ORIGINAL ARTICLE

AFRICAN JOURNAL OF CLINICAL AND EXPERIMENTAL MICROBIOLOGY SEPTMBER 2010 ISBN 1595-689X VOL 11(3)

AJCEM/201008/**21025** -http://www.ajol.info/journals/ajcem

AFR. J. CLN. EXPER. MICROBIOL 11(3): 182-194

USERS' PERCEPTIONS AND EFFICACY OF INDIGENOUS ADJUNCT TEETH-CLEANSING AGENTS ON THE BACTERIAL FLORA OF HUMAN DENTAL CARIES

***Adenike A. O. Ogunshe PhD[1] and Olubukonla G. Odumesi ND[2]**

1 Applied Microbiology and Infectious Diseases, Department of Microbiology, University of Ibadan, IBADAN, Nigeria. and 2 Department of Science Laboratory Technology, School of Pure and Applied Sciences, Moshood Abiola Polytechnic, ABEOKUTA, Nigeria.

*** Correspondence: Applied Microbiology and Infectious Diseases, Department of Microbiology, University of Ibadan, IBADAN, Nigeria. Fax: (234)-2-8103043 E-mail: adenikemicro@yahoo.com**

RUNNING TITLE: INDIGENOUS TEETH-CLEANSING AGENTS

ABSTRACT

Background and Aim: The aim of this study was to conduct *in vitro* and *in vivo* studies for the evaluation of antimicrobial potential of four Nigerian indigenous chewing sticks and to compare the oral effects of the popular Nigerian chewing sticks with toothpastes and other teeth-cleansing agents on a total of 21 and 17 oral bacterial strains isolated from children and adult dental caries respectively.

Materials and Methods: Teeth cleansing agents were assayed *in vitro* against the isolated bacterial carries flora on de Man Rogosa and Sharpe (MRS) agar using modified agar well-diffusion methods. The simulated *in vivo* studies were carried out using twenty four human subjects.

Results: The results obtained indicated that the bacterial flora exhibited different degrees of *in vitro* inhibitions of between low and moderate susceptibility against the teeth-cleansing agents. Toothpastes recorded the highest rates of inhibition against the dental caries isolates, while H_2O_2 was the most inhibitory among the chemical teeth-cleansing agents against the dental caries isolates from children (47.6%) and adults (52.9%). No species differential susceptibility to the teeth-cleansing agents was observed among the dental caries isolates.

Conclusions: This study showed that combined teeth cleansing hygiene with toothpastes, chewing sticks and chemical teeth cleansing agent (H_2O_2) gave the best mouth feel and were found to be the most preferred.

Keywords: Chewing sticks, dental caries, indigenous, oral health and hygiene, teeth-cleansing agents.

INTRODUCTION

The human oral cavity harbours a complex microbial community of diverse range of microorganisms (1, 2, 3), and these comprise of several species of bacteria, fungi and protozoa. The oral cavity is normally relatively stable but under certain circumstances can cause major dental diseases such as caries and periodontitis because many of the commensal oral microorganisms may cause diseases if provided with appropriate conditions. Dental problem is known to be the most common health problem in the human communities (2), while dental diseases are of great economic importance throughout the world, hence, the environmental forces, which both affect and effect the microbial colonisation of the mouth have been the subject of considerable research (4). There is also a growing public recognition of the importance of oral health because once a permanent tooth falls off it cannot be replaced by another tooth. It is therefore, very important to take care of the teeth.

People in rural areas, especially in tropical forest region of the world have utilised plants as part of their primary health care system for millennia (5, 6, 7), and this medical dependence on plants continues till today for at least 80% of the world's rural population (8). Ethnomedicinal plants in form of chewing sticks used for the cure of dental problems include *Zanthoxylum zanthoxyloides*, *Zanthoxylum nitidum*, *Jatropha curaas*, *Azadirachta indica* and *Salvadora persica*, *Acacia senegal* var. Senegal, *Eriosema psoraleoides*, *Ocimum suave*, *Opilia celtidifolia* and *Xerophyta suaveolens*, *Lamium tenuiflorum* etc., *kikar* (*Acacia arabica*) from Tanzania and *arak* (*Salvadora persica*) from Pakistan, as well as *miswak*

(*Salvadora persica*) from Saudi Arabia, among several others (9, 10, 11, 12, 13, 14, 15). In Nigeria, the roots of such plants and others (*Massularia acuminate*, *Sorindeia warneckei*) are traditionally used as chewing sticks for maintaining healthy teeth among indigenous populations (16, 17, 18).

Zanthoxylum xathoxyloides, belonging to the family Rutaceal and the order- Rutales consists of 9 genera, and about 28 species occur in the forest savanna mosaic of the lowland rainforest and sometimes, also abundant in coastal areas of Nigeria, where the roots are traditionally used as chewing sticks for toothache and for maintaining healthy teeth among the indigenous populations (19). The roots and twigs of *Aayan* (*Prosopis africana*), which belongs to the Family Compositae are widely used as tooth-cleaners. Namibian chewing stick, *Diospyros lycioides* commonly known as *mathala* was also found to contain antibacterial compounds against oral pathogens like *S. mutans* and *S. sanguinis* and some periodontal pathogens (20). The purpose of this study is therefore, to investigate the potentials of some of the most popular, local Nigerian chewing sticks and chemical agents as natural tooth cleansing agents or adjuncts to toothpastes in oral hygiene.

