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Comparative Microbiological Study Between the Miswak (*Salvadora persica*) and the Toothpaste

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Abstract: The oral cavity harbors a diverse and abundant number of complex oral pathogens causing different oral diseases. Miswak, a natural toothbrush has been documented as potent antibacterial effect. Also toothpaste is used to promote oral hygiene. Toothpaste active ingredients (Most commonly fluoride) one of the most common symptoms of excess fluoride is "dental fluorosis" which shows discoloration of the teeth. So that this study was to investigate the efficiency of antimicrobial effect of Miswak as natural plant good for oral health confirming by the Hadith and the traditions relating to the life of Prophet Muhammad (PBUH) comparing to the synthetic toothpaste. Therefore, the hot and cold aqueous extracts from Miswak and toothpaste contain sodium fluoride were evaluated for antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* (Oral pathogens) using ordinary disk diffusion agar method and modified agar well diffusion method also comparing the microbial cultural growth before and after using Miswak and toothpaste. The zone of inhibition of tooth paste was less (14mL/26mL) in comparison to Miswak which show the maximum efficiency (25mL/35mL) against the test organism. By culturing method the growth of bacteria was less on the plate after using both the tooth paste and Miswak but Miswak was better in its efficacy. In the present study it has been demonstrated that Miswak is more efficiency antimicrobial agent against *Staphylococcus aureus* and *Candida albicans* tested (Oral pathogen).

Key words: Antimicrobial · Natural Toothpaste · Miswak Miracle · Fluorosis

INTRODUCTION

Man has long been interested in his appearance and maintaining a clean, pleasant appearing mouth and a nice smile.Tooth cleaning aids and toothpicks are traceable back in history [1]. Chewing sticks were used by the Babylonians as early as 3500 BC. Ancient Greek and Roman literature discusses toothpicks that were chewed to help cleaning the teeth and the mouth [2].

Ancient Arabs were accustomed to Miswak to get their teeth white and shiny [3]. The toothbrush was strictly a "novelty sold in Paris for the cleaning of the teeth" [1]. Miswak not only offers economical cleaning of the teeth, but also has been traditionally used by many cultural groups for centuries and religion reinforced these traditions. The influence of Islam on the spread and the use of chewing sticks in different parts of the world are important [4]. Muslims follow the example of the prophet, and according to him (PBUH), the Miswak should be used five times a day before each prayer [3]. In Pakistan more than half of the rural populations use chewing sticks as an oral hygiene tool [5]. Several studies investigating oral hygiene habits in Saudi Arabia highlight the use of Miswak is popular as an oral hygiene aid in different population categories [6-9]. Which are mainly related to age and socio-economic level and to a lesser extent gender [10]. In Africa, the use of chewing sticks is still wide spread and chewing sticks arewideyused in Sudan, Nigeria and Namibia [11-13]. The name Miswak, also called miswaak, Miswaki, siwak, siwaki depending on the arabic dialect and the country, is known in English as the natural toothbrush [14-16]. Throughout the world, 182 species of plants have been used as chewing sticks, with 158 known to Africa aloe. In Ghana and Nigeria, Teclea vardo-ordniana, Garcinia and Acacia species are preferred. Azadirachta indica (Neem tree) is the most popular species used in India, Pakistan and Nepal. In the America, Cornus florida (Dogwood) was used for many dental purposes. The most important species is *Salvadora persica*, also known as Arak, as it is the main

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plant used as chewing sticks from East Africa through to the Asian subcontinent, including Saudi Arabia [14, 17]. *S. persica* is a small tree or a shrub with a crooked trunk; usually one foot in diameter the roots are spongy and easy to crush between the teeth. Pieces of the root usually swell and become soft when soaked in water [18]. For the root to be prepared for cleaning teeth, the stick is typically cut into 15 cm lengths. The width of the stick is usually from 0.7 to 1 cm wide. The stick is washed with water to free it from sand and before use, the bark is removed from the functioning end (About 1 cm long) of the stick, beaten hard or chewed until it becomes frayed. The techn-iques employed to mechanically remove plaque are similar to that of the toothbrush the cleaning movement should always be directed away from the gingival margin of the teeth on both buccal and lingual surfaces. Circular movements can be use to massage the gum, but care should be taken not to damage the soft tissue of the mouth. Fresh soft Miswak is preferred soaked in fresh water for 24 hours before use [17, 19]. The presence of odorous components is considered a good indicator for tooth brushing efficiency [20]. The same head is not recommended to be used for more than 24 hours because of cytotoxic activity [21].

