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Antibacterial Effects of Three Selected Chewing Stick Extracts on *Lactobacillus* Sp.

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Abstract: Studies were carried out on the sensitivity of a predominant bacterium causing oral infections -dental caries in particular - to three local chewing stick extracts. The extracts were from *Fagara xanthoxyloides* (Pako Ata); *Garcinia kola* (Pako Orogbo) and *Anogeissus leiocarpus* (Pako Ayin). The sensitivity of the test organism *Lactobacillus* sp. to the different extracts by the ditch plate method was carried out. Hot and cold water and hot and cold ethanol were used to extract from the chewing sticks. *Anogeissus leiocarpus* was the most effective of all the different extracts used. *Garcinia kola* showed medium effectiveness all through although, the hot ethanolic extract showed the most powerful action while *Fagara xanthoxyloides* was not effective in the water extracts.

Key words: Antibacterial, chewing stick extracts, dental caries, sensitivity, ditch plate method, agar well diffussion

INTRODUCTION

The use of chewing sticks, for oral hygiene, is widely recognised in tropical West Africa. Chewing sticks are non-timber forest products commonly used for oral hygiene, especially for cleaning dental units. The product has prominent market values in many parts of tropical West Africa, where they are generally obtained in the wild Akande and Yamamoto,[1].

They are generally cut to 15-20 cm long and 0.5-1.25 cm diameter for use and are different from disposable toothpicks in that they can be reused for a period lasting up to 1 month. They are chewed and used for tooth brushing. When chewing sticks are used, extracts will inevitably be released but their efficacy for improving oral hygiene is little more than speculative. It has often been speculated that chewing stick extractives could contain compounds capable of inhibiting the activities of tooth-decaying bacteria Akande *et al.*,[2]; Akande and Hayashi.[3], Riose *et al.*,[4].

The mouth or oral cavity of the dentate adult harbours one of the largest most diverse microbial populations found anywhere in the human body because it provides various econiches that enable organisms with differing physiological requirements to grow and reproduce Pelczar *et al.*,[5] Tooth decay or dental caries is a pathological process of external origin and multifactorial nature in which localised destruction of the hard tissues of a tooth occurs Nikiforuk[6] and Dahlen *et al.*[7].

Several theories attempt to explain caries initiation and development. Postulated in the nineteenth century, the chemicoparasitic theory is the oldest and available evidence seems to provide it with the most support Toto,[8]. The acid demineralisation theory proposed in 1882 by Miller has been supported to a high degree by dentists over the years. According to this theory, microorganisms present in the oral cavity produce acids from food carbohydrates at or near the tooth surface. As these organisms come in contact with carbohydrates, they process it through their metabolic machinery resulting in an end product of lactic acid and perhaps other substances capable of damaging tooth structure. The acids produced initiates caries in enamel by dissolution of the crystallites which mineralise the enamel rods Sognnaes[9] and Chavez de Paz,[10].

In fermenting glucose and other sugars, homofermentative species of *Lactobacillus* form only lactic acid. These bacteria ferment dietary carbohydrates with the production of acid which dissolves the mineral component (calcium phosphate or hydroxide) of the enamel and dentine. The lactobacilli are more aciduric than other oral bacteria and can therefore survive and continue to ferment carbohydrate under more strongly acidic conditions Scherp.[11]; Pelczar *et al.*[5] and Pinheiro *et al.*,[12] Various antimicrobial agents have been

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confirmed for prevention of dental caries and notable among them is the use of some chewing stick extracts Fadulu,[13]; Akande and Hayashi[3] and recommended uses of fluorides. Two vitamins; ascorbic acid (vit. C) and pyridoxine (vit. B6) have been employed in plaque and caries control.

The aim of this study is to evaluate the antimicrobial effect of some aqueous chewing stick extracts on a bacteria isolate. The reasoning being that if beneficial properties of chewing sticks are discovered, their inherent potentials could be amalgamated with those of other tooth cleaning formulae for maximum effectiveness.

MATERIALS AND METHODS

The experiment involved three types of chewing sticks; four categories of extracts, one *Lactobacillus* species and two trials.