Materials and methods

Collection of chewing sticks samples
Orin ata (*Zanthoxylum xathoxyloides*) (Engl.). Zepernick & Timter, *Fagara zanthoxyloides* Lam, common name (candle wood); *Aayan* (*Prosopis africana*), common name (mesquite); *ewuro* (*Vernonia amygdalina*) Del., common name (bitter leaf); were all obtained from a local herbs market in Ibadan metropolis, while *dogoyaro* (*Azadirachta indica*), common name (Neem) was obtained from Moshhod Abiola Polytechnic, Abeokuta, Ogun State, Nigeria.

Isolation of bacterial species:
Dental caries specimens were aseptically transferred into 100ml MRS (de Man, Rogosa and Sharpe agar; Lab M, Lab M Ltd., Lancashire, UK; batch no. 092898) broth at pH 5.3 - 5.5 and incubated overnight at 32^0C for the selective isolation of *Streptococcus* and *Lactobacillus* species. 1ml volumes of the overnight broth cultures were separately transferred to sterile Petri dishes by the pour-plate method before incubation aerobically and microaerophically in 5% CO_2 (Gas Pak Anaerobic System, Oxoid, England) at 32^0 -35^0 C for 24h.

Characterisation of the* Streptococcus *and* Lactobacillus *strains

Streptococcus and *Lactobacillus* isolates were characterised based on standard phenotypic taxonomic tools (21, 22, 23, 24, 25). The purity of the strains was checked and the cells were washed twice in 0.9% sterile NaCl solution after centrifuging. The isolates were then stored at 4^oC in Hogness freezing buffer (3.6mM K_2HPO_4; 1.3mM KH_2PO_4; 2.0mM Na-citrate; 1.0mM $MgSO_4$; 12% glycerol) and kept frozen at -12^0C.

Determination of antimicrobial activities of the teeth-cleansing agents against the dental caries flora.
Agar well-diffusion method: Holes, 6 mm in diameter were aseptically punched out of the MRS agar plates, followed by surface flaming of the agar plates. The agar plates when cool were then seeded by streaking the entire surface of the culture plates with the indicator strains isolated from the dental caries. The seeded plates were then incubated at 32^0C for 30 minutes, after which 500µl of each of the chewing stick extracts, toothpastes, and chemical agents were separately dispensed into the agar wells, followed by incubation at 32^0C for 24h (26). The demonstration of antagonism depends on the release of diffusible inhibitory metabolites from the chewing stick extracts, toothpastes and chemical agents into the assay medium during incubation. Inhibitory zones surrounding the agar wells were noted and recorded in mm diameter, while zones less than 10.0 mm diameter and absence of zones of inhibition were recorded as resistant.

Simulated *in vivo* study:
Twenty four adults (Male = 17; Female = 7) who participated in the study were divided into two groups and taught on how to evaluate their oral hygiene by usage of chewing sticks, toothpastes and oral chemical agents, making mouth feel the selection criterion. Twelve of the participants were to use each of the 3 chewing sticks (*Orin ata*, *ewuro* and *dongoyaro* alone for one week each (Group 1). The other twelve participants (Group 2) were to use each of the toothpastes, Close-up [Unilever Nigeria Plc.. with active ingredients – sodium monofluorophopsphate, PEG SCMC synthetic cleaning agent]; Dabur herbal toothpaste with basil [Dabur India Ltd., NEPZ with ingredients – calcium carbonate, glycerin, sodium lauryl sulfate, gum carageenan, liquonine extract, bullet wood extract, *Acacia arabica*, oak galls, tumeric, *Patharman sarsapartilla*, lotus bark, nut grass, etc.,]; Florish gel [PZ, Industries, Nigeria with active ingredients (0.76% sodium monofluorophosphate), Holdent calcium fluoride with menthol [Charmanuel Industries Ltd., London, UK with active ingredients – 0.76% sodium monofluorophosphate, 0.10% sodium fluoride PL 0049/0020, calcium (Ca^{2+}) (1196)] and [Macleans (SmithKline Beecham Nigeria Plc., with active ingredients – 0.8% monofluorophosphate and 0.13% calcium glycorophosphate)] toothpastes alone for one week each. Informed consents were obtained from the subjects prior to the simulated *in vivo* studies.

The 2 groups were to reverse the oral treatments after the third week until the sixth week, when the two groups were to combine chewing sticks and toothpastes in the teeth cleansing study for one week and the chemical agents (ash and H_2O_2) were added for an additional week, making a total of 8 weeks. Mouth feel involves feeling the teeth with the tongue,

especially at the inner teeth surfaces for any feeling of teeth coating, especially on the molars and premolars; breath smell; presence or absence of saliva at the lip sides or minimal spitting while talking for about 15 minutes or more, as well as lightness of the tongue. During the pre-simulated training, the subjects were allowed to use the preferred toothpastes and chewing sticks in avoid bias. Children could not participate in this study because of the oral technicalities involved.

Results

A total of twenty-one oral bacterial strains consisting of *Streptococcus mutans* (7; 33.3%), *Strep. salivarius* (4; 19.0%), *Strep. sanguis* (1; 4.8%), *Lactobacillus acidophilus* (3; 14.3%), *L. casei* (5; 23.8%) and *L. fermentum* (1; 4.8%) were isolated from children caries; while the seventeen bacterial strains obtained from adult dental caries were *Streptococcus mutans* (6; 35.3%), *Strep. salivarius* (4; 23.5%), *L. casei* (5; 29.4%) and *L. fermentum* (2; 11.8%).

