Journal of Applied Biosciences 101:9589 – 9597

ISSN 1997–5902

Mineral compositions, phytochemical constituents and *in vitro* antimicrobial screening of some chewing sticks from Ibadan, South-western Nigeria

***[1]Aworinde D.O., [1]Erinoso S.M and [2]IbukunOluwa M.R.**

[1]Department of Biological Sciences, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria

[2]Department of Biology, Adeyemi College of Education, Ondo State, Nigeria

***Corresponding Author:** daveaworinde@yahoo.com (+234 7038952961, +234 8055361481)

Original submitted in on 17th February 2016. Published online at www.m.elewa.org on 31st May 2016 http://dx.doi.org/10.4314/jab.v101i1.3

ABSTRACT

Background: *Anogeissus leiocarpus* (DC) Guill. & Perr. – Aayin, *Distemonathus benthamianus* Baill. - Eyan, *Prosopis africana* (Guill. & Perr.) Taub. – Ayan, *Terminalia glaucescens* Benth. – Idi, and *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler. – Ata, are common chewing sticks sold in Ibadan, South-western Nigeria.

Objective: This study was aimed at providing scientific justification for the use of chewing sticks in traditional oral health.

Methodology and Results: The powdered plant samples were screened for mineral and phytochemical constituents and the antimicrobial activity was examined by agar well diffusion assay. The test organisms were clinical isolates of *Candida albicans, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Data were statistically analyzed. All the plant samples contained appreciable minerals and phytochemicals. The mineral elements quantified were all highest in *Z. zanthoxyloides* and least in *D. benthamianus* whereas the phytochemicals were mostly highest in *P. africana* and least mostly in *Z. zanthoxyloides. A. leiocarpus* and *D. benthamianus* inhibited the growth of two oral pathogens (*C. albicans* and *S. aureus*) while *P. africana* inhibited the growth of *S. aureus* and *T. glaucescens* was active against *C. albicans*. *Z. zanthoxyloides* was inactive against *C. albicans* and *S. aureus.* However, all the plants showed activity against non-oral pathogenic organisms.

Conclusion and Application of Findings: The activities of *A. leiocarpus* and *D. benthamianus* against *C. albicans* and *S. aureus* have been confirmed in this study and this justifies the traditional use of chewing sticks for oral health. Other plants used in the study inhibited the growth of other pathogens. The findings of this study apply to microbiology and oral health for the development of new or improved oral care products such as herbal toothpaste and mouthwash/rinse for the management of oral pathogens. Further research (*in vivo*) is advocated to evaluate possible toxicity or side effects of these plants in the mouth cavity. Conservation measures should be put in place to ensure sustainable use of these chewing sticks since the roots of the plants are the victims of use.

INTRODUCTION

Oral care is important in the maintenance of good life as it contributes to the general well being of the human body. Of all the human oral diseases, dental caries and periodontal disease are considered the most important (Petersen, 2003). Dental caries is a transmissible infectious disease that remains as a major public health problem in many developing countries and disadvantaged populations of developed nations (Mattos *et al.,* 1998). Dental caries are caused by the activity of Gram-positive bacteria (*Streptococcus mutans, Streptococcus salivarius, Lactobacillus acidophilus,* and *Actinomyces naeslundii*) while periodontal diseases are associated with Gram-negative bacteria (*Actinobacillus actinomycete mycomitans* and *Fusabacterium nucleatum*). *Candida albicans* and *Saccharomyces cerevisiae* are fungal pathogens linked to oral diseases. These oral micro-organisms have also been implicated in tooth decay, gingivitis, periodontitis, and teeth loss (Aneja and Joshi, 2010; Jebashree *et al.,* 2011). Common home practices in the management of dental plagues include good oral hygiene root planning and scaling. In addition, agents such as Chorhexidine, Fluorides and various antibiotics are commercially available that can be used to prevent dental caries. However, these chemicals can alter oral micro-biota and have undesirable side effect, such as vomiting, diarrhoea, and tooth staining (Yadav and Yadav, 2013). Also, in most developed and developing countries, dental treatment usually is expensive and not easily accessible. These challenges have encouraged the continuous search for natural phytochemicals present in plants. These alternative treatment procedures, which include the use of chewing sticks, especially by the rural and semi-urban populations, are now important because of the diversity of plant life, relative cheapness, acclaimed potency, and cultural relevance. The use of chewing sticks to prevent dental diseases (Homer *et al.,* 1990) as they have potential in preventing oral ailments (Ndukwe *et al.* 2004) as received recognition by the World Health Organization (Akande and Ajao, 2011). In African populations, roots formed the major chewing parts of plants; other parts commonly used are slim/sliced stem with bark retained or removed, and twigs. In Nigeria, the use of chewing stick is peculiar to the countryside; however, semi-urban and urban residents also use chewing sticks. The choice of stick depends on the known or perceived cleansing potentials, taste (peppery or bitter), therapeutic values, simplicity in being chewed into brush, and the ability to froth (Akande and Ajao, 2011).

