



# Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

## Original article

### Anti-bacterial effects of chewing sticks on periodontal pathogens

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#### ARTICLE INFO

##### Article history:

Received 3 April 2020

Received in revised form 9 May 2020

Accepted 10 May 2020

##### Keywords:

Acidogenic

Periodontal

Inhibitory-potential

Supra-gingival

Bacteriodes

##### Abbreviations

NTFP: Non-timber forest products

MIC: Minimum inhibitory concentration

#### ABSTRACT

**Background:** From antiquity, several plants with reported effectiveness against periodontal pathogens, and have oral acidogenic effect against bacteria responsible for dental caries and dental plaque is as old as man. **Methods:** The present study focused on the antibacterial activities of the root of *Azadirachta indica* (*A. indica*) (Neem), *Vernonia amygdalina* (*V. amygdalina*) (Bitter leaf), *Fagara xanthoxyloides*, *Prosopis africana* (*P. africana*) (mesquite) and *Anogesissus leiocarpus* were all collected from Saki, Nigeria. The chewing stick were washed, shaded dried for 7 days according to the standard procedure. Five consented individuals, supra-gingival plaques were cultured and subjected to the antibacterial assessment by preparing of the aqueous extracts of the chewing sticks. The test organisms included *Staphylococcus aureus*, *Streptococcus spp*, *Escherichia coli*, *Bacillus subtilis* and *Klebsiella spp* which were isolated from consented individuals. **Results:** All the isolated periodontal strains were inhibited at 2% concentrations of all aqueous extracts except the *Klebsiella* which was inhibited at 8% to 16% concentration of *A. indica*. Both *P. africana* and *V. amygdalina* had no inhibitory effect on *Klebsiella* at all concentrations. Only *A. indica* at 8% and 16% had noticeable inhibition on *Klebsiella*. At 2%, 4%, 8% and 16%, Acacia gum showed remarkable antibacterial activity against *Streptococcus*, *Staphylococcus* and *E. coli*. **Conclusion:** *Fagara xanthoxyloides* and *Anogesissus leiocarpus* were the most efficacious among all the tested chewing stick. In conclusion, the tested chewing sticks were effective as antibacterial agents against all the tested organisms.

#### Introduction

The long and venerable history of the use of plants to improve dental health and promote oral hygiene has been known since antiquity. Cuttings of root, stem or twigs of trees and shrubs have served as traditional tooth brush commonly called chewing sticks [1,2]. It was reported that chewing sticks were in use from as early as some 7000 years ago by the

Babylonians, and later throughout the Greek and Roman empires. It is also believed to be the precursor of the modern day tooth brush and was used in Europe about 3000 years ago. Chewing sticks are important non-timber forest products (NTFP) which has been widely used as dental cleaner in tropical West Africa [3,4].

DOI: 10.21608/MID.2020.29233.1010

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These plants characteristically impart varying taste sensational, a tingling peppery taste, bitter taste and numbness [5]. As it is chewed, the frayed stick is used to clean the teeth, and simultaneously removes plaque by gentle messages to the gums [6]. Human depended on plants for cure of most ailments until scientific advances introduced chemical based toothpaste [7]. The small number of these pathogens is present in the deep layer of mature, supra-gingival plaque and in accordance with their traditional use as an oral hygiene aid; they might assist in the prevention of periodontal diseases by restricting their growth. They can also be used in the treatment of periodontal pockets if it were applied sub gingival, either mixed with gel or absorbed into a solid slow releasing material. Such devices have also been used to deliver conventional antibiotics such as tetracycline [8]. The aim of the study was to assess the antibacterial effects of four locally available commonly used chewing sticks on the periodontal pathogens.

## Materials and Methods

### Chewing stick preparation

The chewing stick selected were obtained locally from the roots of the selected plants which were: *Vernonia amygdalina* (Bitter leaf), *Fagara zanthoxyloides*, *Prosopis africana* (mesquite) and *Anogesissus leiocarpus*. These plants were aseptically from Saki in Oke-Ogun area (woody savanna vegetation) of Oyo state, Nigeria. The plant parts were authenticated by a botanist at the department of Biology. The chewing stick were washed under running tap water, to remove dirty, the sample were shaded dried for 7 days at room temperature to curb distortion in the composition of active principle in the chewing stick [9].

### Collection of plaque samples from the subjects

Dental plaque samples were collected from five different volunteers who had consented to taken part in the study, of age range between 18-79 years. They were swabbed three times with sterile cotton wool to remove debris and saliva. All the samples collected followed the ethical rules. The test organisms were isolated directly from an infected tooth, and subsequently swabbed with sterile cotton wool swab and immediately streaked on a sterile blood agar in triplicate, the plates were incubated at room temperature overnight. Colonial characteristics based on morphological colonial appearance were picked from the plates and purified

by repeated sub culturing until pure colonies were obtained.

### Procedure of chewing stick

The dried samples were well pulverized into a fine powder with a mixer grinder. The powder was stored air tight aseptic container kept at 28 degree per Celsius for subsequent use. 5g of all the plants were poured inside a bottle and filled with 100ml of sterile distilled water for seven days. The supernatant were filtered using Whatman NO.1 filter paper, then labelled and stored until used.

