris-EDTA solution was prepared in accordance to *Aitken A.; 2012*[16]. By weight, 14.114gm of Tris pH 8 equals to 1M and 18.612gm of EDTA equals to 0.5 M were dissolved in 100 ml distilled water. Then, the solution was autoclaved to be sterilized and stored at room temperature. Finally, 107 ml distilled water was added to 4.27gm of NaOH to obtain 0.5M solution. Afterward, 150 µl sterile TE buffer with 100 µl NaOH was placed in each eppendorf tube to receive the wooden toothpicks having the plaque samples.

Selection of patients and collection of samples

This study was conducted in Makkah Al-Mokarammah city, Faculty of dentistry, Umm Al-Qura University. A sample size of 20 female patients was selected according to the following criteria: 1. They were diagnosed with chronic gingivitis and/or periodontitis, 2. Their ages ranged from 20-40 years old, 3. They were systemically free, and 4. They had good oral hygiene.

The intended procedures for each female patient were explained and the informed consent was signed by them. For the plaque samples collection, a proper reflection and isolation of the working field were performed followed by dryness of the teeth with sterile gauze. Then, the plaque samples were pooled from many teeth and gathered using sterile wooden toothpicks and placed in the previously prepared eppendorf tube containing 100 **µl**NaOH and 150 **µl** of sterile TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.6). The tubes were labeled by patient code and stored at -20°C pendantfor further processing. The plaque index was determined for each patient using modified *Quigley-Hein plaque index* where the patient was instructed to use disclosing tablets and the score of each tooth was detected buccally and lingually according to the amount of extension of the plaque as follow; (Figure 1):

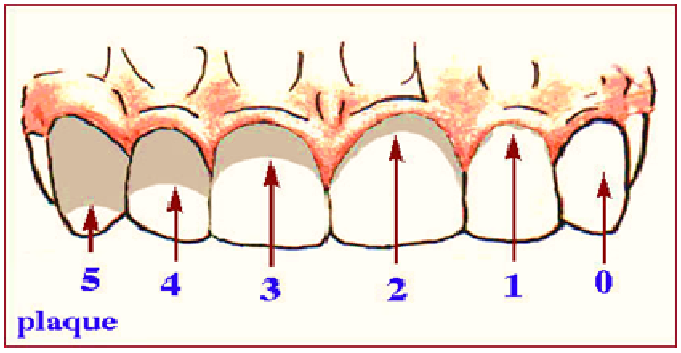

Figure 1.

0: No plaque.
1: Isolated flecks of plaque at the gingival margin.
2: A continuous band of plaque up to 1mm at the gingival margin.
3: Plaque greater than 1mm in width and covering up to one third of the tooth surface.
4: Plaque covering from one thirds to two thirds of the tooth surface.
5: Plaque covering more than two thirds of the tooth surface.

The Plaque Index (PI) was calculated as follow**:**

PI = Total score / Number of the examined surfaces

The experimental mouth wash; Siwak, and the control mouth wash; chlorohexidine (CHX), were placed in glass bottles of

the same color, shape and size and labeled as A and B; respectively and the usage instruction were also written on the label. Each patient was asked to select randomly either a bottle A or a bottle B. Both patients' groups (n=10); experimental (A) and control (B), were instructed to use the mouthwash twice daily for two weeks with their regular oral hygiene measure. After two weeks, plaque samples were recollected again and the plaque indices were re-determined as previously performed.

Table 2. The descriptive statistical analysis of Plaque Index before & afteruse of mouthwash

Mouthwash	Before Mean ± SD	After Mean ± SD	P-value
Siwak	2.58 ± 0.73907	1.93 ± 0.97303	0.002
CHX	2.41 ± 1.04823	1.20 ± 0.97183	0.001
P-value	0.669	0.058	

Subjective assessment of the mouthwash

At the end of the study, the patients were given an electronic questionnaire on GOOGLE (Search Engine) to evaluate their mouthwash subjectively.