Anogeissus leiocarpus ***Distemonathus benthamianus*** ***Prosopis africana*** ***Terminalia glaucescens*** ***Zanthoxylum zanthoxyloides***

Plates a-e: Chewing sticks used in this study.

Numerous reports from different parts of the world exist on the *in vitro* antimicrobial assay of some chewing sticks on oral pathogens (Homer *et al.,* 1990; Cai *et al.,* 2000; Ndukwe *et al.* 2004; Akande and Ajao, 2011; Osho *et al.,* 2011). Some of these plants have been investigated and shown to inhibit

the growth of oral microbial pathogens. Others have broad-spectrum activity against other pathogenic micro-organisms that are not cariogenic. This study was aimed at evaluating the mineral element composition, phytochemical constituents and antimicrobial activity of five chewing sticks sold in Ibadan, South-western Nigeria and their potentials in the management of oral infectious diseases and other pathogenic organisms with a view to validating the traditional use of chewing sticks.

MATERIALS AND METHODS

Collection and Identification of Plants: Fresh roots of *Anogeissus leiocarpus* (DC) Guill. & Perr., *Distemonathus benthamianus* Baill., *Prosopis africana* (Guill. & Perr.) Taub., *Terminalia glaucescens* Benth. and *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler. were collected from a forest around Ladoke Akintola University of Technology (LAUTECH) Botanical Garden, Ogbomoso, Nigeria. Identification was done at University of Ibadan Herbarium (UIH).

Mineral Constituents Analyses: The plant samples were air-dried for six weeks. The dried samples were pulverised into coarse powder and thereafter screened for mineral components. Sodium (Na), calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), fluorine (F), zinc (Zn), iron (Fe), and copper (Cu) were quantified according to AOAC (2005).

Phytochemical Analysis: The powdered samples were screened for phytochemical constituents (alkaloids, flavonoids, saponins, tannins and terpenes) according to Harbone (1973), Evans (2002) and Sofowora (2008).

Preparation of Ethanol Extracts: 300g of each dried powdered root samples was extracted in 80% ethanol (2.5 litres) for 48 hours. The extract was filtered (with Whatman No 1 filter paper) and evaporated to dryness at 40°C using a rotary evaporator. The extract was refrigerated at 4°C prior to use.

Organisms: The test organisms were clinical isolates of *Candida albicans, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* obtained from Medical Microbiology Unit of the University College Hospital (UCH) Ibadan, Nigeria. The isolates were maintained on nutrient agar and grown in nutrient broth for 18 hours at 35 ± 2°C for experiments.

Antimicrobial assay: Graded concentrations (0.5 – 100mg/ml) were prepared and used for the antimicrobial screening. The extracts were screened using agar well diffusion assay (Hood *et al.*, 2003). Nineteen (19) mls of nutrient agar was inoculated with 1ml of overnight cultures (10^{-1} - 10^{-6} cfu/ml) of the test organisms. The inoculated agar was then poured into Petri dishes and allowed to set. From each of the plates, four wells were created using 6mm sterile cork-borer; 100µl of extract was introduced into each of the wells using a micro-pipette. The plates were left at room temperature, long enough for diffusion of the extract into agar. Subsequently, the plates were incubated at 35 ± 2°C for 18-36 hours. Zones of inhibition were recorded in millimetres. Each examination was carried out in triplicates for all the organisms.

Determination of Minimum Inhibitory Concentration (MIC): The MIC was determined using agar dilution assay (Hood *et al.,* 2003). The extracts were prepared at varied concentrations (0.5 – 20mg/ml) for the evaluation of MIC and 2ml of each extract to be tested was added to 18ml of sterile nutrient agar and poured into sterile Petri dishes and allowed to set; the plates were then inoculated by streaking a single colony (10^{-6}cfu/ml) of the organisms onto the surface of the agar. The preparation incubated at 35 ± 2°C overnight was examined for growth. Total suppression of organism growth was taken for activity. The minimum inhibitory concentration in mg/ml was recorded for each extract.

