www.ajbrui.net

Afr. J. Biomed. Res. 13 (January 2010) 61 - 68

Research Article

In-vitro Antimicrobial Activities of Methanol Extracts of *Zanthoxylum xanthoxyloides* and *Pseudocedrela kotschyi*

[1]*Adeniyi C.B.A. Odumosu B.T[1], [2]Ayelaagbe O.O and [3]Kolude B

[1]Departments of Pharmaceutical Microbiology [2]Chemistry and [3]Oral Pathology
P.O. Box 22346, University of Ibadan, Nigeria.

ABSTRACT: The antimicrobial activities of two Nigerian medicinal plant commonly used as chewing sticks *Zanthoxylum xanthoxyloides* and *Pseudocedrela kotschyi* antimicrobial activities were investigated against 7clinical strains of *Streptococcus mutans, Staphylococcus aureus* ATCC 29213*, Candida albicans, Candida tropicalis and Candida krusei* ATCC 6825. The antibacterial and antifungal activities of the extracts were determined using the agar well diffusion and surface plate method respectively. *Zanthoxylum xanthoxyloides,* was active against all the isolates especially on the bacteria with a MIC and MBC of 12.5mg/mL and 25mg/mL respectively. *Pseudocedrela kotschyi* showed no activity on all the tested isolates except on C. krusei ATCC 6825 with a MIC of 6.25mg/mL. . *Streptococcus mutans*, *S. aureus ATCC 29213 and C. albicans* were completely killed within the time period of 4hrs by *Z. xanthoxyloides* extracts in time kill study. A good correlation was found between the killing curves and the MIC of *Z. xanthoxyloides* against the tested isolates. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, saponins and traces of terpenoids. Ability of *Z. xanthoxyloides* crude extracts to inhibit the growth of the bacteria and fungi used in this study is an indication that the plant can be used as a source for antimicrobial agent in the development and formulation of toothpaste, thus justifying the use of the plant in locally as chewing sticks..

Keywords:

INTRODUCTION

Oral care research has gained scientific prominence ever since the discovery of *Streptococcus mutans* by J. Clarke, whose work formed part of the pioneer release of knowledge in the major causative organisms implicated in the dental caries (Clarke 1924). Sequel to this, many researchers have investigated the susceptibility of oral pathogens to various antimicrobial agent and mouth wash solution (Fudulu, 1975; Jeevarathan *et al*., 2007). Chewing stick as a means of tooth cleaning has found a common use in Africa especially in some African countries such as Nigeria, Ghana, Senegal, Addis Ababa etc (Buada sand Boakye-Yiadom, 1973). Researchers have compared the effect of mouth cleaning with chewing sticks and other conventional means like tooth paste and the use of mouth wash solution (Almas and Al-zeid 2004,). Although results obtained have proved that some of the available chewing sticks contain antimicrobial agents which are capable of inhibiting *in- vitro* the growth of most prevalence oral commensal which also include *Streptococcus mutans* and *Staphylococcus aureus* (Vuuren and Viljoen, 2006).

Manuscript received: September 2009; Accepted: October 2009

Address for correspondence:
P.O. Box 22346, University of Ibadan, Nigeria.
E-mail: *baadeniyi@yahoo.co.uk*

The aim of this study is to investigate and establish the antifungal and antibacterial activity of the methanolic stem extract of two chewing sticks against *Streptococcus mutans, Staphylococcus aureus ATCC, Candida albicans, Candida tropicalis and Candida krusei* ATCC 6825. Phytochemical screening of the extract would also be carried out using the convectional methods.

MATERIALS AND METHOD

Plant collection

Zanthoxylum xanthoxyloides FHI 108282 and *Pseudocedrela kotschyi* FHI 108280 stem were collected from different locations within Oyo state and were authenticated at the Forest Research Institute of Nigeria (FRIN) Herbarium. The voucher specimen No. Deposited at Forest Research Institute of Nigeria (FRIN)

Preparation of extracts.

The stems were chopped to bit and air dried at room temperature for 8 weeks and ground to powder and 500g of the powder was exhaustively extracted with methanol in a soxhlet extractor. The extract was later partitioned into hexane and chloroform fractions having kept part of the extracts as the methanol crude extract. Extracts were evaporated to dryness in and weighed.

