MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 6(4) pp. 147-153, April, 2018
Available online http://www.meritresearchjournals.org/mms/index.htm

Original Research Article

The effect of alcoholic and aqueous Miswak extract on oral pathogenic bacteria

Ameena A. AL-Surhanee

Department of Biology, College of Sciences, AlJouf University, AlJouf, Saudi Arabia

Email: ameena.2020@hotmail.com

Abstract

Miswak, a chewing stick obtained from *Salvadora persica*, has been traditionally used in Islamic countries like Saudi Arabia and its use is well supported by religious as well as cultural beliefs. The Prophet (Peace Be Upon Him) also commanded and supported the use of miswak. Recent studies have indicated that toothpastes and antibiotics that kill oral microbes show adverse side effects. In the present work, we examined the inhibition effectiveness of alcoholic and aqueous extracts of miswak, tested at different concentrations (5%-20% w/v), by spreading it by drill on the five isolates of oral pathogenic bacteria that includes, *Streptococcus mutans, Actinomycetem comitans, Lactobacillus casei, Enterobacter aerogenes and Staphylococcus aureus*. We observed that the alcoholic and aqueous extracts of Miswak inhibits the growth of all the pathogenic bacterial isolates as compared to control sample and the inhibition of bacterial isolates increased by using higher concentration of the miswak extracts. It was observed that the inhibition effect of the alcoholic extract was higher as compared to the inhibition effect of the aqueous extract for all isolates and at same concentrations. The highest inhibition (34%, 45%, 59% and 72%) was observed for *Lactobacillus casei* upon the treatment with alcoholic extract of concentration, 5%, 10%, 15% and 20% w/v respectively whereas the percent inhibition upon the application of aqueous extract was observed to be 25%, 41%, 66%, and 54.6% respectively for the same concentrations as mentioned above. The inhibition efficiency of the miswak extracts against *Lactobacillus* was closely followed by *Streptococcus mutans* where the inhibition percentage for alcoholic extract treatment was observed to be 28.5%, 38.7%, 51%, and 69.35 %, whereas the percent inhibition reached to 18.4%, 34.7%, 47% and 65.4% for aqueous extract at 5%, 10%, 15%, 20% w/v concentrations respectively. On the other hand, *Staphylococcus aureus* showed the lowest percent inhibition (4.5%, 11.2%, 17.8%, and 44.4%) when treated with aqueous extract (5% - 20% w/v). This study has thus demonstrated the importance of miswak in the elimination of pathogenic bacteria present in the oral cavity. Therefore, more studies on miswak will definitely help to understand its importance in greater detail.

Key words: Miswak, alcoholic and aqueous extract, oral microbes, bacterial isolates

INTRODUCTION

The oral cavity houses a large and varied group of microbes. These microorganisms are very diverse and inhabit different surfaces of the oral cavity. Bacteria accumulate on oral tissues to form a layer also termed as dental plaques (Rogers, 2008).

Different bacterial species (500-1000), different types

of fungi (70-80), some viruses and parasites inhabit inside the mouth. These oral microorganisms not only exist on our teeth, but are also found on the gums, tongue and the mucous membrane of cheeks and lips. There are some organisms such as fungus Candida, that are present in mouth but don't cause disease unless the person is immunocompromised (Nazim). The two bacteria that are most studied bacteria are *Streptococcus* and *Lactobacillus* that belongs to lactic acid bacteria, and naturally exists inside the mouth. The members of the *Streptococcus* genus are present frequently on the teeth surface, at the junction of two teeth and they play a leading role in teeth decay process. *Streptococci are* gram positive spherical microbes (cocci) that are present in the form of chains and also perform anaerobic fermentation. *Lactobacillus,* on the other hand are rod shaped (bacilli) gram positive bacteria that are present in the form of chains and grow only on acidic medium (Atlas 1995; Turk et al., 1983). Saliva contains almost the same number of oral microorganisms and the most abundant microbes found in saliva are: *Streptococcus, Neisseria, Haemophilus, Staphylococcus, Lactobacillus, Veillonellae, Corynebacterium, Actinomycetes, Micrococcus, Proponibacterium, Escherichia, Proteus, Pasteurella, Treponema, Klebsiella, Pasteurella, Clostridium.*

The numbers of oral microbes, at any given time varies due to the microbial reproduction. Microbial reproduction results in production of toxic metabolites or results in change in pH or oxidation reduction potential (Carlsson, 1967).

