Miswak as an alternative to the modern toothbrush in preventing oral diseases

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Summary

The history and use of miswak (tooth stick) as an oral tool as well as biological effects of *Salvadora persica* extracts are reviewed. An adult Sudanese population using miswak or a modern toothbrush regularly was examined by using clinical and microbial parameters. Use of Freeze-dried extract of miswak was analyzed for antimicrobial components. The results presented in this report showed lower caries experience in the miswak users than in subjects who used a modern toothbrush. There were no significant differences in the periodontal variables of the two groups except for less calculus in the posterior sextants of miswak users. The results also indicate that regular use of miswak has a significant inhibitory effet on the levels of some salivary and subgingival plaque bacteria. Miswak extracts contained antimicrobial components including Cl^-, SO_4^{2-}, SCN^-, NO_3^-. These findings partially supported the hypothesis that adult Sudanese regular miswak users have better oral health and lower levels of oral pathogens than have adult Sudanese who use a modern toothbrush regularly.

(Introduction)

Miswak

Miswak (siwak and several other synonyms are being used) is an Arabic word meaning tooth cleaning stick [1, 2]. In English, miswak has been mentioned as the “natural toothbrush”. The use of miswak can be traced back at least to pre-Islamic times [3]. Presently, many of the world populations including India, Pakistan, several African countries, the Arab countries and most of the Muslim world still use miswak [4]. In geographical regions in which the Arak (Araak) shrub or tree (botanical name *Salvadora persica L*) grows, miswak is interpreted as tooth sticks prepared from this plant. Where *S. persica* is not growing, miswak is prepared from other suitable plants. Miswak is a pencil-sized stick 15-20 cm long and with diameter 1-1.5 cm that is prepared from the root, stem, twigs or bark. The stick is chewed or tapered at one end until it becomes frayed into a brush.

Cleansing efficacy of miswak

Various explanations for the cleansing efficacy of miswak have been offered including: (i) the mechanical effects of its fibers, (ii) its release of beneficial chemicals or (iii) a combination of (i) and (ii) [5]. Also, when the mouth cleaning procedure that includes brushing of teeth, gums and tongue is completed, miswak is removed from or may be left in the mouth for some additional time. Left in the mouth, it will stimulate salivation and thus there may be a better cleansing effect.

Mechanical effect of miswak

Miswak is generally used for a longer period of time than is a modern toothbrush and the cleaning is usually implemented for 5 to 10 min each time [6]. The plant fibers remove plaque and simultaneously massage the gum. Unlike a modern toothbrush, the bristles of miswak are situated along the long axis of its handle. Consequently, the facial surfaces of the teeth can be reached more easily than the lingual surfaces or the interdental spaces. Eid et al. [7] reported that the majority of miswak users applied miswak to both aspects of their teeth and no significant differences in facial plaque scores were noted between the miswak and toothbrush users. Additional studies suggested miswak efficacy to be comparable with that of the conventional tootbrush [8, 9] or demonstrated plaque scores to be significantly lower following the use of miswak as compared with the conventional toothbrush used without toothpaste [10].

Beneficial substances in *S. persica* extracts

A variety of chemical components have been identified in *S. persica* extracts. Some of these have been suggested to contribute to the cleansing efficacy of miswak. Miswak extracts have been shown to inhibit the growth of different microorganisms. Also, decoction of *S. persica* has been used for the treatment of many diseases. These topics have been extensively reviewed elsewhere [11,

2, 12]. It remains, however, to be shown that beneficial substances are leaching out in saliva while miswak is kept in the mouth in amounts that will benefit oral health.

Epidemiological studies

Periodontal disease

Low periodontal treatment needs have been reported among Saudi adults who used miswak [13, 14]. Furtheremore, Gazi et al. [10] compared the periodontal status of habitual miswak users and toothbrush and showed that the former had lower gingival bleeding and interproximal bone height than the toothbrush users. The authors also indicated that there were no significant differences in plaque scores and pocket depths between the two groups. In the northern Kenya, Carl and Zambon [15] suggested that advanced periodontal disease was very rare among persons over the age of 50 years who used miswak for teeth brushing. Eid et al. [16] reported that there was no significant differences in gingival or bleeding indices between miswak and modern toothbrush users.

Caries

In a dental health survey in the Sudan, Emslie [17] reported for the first time less caries in people using chewing sticks than in those using a modern toothbrush. In a controlled clinical study Baghdady and Ghose [18] compared the caries prevalence between Iraqi and Sudanese schoolchildren using the WHO DMFT (diseased, missing, filled teeth) index [19]. They reported that Sudanese schoolchildren showed lower caries prevalence due to the use of miswak and their diet. Similar results were noted in Saudi children aged 13 to 15 years when compared with children in western countries [20]. Again, the main preventive factor reported was miswak use by these children. Carl and Zambon [15] reported that dental caries was relatively rare among Kenyan

primary school children who were using only miswak as an oral hygiene tool. The authors concluded that caries in adults was mostly present in older persons and usually involved the maxillary and mandibular second and first molars, which are difficult to reach for cleaning with miswak. It has also been demonstrated that users of chewing sticks not prepared from *S. persica*, had low caries prevalence compared to modern toothbrush users [21].