The oral bacterial flora were screened for their susceptibility to various teeth-cleansing agents that were commonly used by the rural and urban dwellers in most part of the country as obtained from the results of questionnaire administration (results not shown). The bacterial flora exhibited variable inhibitions to the teeth-cleansing agents, with toothpastes recording the highest rates. The inhibition zones of between 12.0 and 14.0 mm in diameter were considered as being of moderate inhibitory effects, while inhibition zones less than 10.0 mm in diameter were considered as negative (resistant) in this study. Florish (52.5%), Holdent (57.1%) and Close-up (61.9 %) were the most inhibitory among the toothpastes, with the bacterial flora displaying zones of inhibition of between 12.0 and 14.0 mm in diameter against the dental caries isolates from children. Florish (47.0%), Close-up (58.7%) and Holdent (76.4%) exhibited the highest inhibitions against the dental caries isolates from adults, with the bacterial flora also displaying zones of inhibition of between 12.0 and 14.0 mm in diameter against the dental caries isolates.

Table 2 shows the antagonistic effects of the four local chewing sticks on dental caries isolates from children and adults. *Dongoyaro* (47.6 %) and *ayan* (38.1%) were

TABLE 1: ANTAGONISTIC EFFECTS OF TOOTHPASTES ON DENTAL CARIES BACTERIAL ISOLATES

ORAL ISOLATES	TOOTHPASTES MACLEANS	DABUR	FLORISH	CLOSE-UP	HOLDENT
Children					
Streptococcus salivarius *984*	*12.0*	*14.0*	*14.0*	*14.0*	*14.0*
Streptococcus mutans *987*	*12.0*	*10.0*	*10.0*	*10.0*	*10.0*
Streptococcus mutans *989*	*10.0*	*10.0*	*14.0*	*14.0*	*14.0*
Streptococcus sanguis *9812*	*12.0*	*14.0*	*14.0*	*14.0*	*14.0*
Lactobacillus casei *9816*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Lactobacillus casei *9819*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Streptococcus mutans *9820*	*12.0*	*12.0*	*14.0*	*14.0*	*14.0*
Lactobacillus acidophilus *9823*	*10.0*	*12.0*	*12.0*	*12.0*	*12.0*
Lactobacillus casei *9829*	*14.0*	*14.0*	*14.0*	*14.0*	*14.0*
Lactobacillus fermentum *9833*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Streptococcus mutans *9834*	*14.0*	*12.0*	*10.0*	*12.0*	*10.0*
Streptococcus salivarius *9839*	*10.0*	*10.0*	*10.0*	*10.0*	*10.0*
Streptococcus salivarius *9841*	*10.0*	*10.0*	*10.0*	*10.0*	*10.0*
Streptococcus mutans *9849*	*10.0*	*10.0*	*12.0*	*12.0*	*12.0*
Streptococcus mutans *9851*	*14.0*	*14.0*	*14.0*	*14.0*	*14.0*
Lactobacillus casei *9856*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Lactobacillus acidophilus *9861*	*10.0*	*12.0*	*12.0*	*12.0*	*12.0*
Streptococcus salivarius *9865*	*12.0*	*08.0*	*08.0*	*12.0*	*12.0*
Streptococcus mutans *9870*	*08.0*	*08.0*	*08.0*	*08.0*	*08.0*
Lactobacillus acidophilus *9880*	*12.0*	*14.0*	*14.0*	*12.0*	*12.0*
Lactobacillus casei *9882*	*12.0*	*10.0*	*14.0*	*12.0*	*14.0*
Adults					
Lactobacillus casei *A01*	*10.0*	*12.0*	*10.0*	*12.0*	*12.0*
Streptococcus mutans *A02*	*12.0*	*08.0*	*12.0*	*12.0*	*10.0*
Streptococcus mutans *A03*	*10.0*	*10.0*	*10.0*	*10.0*	*12.0*

Lactobacillus casei *A04*	*14.0*	*14.0*	*10.0*	*14.0*	*14.0*
Streptococcus salivarius *A05*	*10.0*	*08.0*	*10.0*	*10.0*	*12.0*
Streptococcus mutans *A06*	*14.0*	*12.0*	*14.0*	*10.0*	*10.0*
Lactobacillus fermentum *A07*	*10.0*	*10.0*	*10.0*	*12.0*	*14.0*
Lactobacillus fermentum *A08*	*08.0*	*10.0*	*12.0*	*12.0*	*12.0*
Streptococcus mutans *A09*	*10.0*	*14.0*	*10.0*	*12.0*	*12.0*
Streptococcus salivarius *A10*	*10.0*	*10.0*	*10.0*	*10.0*	*10.0*
Streptococcus salivarius *A11*	*14.0*	*08.0*	*10.0*	*10.0*	*12.0*
Lactobacillus casei *A12*	*10.0*	*10.0*	*14.0*	*10.0*	*14.0*
Lactobacillus casei *A13*	*10.0*	*14.0*	*10.0*	*14.0*	*14.0*
Lactobacillus casei *A14*	*10.0*	*08.0*	*14.0*	*14.0*	*12.0*
Streptococcus salivarius *A15*	*12.0*	*10.0*	*12.0*	*10.0*	*12.0*
Streptococcus mutans *A16*	*10.0*	*10.0*	*12.0*	*12.0*	*10.0*
Streptococcus mutans *A17*	*12.0*	*08.0*	*14.0*	*14.0*	*14.0*
+14.0	*3 (14.4)*	*5 (23.8)*	*8 (38.1)*	*6 (28.6)*	*7 (33.3)* **}**
+12.0	*7 (33.3)*	*4 (19.0)*	*3 (14.4)*	*7 (33.3)*	*5 (23.8)* **}**
+10.0	*6 (28.6)*	*6 (28.6)*	*4 (19.0)*	*3 (14.4)*	*5 (23.8)*
< 10.0	*5 (23.8)*	*6 (28.6)*	*6 (28.6)*	*5 (23.8)*	*4 (19.0)*
+14.0	*3 (17.6)*	*3 (17.6)*	*4 (23.5)*	*4 (23.5)*	*5 (29.4)* **}**
+12.0	*3 (17.6)*	*2 (11.8)*	*4 (23.5)*	*6 (35.2)*	*8 (47.0)* **}**
+10.0	*10 (47.6)*	*7 (41.1)*	*9 (52.9)*	*7 (41.1)*	*4 (23.5)*
< 10.0	*1 (5.9)*	*5 (29.4)*	*0 (0.0)*	*0 (0.0)*	*0 (0.0)*