Antibiotics have been used to treat these pathogenic microbes, but it results in some common side effects such as sensitivity, which is characterized by irritation and burning sensation that may require stopping of the antibiotic treatment (WHO, 2000). Toothpastes have been used to eliminate oral bacteria, but it is observed that the toothpastes do not treat dental decay and gingivitis but, nonetheless, plays an important role in protecting the teeth from the same (WHO, 2003). Dental caries is a tooth decay process resulting from the deposition of acid on the teeth due to the presence of residual food residue in the teeth. It was shown that no toothpaste can eliminate the presence of caries in the teeth. Dental caries causes partial erosion of the enamel and this erosion cannot be repaired by toothpaste. Toothpaste can only limit the spread of caries besides cleaning the teeth and keeping the mouth fresh (Gazi et al., 1990). Toothpastes contain fluoride that has been reported to affect many tissues and organs. It is also indicated that toothpastes can cause multiple problems for health such as disrupting the functioning of endocrine glands, effect on bone, brain, thyroid gland, blood sugar levels and others (al-Hanafi, 1962). Therefore, an alternative to toothpastes in order to eliminate oral microbes and fungi is required without causing any side effects. Miswak is one such natural product that has shown immense potential to maintain oral health as it produces certain chemicals substances that demonstrate significant biological properties such as anti-bacterial activity (Elvin-Lewis, 1980a; Eid and Selim, 1994; Almas and Al-Bagieh, 1999). Researchers have attempted to obtain the miswak extract from different plants and tested its effect on the overall physiology and functional characteristics of a number of oral bacteria like *Streptococcus mutans and Streptococcus sobrinu*s, which specialize in tooth decay. It was observed that miswak extract prevented the bacteria from producing harmful acids and some enzymes (Fadulu, 1975; Akpata and Akinrimisi, 1977; Wolinsky and Sote, 1983; Taiwo et al., 1999). Studies have shown that the miswak has a significant impact in inhibiting the growth of oral bacteria as it contains effective substances against bacteria. Miswak extract contains sulfur and trimethylamine that works to reduce the pH of the mouth and thus prevents the growth of the bacteria. Miswak extract also contains vitamin C and cetrol, and these two substances play an important role in protection the gums from inflammation whereas chloride, fluoride and silica present in the miswak extracts are known to increase teeth whiteness (Kamel and Yasmine, 2009). In addition to these compounds, the chemical analysis of the miswak extract has shown to contain sulfur compounds and ISO-Isothiocyanate that are responsible for the inhibitory activity of the extract towards different bacteria. Trimethylamine reduces adhesion of the bacteria to teeth surfaces and also removes accumulated bacterial plaques. Tannins, tannic acid, benzyl isothiocyanate, components of miswak, play a significant role in the inhibitory activity against microorganisms and in the treatment of gum infections (Poureslami et al., 2007). Inhibitory effect of the miswak extract that was observed against *Escherichia coli*, *Pseudomonus aeruginosa*, *Enterococcus feacalis* has been attributed due to presence of active nitrate (NO_3^-). Active nitrate (NO_3^-) acts against these bacterial species and also inhibits the oxidation process in both *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Darout et al., 2000). A study has shown that miswak removes the bacterial plaque before it becomes vicious and starts affecting the tissues. Frequent use of miswak leads to a high degree of oral hygiene and prevents pre-existing gum inflammation. Researchers are now recommending the permanent use of miswak to prevent the diseases of mouth and teeth (Hardie and Ahmed, 1995). A study conducted to

measure the effect of miswak on the levels of oral bacteria after the treatment. For the first time, it was shown that the use of Miswak reduces the presence of oral bacteria *Actinobacillus actinomycetemcomitan*, which considered as one of the most harmful microbe and is responsible for a large number of gum diseases and can also cause damage to the surrounding bones (Hattab, 1997).

It was also observed that miswak contains chloride with silica which in turn increases teeth whiteness (a gummy material covering the enamel) and protect teeth from decay. Triglyceride dimethylamine substance, found in miswak, reduces the pH of the mouth which is an important factors for inhibiting the bacterial growth, Vitamin C and Trimethylamine helps in healing gingivitis and maintains healthy gums whereas the presence of a sulfuric substance in the miswak extract prevents dental decay (Darout et al., 2000).