Rationales of own studies

Surprisingly, despite the widespread use of miswak since ancient times, relatively little scientific attention has been paid to its oral health beneficial effects. In 1987, WHO [22] encouraged the developing nations to use miswak for oral hygiene because of tradition, availability and low cost. Recently, it was concluded that chewing sticks may have a role to play in the promotion of oral hygiene and that evaluation of their effectiveness warrants further research [23].

Own hypothesis

As a result of the long-existing tradition and motivation for miswak use as an oral hygiene tool in my home country, I formulated a work hypothesis that habitual miswak users in Sudan have better oral health and lower levels of oral pathogens in their saliva and dental plaque than have subjects who use a modern toothbrush regularly and that the oral health promoting effect of miswak is partly due to its content of thiocyanate.

Aims of my studies

The overall aim was to conduct a systematic evaluation of miswak as an alternative tool to the modern toothbrush in preventing oral diseases. This involved clinical, microbial and chemical assessment using modern scientific methods. Because periodontal diseases and dental caries are the most common

dental diseases in the Sudan, these diseases were selected to test my hypothesis.

Specific aims

1- To assess and compare in a population of adult Sudanese habitual miswak users and toothbrush users:
   a) their periodontal status using prevalence of gingival bleeding, dental calculus, probing pocket depth (PPD) and clinical attachment level (CAL) as clinical parameters,
   b) salivary bacterial levels and their relationships with the periodontal status and experience of caries, respectively, of the test subjects,
   c) levels and associations of subgingival plaque bacteria with respect to oral hygiene and periodontal status at the sampled sites.
2- To identify and quantify some potentially antimicrobial anionic components of *S. persica* root and stem aqueous extracts.

Materials and methods

Study group

The participants of this study were selected among employees and students at the Medical Sciences Campus, University of Khartoum, Khartoum, Sudan. A total of 213 individuals volunteered to participate in the study. Their age ranged between 19 and 65 years (mean 36.6 ± 8.7 years), and they included 201 males (mean age 36.6 years) and 12 females (mean age 35.6 years). The selection criteria used for inclusion in of subjects in the study have been published elsewhere [24]. *S. persica* stems and roots were collected, powdered, extracted and freeze- dried as described previously [25].

Clinical examinations

The periodontal status of the study subjects was assessed by the Community Periodontal Index (CPI) [26] and their caries experience was recorded as present or absent according to the WHO caries criteria [26].

Selection of subjects for biological sampling

Subjects used for biological sampling included those of the study group who had chronic periodontitis as described by Wiebe and Putnins [27]. Criteria for selection required at least one maxillary and one mandibular tooth with PPD ≥4 mm that showed gingival bleeding on probing. Using the measurements of PPD, one maxillary and one mandibular posterior tooth exhibiting 4-5 mm or ≥6 mm PPD and bleeding on probing were selected for bacterial sampling. If the subject had more than 2 qualifying teeth in each jaw, the 2 teeth to be sampled were randomly selected. The teeth numbers were written on pieces of paper, each piece was closed, mixed up and finally 2 were drawn blindly. If posterior teeth were missing, anterior teeth were used instead. Seventy-four subjects were sampled. These included 38 miswak users (27 males and 11 females) and 36 toothbrush users (35 males and 1 female). Fifty-six of the subjects also donated saliva samples; 30 were miswak users (19 males and 11 females) and 26 toothbrush users (25 males and 1 female). There was no significant age difference between the two groups.

Collection of biological samples

Collection of saliva from the selected study subjects has been reported [28]. Immediately after saliva collection, the teeth of each individual selected for subgingival plaque sampling were isolated with cotton rolls, carefully scaled supragingivally with sterile Gracey curettes, cleaned with sterile cotton pellets and dried with air. A sterile curette was then inserted into the pocket and subgingival plaque was collected by multiple scaling strokes of the 6 probing sites per selected tooth. The plaque collected from one maxillary and one mandibular tooth (totally 12 sites) of each subject was pooled and immediately transferred into one Eppendorf tube containing 150 µl of sterile TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 7.6) and the plaque samples were then suspended into the buffer by shaking.

Identification and quantification of bacteria

Handling of the biological samples and identification and quantification of bacteria using whole genomic DNA probes from 28 bacteria (plaque samples) or 25 bacteria (saliva samples) and checkerboard DNA-DNA hybridization [29] have been published [28, 30].

Chemical analysis of *S. persica* freez-dried extract

Aliquots of the powdered extracts were reconstituted in sterile distilled water and filtered through a 0.2mm membrane (Millipore Corp.) and used for identification and quantification of potentially antimicrobial anionic components by using capillary electrophoresis [25].

Results

Periodontal status

Fifty-four percent of the study population had one or more sextants with gingival bleeding and 31.9% dental calculus in one or more sextants. Approximately 10% of the subjects had one or more sextants with PPD 4-5 mm and about 2% had sextants with PPD ≥6 mm. Fifty-one percent of the subjects had CAL ≥4 mm in one or more of the sextants. Subjects in the age group 40-65 years had significantly ($p=0.004$) higher numbers of sextants with gingival bleeding and PPD 4-5 mm ($p=0.03$) and CAL ≥4 mm ($p=0.02$) than had the 30-39 year group. The overall effect of the two oral hygiene methods showed no marked differences as assessed by the periodontal variables used in this population. However, when the data were reanalyzed in order to test the effect of tooth type, the results demonstrated that miswak users had significantly ($p=0.002$) lower numbers of sextants with dental calculus in the posterior sextants than had toothbrush users (Table 1).