moderately inhibitory against the children dental caries isolates while *dongoyaro* (41.1 %) and *ewuro* (35.2%) were also moderately inhibitory against the children and adult dental caries isolates. According to Table 3, H_2O_2 was the most inhibitory among the chemical teeth-cleansing agents against the dental caries isolates from children (47.6 %) and adults (52.9 %). The results of the simulated *in vivo* studies were as shown in Table 4, with the combined teeth cleansing hygiene being the most preferred.

Discussion

A complex ecosystem is formed in the oral cavity, which changes constantly throughout life but some bacteria that are easily recovered from oral cavity upon routine culture have been found to be potentially pathogenic (3, 27, 28, 29, 30). The results obtained in this study in which *Streptococcus mutans* was the most recovered of the culturable bacterial species implicated in children and adult dental caries, followed by

Lactobacillus casei and *S. salivarius* is similar to the findings of the earlier workers; although, the slight observed inter-species differences may be due to differences in diet and geographical location. Franz *et al*. (31), for example, reported that *E. faecium* and *E. faecalis* in humans are dependent on geographical location, while Petti *et al*. (32) and other workers also found different variable affecting the oral microflora.

Since the invention of the first toothpaste, as far back as nearly 4000 years, the market of the toothpaste has never been slowed down (3, 33). Modern toothpaste was invented to aid in the removal of foreign particle and food substances in addition to cleaning of tooth, while fluoride is added to toothpaste to aid in protection from tooth decay; however, many of the innovations made in toothpastes after the fluoride break involve the addition of ingredients with special abilities to toothpaste and toothpaste packaging (34, 35). Almost all the toothpastes in Nigeria claim to contain certain active ingredients, especially calcium and fluoride, which prevent teeth against decay, and also responsible for the total hygiene by cleaning teeth thoroughly down to the gums. Inherent antiseptic properties of some of the toothpastes were claimed to prevent bacterial decomposition of food particles adhering to teeth and gaps between the teeth, hence preventing mouth odour.

Out of twelve toothpastes assayed in this study (based on the results of questionnaires administration, which tested the popularity of the tootthpastes), only the results of the most inhibitory toothpastes were reported in this study. Considering the zones of inhibition, Close-up, Darbur, Florish and Holdent toothpastes had higher *in vitro* inhibitory effects against the dental caries flora. In a similar study carried out in Nigeria (36) and Nepal, only few of their locally available toothpastes (especially, those that have triclosan as a major ingredient), were found to posses efficient antimicrobial properties (35). In spite of the major chemical ingredient(s) in toothpastes used in this study, the total number of the toothpastes with the moderate inhibitory activities was quite low compared with the total number of toothpastes in the country, more especially, the locally manufactured products.

Hydrogen peroxide is an oxidising agent that has effective antiseptic, disinfectant and deodorant properties, with antiseptic action due to its ready release of oxygen when applied to tissues. It was reported that though the effect lasts only as long as the oxygen is being released, it's of short duration and has weak antibacterial activity (37). According to Atlas (38), it was not effective against Gram-positive bacteria but was found in this study to be the most inhibitory teeth-cleansing chemical agent against dental caries isolates from children (47.6 %) and adults (52.9 %). Atlas (38) had also earlier documented that anaerobic bacteria have been found to be particularly sensitive to peroxides because they do not have catalase, an enzyme that degrades peroxides. This concept may therefore, be responsible for the high inhibitory activities of hydrogen-peroxide on the oral isolates which were mostly anaerobes.

Chewing sticks had been the traditional means of teeth cleansing among Nigerians, although Nigeria is not the only nation with traditional inclination for chewing sticks, and different plants are being used to treat different types of oral ailments like toothache, dental plaques, dental caries, pyorrhea and aphthae (9, 10, 13, 14, 16, 39, 40, 41, 42, 43). Various researchers have advocated the use of chewing sticks in community oral health programme because they are readily available, cheaper than the toothbrush and paste, and are found to possess antiplaque properties (16) but there are various findings on the efficacy of chewing sticks on oral health. While some findings reported their efficacy, some did not. The results of the *in vitro* inhibitory effects of four Nigerian local chewing sticks on dental caries bacterial isolates from children and adults in this study indicated moderate inhibitory activities among the children and adults isolates.