Phytochemical screening:

A small portion of the dry powder was used for the preliminary phytochemical screening tests for secondary metabolites such as tannins, alkaloids, saponins, anthraquinones using the standard methods of Trease and Evans (1989) and Harborne (1998) with little modifications.

Test Microorganism and their source

Staphylococcus aureus ATCC 29213, *Candida albicans,* a clinical strain, *Candida tropicalis* were obtained from FIIRO and *Candida krusei* ATCC 6825 is a standard strain while the *Streptococcus mutans* were isolated from saliva and plaque samples of human volunteers using the method of Takada and Hirasawa (2005) with modifications. All other organisms were re-isolated, re-identified and the pure cultures were kept in nutrient agar slant and Sabouraud dextrose agar slant for bacteria and fungi respectively. *Streptococcus mutans* were maintained on blood agar slant and were all stored at 4^{o}C until required for further study. The antimicrobial sensitivity test was also carried out for each of the bacterial isolates (Table 1).

Media and Antimicrobial agent used.

The media used were Mueller Hinton Agar/Broth (OXOID), Tryptone Soya Broth (BIOTEC), Sabouraud Dextrose Agar (ANTEC). The antimicrobial agents used were Gentamycin at 7.8µgmL^{-1} as the positive control and 50% methanol as the negative control.

Determination of antimicrobial activity

This was carried out using agar diffusion method (Adeniyi *et al.,* 2006). A 0.1mL of a 1:100 dilution of an overnight culture of each bacterial and fungi isolate were used to seed sterile molten Mueller Hinton and Sabouraud Dextrose agar medium maintained at 45^{o}C respectively. The medium for *S. mutans* was supplemented with 5% sheep blood. The plates were allowed to dry in the incubator at 37^{o}C for 20mins. A standard cork borer of 8mm diameter was used to cut equidistance well on the surface of the agar. One gramme of the extract was reconstituted with 50% methanol to final concentration of 200mgmL,$^{-1}$, 100 mgmL^{-1}, 50 mgmL^{-1}, 25 mgmL^{-1}, 12.5mgmL^{-1}, 6.25mgmL^{-1}, 3.125mgmL.$^{-1}$ Gentamycin at 7.8µgmL^{-1} served as the positive control while methanol (50%) was used as the negative control. The agar plates were incubated at 37^{o}C for 24h after which diameter of zones of inhibition was measured. Result was average of triplicate experiment.

Determination of Minimum Inhibitory Concentration (MIC):

The minimum inhibitory concentration (MIC) of the extract against the organism was determined by the agar dilution method as described by Okeke *et al*. (1999) and Adeniyi *et al* (2000) with modifications. To exactly 19mL of a sterile molten Mueller Hinton agar maintained at 45^{o}C was added a 1mL of the extracts already diluted to give the following concentrations 200mgmL^{-1},100 mgmL^{-1}, 50 mgmL^{-1}, 25 mgmL^{-1}, 12.5 mgmL^{-1},6.25 mgmL^{-1} and 3.125 mgmL$^{-1.}$ They were properly mixed for even distribution of the extracts within the agar, and the agar allowed to set. The plates were then dried to remove steam. Each plate was divided into six sections. A loopful of the undiluted overnight broth culture was used to inoculate each section of the solidified agar-drug mixture in duplicates. A media/extract plate without an organism and a plate containing only the organism served as positive and negative control respectively. The plates were then examined for the presence of colonies after the incubation period. The least concentration that gave no visible colonies was taken as the minimum inhibitory concentration of the extract for the particular dilution of the organism.