Microbiological study of the plaque samples

The collected plaque samples were microbiologically analyzed using caries risk tests (CRT, IvoclarVivadent AG, Schaan, Liechtenstein). CRT is used to evaluate *Streptococcus mutans* and *lactobacilli* bacterial count in the collected plaque samples by means of selective culture media. Each test vial contains two selective agar surfaces fixed on agar carrier, blue mitis-salivarius-agar with bacitracin on one of the agar carrier surfaces for determination of *Streptococcus mutans* count and bright Rogosa agar on the other surface for determination of *Lactobacilli* count. As recommended by the manufacture instructions; 1. The stored plaque samples were left for two hours for retrieval from freezing. 2. Eppendorf tubes were mixed by vortex for one minute. 3. The agar carriers were removed from the test vialsto peel cautiously the protective foils off both agar surfaces andwet with plaque samples thoroughly using a pipette. The carrier was held obliquely to allow excess plaque solution to drip off. Then, $NaHCO_3$ tablets were placed at the bottom of each vial and carriers were re-inserted into the vial and closed tightly. 4. The patients' serial codes were labeled on each vial; after that, it was placed upright in the incubator at 37°C for 48 hours. 5. Under light source, the agar carrier was held slightly oblique for better evaluation of the density of the *Streptococcus mutans* and *lactobacilli* colonies. They were compared with the corresponding evaluation chart supplied by the manufacturer. They graded into 4 categories according to density of growth where (1) is the least colonized and (4) is the more colonized. The counts of the *Streptococcus mutans* and *Lactobacilli* colonies were expressed in terms of colony forming units per milliliter Colony Forming units (CFU/ml).

RESULTS

Plaque Index (PI)

As shown in *Table 2*, logically, PI of both groups exhibited no significant differences before starting the usage of the mouthwashes; 2.58 ± 0.73907 and 2.41 ± 1.04823 for Siwak and CHX respectively. As well, there is no significant differences between them after their use; 1.93 ± 0.97303 and 1.2 ± 0.97183 for Siwak and CHX respectively. A significantly different reduction of PI at P-level<0.05 was detected after the use of any of the investigated mouthwash denoting thatexperimental Siwak has a comparable effect to that of the gold standard mouthwash; CHX.

Subjective Assessment of mouthwashes

The given questionnaire involved questions about the used mouthwash if it had a bad smell, unpleasant taste, changed teeth color, altered taste sensation or caused burning sensation of tongue. The patients who used Siwak mouthwash did not complain of changing teeth color, changing taste or burning sensation of tongue. However, 60% of them complained of its bad taste and 10% complained of bad smell. On the other hand, the patients who used CHX did not complain of bad smell. Yet, 20% of them complained ofbad taste, 30% changing teeth color, 60% tongue burning sensation, and 80% changing in taste sensation, (Figure 2).

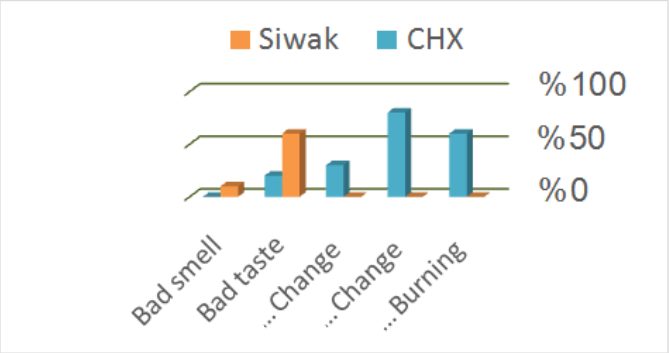

Fig.2. A histogram shows the subjective assessment criteria of siwak and CHX mouthwashes

Table 3. The descriptive statistical analysis of the bacterial counts before & after use of mouthwash