Data Analysis: Data were statistically analyzed and expressed as mean ± SD. Differences in means were assessed for significance by Duncan's Multiple Range Test at $p>0.05$.

RESULTS

The percentage yields of the ethanol extract of the plant samples are presented in Figure 1. The profile of the plants used in this study is shown in Table 1. All the plant samples contained appreciable mineral elements (Table 2). Table 3 shows the correlation coefficient matrix between the mineral elements in the chewing sticks. All values of correlation are positive and significant at 0.01 level. The quantitative phytochemical constituents of the plants are shown in table 4.

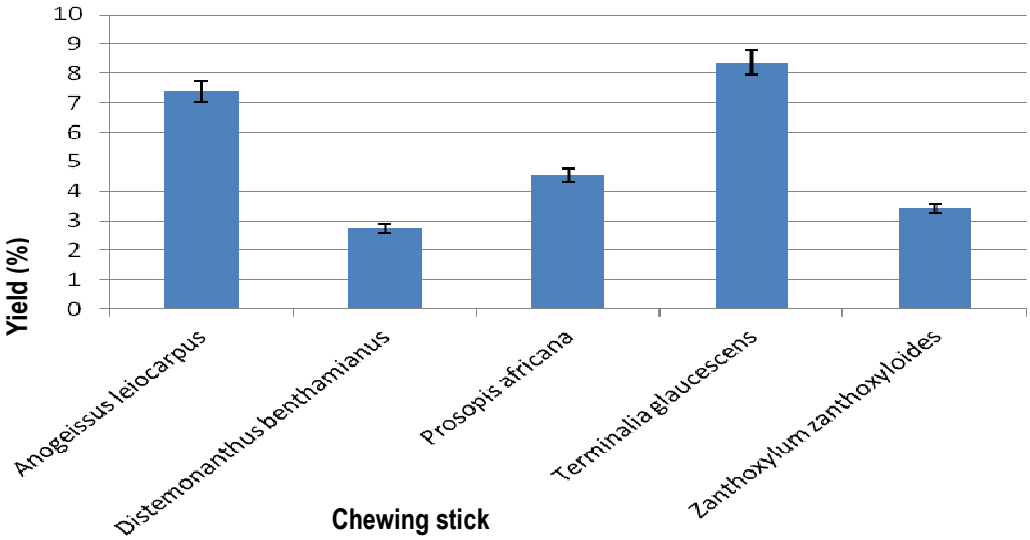

Fig. 1: Percentage yields of 300g pulverised chewing sticks extracted with 2.5 litres of ethanol.

Table 1: Profile of the chewing sticks used in this study

Plant	Family	Local Name (Yoruba)	Plant Habit	Part Used
Anogeissus leiocarpus (DC.) Guill. & Perr.	Combretaceae	Aayin/Orin-odan	Tree	Root
Distemonanthus benthamianus Baill.	Fabaceae	Eyan/Ayan	Tree	Root
Prosopis africana (Guill. & Perr.) Taub.	Combretaceae	Ayan	Tree	Root
Terminalia glaucescens Planch. ex Benth.	Combretaceae	Idi	Tree	Root
Zanthoxylum zanthoxyloides (Lam.) Zepern. & Timler.	Rutaceae	Ata	Tree	Root

Table 2: Mineral element composition of five chewing sticks from Ibadan, South-western Nigeria

Minerals	A	B	C	D	E
Na (%)	$0.059^{d}\pm0.002$	$0.027^{a}\pm0.001$	$0.050^{c}\pm0.005$	$0.037^{b}\pm0.006$	$0.067^{e}\pm0.002$
Ca (%)	$0.165^{c}\pm0.002$	$0.135^{a}\pm0.001$	$0.178^{d}\pm0.003$	$0.150^{b}\pm0.001$	$0.186^{e}\pm0.001$
Mg (%)	$0.253^{d}\pm0.002$	$0.194^{a}\pm0.001$	$0.243^{c}\pm0.03$	$0.218^{b}\pm0.001$	$0.262^{e}\pm0.002$
P (%)	$0.278^{c}\pm0.008$	$0.247^{a}\pm0.001$	$0.299^{d}\pm0.002$	$0.268^{b}\pm0.001$	$0.323^{e}\pm0.001$
K (%)	$0.260^{c}\pm0.002$	$0.222^{a}\pm0.001$	$0.263^{c}\pm0.002$	$0.237^{b}\pm0.001$	$0.282^{d}\pm0.002$
F (mg/kg)	$13.640^{c}\pm0.040$	$8.660^{a}\pm1.723$	$13.947^{c}\pm0.015$	$11.380^{b}\pm0.010$	$14.750^{c}\pm0.010$
Zn (mg/kg)	$28.657^{c}\pm0.031$	$21.300^{a}\pm0.010$	$29.140^{d}\pm0.010$	$23.687^{b}\pm0.015$	$31.457^{e}\pm0.006$
Fe (mg/kg)	$16.233^{cd}\pm0.031$	$8.713^{a}\pm1.553$	$15.813^{c}\pm0.012$	$11.507^{b}\pm0.116$	$17.287^{d}\pm0.012$
Cu (mg/kg)	$3.757^{d}\pm0.060$	$2.210^{a}\pm0.010$	$3.393^{c}\pm0.006$	$2.703^{b}\pm0.006$	$4.503^{d}\pm0.006$