Salivary bacteria

Subjects with one or more sextants with PPD 4-5 mm or two or more sextants with CAL ≥4 mm had similar levels of most salivary bacteria compared to subjects without attachment loss or deep PPD. Presence of $\geq 10^5$ *L. acidophilus* bacterial cells in saliva was significantly correlated with the subject's caries score (p=0.02). The percentages of subjects with detectable levels of *A. actinomycetemcomitans, P. melaninogenica, C. rectus, P. micros, V. parvula, S. mutans, S. anginosus, A. israelii, C. sputigena*, and *C. gingivalis* were significantly higher in the miswak group whereas for *P. intermedia, F. nucleatum, S. sputigena, E. corrodens, L. acidophilus, S. sanguis, S. salivarius, S. oralis,* and *S. mitis* the percentages of subjects were significantly higher in the toothbrush group (Table 2). In the miswak group 16 (53.3%) subjects had one or more teeth with primary or recurrent caries score (1 or 2) as compared to 36 (76.9%) subjects among the toothbrush users (p=0.03).

The findings suggest that miswak may have a selective inhibitory effect on the level of certain bacteria in saliva, particularly several oral streptococci species.

Subgingival bacteria

The detection frequencies of the 28 investigated species at $<10^5$, 10^5 and $\geq 10^6$ bacterial cells varied between 33.8% and 100%, 1.4% and 37.8%, and 1.4 and 41.9%, respectively. Small percentages of subjects had 10^5 bacterial cells of *S. mutans* (1.4%) and *S. sobrinus* (5.4%). At 10^5 bacterial cells threshold, the detection frequencies of the investigated species varied between 2.6% and 47.4% in the miswak group and between 2.8% and 36.1% in the toothbrush group. Similarly, the prevalences of periodontopathic species including *P. gingivalis, T. denticola, B. forsythus, P. intermedia*, *A. actinomycetemocmitans*, and *V. parvula* were between 10.5% and 36.8%, and between 2.8% and 19.4% in the two groups. There were significantly more miswak users than toothbrush users harboring *S. intermedius* (p=0.05), *A. actinomycetemocmitans* (p=0.03), *V. parvula* (p=0.04), *A. israelii* (p=0.02)

and *C. gingivalis* (p=0.03), and significantly fewer of the former group harboring *S. sputigena* (p=0.01), *S. salivarius* (p=0.04), *A. naeslundii* (p=0.02) and *S. oralis* (p=0.001). Significantly more subjects with PPD ≥6mm harbored *P. gingivalis* (p=0.001), *T. denticola* (p=0.002), *B. forsythus* (p=0.04), *F. nucleatum* (p=0.003) and *V. parvula* (p=0.001) than did the subjects with PPD 4-5 mm. Comparison of the subgingival plaque species at 10^5 and 10^6 bacterial cells by gender and oral hygiene type showed no significant differences except for *A. actinomycetemcomitans* (p=0.01) and *S. mitis* (p=0.04) which were present in higher percentage in females than males. Subjects in the age group 40-53 years had significantly higher number of *P. gingivalis* (p=0.001), *B. forsythus* (p=0.04), *P. intermedia* (p=0.05), *C. rectus* (p=0.02) and *C. sputigena* (p=0.02) than had the 19-39 years group. The findings indicate that the type of oral hygiene had a significant effect on levels of 11 out of the 28 species, and that the type of effect was also dependent on type of bacteria and PPD.

Correlations between salivary and subgingival bacteria in autologous samples

There were significantly higher percentages of subjects with $\geq 10^5$ bacterial cells of *P. intermedia, C. rectus, V. parvula, S. mutans, L. acidophilus, S. anginosus, S. salivarius*, and *L. buccalis*, and significantly lower percentages of subjects with *T. denticola* in saliva than in subgingival plaque ($p \leq 0.01$). Significantly higher percentages of subjects demonstrated $\geq 10^6$ bacterial cells of *S. sputigena, S. anginosus, S. sangius* and *S. salivarius* while significantly lower percentages of subjects showed *P. gingivalis* in saliva than in subgingival plaque ($p \leq 0.01$). Significant correlations between the levels of salivary and subgingival plaque bacteria were exhibited between *P. gingivalis, F. nucleatum, S. sputigena, S. sangius*, and *S. mitis* ($p \leq 0.05$) (Table 3). When such correlations were made separately for miswak users and toothbrush users, the former group demonstrated significant correlations between the levels of *F. nucleatum* and *S. oralis* ($p \leq 0.01$). No significant correlations were

shown between these bacterial levels in the toothbrush users. The results indicate that the levels of *P. gingivalis, F. nucleatum, S. sputigena, S. sangius* and *S. mitis* were significantly correlated in autologous saliva and subgingival plaque. A higher accuracy of detection and assessment of the levels of these bacteria in the oral cavity may be achieved by concurrent sampling of saliva and gingival plaque.

Identification and quantification of some potentially antimicrobial anionic components of miswak extracts

The results showed that *S. persica* root and stem deionized distilled water extracts contained Cl^-, SO_4^{2-}, SCN^-, and NO_3^- (Figs 1 and 2). However, the concentrations of these four anionic components differed considerably; stem extract contained more chloride (6.84%), sulphate (20.1%), and thiocyanate (0.38%) than did the root extracts (chloride 4.64%, sulphate 19.85%, thiocyanate 0.28%). Nitrate concentration was 0.05% in both extracts. The % values express w/w % of reconstituted freeze-dried extract.