Additionally, a follow-up *in vivo* experiment on the teeth-cleansing agents to evaluate the oral hygiene on habitual chewing stick and toothbrush was conducted in this present study. The *in vivo* results showed the

TABLE 2: **ANTAGONISTIC EFFECTS OF LOCAL CHEWING STICKS ON DENTAL CARIES BACTERIAL ISOLATES**

	Local chewing sticks			
Isolates	**Orin ata**	**Ayan**	**Ewuro**	**Dongoyaro**
Children				
Streptococcus salivarius *984*	*08.0*	*10.0*	*14.0*	*10.0*
Streptococcus mutans *987*	*10.0*	*10.0*	*10.0*	*14.0*
Streptococcus mutans *989*	*12.0*	*10.0*	*10.0*	*12.0*
Streptococcus sanguis *9812*	*10.0*	*12.0*	*08.0*	*08.0*
Lactobacillus casei *9816*	*12.0*	*12.0*	*10.0*	*10.0*
Lactobacillus casei *9819*	*10.0*	*10.0*	*08.0*	*14.0*
Streptococcus mutans *9820*	*08.0*	*10.0*	*14.0*	*10.0*
Lactobacillus acidophilus *9823*	*12.0*	*12.0*	*10.0*	*12.0*
Lactobacillus casei *9829*	*12.0*	*10.0*	*10.0*	*12.0*
Lactobacillus fermentum *9833*	*08.0*	*10.0*	*08.0*	*10.0*
Streptococcus mutans *9834*	*08.0*	*10.0*	*12.0*	*12.0*
Streptococcus salivarius *9839*	*12.0*	*10.0*	*12.0*	*10.0*
Streptococcus salivarius *9841*	*10.0*	*12.0*	*10.0*	*12.0*
***Streptococcus mutans* 9849**	**10.0**	**12.0**	**10.0**	**14.0**
***Streptococcus mutans* 9851**	**10.0**	**12.0**	**10.0**	**14.0**
***Lactobacillus casei* 9856**	**08.0**	**08.0**	**12.0**	**10.0**
***Lactobacillus acidophilus* 9861**	**10.0**	**10.0**	**08.0**	**10.0**
***Streptococcus salivarius* 9865**	**12.0**	**10.0**	**12.0**	**10.0**
***Streptococcus mutans* 9870**	**10.0**	**12.0**	**10.0**	**10.0**
***Lactobacillus acidophilus* 9880**	**08.0**	**10.0**	**10.0**	**12.0**
***Lactobacillus casei* 9882**	**08.0**	**12.0**	**10.0**	**10.0**
Adults				
***Lactobacillus casei* A01**	**10.0**	**10.0**	**10.0**	**12.0**
***Streptococcus mutans* A02**	**10.0**	**08.0**	**12.0**	**12.0**
***Streptococcus mutans* A03**	**08.0**	**10.0**	**10.0**	**10.0**

Lactobacillus casei *A04*	***10.0***	***10.0***	***12.0***	***12.0***
Streptococcus salivarius *A05*	***12.0***	***10.0***	***08.0***	***10.0***
Streptococcus mutans *A06*	***12.0***	***12.0***	***10.0***	***12.0***
Lactobacillus fermentum *A07*	***10.0***	***10.0***	***10.0***	***10.0***
Lactobacillus fermentum *A08*	***12.0***	***10.0***	***12.0***	***08.0***
Streptococcus mutans *A09*	***10.0***	***08.0***	***10.0***	***10.0***
Streptococcus salivarius *A10*	***10.0***	***10.0***	***08.0***	***10.0***
Streptococcus salivarius *A11*	***08.0***	***10.0***	***10.0***	***10.0***
Lactobacillus casei *A12*	***10.0***	***10.0***	***10.0***	***12.0***
Lactobacillus casei *A13*	***10.0***	***12.0***	***12.0***	***12.0***
Lactobacillus casei *A14*	***10.0***	***10.0***	***10.0***	***10.0***
Streptococcus salivarius *A15*	***12.0***	***10.0***	***12.0***	***09.0***
Streptococcus mutans *A16*	***10.0***	***10.0***	***12.0***	***10.0***
Streptococcus mutans *A17*	***12.0***	***10.0***	***08.0***	***12.0***

+14.0	***- (0.0)***	***- (0.0)***	***2 (9.5)***	***4 (19.0) }***
+12.0	***6 (28.6)***	***8 (38.1)***	***4 (19.0)***	***6 (28.6) }***
+10.0	***8 (38.1)***	***12 (57.1)***	***11 (52.3)***	***10 (47.6)***
<10.0	***7 (33.3)***	***1 (4.76)***	***4 (19.0)***	***1 (4.76)***
+14.0	***- (0.0)***	***- (0.0)***	***- (0.0)***	***- (0.0) }***
+12.0	***5 (29.4)***	***2 (11.8)***	***6 (35.2)***	***7 (41.1) }***
+10.0	***10 (46.6)***	***13 (76.4)***	***8 (38.1)***	***8 (47.0)***
<10.0	***2 (11.8)***	***2 (11.8)***	***3 (17.6)***	***2 (11.8)***

local chewing stick, *orin ata* (*Zanthoxylum zanthoxyloides*) to be the most preferred, based on the mouth-feel effect by the adult study group. The simulated experiment among the control adult group indicated that the combined cleansing effect of *Zanthoxylum zanthoxyloides* before or after brushing with any of the toothpastes, followed by rinsing with

hydrogen peroxide gave the best mouth-feel effect for a period of 12-18 hours after the oral hygiene treatments.

The combined cleansing effect of *ewuro* (*Vernonia amygdalina*) and then brushing with any of the toothpastes followed by rinsing with hydrogen peroxide also gave a better mouth feel effect for a period of 8 hours. The antimicrobial property of *Zanthoxylum zanthoxyloides* has also been investigated by Muhammad and Shinkafi (44), and the extracted essential oils from this chewing stick were found to posses antiseptic and anti-carcinogenic actions.