A = *Anogeissus leiocarpus*, B = *Distemonanthus benthamianus*, C = *Prosopis africana*, D = *Terminalia glaucescens*, E = *Zanthoxylum zanthoxyloides*. Values are mean ± SD; n=3. Means followed by the same letter in the same row are not significantly different by Duncan's Multiple Range Test ($p>0.05$).

Table 3: Correlation coefficient matrix between the mineral elements of five chewing sticks from Ibadan, South-western Nigeria

	Mineral	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	Na (%)	0.899	0.986	0.866	0.968	0.893	0.959	0.950	0.984
2	Ca (%)		0.930	0.968	0.975	0.933	0.981	0.939	0.924
3	Mg (%)			0.870	0.972	0.937	0.977	0.977	0.968
4	P (%)				0.954	0.874	0.932	0.866	0.907
5	K (%)					0.924	0.991	0.956	0.979
6	F (mg/kg)						0.940	0.913	0.891
7	Zn (mg/kg)							0.974	0.956
8	Fe (mg/kg)								0.935
9	Cu (mg/kg)								

All values of correlation are significant at the 1% level

Table 4: Phytochemical constituents (mg/100g) of five chewing sticks commonly sold in Ibadan, South-western Nigeria

Phytochemicals	***A. leiocarpus***	***D. benthamianus***	***P. africana***	***T. glaucescens***	***Z. zanthoxyloides***
Alkaloids	$766.67^{d}\pm12.58$	$581.67^{b}\pm12.58$	$1235.00^{e}\pm0.00$	$640.00^{c}\pm18.03$	$370.00^{a}\pm22.91$
Flavonoids	$156.67^{d}\pm10.41$	$46.67^{a}\pm7.64$	$188.33^{e}\pm7.64$	$125.00^{c}\pm0.00$	$81.67^{b}\pm2.89$
Saponins	$475.00^{c}\pm13.23$	$558.33^{d}\pm17.56$	$675.00^{e}\pm15.00$	$416.67^{b}\pm10.41$	$378.33^{a}\pm12.58$
Tannins	$616.67^{d}\pm7.64$	$418.33^{a}\pm12.58$	$951.67^{e}\pm17.56$	$585.00^{c}\pm8.66$	$481.67^{b}\pm10.41$
Terpenes	$75.00^{b}\pm5.00$	$100.00^{c}\pm5.00$	$83.33^{b}\pm7.64$	$48.33^{a}\pm7.64$	$188.33^{d}\pm7.64$

Values are mean ± SD; n=3. Means followed by the same letter in the same row are not significantly different by Duncan Multiple Range Test ($p>0.05$).

Table 5: Correlation coefficient matrix between the phytochemical constituents of five chewing sticks from Ibadan, South-western Nigeria

	Phytochemicals	**2**	**3**	**4**	**5**
1	Alkaloids	0.815**	0.846**	0.936**	-0.527*
2	Flavonoids		0.410	0.897**	-0.464
3	Saponins			0.666**	-0.338
4	Tannins				-0.359
5	Terpenes				

**Correlation is significant at 0.01 level; *correlation is significant at 0.05 level

Table 6: Inhibitory effects of *Anogeissus leiocarpus* on five pathogenic organisms