Discussion

Resources for oral health care are limited in many developing countries and the need to explore and test easily available and inexpensive traditional preventive tools is recognized and supported by WHO [22]. This is also in line with a recent consensus [23]) stating that "chewing sticks may have a role to play in the promotion of oral hygiene" and that "evaluation of their effectiveness warrants further research". My hypothesis that habitual miswak users have better oral health and lower levels of oral pathogens than have individuals who use modern toothbrush regularly was not completely supported by the results of this study. The overall effect of the two oral hygiene methods showed no significant differences regarding the periodontal variables assessed in the Sudanese population. However, in order to see if tooth type may have influenced the results, the data were re-analyzed using a

bi-variate table which is somewhat similar to the analysis of co-variance. The results demonstrated that miswak users had significantly lower numbers of sextants with dental calculus in the posterior sextants than had the modern toothbrush users. This is in line with Almas and al-Lafi [31] who indicated that miswak chemical components contribute to its mechanical action in dental calculus removal. Miswak extracts contained high amounts of chloride [25] and substantial amounts of silica [32]. Recently, it has been shown that the commercially available dentifrice (Whitening Toothpaste) which contains 10% silica is efficacious for control of supragingival calculus formation [33]. Furthermore, miswak is generally used for a longer period of time than is the modern toothbrush, the cleaning is usually implemented for 5 to 10 min each time [6], and the plant fibers remove plaque and simultaneously massage the gum. Thus, my finding that miswak users had lower numbers of posterior sextants with dental calculus than had toothbrush users may be attributed to miswak`s chemical components and/or due to the differences in frequency and duration of brushing between the two methods. It has been suggested that the level of supragingival calculus is a fairly good measure of the oral hygiene level and the frequency of professional dental care in a population [34]. Calculus promotes and retains dental plaque on its outer surface and consequently is an important risk factor of progression of attachment loss [35]. My finding that there were no significant differences in the pocket depths between habitual miswak and toothbrush users is consistent with previous reports. Thus, Gazi et al. [10] demonstrated that there were no significant differences in plaque scores and PPD measurements between habitual miswak and toothbrush users. Eid et al. [16] also indicated that there were no significant differences in plaque scores and attachment loss between habitual miswak and toothbrush users.

The lower caries experience in miswak users [28] partially supports my work hypothesis of better oral health in the miswak group. This is also in agreement with previous epidemiological studies [17,18, 20, 15]. The lower caries

experience in the miswak users may be explained by the results of previous study [25], which demonstrated that miswak extract contained thiocyanate (SCN^-). Tenovuo et al. [36] showed *in vitro* that acid production by *S. mutans* in human dental plaque was almost totally inhibited when supplementing saliva with SCN^- and hydrogen peroxide. This is also in agreement with observations by Lenander-Lumikari et al. [37]. The finding of lower caries experience of miswak users can also be explained by the cleansing effect of miswak. When the mouth cleaning procedure is completed, miswak is often left in the mouth for some additional time. Left in the mouth, it will stimulate salivation and thus promoting a better cleansing and anti-cariogenic effect.

The present study compared miswak and toothbrush users among students and staff members of the University of Khartoum. This because of their wide age range and since their food habits and life style were more similar than those of the various Sudanese ethnic groups. To compare the clinical effect of miswak and toothbrush [24], I used the CPI [26], which is a system that uses a hierarchical and partial recording methodology. Recently, Baelum and Scheutz [38] indicated that the usefulness of the CPI/CPITN data for understanding the epidemiology of periodontal diseases is limited.

Darout et al. [28] is the first report applying the checkerboard DNA-DNA hybridization method [29] to assess bacterial levels in saliva. The results showed that several bacterial species including periodontitis-associated bacteria were detectable. This is in agreement with previous reports using different methods showing frequent detection of various bacterial species including periodontitis-associated species in saliva [39, 40]. The paper also demonstrated that the type of oral hygiene had a significant effect on the salivary levels of 19 out of the 25 bacterial species investigated, and that the type of effect also depended on the type of bacteria. Thus, 10 of these species were present in significantly higher numbers, and 9 were found in significantly lower numbers in the saliva of miswak users than of toothbrush users. These microbial differences may be due to release of antimicrobial

substances of miswak or other factors.

My finding that four out of the six *Streptococcus* spp. examined were detectable in significantly lower levels in the miswak group can be explained by the results reported in publication [25]. Several of the anionic components detected in miswak are known to have antimicrobial effects. Silva Mendez et al. [41] reported that nitrite exerted *in vitro* inhibitory effects against cariogenic bacteria including *S. mutans*, *L. casei* and *A. naeslundii* at acidic pH. The authors also demonstrated that the ability of these bacteria to recover from nitrite exposure was markedly affected by nitrite concentration. At acidity levels below pH 7, low concentrations of nitrite (0.2 mM) caused complete killing of the test bacteria. Gazi et al. [42] demonstrated that the use of miswak significantly decreased salivary pH due to its high chloride content. This condition may increase the bactericidal effect of nitrite in the mouth. Moreover, the bactericidal effect of nitrite is significantly enhanced by SCN^- [43]. The higher levels of some periodontal pathogens in the saliva of miswak users [28] may be due to a microbial shift from more streptococci to more periodontitis-associated species. If so, this would be in line with the ecological plaque hypothesis [44, 45]. Also, Hillman et al. [46] reported an antagonistic interrelationship between streptococci species and periodontitis-associated species. Thus, it has been suggested that growth of *A. actinomycetemcomitans* was inhibited by the potentially beneficial species such as *S. sanguis*. This may explain the weak effect of miswak use on some oral anaerobic (*P. gingivalis, E. corrodens, S. sputigena P. micros, T. denticola, C. rectus*) and facultative species (*C. sputigena, C. gingivalis, A. actinomycetemcomitans*).

