



# Antimicrobial Activities of Some Selected Indigenous Chewing Stick on Bacteria Isolated from the Mouth among Patients Attending State Specialist Hospital, Akure, South-West Nigeria

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## Abstract

The use of chewing sticks to brush the mouth has been the major practice in Nigeria both in the ancient and modern days. This study was designed to determine the antimicrobial activities of the ethanol and aqueous extracts of four different Nigerian chewing sticks namely: *Fagara zanthoxyloides*, *Anogeissus leiocarpus*, *Distemorantus benthamianus* and *Psedocedrela kotschyi*. Isolation and identification of microorganisms present in the mouth were carried out using conventional method, however, antimicrobial activities and Minimum inhibitory concentration of the ethanol and aqueous extracts of *F. zanthoxyloides*, *A. leiocarpus*, *D. benthamianus* and *P. kotschyi* were determined using standard microbiological method. *Staphylococcus aureus*, *Streptococcus mutans*, *S. albus*, *S. epidermidis* and *Strep. salivans* were isolated from the saliva of patient sampled. The ethanol extracts of all the chewing stick used had higher zone of inhibition when compared with aqueous extracts. However, the extract of *D. benthamianus* showed the highest inhibitory activity on *S. aureus* (32 mm), *Strep. salivans* (13 mm) and *S. albus* (13 mm); *F. zanthoxyloide* and *A. leiocarpus* on *Streptococcus mutans* (20 mm); *P. kotschyi* on *S. epidermidis* (20mm) and *F. zanthoxyloide* on *Strep. salivans* (13 mm) isolates. The physiochemical assay reveals the presence of tannin, saponin, steroid, phenol, flavonoid and alkaloid. It has been show from this study that the ethanol extracts of some indigenous chewing sticks commonly used in Nigeria showed significant antimicrobial activities against the tested organisms.

**Keywords:** Chewing Stick; Antimicrobial Activities; Extracts; Physiochemical

## Introduction

The normal microbiota of the mouth consists of organisms that resist mechanical removal by adhering to the surface such as the gums and the teeth [1]. There is therefore need to remove these organisms by cleansing effect through chewing stick that contains antibacterial property. However, the use of indigenous chewing stick is a common practice in Nigeria but has the limitation of social status due to lack of proper sensitization which is due to the fact that insufficient knowledge of their antibacterial potential are known [1]. Plants are important in man day today activities. They can be used as cosmetics, food, flavors, ornamental, and for medicinal purposes. Medicinal plants have been used as alternative to conventional drugs, because of their potential health benefits. Various plant extract has great antibacterial potential against infectious agents and can be used for therapeutic purposes [2]. Investigation carried out on *F. zanthoxyloides* ("Orin ata"), *A. leiocarpus* "Orin ayin", *D. benthamianus* ("Orin ayan") and *P. kotschyi* ("Emi gbegiri"), by Akande and Hayashi (1998) revealed their antibacterial activity on *S. aureus* [1].

Dental plaque formation and gingivitis are associated with the early stages of development of periodontal disease [3]. There are numerous various report of studies of the micro flora of the gingival crevice, dental plaque and chronic destructive periodontal disease. There have been more emphases on the importance of the anaerobes on the etiology of periodontal disease [4,5]. About 80% to 90% of the Nigerian middle class to lower class population use chewing sticks mainly because they are readily available, cheap and efficacious, a few of them uses a combination of two or more chewing sticks simultaneously. Medicinal properties of chewing stick include gum healing, analgesia, anti-sickling, homeostasis and astringency as well as antimicrobial and plaque inhibiting effect [6].