Interesting, a scientific study confirmed that the miswak plant (Arak) effectively eliminates all bacteria types that cause teeth decay and gum diseases, and the two ways by which the Miswak plant does that is via mechanical and chemical effects. The mechanical effect of miswak is that it helps in gum massage that in turn enhances the blood circulation and keeps them away from inflammation along with keeping the teeth clean. The chemical effect of miswak is due to a range of active substances. Most important of them is the substance "Thiocyanidetinovio" that has been shown to have a distinctive effect on acid production in saliva (al-Bagieh and Almas, 1997). Recent reports have indicated that the miswak, prepared from the arak stick, contains a large amount of tannins. Presence of tannins in miswak makes it a potent anti-fungal, anti-septic and an astringent agent that not only helps to stop the bleeding of the gums, but also makes them stronger. Miswak contains a mustard substance called Sinnigrin that acts as a disinfectant and astringent substance that also helps to kill microbes. It also contains glues, starch and salts, that in turn makes the saliva viscose and that further assists in oral cleaning process (Almas and Al-Bagieh, 1999).

An international scientific team that conducts scientific research on the use of miswak sticks found that it contains natural substances that have an effect that is similar to the antibiotics in the elimination of bacteria, pathogenic agents that exist in mouth and on teeth surface. Thus, using miswak regularly keeps mouth clean and safe from any microbial infection and prevents teeth from decay, and cavities (Almas, 1999). In a study it was indicated that the miswak sticks contain a set of soft fibers which rub the teeth and gums and works to prevent the propagation of any fungi or microbes inside the mouth. Miswak contains acids, salicylic crystals, starch granules, and other cleaning and antiseptics substances that penetrate inside teeth and gums layers and thus, significantly protects the oral cavity (Ezmirly et al., 1979). In Pakistan, a center for research conducted a study aimed to understand the application of miswak in the prevention of oral cancer. Recently, it has been indicated that the miswak comprises elements that have the ability to limit the growth of malignant cells (Hattab, 1997).

The present study is aimed to determine the anti-bacterial activity of the alcoholic and aqueous extracts of miswak plant by using different concentrations of the respective extract on different types of oral pathogenic bacteria. This study is also important as it is aimed to find a potent alternative to different anti-microbial agents like antibiotics to which the bacteria has become resistant.

MATERIALS AND METHODS

Preparation of the plant sample

Miswak sticks were bought from the local market in JOUF area. They were transported to the lab and were washed with distilled sterilized water. Miswak sticks were further dried on sterilized filter papers at room temperature. Finally, the dried sticks were crushed and were ground with the help of a blender. The resulting powder was kept in polyethylene bags and stored at 4^0 C until further use.

Aqueous extract preparation

The plant aqueous extract was prepared by steeping method where different amounts of miswak powder viz 2 g, 4 g, 6 g and 8g was taken and subsequently were put in 100 ml of distilled and sterilized water for five days. The solid materials were removed by filtration using Whatman filter papers No.1 and the resulting extract was further filtered through a bacterial filter. The filtrated was then centrifuged at a speed equal to 4000 cycles per minute for duration of ten minutes. The filtrate was then taken out and put into sterilizing bottles and were stored at 4^0 C until further use.

Alcoholic extract preparation

The plant alcoholic extract was prepared by steeping where different amounts of miswak powder viz 2 g, 4 g, 6 g and 8g was taken and was put into 100 ml of 70% ethanol alcohol for five days. Solid materials were

removed by filtration by using Whatman filter papers No.1 and the resulting extract was further filtered through a bacterial filter. The filtrated was then centrifuged at a speed equal to 4000 cycles per minute for duration of ten minutes. The filtrate was then taken out and put into sterilizing bottles and were stored at 4^0 C until further use (Majid and Muhannad, 1988).

Isolation of bacteria

The isolation of the bacteria was performed as described in (Li et al., 2007; Ted and Christine, 1995). Briefly, the following protocol was followed for the isolation of bacteria:

1- In the firstly step, the plaques present on the teeth were removed with the help of tooth sticks and the sticks were then placed in plastic plate (Aebi, 1974; Quigley and Hein, 1962). It is important to note that the plaque samples were taken before cleaning the teeth.

2- 10 ml of phosphate buffer solution was added to the plastic plate containing the plaque sample. The plaque sample was then crushed using sterilized pestle and mortar until a homogenous suspension was obtained.

3- Serial dilutions of the homogenous suspension obtained during the previous step were prepared.