This is the first report on detection frequencies and levels of subgingival bacteria and their associations in adult Sudanese habitual miswak users and toothbrush users [47]. My results showed that the type of oral hygiene had a significant effect on the subgingival plaque levels of 11 out of the 28 bacterial species investigated, and that the effect depended on the type of bacteria. Using 10^5 bacterial cells threshold, *S. intermedius*, *A. actinomycetemcomitans*,

V. parvula, *A. israelii* and *C. gingivalis* were present in significantly higher numbers in subgingival plaque of the miswak than of the toothbrush group. *S. sputigena*, *S. salivarius*, *A. naeslundii* and *S. oralis* were found in significantly lower numbers in the miswak group. When I used 10^6 bacterial cells threshold, *P. intermedia* and *S. mitis* were significantly higher in the toothbrush group than in the miswak group. The results comply with the levels of 25 of these bacterial species in salivary samples from the same individuals .

Similar to publication [28], the results of Darout et al. [47] showed that species including *P. gingivalis, T. denticola, C. rectus*, *F. nucleatum* and *L. buccalis* did not seem to be influenced by the type of oral hygiene used. This may suggest that the two oral hygiene methods had similar effects on the levels of these species. This is consistent with the findings of studies showing that supragingival plaque control had little or no effect on the levels of subgingival species, at least in sites with deeper probing depths [48, 49].

My work hypothesis that habitual miswak users have lower levels of oral pathogens than have individuals who use a modern toothbrush regularly was partially supported by the lower level of *L. acidophilus*, *P. intermedia, F. nucleatum, S. sputigena, E. corrodens, L. acidophilus, S. sanguis, S. oralis, S. salivarius, A. naeslundii,* and *S. mitis* in miswak users [28, 47].

Darout et al. [30] is the first report about the checkerboard DNA-DNA hybridization method being used to assess bacterial levels and their correlations in autologous saliva and subgingival plaque samples. Previously, Kononen et al. [50] correlated gram-negative anaerobes recovered by culture in saliva and subgingival samples of a group of young women. A relatively high percentage of the study group had detectable levels of several of the examined opportunistic and commensal bacteria in their paired saliva and subgingival plaque samples. The results of Darout et al. [30] showed that species including *P. intermedia, T. denticola* and *P. gingivalis* were more frequently detected in saliva than in subgingival plaque. This is consistent with a study that used PCR to assess the frequencies of six oral bacteria in

paired samples of unstimulated saliva and subgingival plaque of adult subjects in the USA [39]. The latter study showed that *P. gingivalis*, *P. intermedia*, *P. nigrescens*, and *T. denticola* were detected more frequently in saliva than in periodontal pockets. It has been shown that a large increase in the number of bacterial species on the teeth was reflected by an increase of these species in saliva [51]. The cariogenic bacteria *S. mutans* and *L. acidophilus* were demonstrated at detectable ($\geq 10^5$ bacterial cells) levels but not at high ($\geq 10^6$ bacterial cells) levels [28, 30]. Usually, high *S. mutans and L. acidophilus* counts indicate active caries or a high caries risk. This may not always be valid for Sudanese subjects who have been shown to have high prevalence of mutans streptococci even in populations with extremely low prevalence of dental caries [52]. The results of publication [30] showed that *S. mutans* and *L. acidophilus* occurred significantly more frequently in saliva than in subgingival plaque. This is in general agreement with the findings of Beighton [53]. Furthermore, Van Der Reijden et al. [54] using bacterial culture demonstrated in a Dutch population that the prevalences of mutans streptococci in subgingival plaque varied from 82% in untreated periodontitis patients to 94% in maintenance patients. The lower detection frequencies of mutans streptococci reported in study [47] may be attributed to the difference in the threshold levels of detection frequencies used in the two studies as well as ethnic differences.

Darout et al. [30] showed significant positive correlations between levels of *P. gingivalis*, *F. nucleatum*, *S. sputigena*, *S. sanguis* and *S. mitis*. Umeda et al. [39] used kappa statistics to estimate the agreement between bacterial levels in paired samples of saliva and pooled subgingival plaque. They found a fair agreement between saliva and plaque samples for *P. gingivalis*, *P. intermedia* and *T. denticola*, and a poor agreement for *A. actinomycetemcomitans*, *B. forsythus* and *P. nigrescens*. Except for *P. gingivalis*, my data did not support their findings.