Organism (10^{-5}cfu/ml)	Concentration of Extract and Control/Zone of Inhibition (mm)						
	0.5mg/ml	1mg/ml	2mg/ml	10mg/ml	20mg/ml	100mg/ml	Gentamycin 10µg/ml)
C. albicans	$4.38^{a} \pm 0.12$	$11.67^{b} \pm 0.58$	$12.33^{b} \pm 0.58$	$16.00^{c} \pm 0.00$	$16.33^{c} \pm 4.51$	$18.33^{c} \pm 0.58$	24.67±2.08
E. coli	Na	Na	Na	Na	Na	Na	30.00±1.00
K. pneumoniae	$3.80^{a} \pm 0.20$	$8.33^{b} \pm 0.58$	$10.33^{b} \pm 0.57$	$13.00^{c} \pm 1.00$	$14.33^{c} \pm 2.08$	$20.33^{d} \pm 2.08$	29.00±1.00
P. aeruginosa	$13.20^{b} \pm 0.27$	$10.67^{a} \pm 0.58$	$14.67^{c} \pm 1.53$	$16.67^{d} \pm 0.58$	$20.00^{e} \pm 0.00$	$21.33^{e} \pm 0.58$	21.33±0.58
S. aureus	$2.43^{a} \pm 0.15$	$4.17^{b} \pm 0.29$	$5.67^{c} \pm 0.58$	$8.33^{d} \pm 0.58$	$10.33^{e} \pm 0.58$	$13.67^{f} \pm 1.53$	31.33±1.53

Values are mean **±** SD; n=3. Means followed by the same letter in the same row are not significantly different by Duncan's Multiple Range Test ($p>0.05$). Na = Not active; diameter of cork-borer = 6mm

Table 7: Inhibitory effects of *Distemonanthus benthamianus* on five pathogenic organisms

Organism (10^{-5}cfu/ml)	Concentration of Extract and Control/Zone of Inhibition (mm)						
	0.5mg/ml	1mg/ml	2mg/ml	10mg/ml	20mg/ml	100mg/ml	Gentamycin (10µg/ml)
C. albicans	$10.53^{a} \pm 0.25$	$10.67^{a} \pm 1.15$	$11.67^{a} \pm 1.53$	$14.67^{b} \pm 1.53$	$16.67^{b} \pm 1.53$	$21.33^{c} \pm 1.15$	24.67±2.08
E. coli	$3.67^{a} \pm 0.12$	$5.33^{a} \pm 0.58$	$10.00^{b} \pm 2.00$	$13.00^{c} \pm 1.00$	$13.00^{c} \pm 1.73$	$19.33^{d} \pm 1.53$	30.00±1.00
K. pneumoniae	$5.77^{a} \pm 0.25$	$8.33^{b} \pm 0.58$	$10.33^{c} \pm 0.58$	$11.33^{d} \pm 0.58$	$13.00^{e} \pm 0.00$	$14.33^{f} \pm 0.58$	29.00±1.00
P. aeruginosa	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$12.67^{b} \pm 0.58$	$15.33^{c} \pm 1.53$	$17.00^{d} \pm 1.00$	21.33±0.58
S. aureus	$2.63^{a} \pm 0.15$	$5.00^{b} \pm 1.00$	$8.33^{c} \pm 0.58$	$11.33^{d} \pm 1.15$	$11.00^{d} \pm 1.00$	$15.67^{e} \pm 2.08$	31.33±1.53

Values are mean **±** SD; n=3. Means followed by the same letter in the same row are not significantly different by Duncan's Multiple Range Test ($p>0.05$); diameter of cork-borer = 6mm

Table 8: Inhibitory effects of *Prosopis africana* on five pathogenic organisms

Organism (10^{-5}cfu/ml)	Concentration of Extract and Control/Zone of Inhibition (mm)						
	0.5mg/ml	1mg/ml	2mg/ml	10mg/ml	20mg/ml	100mg/ml	Gentamycin (10µg/ml)
C. albicans	Na	Na	Na	Na	Na	Na	24.67±2.08
E. coli	Na	Na	Na	Na	Na	Na	30.00±1.00
K. pneumoniae	$0.00^{a} \pm 0.00$	$4.00^{b} \pm 1.00$	$8.67^{c} \pm 1.16$	$10.00^{c} \pm 2.00$	$13.67^{d} \pm 3.22$	$20.00^{e} \pm 2.00$	29.00±1.00
P. aeruginosa	$12.13^{ab} \pm 0.23$	$12.33^{ab} \pm 0.58$	$12.67^{b} \pm 1.16$	$12.33^{ab} \pm 2.52$	$9.67^{a} \pm 1.53$	$16.33^{c} \pm 1.53$	21.33±0.58
S. aureus	$6.80^{a} \pm 0.20$	$10.00^{b} \pm 2.00$	$14.67^{c} \pm 3.06$	$16.00^{c} \pm 1.00$	$19.67^{d} \pm 1.53$	$23.33^{e} \pm 1.53$	31.33±1.53