Much effort has focused on examining the inhibitory effect of chewing sticks on oral organisms, but information concerning the antibacterial activity of *F. zanthoxyloides* (“Orin ata”), *A. leiocarpus* “Orin ayin”, *D. benthamianus* (“Orin ayan”) and *P. kotschyi* (“Emi gbegiri”) against other human pathogens is scarce [7,8]. Therefore, this study aimed at presenting the antibacterial potential of the ethanol and aqueous extract of *F. zanthoxyloides* (“Orin ata”), *A. leiocarpus* “Orin ayin”, *D. benthamianus* (“Orin ayan”) and *P. kotschyi* (“Emi gbegiri”) on bacteria isolated from the mouth among patients attending State Specialist Hospital, Akure, South-West Nigeria.

## Materials and Methods

### Collection and Identification of chewing stick

*Fagara zanthoxyloides* (“Orin ata”), *A. leiocarpus* “Orin ayin”, *D. benthamianus* (“Orin ayan”) and *P. kotschyi* (“Emi gbegiri”) were collected from “Odo petu” along Arakale road in Akure, Ondo-state. The plants were collected in early April during the beginning of raining season. They were identified at the Department of Forestry and Wood Technology, Federal University of Technology, Akure.

### Collection of samples from patient

Thirty two (32) samples were collected from Dental Department, State Specialist Hospital Akure, Ondo State using swab stick. The samples collected were transported to Microbiology Laboratory and stored at the refrigerator for further research.

### Identification of bacteria collected

Identification of bacterial collected from Dental Department, State Specialist Hospital, Akure. Was carried out using streaking method. The samples were streaked on Eosine methylene blue agar, NA, CLED, chocolate agar and MSA and immediately incubated at 37 °C for 24 hours. Morphological characteristics were observed and biochemical tests were carried out for the identification of the bacteria as described by Olutiola *et al.* (1991) [6].

### Preparation of extracts

Preparation of aqueous and ethanol extracts was carried out by mixing 100 g of *F. zanthoxyloides*, *A. leiocarpus*, *D. benthamianus* and *P. kotschyi* powder with 1 L of distilled water (for aqueous extract) and 95% ethanol according to the method described by Ogundiya *et al.* (2008) (for ethanol extract) for 24 h [9]. The mixture was then filtered using Whatman filter paper number 1 filter paper, and the filtrate was then evaporated in vacuum evaporator at 60°C (for aqueous) and 40 °C (for ethanol). The extracts were stored in sterile bottles and kept frozen at –20 °C until further use. Before testing, the extracts were freshly reconstituted in ethanol (for ethanolic extract) and water (for aqueous extract) at a final concentration of 400 mg/mL which was used to further prepare serial dilutions (400–50 mg/mL)

### Inoculum Preparation

All bacterial isolates were grown to the exponential phase in tryptic soy broth (TSB) at 37 °C for 18 h. The bacterial growth was estimated as turbidity using spectrophotometer to measure the light absorption of the microbial mass as determined by the optical density readings at 620 nm. Growth was checked every 30 minutes, and the exponential phase of bacterial growth was identified by the increased OD<sub>620</sub> reading. Then, the inoculum density of each bacterial suspension was adjusted to a final density equivalent to 0.5 McFarland Standard ( $1.5 \times 10^8$  CFU/mL) in sterile saline (0.84% NaCl).

### Antimicrobial Testing

The antimicrobial activity of the extracts was carried out using the agar diffusion and minimal inhibitory concentration (MIC) methods. The antimicrobial testing was performed on Mueller Hinton agar plates (Difco Laboratories) using the agar diffusion method. Briefly, 100  $\mu$ L of bacterial suspension was spread smoothly on the agar plates. The required numbers of wells, each 3 mm in diameter, were cut out of the agar using a sterile glass capillary ensuring proper distribution of holes in the periphery and one in the center for each agar plate. Then, wells were filled with 50  $\mu$ L of sterile extract (aqueous or ethanol) made from extract stock solution (400, 200, 100, and 50 mg/mL) was followed by 2 h preincubation at room temperature for proper diffusion of the plant extract into the media. Then, the plates were incubated at 37°C for 24 h. The mean diameter of complete growth inhibition zone (in mm) was measured without the well’s diameter and considered as the inhibition zone. The test for each microorganism was repeated three times to ensure reproducibility. The average zones diameter values from three repeats were taken in determination of the final inhibition zones. This was done to ensure that all inhibition zones within each experiment were obtained under the same experimental conditions.