Darout et al. [28, 47, 30] I used cutoff threshold levels based on comparisons of hybridization results to that of known concentrations of positive controls (10^5 and 10^6 cells). The scoring is in accordance with reports by others [55, 56, 57]. Miswak aqueous extracts contained potentially antimicrobial anionic components including Cl^-, SO_4^{2-}, NO_3^- and SCN^-. The finding of Cl^- in *S. persica* root and stem extracts is consistent with observations of Farooqui and Srivastava [56]. SO_4^{2-} and SCN^- in *S. persica* root and stem water extracts are released from hydrolysis of glucosinolates by myrosinase enzyme in the plant tissue [58, 59, 60]. Certain isothiocyanates under neutral conditions and in the presence of the enzyme myrosinase may decompose into their respective alcohol derivatives and SCN^- [61]. Darout et al. [25] indicate that the root and stem of *S. persica* are rich in SO_4^{2-}. The presence of SO_4^{2-} compounds in *S. persica* root and twigs has previously been reported [60]. Chemical analysis of air-dried *S. persica* stem extract showed high SO_4^{2-} content [32]. Antibacterial and weak anti-inflammatory effects of *S. persica* root and twig extracts have been attributed to their content of beta-sitosterol, SO_4^{2-} compounds and Cl^- [62]. In addition, Cl^- leaching into saliva from miswak while in the mouth may mediate the innate host defense systems in human saliva. Cl^-, I^- and SCN^- (pseudohalides) are substrates for salivary peroxidase and/or the myeloperoxidase hydrogen peroxide antimicrobial system. The peroxidase-hydrogen-peroxide-chloride system is a part of the innate host defense that is mediated by polymorphonuclear leukocytes in humans [63]. It has been shown that the latter system was more bactericidal against *A. actinomycetemcomitans* than with the myeloperoxidase-thiocyanate and hydrogen peroxide system [63]. Recently, Ihalin et al. [64] indicated that the oxidation product of lactoperoxidase and myloperoxidase with I^- and/or Cl^- was bactericidal against *P. gingivalis, F. nucleatum* and *S. mutans*. The lower levels of *P. intermedia* and *F. nucleatum* in the miswak users [28, 47] may be attributed to its Cl^- and SCN^- content.

NO_3^- in *S. persica* root and stem water extracts may be released from the residual nitrate ions taken up by the *S. persica* plant or from the oxidation of ammonia and other nitrogen compounds. Nitrate, nitrite and nitrosamines have been demonstrated to occur naturally in vegetables [41]. The antimicrobial agent nitric oxide (NO) is formed in the mouth and its concentration is directly related to salivary nitrite, which in turn is related to dietary nitrate intake [41]. It has been demonstrated that nitrite, upon its ingestion and mixture with gastric acid, is a potent bacteristatic and/or bactericidal agent. Acidified nitrite is bactericidal against gastrointestinal, oral, and skin pathogens [65]. Recently, Allaker et al. [66] reported that acidified nitrite exhibited growth-inhibitory effect on *F. nucleatum, E. corrodens* and *P. gingivalis*. Furthermore, it has been shown that the salivary nitrite exerted bactericidal effect on several pathogens such as *Escherichia coli*, *Salmonella typhimurium*, *Yersinia entrocolitica*, *Shigella sonnei* and *C. albicans* at acidic pH [66].

Darout et al. [25] demonstrated that miswak water extracts contained 0.05% NO_3^-. The salivary generation of nitrite is accomplished by a symbiotic relationship involving nitrate-reducing bacteria on the tongue surface, which are designated to provide a host defense against microbial pathogens in the mouth and lower gut via NO production [67]. Thus, the finding that miswak extract exhibited antimicrobial effects against *S. faecalis*, *P. aeruginosa, Actinomyces* spp. and *Staph. aureus* [68] may be due to its nitrate and high Cl^- content that creates acidified conditions for antimicrobial action of nitrate similar to that in the mouth and the stomach.

Furthermore, leachable SCN^- from miswak during use may also contribute to its antimicrobial effect [25]. This supports my work hypothesis that SCN^- may contribute to the antimicrobial effect of miswak. Elvin-Lewis [69] suggested that the antimicrobial effect of miswak may involve salivary peroxidase. I therefore hypothesized that SCN^- leaching out from miswak while in the oral cavity may lead to an elevated level of salivary SCN^-. This in turn may enhance the efficacy of the salivary peroxidase-thiocyanate and hydrogen

peroxide system, a known innate antimicrobial component of human saliva. There are data showing that the most susceptible bacteria to this antimicrobial system are the oral streptococci [70, 71], whereas other anaerobic oral bacteria (*C. sputigena, C. gingivalis, P. gingivalis, E. corrodens, S. sputigena P. micros, T. denticola,* and *C. rectus*) were less affected [72]. The susceptibility of oral streptococci to the salivary peroxidase antimicrobial system may vary with the species studied. For instance, *S. salivarius* and *S. mutans* were found to be more susceptible than other *Streptococcus* species [70]. The low pH of saliva found in miswak users [42] may decrease the proportion of acid-sensitive streptococci species such as *S. sanguis*, *S. oralis* and *S. mitis* and increase the proportion of *S. mutans*. This may explain the presence of higher levels of *S. mutans* in miswak users [30]. SCN^- in human saliva acts as a substrate for salivary peroxidase to generate hypothiocyanite ($OSCN^-$) in the presence of hydrogen peroxide (H_2O_2) produced by oral bacteria and leukocytes [71]. This principle is demonstrated by the following reaction:

$$H_2O_2 + SCN^- \xrightarrow{\text{Peroxidase}} OSCN^- + H_2O$$

$OSCN^-$ has antibacterial properties and other important protective functions for the host [73, 74]. For example, low concentrations of $OSCN^-$ *in vitro* have been shown to kill orally transmitted viruses and echovrius type 11 [75], human immunodeficiency virus [76] as well as *C. albicans* [77]. Furthermore, the salivary peroxidase-thiocyanate and hydrogen peroxide system protects oral mucosal cells from the toxic accumulation of H_2O_2 by converting it into non-toxic $OSCN^-$ [73]. SCN^- is not merely a co-factor in the salivary peroxidase antimicrobial system; it also acts as a ubiquitous vitaminoid and promotes unspecific and specific resistance mechanisms of the tissues against infections. Therefore, it has been used as an antiseptic for suppurating wounds, ulcers, infectious skin disorders, infections of the mouth and throat, and for caries prevention [78].

In saliva up to 5mM endogenous SCN^- is secreted by the salivary glands as the result of catabolism of thiols [79]. Rather high concentrations of SCN^- in

aqueous extract of *S. persica* were found in the present study. Miswak exposed to saliva during use may provide saliva with adjunct SCN^- which may serve as an external source for the salivary peroxidase-thiocyanate and hydrogen peroxide antimicrobial system [80]. It has been suggested that the possible enzymatic action of saliva on miswak isothiocyanate may release unstable products that exert antimicrobial activity *in vivo* [69]. In a clinical trial, Gazi et al. [42] found a significant increase in the salivary calcium and chloride levels with a significant decrease in pH of saliva and phosphate immediately after chewing miswak. The authors also showed that dental plaque and gingival bleeding indices were significantly lower after immediate use of miswak in comparison with immediate use of conventional toothbrush. Moreover, SCN^- from miswak together with low salivary pH may enhance bacteriocidal activity of nitrate against cariogenic bacteria. Rosin et al. [81] claimed that a dentifrice containing 0.5% SCN^- and 0.1% H_2O_2 inhibited dental plaque and gingivitis. My study shows for the first time that miswak aqueous extracts contain potential anionic components including SCN^- in both free and bound forms and above I postulate their possible mode of action against oral bacteria. If my work hypothesis on the contribution of endogenous SCN^- to the antimicrobial effect of miswak is correct, this implies that miswak, when in the mouth, may represent an external source of leachable SCN^-. However, it remains to be shown *in vivo* that saliva extracts SCN^- from miswak in adequate amounts and that such additional SCN^- really results in a more efficient peroxidase-thiocyanate and hydrogen peroxide antimicrobial system.

Conclusions

1. The periodontal status of miswak users in this Sudanese population was similar to that of the toothbrush users, suggesting that the efficacy of miswak use for oral hygiene was comparable to that of the modern toothbrush.

2. The miswak users had significantly less calculus in their posterial sextants than had the toothbrush users which may be due to anti-calculus effect of miswak.
3. The findings suggest that miswak may also have a selective inhibitory effect on the level of certain bacteria in saliva, particularly several oral S*treptococcus* species.
4. The results indicate that the type of oral hygiene used had a significant effect on the levels of 11 out of the 28 species investigated and that the effect was also dependent on type of bacteria and probing pocket depth.
5. This study indicates that the levels of *P. gingivalis, F. nucleatum, S. sputigena, S. sanguis* and *S. mitis* are correlated significantly in autologous saliva and subgingival plaque.
6. Miswak users showed lower caries experience and lower levels of the oral pathogens *P. intermedia*, *F. nucleatum*, *S. sputigena*, *E. corrodens*, *L. acidophilus*, *S. sanguis*, *S. salivarius*, *S. oralis*, and *S. mitis*. This is in support of my hypothesis that adult Sudanese habitual miswak users have significantly better oral health and lower levels of oral pathogens than have adult Sudanese who use a modren toothbrush regularly.
7. Demonstration of high levels of thiocyanate in aqueous miswak extracts complies with my hypothesis that antimicrobial effect of miswak may be due to its thiocyanate content.

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Table 1. The mean number of sextants per subject with gingival bleeding, supragingival calculus, probing depth, and clinical attachment loss adjusted for age, by oral hygiene group and tooth type

Variables	Oral hygiene group	Mean	S.E.	p-values
Anterior teeth				
Gingival bleeding	miswak	0.30	0.05	0.5
	toothbrush	0.34	0.05	
Dental calculus	miswak	0.22	0.04	0.4
	toothbrush	0.17	0.04	
Probing depth ≥4 mm	miswak	0.04	0.01	0.05
	toothbrush	0.009	0.01	
Attachment loss ≥4 mm	miswak	0.53	0.06	0.2
	toothbrush	0.42	0.07	
Posterior teeth				
Gingival bleeding	miswak	0.94	0.12	0.09
	toothbrush	1.22	0.12	
Dental calculus	miswak	0.11	0.05	0.002
	toothbrush	0.35	0.06	
Probing depth ≥4 mm	miswak	0.10	0.05	0.057
	toothbrush	0.23	0.05	
Attachment loss ≥4 mm	miswak	0.64	0.04	0.20
	toothbrush	0.55	0.04	

Table 2. Percentage of subjects showing detectable levels of bacteria in saliva, by type of oral hygiene habit and type of bacterial species.