Bacteria / Mouthwash	*Lactobacilli*			*Streptococcus mutans*		
	Mean ± SD		P-value	Mean ± SD		P-value
	Before	After		Before	After	
Siwak	3.1 ± 0.994	1.7 ± 0.823	0.003	2.7 ± 0.949	1.2 ± 0.422	0.0001
CHX	2.8 ± 0.919	1.8 ± 0.789	0.001	2.5 ± 0.973	1.2 ± 0.422	0.002
P- Value	0.496	0.823		0.555	1	

Quantitative Evaluation of the Siwak Mouth Wash (Bacterial counts)

As shown in *(Table 3)*, the starting *Lactobacilli* meanbacterial count for both mouthwashes was insignificantly different before using the mouthwashes. *Lactobacilli* bacterial count is affected by the use of both mouthwashes significantly. For Siwak *Lactobacilli*mean bacterial count was 3.1 ± 0.99 and reduced to 1.7 ± 0.82. For CHX, *Lactobacilli* bacterial count was 2.8 ± 0.92 and reduced to 1.8 ± 0.79 at P-level < 0.05. As well, it has no significant differences between the two

mouthwashes after their use, *(Figure 3)*. Similarly, as regards tomeanbacterial count of *Streptococcus mutans* shown in *(Table 3)*,no significant differences between Siwak and CHX either before or after using both mouthwashes.Before mouthwash use,*Streptococcus mutans*mean bacterial count was 2.7 ± 0.95 and 2.5 ± 0.97 for Siwak and CHX respectively butafter their use,both recorded identical values (1.2 ± 0.42). However, there is an individual significant reduction in the bacterial count for each mouthwashbefore and after their usage at P-level < 0.05, *(Figure 4)*.

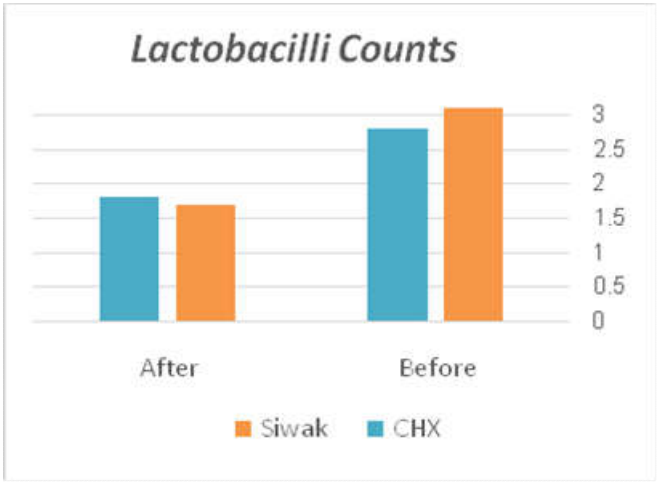

Fig.3. The mean values of Lactobaciili counts before and after mouthwash use

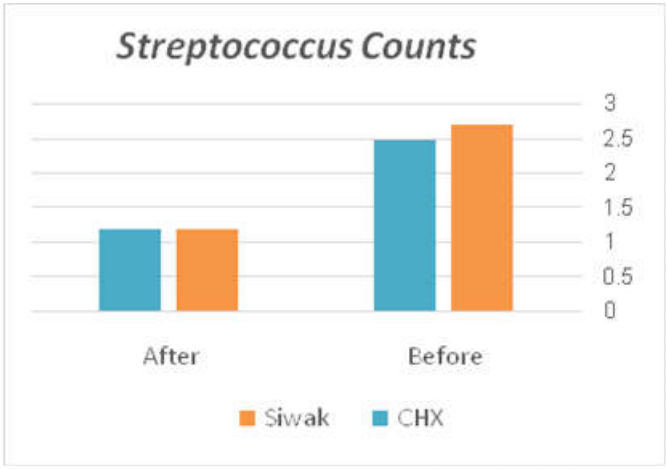

Fig. 4. The mean values of Streptococcus mutans counts before and after mouthwash use