Usually, the teeth are brushed for 5 to 10 minutes but some users chew on the stick for several hours while involved in other activities: this habit lead to the name "Chewing stick" [15]. However, the term chewing stick is considered misleading as the stick is chewed only briefly to fray its fibres before its common use as a tooth-brush [22].

Toothpaste is a paste or gel dentifrice used with a toothbrush as an accessory to clean and maintain the aesthetics and health of teeth. Toothpaste is used to promote oral hygiene: it serves as an abrasive that aids in removing the dental plaque and food from the teeth, assists in suppressing halitosis and delivers active ingredients (Most commonly fluoride) to help prevent tooth and gum disease (Gingivitis) so toothpaste is not intended to be swallowed due to the fluoride content, but is generally not very harmful if accidentally swallowed in small amounts; however, one should seek medical attention after swallowing abnormally large amounts lead to dental flourosis [23]. So this study aim to investigate the efficiency of antimicrobial effect of Miswak as natural plant good for oral health confirming by the Hadith and the traditions relating to the life of Prophet Muhammad (PBUH) comparing to the synthetic harmful toothpaste containing fluoride.

MATERIALS AND METHODES

- Collection of materials: Miswak and toothpaste containing flouride (Signal toothpaste) were purchased from Cairo markets.
- Aqueous extraction:About 1gm of each respective selected Miswak,toothpaste and10 ml of hot and cold water were added to them in sterile test tubes kept for 1 week in room temperature until use.
- Preparation of inoculums: The *Staphylococcus aureus* (S.aureus) *Candida albicans* (C.albicans) microorganisms were isolated from mouth by oral swab and were cultured on both nutrient agar and sabaroud agar for overnight at 37°C for bacteria and 25°C for fungi. then the growing microorganisms were examined microscopically using Gram stain and biochemical tests were made for identification.
- Antimicrobial screening:The antibacterial activity of the Miswak (*S. persica*) extract and toothpaste was evaluated by using the disk diffusion test technique where the sterile paper disks were moisten for15min in 10% solution of extract. Oral swab streaked on nutrient agar plate then, these disks were placed on the streaked plate; the plate was incubated at 37°C for 24 h and examined for zone of inhibition. This procedure was duplicated using modified agar well diffusion method. In this method, nutrient agar plates were seeded oral swab. A sterile 4 mm cork-borer was used to cut four wells at equidistance in each of the plates. 0.2 ml of each extract was introduced into each of the four wells. The plates were incubated at 37°C for 24 h (48 h for yeast species). The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition (In mm) [24].
- Cultural examination:Oral swab was cultivated on nutrient agar plate before and after use of both Miswak and toothpaste respectively incubated at 37°C for 48 hr.

RESULTS

This investigation of antimicrobial activity was performed on Miswak and toothpaste (Table 1) Showed comparison in between the two antimicrobial activity examination methods and the result of examined extracts obtained by modified agar well diffusion method is better than that obtained by disk diffusion agar method.

Table 1: Comparison in between the two antimicrobial activity examination methods.

Examined extracts	Disk diffusion method	Modified agar well diffusion method
Miswak	25/30mm	26/ 35- 32/ 37mm
Toothpaste	14/ 19mm	26/ 29mm

Table 2: The diameter (mm) of zone of inhibition produced by Miswak and toothpaste

	Miswak extract				Toothpaste extract				
	Disk diffusion method		Modified agar well diffusion method		Disk diffusion method	Modified agar well diffusion method	Control+ve	Control+ve	Control-ve
Oral pathogens	Hot	Cold	Hot	Cold	Cold	Cold	Ciprofluxacin	Nystatin	Distelled water
Staphylococcus aureus	0	25	26	35	14	26	25	0	0
Candida *albicans*	0	30	32	37	19	29	0	16	0

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

	Using Toothpaste		Using Miswak	
Oral pathogen Cultural growth	Before	After	Before	After
Staphylococcus aureus	++ve	+ve	++ve	-ve
Candida albicans	++ve	+ve	++ve	-ve

(++ve = heavy growth)

(Table2) Showed the screening step in the preliminary studyfor antimicrobial activity was the disk diffusion test on agar diffusion method and by modefied well diffusion method these two tests were used in order to determine antimicrobial activityof hot and cold aqueous extracts. The diameter of the clear zone indicated the inhibition activity. Both toothpaste and Miswak showed antibacterial effect against *S.aureus* and *C. albicans* in cold extracts and only Miwak hot aqueous extract indicated antimicrobial effect by modified agar well diffusion method and it was more effecient also Miwak is more efficient antifungal than antibacterial.