4- The samples were then cultivated by spread-plating 0.1 ml of the sample on blood agar , mannitol salt agar media. Samples were taken from an appropriate dilution (third or fourth dilution).

5- The plates were incubated in aerobic and microaerophilic conditions at 37°C for 24 -48 hours.

6- After incubation, the colonies were counted and were calculated by the ratio 1ml / plaque or growing bacteria wherein the diagnosis was performed with respect to the diagnostics tables mentioned in (Macfaddin, 2002).

Preparation of active pure bacterial cultures

An active bacterial culture of the previously isolated bacteria was established first before performing the antimicrobial activity assay with the miswak extracts. The bacterial samples were activated 24 hours before experiment and the samples were kept at 37 ° C. Blood agar was used as a nutrient source for the bacteria (Al-Delaimy and Ali, 1970) (34).

Preparation of bacterial samples

From the active bacterial culture, propagation of bacteria was allowed on a suitable nutrient medium by incubating it at 37°C for 24 hours. Ten colonies for each type of bacteria were used in the experiment. The colonies were transferred to a test tube containing 5 ml Nutrient Broth medium under aseptic conditions. The test tubes were then incubated at a temperature 37°C to allow the bacteria growth. Appropriate dilutions for each type of bacteria was prepared, hence the total number of cells approximately 10 milliliters (Al-Delaimy and Ali, 1970).

Antimicrobial activity test

To test the anti-bacterial sensitivity upon treatment of the plant extract, 0.1 ml the bacterial suspension was spread-plated on Nutrient Agar Petri dishes. The Petri dishes were then kept at room temperature for 15 minutes to let the bacterial suspension dry. Equal sized wells of 7mm diameter were drilled in the Petri dishes, saturated with dried bacterial suspension, using a sterilized crock perforator. Subsequently, 0.1 ml from plant aqueous and alcoholic extract of different concentrations was carefully transferred inside the wells with the help of a thin pipette. The Petri dishes were then incubated at 37°C for 24-48 hours. The bacterial inhibition of the extracts tested was estimated by measuring the growth diameter of the treated well as compared to the control sample (Baron and Finegold). The experiment was performed in three replicates per treatment.

Statistical analysis

The statistical analyses were performed using a completely randomized design with three replicates per treatment. Significance of the data was determined by performing Least Significant Difference Test (L.S.D) (Al-RawiKhasha and Abdul, 1980) [(36)].

RESULTS AND DISCUSSION

Determination of the bacterial isolates from the dental plaques

In the first part of our study, we attempted to understand the multiplicity and diversity of the bacterial community present in the single isolated plaque sample. It has been noted that the conditions surrounding the teeth of different individuals vary are not the same for two individuals and are controlled by some physical and chemical factors that affect the microbial component of

Table 1. Represents the multiplicity and diversity of bacterial isolates identified from a single sample

Bacterial species isolated	Percentage
Streptococcus mutans (gram positive)	39.9
Lactobacillus casei (gram positive)	27.1
Staphylococcus aureus (gram positive)	21.2
Actinomycetem comitans (gram negative)	9.3
Enterobacter aerogenes (gram negative)	2.5
Total	100

Table 2. The effect of miswak aqueous extract on growth of different species of bacteria.

Concentration / Bacterial isolates	Average of bacterial growth by ml					Percentage of bacterial growth inhibition				
	5%	10%	15%	20%	0%	5%	10%	15%	20%	0%
Streptococcus mutans	40	32	26	17	49	18.4	34.7	47	65.4	0
Lactobacillus casei	33	26	20	15	44	25	41	54.6	66	0
Staphylococcus aureus	43	40	37	25	45	4.5	11.2	17.8	44.4	0
Actinomycetem comitans	55	45	42	33	65	15.4	30.8	35.4	49.3	0
Enterobacter aerogenes	35	35	25	20	45	17.7	22.2	44.4	55.5	0