Values are mean **±** SD; n=3. Means followed by the same letter in the same row are not significantly different by Duncan's Multiple Range Test ($p>0.05$). Na = Not active; diameter of cork-borer = 6mm

Table 9: Inhibitory effects of *Terminalia glaucescens* on five pathogenic organisms

Organism (10^{-5}cfu/ml)	Concentration of Extract and Control/Zone of Inhibition (mm)						
	0.5mg/ml	1mg/ml	2mg/ml	10mg/ml	20mg/ml	100mg/ml	Gentamycin (10µg/ml)
C. albicans	$25.27^{a} \pm 0.64$	$25.33^{a} \pm 0.58$	$26.00^{a} \pm 2.00$	$22.67^{a} \pm 2.52$	$23.33^{a} \pm 3.06$	$25.33^{a} \pm 0.58$	24.67±2.08
E. coli	$6.77^{a} \pm 0.25$	$11.67^{b} \pm 0.58$	$14.33^{c} \pm 1.53$	$18.33^{d} \pm 0.58$	$21.00^{e} \pm 1.00$	$22.67^{d} \pm 1.15$	30.00±1.00
K. pneumoniae	$2.77^{a} \pm 0.25$	$9.67^{b} \pm 2.08$	$11.00^{b} \pm 1.00$	$17.67^{c} \pm 2.52$	$16.67^{c} \pm 1.15$	$22.67^{d} \pm 1.53$	29.00±1.00
P. aeruginosa	$18.83^{b} \pm 0.76$	$18.33^{b} \pm 0.58$	$11.00^{a} \pm 1.00$	$16.67^{b} \pm 1.15$	$22.00^{c} \pm 1.73$	$27.00^{d} \pm 1.73$	21.33±0.58
S. aureus	Na	Na	Na	Na	Na	Na	31.33±1.53

Values are mean **±** SD; n=3. Means followed by the same letter in the same row are not significantly different by Duncan's Multiple Range Test ($p>0.05$). Na = Not active; diameter of cork-borer = 6mm

Table 10: Inhibitory effects of *Zanthoxylum zanthoxyloides* on five pathogenic organisms

Organism (10^{-5}cfu/ml)	Concentration of Extract and Control/Zone of Inhibition (mm)						
	0.5mg/ml	1mg/ml	2mg/ml	10mg/ml	20mg/ml	100mg/ml	Gentamycin (10µg/ml)
C. albicans	Na	Na	Na	Na	Na	Na	24.67±2.08
E. coli	Na	Na	Na	Na	Na	Na	30.00±1.00
K. pneumoniae	$3.23^{a} \pm 0.25$	$10.33^{cd} \pm 0.58$	$8.67^{bc} \pm 3.06$	$6.00^{ab} \pm 2.00$	$10.00^{c} \pm 2.00$	$13.33^{d} \pm 1.16$	29.00±1.00
P. aeruginosa	$21.00^{ab} \pm 1.00$	$19.33^{a} \pm 1.16$	$22.00^{abc} \pm 1.00$	$25.00^{c} \pm 1.00$	$24.00^{bc} \pm 4.00$	$28.33^{d} \pm 0.58$	21.33±0.58
S. aureus	Na	Na	Na	Na	Na	Na	31.33±1.53

Values are mean **±** SD; n=3. Means followed by the same letter in the same row are not significantly different by Duncan's Multiple Range Test ($p>0.05$). Na = Not active; diameter of cork-borer = 6mm.

Table 11: Minimum inhibitory concentration (MIC) of test extracts on five pathogenic organisms

	Organism / MIC (mg/ml)				
Plant Extract	*C. albicans*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*
A. leiocarpus	10.0	-	50.0	20.0	100.0
D. benthamianus	20.0	50.0	100.0	50.0	50.0
P. africana	-	-	50.0	50.0	20.0
T. glaucescens	10.0	1.0	10.0	10.0	-
Z. zanthoxyloides	-	-	100.0	1.0	-