## The Minimal Inhibitory Concentration (MIC)

The MIC of the extracts was determined using the standard microdilution method in 96 multi-well microtiter plates, with slight modifications. Briefly, the dissolved extracts were first diluted to a concentration of 50 mg/mL, then 50  $\mu$ L from each of the aqueous and ethanol extracts was pipetted into the first well of each microtiter plate row, and 50  $\mu$ L of TSB was distributed from the 1st to the 12th well of each row. Twofold serial dilution was achieved by transferring 50  $\mu$ L of scalar dilution from the first to the subsequent wells of each row. The final concentration of the extracts adopted to evaluate antibacterial activity was included from 25 mg/mL to 0.003 mg/mL. Finally, 10  $\mu$ L of each bacterial suspension was added to each well. The lowest concentration at which no turbidity occurred was taken as the MIC value. Plates were analyzed individually to determine MIC and the average MIC values from three repeats were taken in determination of the final MIC values for each extract to ensure accuracy and reproducibility.

## Data analysis

All experiments were carried out in triplicates. Data obtained were analysed using one way analysis of variance (ANOVA) and means were compared by Duncan multiple range test (DMRT) using SPSS statistical version 16.0 software. Differences were considered significant at  $p \leq 0.05$ .

## Results

### Isolation and Identification of bacteria from patients sampled

Different strains of bacteria that were collected and identified are *S. aureus*, *Strep. mutans*, *S. albus*, *S. epidermidis* and *Strep. salivans*. The morphological and biochemical characteristics can be found on Table 1.

### Percentage frequency of bacteria isolated

The most frequently occurred bacteria isolated was *S. albus* (16; 50.0%) followed by *S. aureus* (7; 21.9%), *Strep. mutans* (4; 12.5), *S. epidermidis* (3; 9.4%) and *Strep. salivans* (2; 6.3%). This is shown in Table 2.

### Antibacterial activities of aqueous and ethanol extracts of the chewing sticks

The ethanol extracts of all the chewing stick used had higher zone of inhibition when compared with aqueous extracts. However, the extract of *D. benthamianus* showed the highest inhibitory activity on *S. aureus* (32 mm), *Strep. salivans* (13 mm) and *S. albus* (13 mm); *F. zanthoxyloide* and *A. leiocarpus* on *Streptococcus mutans* (20 mm); *P. kotschy* on *S. epidermidis* (20mm) and *F. zanthoxyloide* on *Strep. salivans* (13 mm). This can be seen in Table 3.

Biochemical characteristics	Bacterial isolates			
	<i>Streptococcus mutans</i>	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>Strep. salivans</i>
Indole test	-	-	-	+
Catalase test	+	+	+	+
Urea Hydrolysis	-	+	+	-
Nitrate test	+	+	+	+
Starch Hydrolysis	+	-	-	+
Hydrogen sulphide production	-	-	+	-

- = negative to the test, + = positive to the test

**Table 1:** Biochemical characterization of bacteria isolated from the saliva of patients

Bacterial isolates	Number of bacteria isolated	Percentage occurrence of the bacteria isolate (%)
<i>S. albus</i>	16	50
<i>S. aureus</i>	7	21.9
<i>Strep. mutans</i>	4	12.5
<i>S. epidermidis</i>	3	9.4
<i>Strep. salivans</i>	2	6.3
<b>Total</b>	<b>32</b>	<b>100</b>