Table 3. The effect of miswak alcoholic extract on different species of bacteria

Concentration / Bacterial isolates	Average of bacterial growth by ml					Percentage of bacterial growth inhibition				
	5%	10%	15%	20%	0%	5%	10%	15%	20%	0%
Streptococcus mutans,	35	30	24	15	49	28.5	38.7	51	69.3	0
Lactobacillus casei	29	24	18	12	44	34	45.4	59	72.7	0
Staphylococcus aureus	40	38	33	22	45	11	15.5	26.6	51.1	0
Actinomycetemcomitans	51	42	39	31	65	21.5	35.3	40	52.3	0
Enterobacter aerogenes	35	28	22	18	45	22.2	37.7	51.1	60	0

the dental plaque (Li et al., 2007; Noguchi et al., 2005). The sample that is tested was taken from a formation of thin membrane on tooth that is indicative of dental plaque. Table (1) shows the multiplicity and diversity of bacterial isolates that were isolated from a single sample. It was interesting to note that *Streptococcus mutants* was the most abundant bacterial species followed by *Lactobacillus casei*. The contribution of *Staphylococcus aureus* in the total bacterial population was 21.2 % whereas *Actinomycetem comitans* population abundance was 9.3% of the total population. *Enterobacter aerogenes* had the lowest contribution of 2.5% in the total bacterial population.

Average of bacterial growth by ml

The results presented in table1 and 2 show the effectiveness of alcoholic and aqueous extracts of miswak on bacterial growth inhibition for all the isolates. It was evident from the data that alcoholic extract of miswak has higher inhibition activity against the bacterial isolates as compared to the aqueous extract at all concentrations tested. We also observed that the percent inhibition of the miswak extracts on the experimental isolates increased with the application of higher extract concentration as compared to the control sample. The highest percent inhibition was obtained for *Lactobacillus casei* at 34%, 45%, 59%, and 72% for alcoholic extract treatment and 25%, 41%, 66%, and 54.6% for aqueous extract treatment at 5, 10, 15 and 20 g/100 ml concentrations respectively. Whereas the treatment of *Streptococcus mutans* with the same concentrations (5, 10, 15 and 20 g/100 ml) of alcoholic and aqueous extracts led to percent inhibition at 28.5%, 38.7%, 51%, and 69.35% for alcoholic extract treatment whereas

treatment with aqueous extract led to percent inhibition at 18.4%,34.7%,47%, and 65.4%). *Staphylococcus aureus* showed the lowest percent inhibition upon treatment with the aqueous extract with percent inhibition at 4.5%, 11.2%, 17.8%, and 44.4% as compared to the control sample. The ability of alcoholic and aqueous extracts of miswak to inhibit the growth of pathogenic bacterial isolates can be attributed due to the contents present in the extract that contains many effective substances against bacteria such as sulphur and trimethyamine that reduces the oral pH and also inhibits the bacterial growth (Elvin-Lewis, 1980a). Nitrates, that have an effective inhibitory activity against different bacterial species, are also present in the miswak extract (Almas and Al-Bagieh, 1999). It was also discovered that the miswak contains natural substances that have an effect similar to that of the antibiotics in a way that both eliminates bacteria and pathogens present in the oral cavity (Poureslami et al., 2007). This study also confirmed that miswak is an effective agent that eliminates all bacterial species that causes teeth decay and periodontal disease as a result of its mechanical and chemical effects. The mechanical effect because of the miswak usage helps in gums massage resulting in better circulation of the blood, thus helps to keep the teeth clean and keeps them away from any inflammation. The chemical effect of miswak on the other hand is due to the fact that it contains a group of active substances, most important of which is thio-cyanide-tino-vio, which has been shown to have a disincentive effect for acid production in saliva (al-Bagieh and Almas, 1997).

CONCLUSION

In conclusion, we demonstrate the effectiveness of miswak in maintaining the oral hygiene and health. Regular use of miswak helps to keep the oral pathogens away and also maintains sound dental health.

REFERENCES

Aebi H (1974). Methods of enzymatic analysis. Academic Press, New York 2: 674-84.

Akpata ES, Akinrimisi EO (1977). Antibacterial activity of extracts from some African chewing sticks. Oral Surg Oral Med Oral Pathol 44(5): 717-722.

al-Bagieh NH, Almas K (1997). In vitro antibacterial effects of aqueous and alcohol extracts of miswak (chewing sticks). Cairo Dent J 13: 221-224.

Al-Delaimy KS and Ali SH (1970). Antibacterial action of vegetable extracts on the growth of pathogenic bacteria. J Sci Fd Agric 21: 110-112.

al-Hanafi Salah (1962).The Sawak, University of Damascus.

Almas K (1999). The antimicrobial effects of extracts of *Azadirachta indica* (Neem) and *Salvadora persica* (Arak) chewing sticks. Indian J Dent Res 10(1): 23-26.