Extracts: Three different chewing stick samples were investigated for their antibacterial effects. They are: *Fagara xanthoxyloides* (Pako Ata); *Garcinia kola* (Pako Orogbo) and *Anogeissus leiocarpus* (Pako Ayin).

Aqueous extracts were obtained from each of the chewing sticks with their barks intact. The chewing sticks were oven dried at 100°C for 4 h after which they were ground into fine powder.

Four categories of extracts were prepared

Hot ethanol extract: One gram of each species was extracted on a soxhlet apparatus using ethanol as solvent. The concentrated extract was recovered and stored in sterile McCartney bottles until ready for use.

Cold ethanol extract: Two grams of each species was poured into McCartney bottles and filled with 20 mL of ethanol. The bottles were placed on a shaker for about 4 h. The extracts were centrifuged at 2000 rpm for 20 min. The supernatants were decanted, labelled and stored in the refrigerator.

Hot water extract: Two grams of each species was poured into sterile McCartney bottles, filled with 20 mLs of sterile distilled water and placed in a water bath at 100°C for 3 h. After which the bottles were allowed to cool. The extracts were centrifuged at 2000 rpm for 20 min. The supernatants were decanted, labelled and stored in the refrigerator.

Cold water extract: Two grams of each species was poured into McCartney bottles, filled with 20 mL of distilled water and left to soak overnight 24 h. The supernatants were centrifuged at 2000 rmp for 20 min. The supernatants were decanted, labelled and stored in the refrigerator.

Isolation of bacteria: The test organism was isolated directly from an infected tooth. The infected area of the tooth was swabbed three times with sterile cotton wool to remove debris and saliva. The tooth was then swabbed with sterile cotton wool swab and immediately streaked on sterile blood agar plates in duplicates. The plates were incubated at 37°C for 24 h. Characteristic colonies were picked from the plates and purified by repeated subculturing. Pure colonies were streaked on nutrient agar slopes in McCartney bottles, incubated at 37°C for 24 h. These slants were used as stock cultures and were kept in the refrigerator.

Characterisation of bacterial isolates: The bacterial colonies were differentiated first on basis of colonial morphology followed by microscopic examination after Gram staining and spore staining. Gram staining followed the procedure of Conn *et al.*,[14] while spore staining followed the procedure as described in Fawole and Oso[15], Biochemical tests were carried out to characterise the isolates as described by Collins *et al.*[16], Olutiola *et al.*,[17] and Pollack *et al.*[18].

Determination of inhibitory properties

Ability of isolate to grow in extract: This was to establish if the different extracts had inhibitory properties. 0.25m of the isolate obtained from a 24 h broth culture was introduced into sterile test tubes containing 2.5 mL undiluted extracts. The tubes were incubated at 37°C for 24 h. The broth cultures were sub cultured onto agar plates by the streaking method to observe for growth.

Bioassay: The test organism was sub cultured three times in fresh media to obtain a more vigorous population. 1 mL of the culture was aseptically transferred into sterile Petri dishes. 15 mL of molten nutrient agar was poured into the same plate. 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 and agar plugs were removed creating a well/ditch. To each ditch was added chewing stick extracts from Hot ethanol, Cold ethanol, Hot water and Cold water while sterile distilled water and ethanol were used as control.

All plates were labelled and allowed 2 h for proper diffusion of the extracts before incubation at 37°C for 24 h. The mean zones of inhibition were measured and recorded to the nearest mm. A mean inhibition zone greater than 2 mm (in two trials) was used as the minimum threshold.[3]

Table 1: Inhibitory properties of extract on *Lactobacillus* sp

Extracts	F. xanthoxyloides (Pako ata)	A. leiocarpus (Pako ayin)	G. kola (Pako orogbo)
Hot Water	+++	+	++
Cold Water	+++	+	++
Hot Ethanolic	-	-	-
Cold Ethanolic	-	-	++