Determination of Minimum Bactericidal/Fungicidal Cconcentration (MBC/MFC):

Minimum bactericidal concentration (MBC) of the plant extracts was determined by a modification of the method of Aibinu *et al.* (2007). To a 0.5mL. extract at different concentration as used in the MIC assay that showed no visible growth on the agar plates, was added 0.5ml. of test organism in tubes. These were incubated at 37^{o}C for 24-48hr. Samples were streaked out from the tubes on to Mueller Hinton/ blood agar and Sabouraud dextrose agar to determine the minimum concentration of the extract required to kill bacterial and fungi respectively. These concentrations were indicated by failure of the organism to grow on transfer to these media plates. The lowest concentration that prevented bacterial/fungal growth after days of incubation was recorded as the minimum bactericidal/ fungicidal concentration (MBC/MFC). All tests were performed in duplicates to ensure accuracy. Agar plates without extracts and another agar plates without any inoculated organism were also incubated serving as positive and negative controls plates respectively.

Bactericidal activity of the extract: The killing rate of the extract against the organisms (*S.aureus ATCC 29213, S. mutans and C. albicans*) were carried out using the method of Adeniyi *et al*. (2000). A 0.1mL of an approximately 1x10^{7} cfu of test bacterial strain was introduced into a 4.5mL of Mueller Hinton Broth containing MIC. An appropriate quantity of the test sample (extract-culture mixture) was withdrawn immediately, diluted out in normal saline and two drops of each dilution plated into a sixth portion of an oven dried Mueller Hinton agar to give control time 0 minutes count. Samples were taken at an interval of 30minutes, 1hour, 2hours, 3hours, 4hours and 5 hours. Each plate was divided into 6 portions and two drops of each dilution were placed on each portion per plate. The media for *S. mutans* was supplemented with 5% sheep blood. The procedure was carried out in duplicate to ensure reproducibility. Plates were incubated at 30 ^{o}C for 24-48 hours before counting the colonies. Control plates for positive and negative control were also incubated. The number of colony forming unit were counted after the period of incubation. The numbers of surviving bacterial cells per ml were calculated by taking into consideration the diluting factor and the volume of the inoculums. All the procedure was repeated for 2 × MIC, 4 × MIC and 8 × MIC. A graph of percentage viable count against time in minutes was plotted on a semi-logarithm graph.

RESULTS

The phytochemical screening of the two extracts revealed the presence of tannins, saponins, flavonoids. Alkaloids and traces of terpenoides were present in only *Z. xanthoxyloides*. The fractions of hexane and chloroform gave a minute yield hence there was no antimicrobial assay for their fractions.

Table1.
List of microorganisms and their antibiogram.

Organisms	Antibiogram
Streptococcus mutans	CHL^{S} AUG^{R} AMX^{R} ERY^{R}
BAA 001	COT^{S} GEN^{R} CXC^{R} TET^{S}
Streptococcus mutans	CHL^{S} AUG^{R} AMX^{R} ERY^{R}
BAA 002	COT^{S} GEN^{R} CXC^{R} TET^{S}
Streptococcus mutans	CHL^{S} AUG^{R} AMX^{R} ERY^{R}
BAA 003	C OT^{S} GEN^{R} CXC^{R} TET^{R}
Streptococcus mutans	CHL^{S} AUG^{R} AMX^{R} ERY^{R}
BAA 004	CO T^{S} GEN^{R} CXC^{R} TET^{R}
Streptococcus mutans	CHL^{S} AUG^{R} AMX^{R} ERY^{R}
BAA 005	COT^{R} GEN^{R} CXC^{R} TET^{R}
Streptococcus mutans	CHL^{S} AUG^{R} AMX^{R} ERY^{R}
BAA 006	COT^{S} GEN^{R} CXC^{R} TET^{S}
Streptococcus mutans	CHL^{S} AUG^{R} AMX^{R} ERY^{R}
BAA 007	COT^{S} GEN^{S} CXC^{R} TET^{S}
Staphylococcus aureus	CHL^{S} AUG^{R} AMX^{S} ERY^{S}
ATCC	COT^{S} G EN^{R} CXC^{R} TET^{S}

Note R=Resistance, S=Sensitive

The result of the antimicrobial susceptibility of the crude methanol extracts of the two plants showed that *Z. xanthoxyloides* was active against all the isolates especially on the bacteria with a MIC and MBC of 12.5mg/mL and 25mg/mL respectively. *Pseudocedrela kotschyi* showed no activity on all the tested isolates except on *C. krusei* ATCC 6825 with a MIC of 6.25mg/mL.