**Table 2:** Percentage frequency of bacteria isolated

Extracts		Diameter zones inhibition (mm)				
		<i>S. aureus</i>	<i>Strep. mutans</i>	<i>S. albus</i>	<i>S. epidermidis</i>	<i>S. salivans</i>
<i>A. leiocarpus</i>	Aqueous	0.00±0.0 <sup>a</sup>	13.0±1.0 <sup>b</sup>	11.0±0.0 <sup>b</sup>	10.0±0.5 <sup>b</sup>	0.00±0.0 <sup>a</sup>
	Ethanol	0.00±0.0 <sup>a</sup>	20.0±0.0 <sup>c</sup>	12.0±0.0 <sup>b</sup>	14.0±1.0 <sup>b</sup>	9.0±1.5 <sup>b</sup>
	Distilled water	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>
<i>F. zanthoxyloides</i>	Aqueous	14.0±1.5 <sup>c</sup>	10.0±0.5 <sup>b</sup>	8.0±0.0 <sup>b</sup>	0.00±0.0 <sup>a</sup>	10.00±0.0 <sup>b</sup>
	Ethanol	19.0±1.0 <sup>b</sup>	20.0±1.0 <sup>c</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	13.00±0.0 <sup>b</sup>
	Distilled water	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>
<i>P. kotschyi</i>	Aqueous	16.00±0.0 <sup>c</sup>	0.00±0.0 <sup>a</sup>	9.00±0.0 <sup>b</sup>	15.00±0.0 <sup>bc</sup>	0.00±0.0 <sup>a</sup>
	Ethanol	25.00±0.0 <sup>d</sup>	12.0±1.0 <sup>b</sup>	10.0±1.5 <sup>b</sup>	20.0±1.0 <sup>c</sup>	10.0±1.5 <sup>b</sup>
	Distilled water	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>
<i>D. benthamianus</i>	Aqueous	10.0±0.5 <sup>b</sup>	15.00±0.0 <sup>b</sup>	0.00±0.0 <sup>a</sup>	14.0±1.5 <sup>b</sup>	0.00±0.0 <sup>a</sup>
	Ethanol	32.0±1.5 <sup>d</sup>	18.0±0.0 <sup>c</sup>	13.00±0.0 <sup>b</sup>	0.00±0.0 <sup>a</sup>	13.00±0.0 <sup>b</sup>
	Distilled water	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>	0.00±0.0 <sup>a</sup>

**Table 3:** Antibacterial activities of aqueous and ethanol extracts of the chewing sticks

Values in the same row carrying the same superscript are not significantly different according to Duncan's multiple range tests at ( $P \leq 0.05$ ).

### Minimum inhibitory concentration of the plants extracts

The ethanolic extract of *A. leiocarpus* displayed inhibitory activity at 30 mg/ml on *S. aureus* and *Strep. mutans* which is the least compared to others. Also, ethanolic extract of *P. kotschyi* displayed inhibitory activity on *Strep. mutans* and *S. albus* at the same concentration. *Fagara zanthoxyloides* had its own MIC at 40 mg/ml on *S. albus* and *Strep. salivans* whereas, the MIC of *D. benthamianus* was 30 mg/ml which was on *Strep. mutans*. This is presented in Table 4.

### Qualitative phytochemical constituents of ethanol extracts of the chewing stick

The result obtained showed that the extract of the plants possessed tannin, saponin, phenol, alkaloid, steroid and flavonoid and are shown in Table 5.

Extracts		MIC (mg/ml)				
		<i>S. aureus</i>	<i>Strep. mutans</i>	<i>S. albus</i>	<i>S. epidermidis</i>	<i>S. salivans</i>
<i>A. leiocarpus</i>	Aqueous	ND	50	40	50	ND
	Ethanol	ND	30	50	50	50
<i>F. zanthoxyloides</i>	Aqueous	50	100	100	ND	40
	Ethanol	50	50	ND	ND	50
<i>P. kotschyi</i>	Aqueous	50	ND	50	30	ND
	Ethanol	40	30	30	ND	40
<i>D. benthamianus</i>	Aqueous	50	30	ND	50	ND
	Ethanol	50	100	50	ND	40