### Determination of inhibitory properties

Susceptibility is an ability of isolate to grow in extract: This was establishing if the different extracts had inhibitory properties. 0.25m of the isolate obtained from a 24 hours broth culture was introduced into sterile test tubes containing 2.5ml undiluted extracts. The tubes were incubated at 37 degree Per Celsius for 24 hours. The broth cultures were sub cultured onto agar plates by the streaking method to observe for growth. The test organisms were sub cultured three times in fresh media to obtain a more vigorous population. 1ml of cultured was aseptically transferred into a sterile petri dishes. 15ml of molten nutrient agar was poured into the same plates. It was allowed to gel and dry. This was done in duplicates. A sterile cork borer of size 5mm in diameter was pushed into the agar was pushed into the agar and agar plugs were removed creating a well /ditch. To each ditch was added chewing stick extracts from cold water while sterile distilled water were used as control. All plates were labeled and allowed for 2 hours for proper diffusion of the extracts before incubated at 37 degree per Celsius for 24 hours. The mean zone of inhibition were measured and recorded to the nearest mm diameter. A mean inhibition zone greater than 2mm was used as the minimum threshold [3,9].

## Results

**Table 1.** Susceptibility (MIC) of plaque bacteria to the extracts of various chewing sticks.

Pathogen	Susceptibility to the <i>P. Africana</i> extract %				Susceptibility to the <i>A. indica</i> extract %				Susceptibility to the <i>F. xanthoxyloides</i> extract %				Susceptibility to the <i>V. amygdalina</i> extract %			
	2	4	8	16	2	4	8	16	2	4	8	16	2	4	8	16
A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-
E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

**Legend:** A = *Staphylococci* spp, B= *Streptococci* spp, C= *E. coli*, D= *Klebsiella* spp, E= *Bacillus subtilis*

## Discussion

Neem extract showed inhibitory potential at 2% against *Staphylococcus*, *Streptococcus* and *E. coli* whereas there was no noticeable difference at 2 to 8% of neem against *Klebsiella* spp and *Bacillus subtilis*. Similar results was obtained in [10, 11]. The susceptibility of *Streptococcus* and *E. coli* could be a function of available binding sites on the bacterial cell walls; these are probably bacterial surface protein. Tannins have been shown to form irreversible complexes with proline rich proteins which would lead to inhibition of cell wall protein synthesis.

At 2%, 4%, 8% and 16%, Acacia gum showed remarkable antibacterial activity against *Streptococcus*, *Staphylococcus* and *E. coli*. This result re-affirmed earlier study of [3,10]. The susceptibility of *Staphylococcus*, *Streptococcus* and *E.coli* to 2% of *Prosopis africana*, *Fagara xanthoxyloides*, *Azadirachta indica*, and *Vernonia amygdalina* extracts showed susceptibility of the same strain to 4%, 8% and 16% while *Prosopis africana* showed no antibacterial spectrum even with increasing concentrations against *Klebsiella* and *Bacillus* spp. The susceptibility of *Klebsiella* spp at 8% and 16% is reported in this study, whereas at 2% and 4% of *Azadirachta indica* against *Staphylococcus*, *Streptococcus* and *E.coli* was noticed. However, against all norms from earlier studies, it was documented that the bacteria of the general *Streptococcus*, *Lactobacillus*, *Corynebacterium*, and *Staphylococcus* are normal flora of the mouth and can as well cause dental caries [12, 13]. The observations made in this study are consistent with the existing reports. In some earlier studies of dental caries, the most implicated organisms in periodontal infection were *Streptococcus mutans* and *Lactobacillus* spp. *Lactobacillus* thrives in the acidic environment

created by *S. mutans* [14,15]. This study confirmed the earlier reports of [11,13]. Conclusively, *V. amygdalina* showed a minimal antibacterial activity from all the extracts used. *Anogeissus Leiocarpus* produced a relatively intense antibacterial activity against the *Lactobacillus* spp. This study complements earlier observation by [12,16]. There was no appreciable inhibition with *F. Xanthoxyloides* against *Klebsiella* and *Bacillus* spp. The same result was also reported by [17,18] in *Zanthoxyleim giletti* which is generally the same as *F. xanthoxyloides* [5,11,12] which showed no inhibition or antibiotic effect on *Staphylococcus* spp. *Anogeissus leiocarpus* was efficacious against all the tested pathogens. While some findings reported their efficiency, some did not. The antimicrobial property of *Zanthoxylum xanthoxyloides* has also been re-investigated by [16, 17] where the extracted essential oils from this chewing stick were found to possess antiseptic and anti-carcinogenic actions. The present study has however broaden our knowledge towards harnessing our natural plants products for their potential antimicrobial potency for their holistic oral health.

## Conclusion

Local herbs are essentially effective as antibacterial as well as being supragingival. The use of chewing sticks as an alternative to toothpastes which are chemical-based should be encouraged in industrial quantity.

## Disclosure of potential conflict of interests

The authors hereby declare that there is no conflict of interest whatsoever.

## Acknowledgements

We hereby acknowledge the supports of all the consented subjects in this study.

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