DISCUSSION

Primarily, the biocompatibility and anti-mutagenicity of Salvadora Persica extract were proven after histopathological and immunohistochemical investigation carried out by *Shafie and Mubark, 2004*. The results were supported by *Al-Bayaty et al, 2010*,who evaluated the toxicological profile of the Miswakextract through intra peritoneal injection of albino mice with varying concentration of plant extract ranged from10 to 100 mg/ml. They reported no mortality among the experimental animals, which, in turn, indicated the well-systemic tolerance of the extract. Accordingly, the siwak extract was thoroughly investigated in this study. In agreement with numerous studies, the present investigation provided considerable evidence about beneficial effects of Siwak (Salvadora Persica) mouthwash on the oral tissues, besides, its well-known mechanical cleansing action. *Abo Al-Samh & Al-Bagieh, 1996*, reported that aqueous extracts of Miswak has bactericidal effect against several oral periodontal pathogens including Gram-negative bacteria but Gram-positive bacteria displayed either growth inhibition; bacteriostatic action, or even remained unaffected. *Almas & Al-Zeid, 2004,* assessed the antimicrobial activity of miswak*in vivo* particularly on salivary *streptococcus mutans* and *lactobacilli* count using CRT. Their results showed that there was a marked reduction of *Streptococcus mutans*among miswak (chewing stick), 50% miswak extract mouthwash, toothbrush, and normal saline. The reduction of *streptococcus mutans* was significantly greater using miswak in comparison to tooth brushing and there was no significant difference for *lactobacilli* reduction. They also concluded that miswak has an immediate antimicrobial effect; however, *Streptococcus mutans* were more susceptible to miswak antimicrobial activity than *lactobacilli*. Later, the potential beneficial effect of 50% miswak extract in oral hygiene had been confirmed by *Bhat et al., 2012*. It has very significant detrimental effect on both the dental caries causing micro-organisms; *Streptococcus mutans* and *lactobacilli*, present in saliva more than toothbrushing and saline mouthwash. *Al-Bahieh et al, 2012*, reviewed the antimicrobial andanticariogenic effects of Miswak on various aspects of oral health such as dental plaque, gingival health, and periodontal status and overall oral hygiene.

They guaranteed the regular use of Miswak as an effective oral hygiene aid. More recently, *Al-Dabbagh et al, 2016* carried out a randomized controlled clinical trial to evaluate the efficacy of Salvadorapersica (Miswak) products; tooth paste and mouthwash, on cariogenic bacteria; *Streptococcus mutans* and *Lactobacilli* using CRT. Miswak products, especially mouth wash, were more effective in reducing the growth of cariogenic bacteria than ordinary toothpaste and saline. The efficacy of Siwak extract could be attributed to the important chemical substances of Salvadora Persica that most likely affect the plaque and bacterial counts.*Farooqi & Srivastava, 1968 and Lewis & Elvin-Lewis, 1977*, isolated benzyl isothiocyanate from Salvadora Persica.Benzyl isothiocyanateis considered the main antibiotic component of the extracts against several types of cariogenic oral bacteria, which are specific for development of dental plaque. It can also inhibit the bacterial growth and its conjugated acid production (Sote, 1987). Moreover, Salvadora Persica contains salvadorine which has bactericidal effect and stimulatory action on the gingiva (Abo Al-Samh, 1997). The plant fibers contain sodium bicarbonate which is beneficial for healthy teeth, trimethylamine and vitamin C that help in healing of gum tissue, silica that can act as polisher, in addition to sulfur that permits warm feel and distinctive smell and fluoride which has anti-cariogenic effect, *Elmostehy et al, 1983,* confirmed the presence of fluoride and vitamin C.A plenty of Vitamin C in Salvadora Persica plays a role in reducing the plaque quantity, healing and repairing of soft tissue, *Al-Bagieh& Almas, 1997,* and*Mohana&Lindequist, 2005,* extracted Tannins (tannic acid) which has an astringent effect on the mucous membrane, thus reducing gingivitis and also hinder the action of glucosyl transferase resulting in reducing plaque and gingivitis. *Ghazi et al, 1990*; detected the presence of calcium and chloride; where calcium inhibits demineralization and promotes remineralization of the enamel and chloride inhibits calculus formation and removes the teeth stains. *In 2012, Saad, et al.* extracted Siwak oil from Miswak sticks to be analyzed chromatographically. Among 18 detected