(Table3) For cultural growth before and after use of Miswak and toothpaste the table showed that using Miswak and toothpaste were effective in decreasing the microbial growth on nutrient agar plate but Miswak is more effective than toothpaste.

DISCUSSION

Maintenance of good oral hygiene is the key of the prevention of dental disease [25]. The activities of oral micro flora being responsible for mouth odor and most oral disease are not in doubt. The need to keep these oral organisms to a level consists with oral healthy by antimicrobial agent inclusion in dentifrice has been stressed [26].

Data from the present study is in support of this assertion as all the investigated dental care product toothpaste and Miswak exhibited wide variations in their effectiveness against the tested oral microorganisms, feature that may have been largely due to their antimicrobial active ingredients (Table 1 and Table 2). Among the investigated toothpaste and Miswak our finding supported the hypothesis that Miswak is the most effective, based on the mean diameter of the zone of microbial inhibition produced by the Miswak in agar well diffusion method, against the tested oral pathogen *S.aureus* and *C .albicans* microorganisms this exceptional ability of Miswak is in agreement with (Sofrata) that suggesting the presence of volatile active antibacterial compounds in Miswak and the inhibition zones associated with the Miswak pieces clearly demonstrated much stronger inhibitory effects than the aqueous Miswak extract when it have been used on *S. mutans*, the Miswak pieces caused unexpectedly large inhibition zones of 3.4cm [27]. However, our preliminary test using 10% Miswak aqueous extract yielded an inhibition zone against *S. aureus* and *C. albicans* range of 2.5 / 3.7cm. This result was more than the result obtained in earlier studies in which 50% Miswak aqueous extract achieved an inhibition zone of 0.2 / 0.3cm [28-30]. The antibacterial effect of Miswak pieces on *A. actinomycetemcomitans, P. gingivalis, H. influenzae* and *L. acidophilus* cannot be compared with the crude extract, as there as no published studies on the effect of Miswak extract on these bacterial strains. It was also difficult to compare the antibacterial effect of Miswak pieces with that of known antibacterial substancesas the exact content and amount of Miswak pieces is unknown. Thus, the need to evaluate Miswak