DISCUSSION

The ethanolic extracts of chewing sticks have been reported to be more active compared to water extracts of the plants (Akande and Ajao, 2011; Osho *et al.,* 2011). This observation might be of better dissolution of active constituents of the plants in ethanol than in water. Of interest are the high compositions of calcium, magnesium, phosphorus, fluorine and iron in *Z. zanthoxyloides* and low values in *D. benthamianus.* One would expect highest bioactivity in *Z. zanthoxyloides* because of the high content of important minerals; however, the reverse was the case *A. leiocarpus* and *D. benthamianus* with minerals but high phytochemicals showed activity against the oral pathogens. This finding corroborates the submission by various workers in the field that secondary metabolites (phytochemicals) are responsible for the therapeutic effects observed in medicinal plants (Edeoga *et al.,* 2002; Evans, 2002; Sofowora, 2008). Nonetheless, the role of minerals in oral health cannot be overlooked, however accessory. For example, calcium, magnesium, phosphorus and silica are minerals important for oral health. Calcium is associated with strength and health of the jaw bones. Magnesium plays a key role in the strength and formation of teeth, and in conjunction with calcium to mineralize teeth. Phosphorus, on the other hand, collaborates with calcium on the formation of teeth (Artemis, 2011). Fluoride is important as a nutrient at optimal levels for the mineralization and re-mineralization of teeth and bone as well as the reverse of demineralization and inhibition of active metabolism of acid-producing dental caries-causing bacteria (Wynn, 2002). Although controversies surround the importance of fluoride in oral health, recent reports suggest its significance in oral care and general well being (Palmer and Gilbert, 2012). Low levels of fluoride in drinking water is connected to tooth decay while excess fluoride ingestion results in toxicity and mottling of tooth enamel (Kulkarni *et al.,* 2014). The position of the Academy of Nutrition and Dietetics is that optimal and systemic use of topical fluoride is important for oral health and overall well being, and that its improper use has resulted in the reported cases of dental caries and allied disability (Palmer and Gilbert, 2012). The positive are an indication that association exists among the elements and that each element serves a complementary role. Medicinal plants rich in alkaloids and tannins are potential health-promoting plants (Jigam *et al.,* 2010). High saponin content in *P. africana* confirms the frothing nature of the chewing stick. The colour and organoleptic properties of *Z. zanthoxyloides* probably came about as a result of the concentration of terpenes in the plant. The activity of the plant extracts is concentration-dependent. This implies that activity is pronounced at high concentrations and vice versa. All the plants could also be explored for activity against pathogenic organisms other than oral microbes. Osho *et al.* (2011) evaluated the antibacterial activity of *Fagara* (*Zanthoxylum*) *zanthoxyloides* and *Prosopis africana* on *Pseudomonas aeruginosa* and *Klebsiela pneumoniae.* The authors reported *s*, and 22mm on *P. aeruginosa* and 25mm on *K. pneumoniae* with cold ethanol extract of *P. africana.* These findings of Osho *et al.* (2011) are in line with results obtained in this study and agree with the activity reported with 100mg/ml concentration of extracts. *Staphyloccocus aureus* was resistant to *Z. zanthoxyloides* whereas Osho *et al.* (2011) reported the inhibitory effect of the plant on *S. albus.* Several other plants besides the common chewing sticks have also been screened and found to be effective in the management of oral micro-organisms. Notable among them are: *Syzygium aromaticum* (Aneja and Joshi, 2010), *Psidium guajava* (Jebashree *et al.,* 2011), *Zingiber officinale* (Patel *et al.,* 2011), *Newbouldia officinale* (Okeke, 2003), *Azadirachta indica* (Lekshmi *et al.,* 2012), and *Vernonia amygdalina* (Osho *et al.,* 2011). These plants or their extracts could be formulated into preventive mouth wash or herbal toothpaste either singly or combined (for possible synergistic effect) as antimicrobial agents in the prevention or cure of dental caries. In this present study, *A. leiocarpus* and *D. benthamianus* showed activity against the oral pathogens *C. albicans* and *S. aureus.* This finding confirms the traditional use of these plants. Other plants used in the study inhibited the growth of non-oral pathogens. This suggests that the use of the chewing sticks by traditional societies has been based on claimed ethnobotanical use and not on informed ethnopharmacological importance. *A. leiocarpus* and *D. benthamianus* also inhibited the growth of other micro-organisms such as *E. coli, K pneumoniae,* and *P. aeruginosa.* This research finds application in microbiology and in oral health for the development of new or improved oral care products such as herbal toothpaste and mouthwash/rinse for the management of oral pathogens. Further research (*in vivo*) is advocated to evaluate possible toxicity or side effects of these plants in the mouth cavity. Conservation measures should be put in place to ensure sustainable use of these chewing sticks since the roots of the plants are the victims of use.