compounds, thymol (isopropyl-5-methyl phenol) was present by 7.21% and carvacrol was present by 7.31%. Thymol is part of a naturally occurring class of compounds known as biocideswith strong antimicrobial characteristic when used alone or with other biocides such as carvacrol. In addition, thymol can reduce bacterial resistance to common drugs such as penicillin Numerous studies have demonstrated the antimicrobial effects of thymol, ranging from inducing antibiotic susceptibility in drug-resistant pathogens to powerful antioxidant properties (Ündeğer, 2009). Thymol and carvacrol reduce bacterial resistance to antibiotics through a synergistic effect, and thymol has fungicidal effect, particularly against fluconazole-resistant strains. This is especially relevant to *Candida* infections (https://en.wikipedia.org/wiki/Thymol). Also, eugenol was detected by 7.88%. It has well-known local antiseptic and analgesic effect (http://www.indepthinfo.com/ nutrition/eugenol.htm). They also confirmed the presence of 1.062 mg/L fluorine, and 34.3 mg/ 100gm ascorbic acid (Vit. C) in Siwak oil.

The present investigation has been confirmed the antimicrobial effect of CHX. CHX is considered the gold standard mouth rinse since few decades because of its safety and efficacy [37].CHX is able to bind to salivary mucins, tooth structure, dental plaque, and oral soft tissues and then released slowly into the mouth. Its long lasting antibacterial activity is accompanied with broad spectrum action as it is active against a wide range of Gram-positive and Gram-negative microorganisms. Its antibacterial action is most likely due to the immediate bacteriocidal action, followed by prolonged bacteriostatic action. It can also penetrate plaque biofilm and produce changes in microbial cell surface morphology that alter co-aggregation and re-colonization [38].

Based on the obtained results of the subjective questionnairesurveyed inthe present investigation, Siwak has better tolerance as mouthwash rather than CHX as the patients who used CHX complained from teeth discoloration, taste alteration, burning sensation. This confirmed the results of *Farah et al. in 2009*[12], who recommended CHX use twice-daily at only a short-term, as long-term use has numerous adverse effects, which include tooth and restoration staining, increased calculus deposition, unpleasant taste, taste alteration, and burning sensation. At last, it should be pointed out that the benefits of Miswak have been proved a long time ago religiously, as our prophet Mohammed, peace be upon him recommended us to use Miswak saying “Were it not that I might over-burden the Believers I would have ordered them to use Siwak at the time of every prayer”[8].

Conclusions

Within the confronted limitation in this study, the following could be concluded:

- Using Siwak extract is a safe, effective, has no side effects, and very well tolerated mouthwash.
- Siwak extract mouthwash has demonstrated a significant effect in diminishing plaque index and counts' reduction of cariogenic bacteria that aid in dental plaque formation comparable to that of the gold standard mouthwash; CHX.
- Qualitative assessment of the experimental Siwak mouthwash proved better patient's tolerance than CHX.

Recommendations

- Some ingredients might be added to the formula of the experimental Siwak mouthwash to improve taste and smell.
- The experimental mouthwash; Siwak, needs further investigations regarding product validity periods, different concentration rather than the investigated percentage (10%), and the effect of its prolonged use.
- A cross sectional study of a larger sample size is necessary for better assessment of Siwak experimental mouthwash besides evaluation of other oral health indices e.g. bleeding index.
- Moreover, Siwak extract is considered a new era for further oral and dental applications.

Conflicts of Interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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