TABLE 3: *ANTAGONISTIC EFFECTS OF CHEMICAL AGENTS ON DENTAL CARIES BACTERIAL ISOLATES*

	Chemical agents		
Isolates	***Ash***	***NaCl***	***H_2O_2***
Children			
Streptococcus salivarius ***984***	***10.0***	***12.0***	***10.0***
Streptococcus mutans ***987***	***12.0***	***10.0***	***10.0***
Streptococcus mutans ***989***	***10.0***	***10.0***	***10.0***
Streptococcus sanguis ***9812***	***NDG***	***NDG***	***NDG***
Lactobacillus casei ***9816***	***10.0***	***10.0***	***14.0***
Lactobacillus casei ***9819***	***10.0***	***10.0***	***12.0***
Streptococcus mutans ***9820***	***08.0***	***10.0***	***12.0***
Lactobacillus acidophilus ***9823***	***12.0***	***10.0***	***12.0***
Lactobacillus casei ***9829***	***10.0***	***10.0***	***12.0***
Lactobacillus fermentum ***9833***	***10.0***	***10.0***	***10.0***
Streptococcus mutans ***9834***	***12.0***	***12.0***	***10.0***
Streptococcus salivarius ***9839***	***10.0***	***12.0***	***14.0***
Streptococcus salivarius ***9841***	***10.0***	***10.0***	***10.0***
Streptococcus mutans ***9849***	***10.0***	***08.0***	***10.0***
Streptococcus mutans **9851**	**12.0**	**12.0**	**10.0**
Lactobacillus casei **9856**	**08.0**	**10.0**	**10.0**
Lactobacillus acidophilus **9861**	**10.0**	**14.0**	**14.0**
Streptococcus salivarius **9865**	**12.0**	**10.0**	**12.0**
Streptococcus mutans **9870**	**10.0**	**10.0**	**14.0**
Lactobacillus acidophilus **9880**	**12.0**	**12.0**	**12.0**
Lactobacillus casei **9882**	**10.0**	**10.0**	**10.0**
Adults			
Lactobacillus casei **A01**	**10.0**	**12.0**	**14.0**
Streptococcus mutans **A02**	**12.0**	**10.0**	**12.0**
Streptococcus mutans **A03**	**10.0**	**10.0**	**10.0**
Lactobacillus casei **A04**	**10.0**	**10.0**	**12.0**
Streptococcus salivarius **A05**	**12.0**	**08.0**	**10.0**
Streptococcus mutans **A06**	**08.0**	**12.0**	**12.0**
Lactobacillus fermentum **A07**	**10.0**	**10.0**	**10.0**
Lactobacillus fermentum **A08**	**12.0**	**10.0**	**08.0**
Streptococcus mutans **A09**	**10.0**	**08.0**	**10.0**
Streptococcus salivarius **A10**	**10.0**	**12.0**	**12.0**
Streptococcus salivarius **A11**	**10.0**	**10.0**	**12.0**
Lactobacillus casei **A12**	**08.0**	**10.0**	**10.0**

Lactobacillus casei ***A13***	***10.0***	***12.0***	***12.0***
Lactobacillus casei ***A14***	***08.0***	***10.0***	***10.0***
Streptococcus salivarius ***A15***	***12.0***	***08.0***	***14.0***
Streptococcus mutans ***A16***	***10.0***	***10.0***	***10.0***
Streptococcus mutans ***A17***	***08.0***	***12.0***	***12.0***
+14.0	***- (0.0)***	***1 (4.76)***	***4 (19.0) }***
+12.0	***6 (28.6)***	***5 (23.8)***	***6 (28.6) }***
+10.0	***12 (57.1)***	***13 (61.9)***	***10 (47.6)***
<10.0 / NDG	***3 (14.2)***	***2 (9.5)***	***1 (4.76)***
+14.0	***- (0.0)***	***- (0.0)***	***2 (11.8) }***
+12.0	***4 (23.5)***	***5 (29.4)***	***7 (41.1) }***
+10.0	***9 (59.4)***	***9 (52.9)***	***7 (41.1)***
<10.0	***4 (23.5)***	***3 (17.6)***	***1 (5.88)***

Keys: NDG = No determinable growth

The observed *in vitro* results of the teeth cleansing agents are not exclusively the same results obtained *in vivo* and this can be easily explained by the fact that the oral bacterial pathogens usually form a layer (biofilm) over the teeth enamel, and the time interval for the teeth cleansing agents to come in contact with them and brush them off are usually about 5-10 minutes brushing periods. In the determination of the mouth feel (checking the teeth with the tongue, especially at the inner teeth surfaces for any feeling of teeth coating, especially on the molars and premolars; breath smell; presence or absence of saliva foam at the lip sides or minimal spitting while talking for about 15 minutes or more, as well as lightness of the tongue), and it was generally accepted by the subjects that the combined teeth cleansing hygiene is the most preferred. Continuous slight spitting and or accumulation of foamy or non-foamy but thick whitish saliva at the corners of the lips were prevented or very minimal among the subjects during the study. It was also agreed that teeth cleansing, at least twice a day is the best mode of good oral hygiene.

In addition to the teeth cleansing effects, the subjects used in the present study also supported the usage of local chewing sticks as natural means of aiding mastication, being the only oral hygiene agent that can be daily chewed for periods of time. It was also advocated that chewing sticks aid in inducing salivation and thereby can also be responsible for cleansing of the salivary organs. Further studies on the effect of the teeth cleansing agents on the microbial loads of saliva are also on-going in our laboratories.

Since there could be varied human oral bacterial species among different nations, it is necessary that differentiation in oral bacterial flora associated with dental carries be put into consideration when preparing teeth-cleansing agents such as toothpastes. According to Tiwari *et al*. (3), fluoride and triclosan are the most commonly used and recommend active compounds by WHO, ADA and FDI, and regular evaluation of the efficacy of the fluoridated toothpaste by the private laboratory have been recommended by the WHO (45) but excess use of the fluoride can cause dental fluorisis, so the recommended amount of the fluoride was advised to be used as the ingredients in the toothpaste.