KEY: ND- No zone of inhibition

**Table 4:** Minimum inhibitory concentration of the plants extracts

Chewing stick	Alkaloids	Steroid	Phenol	Tannin	Saponin	flavonoid
<i>A. leiocarpus</i>	+	+	-	-	+	+
<i>F. zanthoxyloides</i>	+	+	+	+	+	+
<i>P. kotschyi</i>	+	+	+	+	+	-
<i>D. benthamianus</i>	+	+	+	+	+	+

KEY(-)= Absent, (+)= Present

**Table 5:** Qualitative estimation of the phytochemical constituents present in the ethanol extracts of the chewing stick

## Discussion

Nigerians use chewing sticks for their mechanical cleansing effect. The choice of stick depends largely on traditional preference rather than clinical effectiveness [9]. However, there have been documented report of antimicrobial properties of these sticks although few report of the selected indigenous chewing sticks has been documented.

Ethanol extracts of the selected chewing sticks had greater inhibitory effect than the aqueous extract [6,10]. This is in agreement with the observation of Karou et al. 2005 who stated that ethanolic extract of medicinal plants are more effective than aqueous extracts [11].

The observation that *D. benthamianus* has the most consistent inhibitory effect on almost all the isolates tested (*S. aureus*, *Strep. mutans*, *S. albus*, and *Strep. salivans*) except for *S. epidermidis* is in agreement with the study on the inhibitory activity of ethanomedicinal plant extract on microbial pathogens associated with the mouth by Karou et al. 2005 [11]. However, *A. leiocarpus* had low inhibitory effect against almost all the tested strains except *Strep. mutans* which is agreement with the observation by Wolinsky and Sote (1994), in his report which state the lack of effect of *A. leiocarpus* on the adherence and growth of the oral opportunistic pathogen, *Strep. Mutans* [12]. Although, this particular stick is commonly used by Nigerians for dental hygiene because of its other medicinal property such as the anti-stickling effect on red blood cells [13]. Moreover, this study also showed that *F. zanthoxyloides* had less inhibitory effect on the tested isolates in comparison with *P. kotschyi*.

In this study, all the extracts inhibited all the isolated oral associated microorganisms at 100 mg/ml with more effectiveness by the ethanol extract. However, *D. benthamianus*, *P. kotschyi* and *A. leiocarpus* still had inhibitory effect at concentration as low as 30 mg/ml. This report is contrary to the report of Al-Ayed et al. 2016 who reported that the minimum inhibitory concentration of the chewing stick studied on oral pathogens was 400 mg/ml.

It is well known that the antimicrobial properties of *F. zanthoxyloides*, *D. benthamianus*, *P. kotschyi* and *A. leiocarpus* extracts are attributed to the different phytochemical constituents. From the report of Wolinsky and Sote (1994), the phytochemical constituents of extracts consist of flavonoids, sterols, saponins, tannins, basic alkaloids, and reducing components in ethanol extract and saponins, tannins, and reducing components in aqueous extract which could be responsible for the observed antimicrobial property of ethanol extract compared with aqueous extract. This is in line with the observations of this study. The qualitative screening of the ethanol extracts of the chewing sticks showed the presence of alkaloid, steroids, flavonoids and Saponin in *D. benthamianus*, *A. leiocarpus*, *F. zanthoxyloides* and *P. kotschyi*. Tannin and phenol are also present in all the ethanol extracts of the selected chewing sticks except for *A. leiocarpus*. The presence of these bioactive components in these plants extracts makes them a potential source of antibacterial agents and incorporation in the production of toothpastes [14].

## Conclusion and Recommendation

The use of chewing stick conforms to the notion of primary health care approach (PHCA) and well established associations with certain cultural and religious belief. Considering the antibacterial properties of *F. zanthoxyloides*, *D. benthamianus*, *P. kotschyi* and *A. leiocarpus* as observed in this study, the use of these type chewing sticks in developing countries will be of great help because of financial constraints and limited oral care facilities. Moreover, the use of the extracts of these sticks can be incorporated in the conventional toothpaste to improve their effectiveness.

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