Acknowledgement: The technical assistance rendered by Mrs Lola Ogunleye (Department of Medical Microbiology, University College Hospital, Ibadan, Nigeria) is highly appreciated.

REFERENCES

Akande TA and Ajao AT, 2011. Chemotherapeutic values of four Nigerian chewing sticks on bacterial isolates of dental infection. Global Journal of Science Frontier Research 11(8): 91-95.

Aneja KR and Joshi R, 2010. Antimicrobial activity of *Syzygium aromaticum* and its bud oil against dental-caries-causing microorganisms. Issued: August 01, 2010. 14pp.

Artemis N, 2011. Holistic dental care. http://www.eidon.com/silica article/. Accessed and retrieved: 9th December, 2015.

Association of Analytical Chemists (AOAC), 1990. Official Methods of Analysis, 15th Edition. Washington, DC, USA. pp. 112-135.

Cai L, Wei G, Van der Bijl P, Wu CD, 2000. Namibian chewing stick, *Diospyros lycioides,* contains antibacterial compounds against oral pathogens. Journal of Agricultural Food Chemistry 48: 909-914.

Edeoga HO, Okwu EO, Mbaebie MO, 2002. Phytochemical constituents of some Nigerian Medicinal plants. African Journal of Biotechnology. 4(7): 685-688.

Evans WC, 2002. Trease and Evans Pharmacognosy, 15th Edition. W.B. Saunders, London. pp. 214-393.

Harbone JB, 1973. Phytochemical Methods, Chapman and Hall Ltd., London. pp. 49-188.

Homer KA, Manji F, Beighton D, 1990. Inhibition of protease activities of periondontopathic bacteria by extracts of plants used in Kenya as chewing sticks (mswaki). Archives of Oral Biology 35: 421-424.

Hood JR, Wilkinson JM, Cavanagh HMA 2003. Evaluation of common antibacterial screening methods utilized in essential oil research. Journal of Essential Oil Research 15(6): 428-433.

Jebashree HS, Kingsley SJ, Sathish ES, Devapriya E, 2011. Antimicrobial activity of few medicinal plants against clinically isolated cariogenic pathogens – an *in vitro* study. International Scholarly Research Network 1-6.

Kulkarni N, Kalele K, Kulkarni M, Kathariya R 2014. Trace elements in oral health and disease: An updated review. Journal of Dental Research and Review 1(2): 100-104.

Lekshmi NCJ, Sowmia N, Viveka S, Brindha JR and Jeeva S, 2012. The inhibiting effect of *Azadirachta indica* against dental pathogens. Asian Journal of Plant Science and Research 2(1): 6-10.

Ndukwe KC, Lamikanra A, Okeke IN, 2004. Antibacterial activity in plants used as chewing sticks in Africa. Drugs of the Future 29: 1221-1233.

Okeke AO, 2003. Three-minute herbal treatment to reduce dental caries with a *Newbouldia laevis* based extract. American Journal of Undergraduate Research 2(2): 1-4.

Osho A, Bello OO, Fayemi SO, Adetunji T, 2011. *In vitro* screening of some selected Nigerian medicinal plants (*Fagara zanthoxyloides, Vernonia amygdalina, Prosopis africana* and *Azadirachta indica*) for antibacterial activity. Advances in Bioresearch 2(1): 190-195.

Palmer CA and Gilbert JA, 2012. Position of the Academy of Nutrition and dietetics: The impact of fluoride on health. Journal of the Academy of Nutrition and Dietetics 112(9): 1443-1453.

Patel RV, Thaker VT, Patel VK, 2011. Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment. Asian Pacific Journal of Tropical Medicine S58-S61.

Petersen PE, 2003. The World Oral health Report 2003: Continuous improvement of oral health in the 21st century – the approach of the WHO Global Oral health Programme. Community Dentistry and Oral Epidemiology 83(1): 3.

Sofowora A, 2008. Medicinal plants and traditional medicine in Africa. Spectrum Book Ltd., Ibadan, Nigeria. p. 289.

Wynn R, 2002. Fluoride after 50 years, a clearer picture of its mechanism. General Dentistry 50(2): 118-122.

Yadav R and Yadav SK, 2013. Dental disease and its cure: A review. Asian Journal of Pharmaceutical and Clinical Research 6(2): 16-20.