TABLE 4: SIMULATED IN VIVO *EFFECTS OF TEETH CLEANSING AGENTS ON MOUTH FEEL OF SUBJECTS*

Period of study	Best preferred	Better preferred	Preferred	
Weeks 1 & 4	***orin ata* [14]**	***ewuro* [7]**	***dongoyaro* [3]**	**Grp. 1**
Weeks 2 & 5	***orin ata* [12]**	***ewuro* [8]**	***dongoyaro* [4]**	**Grp. 2**
Weeks 3 & 6	***orin ata* [12]**	***ewuro* [9]**	***dongoyaro* [3]**	**Grp. 3**
Weeks 1 & 4	**Close up [10]**	**Holdent [9]**	**Florish [5]**	**Grp. 1**
Weeks 2 & 5	**Holdent [11]**	**Close up [9]**	**Florish [5]**	**Grp. 2**
Weeks 3 & 6	**Close up [12]**	**Holdent [9]**	**Florish [3]**	**Grp. 3**
Week 7	**Close up + *orin ata* [14]**	**Holdent + *orin ata* [6]**	**Close up + *ewuro* [4]**	**All Grps.**
Week 8*	**NaCl + toothpastes + Chw stck [14]**	**H_2O_2 + toothpastes + Chw stck**	**Ash + toothpastes +Chw stck**	

Keys: **Chw. stck = chewing sticks; Grp. = group; Grps. = groups**

Abiding by regulations, even in the production of household toilet products like toothpastes has been a running battle in the country, it is therefore, strongly recommended that all toothpastes in the country, including the locally produced ones be subjected to regular evaluation of their efficacy. Similarly, results on alternative teeth cleansing agents such as chewing sticks or herbal regimens would also have to consider this concept. This will aid in investigating into the indigenous chewing sticks of various countries, and there may ultimately be internationally recognised broad spectrum chewing sticks that cut across many nations. The active ingredients of these chewing sticks can then be extracted and prepared into industrial herbal toothpastes just as in the case of Darbur, an Indian toothpaste and a Korean toothpaste known as Herb & Bio.Salt, a gum care toothpaste consisting of natural herbal extracts from *Lonicera japonica*, *Taraxacum platycarpum*, green tea and sodium chloride.

REFERENCES

1. Black JG. Microbiology: Principles and Application, 2nd ed. Prentice Hall, Englewood Cliffs, New Jersey, USA. 1993; pp. 778.
2. Loesche WJ. Microbiology of dental decay and periodontal disease. In: Baron's Medical Microbiology. 4th Ed. University of Texas Medical Branch. 1996. pp. 1273.
3. Tiwari KB, Shrestha UT, Acharya AM. Subedi B, Paudyal B, Jnawali M, Shakya PUKC, Agrawa VP. Antibacterial activities of locally used toothpastes against dental pathogens. Braz Dent J. 2002; 13: 155-61.
4. Hogg SD. The lactic microflora of the oral cavity. In: The lactic acid bacteria vol. 1, Wood, B.J.B. (Ed.). Elsevier Science Publishers Ltd., England 1992; 115-147.
5. Farmsworth NR, Saejarto DD. The global importance of medicinal plants. In: The conservation of medicinal plants. *Proceedings of an International Consultation*. Akerele, O. Heywood, V. and Synge, H. (Eds.). Chiang Mai, Thailand. Cambridge University Press, Cambridge. 1991; 25-51
6. Abu-Rabia A: Urinary diseases and ethnobotany among pastoral nomads in the Middle East. *Journal of Ethnobiology and Ethnomedicine* 1:4: http://www.ethnobiomed.com/content/1/1 4
7. Busia K. Medical provision in Africa - past and present. Phytoth Res. 2005; 19 (11):919-23.
8. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *J. Ethnobiol Ethnomed*. 2006; **2:**43 .
9. van Palenstein H. WH, Munck L, Mushendwa S, Mrema FG. Cleaning effectiveness of chewing sticks among Tanzanian schoolchildren. J Clin Periodontol. 1992; 19(7): 460-463.
10. Almas K. The antimicrobial effects of seven different types of Asian chewing sticks. Odontostomatol Trop. 2001; 24(96): 17-20.
11. Wu CD, Darout IA, Skaug N. Chewing sticks: timeless natural toothbrushes for oral cleansing. J Periodontal Res. 2001; 36(5): 275-284.
12. Al-Bagieh NH, Idowu A, Salako NO. Effect of aqueous extract of *miswak* on the *in vitro* growth of *Candida albicans*. Oral Health Prev Dent. 2003; 1(4): 301-307.
13. Al-Otaibi M, Al-Harthy M, Söder B, Gustafsson A, Angmar-Månsson B. Comparative effect of chewing sticks and toothbrushing on plaque removal and gingival health. Oral Health Prev Dent. 2003; 1(4): 301-307.
14. **Al-Otaibi M. The *miswak* (chewing stick) and oral health: studies on oral hygiene practices of urban Saudi Arabians. Swed Dent J Suppl. 2004; (167): 2-75.**
15. **Dulger, B. Antifungal activity of *Lamium tenuiflorum* against some medical yeast**

***Candida* and *Cryptococcus species*. Pharm Biol 2009; (5): 467 – 470.**

16. ote EO. Oral prophylactic procedures and gingival health among Nigerian school children. Afr Dent J. 1991; 5: 15-20.

17. Adesina SK, Olugbade TA, Akinwusi DD., Bergenthal D. Extractives from *Zanthoxylum lemairie* root and stem. Pharmaz. 1997; 52: 720-724.