Bacterial species	Miswak (n=30)		Toothbrush (n=26)		p
	No. of bacteria		No. of bacteria		
	10^5	$\geq 10^6$	10^5	$\geq 10^6$	
P. gingivalis	56.7	0	50.0	7.7	0.7
A. actinomycetemcomitans	33.3	30.0	11.5	0	0.0001
P. intermedia	56.7	3.3	50.0	26.9	0.03
P. melaninogenica	40.0	30.0	0.0	0	0.0001
F. nucleatum	20.0	0	42.3	15.4	0.002
T. denticola	20.0	0	8.0	4.0	0.5
C. rectus	73.3	13.3	23.1	11.5	0.001
P. micros	53.3	0	0.0	0	0.0001
S. sputigena	60.0	10.0	34.6	57.7	0.0002
S. intermedius	26.7	0	34.6	0	0.5
E. corrodens	40.0	0	34.6	30.8	0.01
V. parvula	60.0	13.3	11.5	3.9	0.0001
S. mutans	26.7	0	3.9	0	0.03
S. sobrinus	3.3	0	0.0	0	0.4
L. acidophilus	13.3	0	46.2	0	0.01
S. anginosus	50.0	46.7	19.2	19.2	0.0001
S. sanguis	53.3	3.3	30.8	57.7	0.0001
S. salivarius	70.0	3.3	57.7	34.6	0.002
S. oralis	20.0	0	57.7	7.7	0.0005
S. mitis	0	0	19.2	0	0.02
A. israelii	50.0	0	7.7	0	0.001
C. sputigena	73.3	3.3	11.5	0	0.0001
C. gingivalis	66.7	0	7.7	0	0.0001
C. gracilis	46.7	0	26.9	7.7	0.6
L. buccalis	30.0	0	19.2	0	0.4

Table 3. The correlations between the levels of autologous bacteria in intra-subject subgingival plaque and saliva samples.

Bacterial species	r-values	p-values
Opportunistic bacteria		
P. gingivalis	0.27	0.04*
A. actinomycetemcomitans	0.16	0.22
P. intermedia	0.25	0.06
P. melaninogenica	0.23	0.08
F. nucleatum	0.29	0.03*
T. denticola	0.10	0.46
C. rectus	0.05	0.71
P. micros	-0.04	0.78
S. sputigena	0.31	0.02*
S. intermedius	0.26	0.06
E. corrodens	-0.12	0.38
V. parvula	-0.12	0.39
C. sputigena	-0.14	0.29
C. gingivalis	-0.10	0.45
S. mutans	N.c.	N.c.
S. sobrinus	N.c.	N.c.
L. acidophilus	-0.02	0.89
Commensal bacteria		
S. anginosus	0.23	0.09
S. sanguis	0.30	0.02*
S. salivarius	N.c.	N.c.
S. oralis	0.25	0.07
S. mitis	0.26	0.05*
A. israelii	-0.02	0.89
L. buccalis	-0.03	0.81

N.c.= no correlation
*Statistically significant

Fig. 1. Electropherogram of a mixture of Br-, Cl -, So42, No3- and SCN- (ca 40 ppm each).

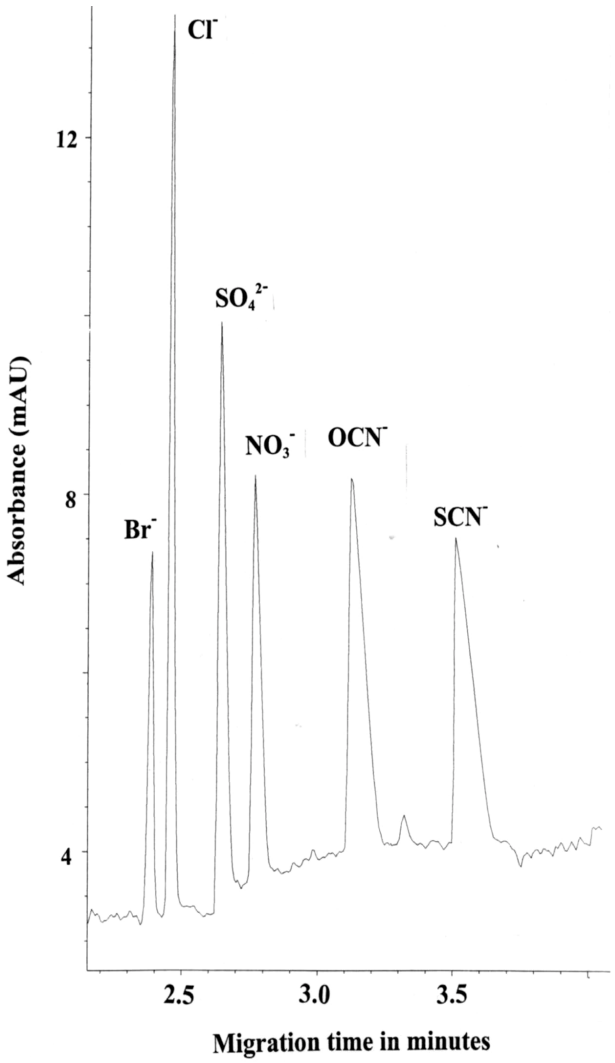

Fig. 2. Electropherogram of a solution of stem of *S. persica.*