antibacterial effect by standard methods of evaluating antibacterial substances prompted an alternative extraction method for obtaining an active Miswak extract Pharmacological studies indicated that with steam distillation it is possible to obtain essential volatile oil from the roots, stems and leaves of *Salvadora Persica* [20, 31, 32]. From GCMS analysis, the root oil comprises of Benzylisothiocyanate (BT) (70%), limonene (9.4%), pinene (8.7%) and flavonoids (2.55%) [20, 33]. Some of these compounds are known to have antibacterial, antifungal and antiviral activities [33-37] The Miswak exhibited stronger antibacterial activity against Gram –ve bacteria than Gram +ve bacteria, as evidenced by the pronounced differences in inhibition zones associated with the Gram-ve speices *A. actinomycetem-comitans*, *P. gingivalis*, *H. influenzae* and the Gram positive species *S. mutans* and *L. acidophilus*. Studies on the effects of BITC and flavonoids on Gram negative and Gram positive bacteria present contradictory results [37]. This may be due to the different assays used to test antibacterial effects and to variations within each assay. Well standardised studies are needed to identify which components of the oil extract have an antibacterial effect against Gram negative and Gram positive species. Comparison of the effect of suspended and embedded Miswak pieces disclosed that these suspended Miswak pieces had similar or stronger effects on Gram negative bacteria,whereas, the opposite was true for Gram positive bacteria where the effect of the suspended Miswak was substantially reduced. Most although there are naturally other, more polar, components in the roots of Miswak that could be obtained through water or alcohol extraction and which may contribute to the bactericidal activity, these components are far less potentin antimicrbial capacity and have low activity against Gram negative bacteria [38-42]. In our study the invistigation of antimicrobial activity was performed on Miswak and toothpaste. (Table 1) showed comparison in between the two antimicrobial activity examination methods and the result of examined extracts obtained by modified agar well diffusion method is better than that obtained by disk diffusion agar method. (Table 2) showed the screening step in the preliminary study for antimicrobial activity was the disk diffusion test on agar diffusion method and by modefied well diffusion method these two tests were used in order to determine antimicrobial activity of hot and cold aqueous extracts. The diameter of the clear zone indicated the inhibition activity. Both toothpaste and Miswak showed antibacterial effects against *S. aureus* and *C. albicans* in cold extracts (Table 3). For cultural growth before and after use of Miswak and toothpaste the table showed that using Miswak and toothpaste were effective in decreasing the microbial growth on nutrient agar plate but Miswak is more effective than toothpaste especialy the cold extract. This result which appearing the effectivness of cold aquoes extract of Miswak depending upon consumption of uncooked cruciferous vegetables. That is confirmed with the study of Aires *et al.* [43] and Beevi *et al*. [44] where suggesting that the same mechanism appears active in the release of BITC when a Miswak stick chewed on prior to mechanical cleansing of the teeth. As boiled Miswak sticks lose antibacterial activity, most likely due to inactivation of the myrosinase, this assumption is confirmed. It has previously been shown that BITC exerts antimicrobial activity; however, there ported repertoire of susceptible bacteria and killing efficiency differs, probably due to variations in the methods used for antimicrobial testing. So in our finding (Table 1) the modified well agar diffusion method is more better readiable method than the disk diffusion agar this is due to high concentration and amount of extract used in the modified one because the medium containing agar appeared to inhibit the diffusion of the substances released from pieces of Miswak sticks. To avoid the inhibitory effect [13, 45]. In addition to that the use of Miswak chewing sticks for cleaning teeth may be protective against oral pathogens strongly associated with the pathogenesis of periodontal disease and tooth loss. The kinetics of bacterial killing mediated by the essential oil was rapid. Within minutes the bacteria load was diminished by 1000 times. The short exposure required for bactericidal effect supported the assumption Miswak chewing sticks may be efficient for improving oral health. There are few studies evaluating the *in vivo* effect of the practice of Miswak sticks on oral health and conclusions vary with study design. Larger studies with DNA based analysis of the microbiota would be necessary in order to evaluate the benefit of Miswak chewing sticks in oral hygiene. The rapid killing mediated by *S. persica* root essential oil suggested BITC containing oil might target the bacterial membrane. Electron micrographs of the Gram negative bacteria *A.actinomycetem comitans* displayed protrusions of the bacteria cell membrane.This membrane effect resembled those reported for antimicrobial peptide treated bacteria [46]. And the rapid antimicrobial effect [47]. Antimicrobial peptides bind to and destabilise bacterial membranes and during this process the peptides are essentially consumed [48]. This mechanism of killing confers a stochiometric relation between required numbers of bound peptides

molecules in order to kill a certain number of bacteria [49]. Our results on Miswak and toothpaste suggested that Miswak is more effective and safe antimicrobial toothbrush than toothpaste especialy that contain flouride. Interestingly, in a recent publication because young infants and children under age 2 years can swallow most, if not all, of the toothpaste when brushing, there has been concern that the use of fluoride toothpaste containing 1,000-1,500 ppm F could give rise to enamel fluorosis of the front permanent incisors. Enamel fluorosis is a condition which can vary from minor white spots to unsightly yellow /brown of the enamel due to excessive intake of fluoride [50].

CONCLUSIONS

Miswak extract from *Salvadora persica* was more effective as antimicrobial against *S. aureus* and *C. albicans* than toothpaste. These findings determined in this study suggested Miswak might play a roll in periodontal disease prevention and for maintaining good oral hygiene. In our study the hot extract was less in its antimicrobial effect than the cold one this is may be due to loss of some active ingredients by hot water. So it is recomonded to use fresh Miswak and if soaked in cold not hot water before use. Further studies are warranted for exploring and identifying the underlying mechanisms of action of Miswak on bacteria. In favour of investigating the potential of Benzyl isothiocyanate as an antimicrobial substance for therapeutic use.The cytotoxic activities of fresh Miswak and Miswak oil need to be evaluated before the development of oral applications becomes a future reality.Further studies on active compound identification and suitable purification of these medicinal plants are suggested.with the increase of the awareness of people using this kind of plant as treatment by the suitable sterilization method to use to be more effective with taking in consideration that there aresome highly pathogenic strains which have high resistancy to a lot of antibiotics even the effect of the Miswak. The toothpastes should be kept out of reach of children and not to give them more than pea sized. In case of adults, check for properly labelled toothpastes with safe levels of fluoride content. It is recommended to use Miswak instead of toothpaste which confirmed by our Prophet Muhammad (PBUH).

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