18. Ndukwe KC, Lamikanra A, Okeke IN. Antibacterial activity in plants used as chewing sticks in Africa. Drugs Future, 2004; 29(12): 1221.

19. Adesina SK. The Nigerian *Zanthoxylum*: Chemical and biological values. Afr J Med. 2005; 2 (3): 282-301.

20. Sharpe M.E. Lactobacilli in meat products. Food Manuf. 1962; 37: 582-589.

21. Vera HD, Power DA. Culture media. In: Manual of Clinical microbiology, (Eds.), Lennette, E.H., Balows, A., Hausler, W.J. and Truant, J.P. Washington: American Society for Microbiology 3rd edition, 1980; 992-993.

22. Molin G, Jeppsson B, Ahrné S, Johansson ML, Nobaek S, Ståhl M, Bengmark S. Numerical taxonomy of *Lactobacillus* spp. associated with healthy and diseased mucosa of the human intestines. J Appl Bacteriol. 1993; 74: 314-323.

23. Schleifer KH, Ludwig W. Phylogenetic relationships of lactic acid bacteria. In: *The genera of Lactic Acid Bacteria* ed. Wood, B.J.B. and Holzapfel, W.H. Blackie Academic and Professional. 1995; Vol. 2, 7-18.

24. Hardie JM, Whiley RA. Classification and overview of the genera *Streptococcus* and *Enterococcus*. J Apppl Microbiol Symp Suppl. 1997; 83: 1S-11S.

25. Ammor S, Rachman C. Chaillou S. Prévost H. Dousset X. Zagorec M. Dufour E. Chevallier I. Phenotypic and genotypic identification of lactic acid bacteria isolated from a small-scale facility producing traditional dry sausages. Food Microbiol. 2005; 22 (5): 373-382.

26. Tagg JR, Dajani AS, Wannamaker LW. Bacteriocins of Gram-positive bacteria. Bacteriol Rev. 1976; 40: 722-756.

27. Schupabach P, Osterwalder V, Guggenheim B. Human root caries: microbiota in plaque covering sound, carious and arrested carious root surfaces. Carries Research, 1995; 29(5): 382-95

28. Lopez L, Berkowitz R, Zlotnik H, Moss M, Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries. Paed Dent 1999; 21: 9-11. 20. Lopez L, Berkowitz R, Zlotnik H, Moss M, Weinstein P. Topical antimicrobial therapy in the prevention of early childhood caries. Paed Dent 1999; 21: 9-11.

29. Saini S, Mahajan A, Sharma JK, Arora O, Saini OP. Polymicrobial aetiology of dental caries. Ind J Path Microbiol. 1999; 42: 25-29.

30. Vivacqua-Gomes N, Gurgel-Filho ED, Gomes BPFA, Ferraz CCR, Zaia AA, Souza-Filho FJ. Recovery of *Enterococcus faecalis* after single- or multiple-visit root canal treatments carried out in infected teeth *ex vivo*. Int Endotonl J. 2005; 38 (10): 697-704.

31. Franz CAP, Holzapfel WH, Stiles MEM. Enterococci at the crossroads of food safety? International J Food Microbiol. 1999; 47: 1-24.

32. Lee SS, Zhang W, Li Y. The antimicrobial potential of 14 natural herbal dentifrices results of an *in vitro* diffusion method study. J Am Dent Ass. 2004; 135: 1135-41.

24. Clarke JK. On the bacterial factor in the etiology of dental caries. Br J Exp Path. 1942; 5: 141-7.

33. Clarke JK. On the bacterial factor in the etiology of dental caries. Br J Exp Path. 1942; 5: 141-147.

34. Hawkins R, Locker D, Noble J, Kay EJ. Professionally applied topical fluorides for caries prevention. Br Dent J. 2003; 6: 313-317.

35. Okpalugo, J. Ibrahim, K. Inyang, US Toothpaste formulation efficacy in reducing oral flora. *Trop J Pharm Res*. 2009; 8 (1): 71-77.

36. Reynolds JEF. Disinfectants. In: Martindale - The Extra Pharmacopoeia, 30th ed. The Pharmaceutical Press, London, 1993; 781-805.

37. Atlas RM. Microorganisms in our world. JM. Smith (Ed.). St Louis: Mosby-Year Book, Inc. USA. 1995; 667-670.

38. Olsson B. Efficiency of traditional chewing sticks in oral hygiene programs among Ethiopian schoolchildren. Community Dent Oral Epidemiol. 1978; 6(3):105-109.

39. Etkin NL. A Hausa herbal pharmacopoeia: Biomedical evaluation of commonly used plant medicines. J Ethnopharmacol. 1981; 4 (1): 75-98.

40. Aderinokun GA, Lawoyin JO, Onyeaso CO. Effect of two common Nigerian chewing sticks on gingival health and oral hygiene. Odontostomatol Trop. 1999; 22 (87):13-18.

41. Hebbar SS, Harsha VH, Shripathi V, Hegde GR. Ethnomedicine of Dharwad district in Karnataka, India--plants used in oral health care. J Ethnopharmacol. 2004; 94(2-3):261-266.

42. Tapsoba H, Deschamps JP. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. J Ethnopharmacol. 2006; 8: 104(1-2):68-78.

43. Muhammad S, Shinkafi MA. *In vitro* activity of the extracts of some Nigerian plants used as chewing sticks on human odontopathogens. Int J Pure Appl Sc. 2007; **1**(2): 25 –31.

44. Peterson PE. World Oral Health Report: Oral Health Programme Non-communicable Disease Prevention and Health Promotion, world Health Organization, Geneva, Switzerland; 2003.