-No growth, + Minimum growth, ++ Medium growth, +++ Maximum growth

Table 2: Sensitivity of *Lactobacillus* sp. to the chewing stick extracts

Chewing stick	(Zone of Inhibition (Nearest mm[a]) Hot water	Cold water	Hot ethanol	Cold ethanol
F. Xanthoxyloides	-	-	10±0	20±2
A. leiocarpus	17± 2[b]	15±2	20±4	20±2
G. kola	8±2	4±0	17±2	13±1

- No detectable inhibition; a-each record is a mean of two trials; b-standard error

RESULTS

Three bacterial isolates were identified but only one organism was used as the test organism. This is because of the prevalence of this particular organism on isolation. The test organism used was *Lactobacillus* sp. Table 1 shows the result of the inhibitory properties of the extracts on the growth of the test organism. There was no growth in the hot ethanolic extract of all the chewing sticks. The sterile cold water allowed the most growth in all three chewing sticks. *Anogeissus leiocarpus* inhibited the organism the most while *Fagara xanthoxyloides* was the least inhibitory overall.

The sensitivity of the test organism *Lactobacillus* sp. to the different extracts by the ditch plate method is shown in Table 2. *Anogeissus leiocarpus* is shown to be the most effective with all the different extracts used. *Garcinia kola* showed medium effectiveness all through although, the hot ethanolic extract showed the most powerful action. *Fagara xanthoxyloides* was not effective in both water extracts. Its maximum effect was from the cold ethanolic extract Table 2.

DISCUSSION

The activities of microbial oral flora in causing different types of oral infections most especially dental caries are frequently investigated (Pinheiro *et al.*,[12]). It is well documented that the bacteria of the genera *Streptococcus*, *Lactobacillus*, *Fusobactenun*, *Conynebacterium*, *Staphylococcus* are normal flora of the mouth and can as well cause dental caries (Rowe *et al.*,[19]; Slots,[20]) Many of the observations made in this study are consistent with the existing reports.

In the study of dental caries, the most prevalent organisms in this infection were *Streptococcus mutans* and *Lactobacillus* sp. *Lactobacillus* thrives in the acid environment created by *S. mutans* Caldwell and Leliner,[21]. The data presented in Tables 1 and 2 generally confirmed the earlier reports of Fadulu[13], Akpata and Akinremisi[22], Rotimi *et al.*[23] and Akande and Hayashi[3]. They found out that some African chewing sticks have been found to be effective against some Gram positive organisms. They also found out that most of the Nigerian chewing sticks were effective in preventing and curing many oral infections including dental caries.

Generally, *G. kola* showed a minimal antibacterial activity from all the extracts used. *A. leiocarpus* produced a relatively intense antibacterial activity against the *Lactobacillus* sp. from all the extracts used while *F. xanthoxyloides* showed no inhibition or antibacterial effect on the test bacteria with the water extracts. This result compliments earlier observation by Rotimi *et al.*[24] who tested some Nigerian chewing stick extracts against *Bacteroides gingivalis* and *Bacteroides melaninogenicus*. They reported no appreciable inhibition with *F. xanthoxyloides*. The same result was also reported by Akande and Hayashi[3], *Zanthoxylum giletti* (which is generically the same as *F. xanthoxyloides* Lowe and Soladoye,[25] showed no inhibition or antibiotic effect on *Staphylococcus* sp. The converse was the case in the case of the ethanolic extract in which *F. xanthoxyloides* had an intense antibacterial activity. *A. leiocarpus* proved to be the most effective of the three chewing sticks used on the *Lactobacillus* sp.

Conclusively, in all dental caries experiences, prevention is a worthy aim, which should be safely and effectively used for the attainment of improved dental health. Much research has been carried out regarding the cause, nature, treatment and prevention of dental caries but thus far, this great effort has not provided the means of eliminating the disease.

Study has however provided some methods for the partial control and prevention of these dental diseases. To this end, the study has revealed that dental caries can be prevented by minimizing our sugar intake since the infection is caused by an organism which metabolise sucrose into lactic acid. Due to the inhibitory activities of some chewing sticks most especially *A. leiocarpus* against a microorganism associated with dental caries, it should be cultivated on a large scale and its use encouraged.

Finally, the importance of oral hygiene should be made known to people and if practiced, should greatly reduce or even eliminate the caries experience.

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