Almas K, Al-Bagieh NH (1999). The antimicrobial effects of bark and pulp extracts of miswak Salvadora persica. Biomedical Letters 60(235): 71-75.

Al-RawiKhasha Mahmoud and Abdul Aziz Mahmoud Khalaf Allah (1980). Design and analysis of agricultural experiments. Dar Al Kutob for Printing and Publishing, University of Mosul.

Atlas RM 1995. Principles of Microbiology. Mosby. Comp. London Inc. 633 p.3.

Baron ET and Finegold S. Diagnostic Microbiology, 8 thed.

Carlsson J (1967). Presence of various types of non haemolytic *Streptococci* in dental plaque and in other sites of the oral cavity in man. Odontol Revy 18(1); 55-74.

Darout IA, Albandar JM, Skaug N (2000). Periodontal status of adult Sudanese habitual users of miswak chewing sticks or toothbrushes. Acta Odontol Scand 58(1): 25-30

Darout IA, Christy A, Skaug N (2000). Identification and quantification of some potentially antimicrobial anionic components in miswak extract. Indian J Pharmacol 32: 11-14.

Eid MA, Selim HA (1994). A retrospective study on the relationship between miswak chewing stick and periodontal health. Egypt Dent J 40(1): 589-592.

Elvin-Lewis M (1980a). Plants and dental health. J Prev Dent 6:59-60.

Ezmirly ST, Cheng JC, Wilson SR (1979). Saudi Arabian medicinal plants: Salvadora persica. Planta Med 35(2): 191-192.

Fadulu SO (1975). Antibacterial properties of the buffer extracts of chewing sticks used in Nigeria. Planta Medica 27: 123-126.

Gazi M, Saini T, Ashri N, Lambourne A (1990). Meswak chewing stick versus conventional toothbrush as an oral hygiene aid. Clin Prev Dent 12(4): 19-23

Hardie J, Ahmed K (1995). The Miswak as an aid in oral hygiene. J Philipp Dent Assoc 47(1): 33-38.

Hattab FN (1997). Meswak: the natural toothbrush. J Clin Dent 8(5): 125-129

Kamel ,Yasmine Ahmed (2009). Plants and herbs have been used in treatment by prophet (peace be upon him). The first edition. Dar Hammurabi and Israa for publication and distribution Amman Jordan

Li Y, Ge Y, Saxena D, Caufield PW (2007). Genetic profiling of the oral microbiota associated with severe early childhood caries. J Clin Microbiol 45(1): 81-87.

Macfaddin DF (2002). Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott Williams and Willeins. Philadelphia , USA.

Majid SH, Muhannad JM (1988). The Iraqi Plants and Herbs between traditional Medicine and Scientific Research, Althawra for Printing and Publishing, Baghdad, first edition.

Nazim, Al-Nasimi Mahmoud (1087). Prophetic Medicine and Modern Science, Beirut.

Noguchi N, Noiri Y, Narimatsu M and Ebisu S (2005). Identification and localization of extraradicular biofilm-forming bacteria associated with refractory endodontic pathogens. Appl Environ Microbiol 71(12): 8738-8743.

Poureslami HR, Makarem A, Mojab F (2007). Paraclinical effects of miswak extract on dental plaque. Den Res J 4(2): 106-110 .

Quigley GA and Hein JW (1962). Comparative cleaning efficiency of manual and power brushing. J Am Dent Assoc 65: 26-29.

Rogers AH (2008). Molecular oral Microbiology. Caister Academic press. ISBN: 978-1-904455-24-0.

Taiwo O, Xu HX, Lee SF (1999). Antibacterial activities of extracts from Nigerian chewing stick. Phytother Res 13(8):675-679.

Ted RJ and Christine LC (1995). Laboratory experiment in microbiology. 4th ed. Benjami/Cummings. California, USA.

Turk DC, Porter JA, Dueden BI, Reid TMS 1983. A short Text Book of Medical Microbiology. 5th Ed. Ltd, Bungay.

WHO. Concensus statement on oral hygiene (2000). Int Dent J 50: 139.
WHO. The World Oral Health Report (2003). Continuous improvement of oral health in the 21st century- the approach of the WHO Global Oral Health Programme. Geneva: World Health Organization.
Wolinsky LE, Sote EO (1983). Inhibiting effects of aqueous extracts of eight Nigerian chewing on bacterial properties favouring plaque formation. Caries Res 17: 253-257.