The highest activity of *Z. xanthoxyloides* was observed against *S. mutans* and *C. krusei* with zone diameters of 20mm at 200mg/mL concentrations.

The MIC of *Z. xanthoxyloides* against the fungi ranged from 25 to 50mg/mL. The antibiotic susceptibility pattern of each of the isolate is shown in Table 1. There was an observed multiple resistance to the antibiotic tested. The killing rate of *Z. xanthoxyloides* on *S. mutans, S. aureus ATCC 29213* and *C. albicans* was in the range of 2-4hours (Figures 1-3). Time kill study of the extract on *S. aureus ATCC 29213* showed that the extract killing effect was drastic in the first 1hr and then became gradual 3hr later while

the surviving cells was reduced to zero after 4hr of exposure. The extract has a similar effect on *S. mutans* and *C. albicans* in the time course study, the killing effect was observed to be drastic for the first 2hr then became gradual to until there was no surviving cells.

Table 2 :
Antibacterial activity of *Pseudocedrela kotschyi* and *Zanthoxylum xanthoxyloides* extracts

Extracts	**Concentration mg/mL**	***Streptococcus mutans* strains**							***S. aureus* ATCC 29213**
		Diameter zone of inhibition*							
		BAA 01	BAA 02	BAA 03	BAA 04	BAA 05	BAA 06	BAA 07	
Pseudocedrela kotschyi	200	13±0.00	—	13±0.00	12±0.00	12±0.00	—	13±0.00	—
	100								—
	50	—	—	—	—	—	—	—	—
	25	—	—	—	—	—	—	—	—
	12.5	—	—	—	—	—	—	—	—
	6.25	—	—	—	—	—	—	—	—
	3.125	—	—	—	—	—	—	—	—
Zanthoxylum xanthoxyloides	200	18±0.00	18±0.00	20±0.00	20±0.00	20±0.00	—	20±0.00	19±0.50
	100	15±0.00	16±0.00	18±0.00	18±0.00	18±0.00	—	18±0.00	17±0.50
	50	12±0.00	14±0.00	16±0.00	17±0.00	17±0.00	—	15±0.00	17±0.00
	25	11±0.00	12±0.00	13±0.00	14±0.00	14±0.00	—	13±0.00	15±0.50
	12.5	—	—	—	—	—	—	—	13±0.00
	6.25	—	—	—	—	—	—	—	10±0.00
	3.125	—	—	—	—	—	—	—	—
Gentamycin	7.8*	20	17	22	20	20	22	23	25±0.00
Methanol 50%		—	—	—	—	—	—	—	—

** Gentamycin concentration is in µg/ml, Diameter of cork borer = 8mm, — = No zone of inhibition, All experiment were performed in duplicates*

Table 3.
Antifungal activity of *Pseudocedrela kotschyi* and *Zanthoxylum xanthoxyloides* extracts

Extracts	**Concentration mg/mL**	**Organisms**		
		Diameter zone of inhibition*		
		C. albicans	*C. tropicalis*	*C. krusei*
Zanthoxylum xanthoxyloides	200	17±0.50	14±0.50	20±0.50
	100	16±0.00	12±0.50	18±0.00
	50	14±0.00	10±0.50	16±0.50
	25	13±0.00	—	15±0.00
	12.5	12±0.00	—	13±0.50
	6.25	10±0.00	—	—
	3.125	—	—	—
Pseudocedrela kotschyi	200	—	—	23±0.50
	100	—	—	22±0.50
	50	—	—	21±0.50
	25	—	—	18±0.00
	12.5	—	—	15±0.00
	6.25	—	—	13±0.00
	3.125	—	—	—
Gentamycin	7.8*	28±0.00	30±0.00	35±0.00
Methanol 50%		—	—	—

**Gentamiycin concentration is in µg/ml, Diameter of cork borer = 8mm; — = No zone of inhibition, N.D = Not determined. All experiment were performed in duplicates*

Table 4.
Minimum inhibitory concentration of *Pseudocedrela kotschyi* and *Zanthoxylum xanthoxyloides* extracts on the isolates

	Concentration (mg/mL)*				
Sample	*S. mutans*	*S. aureus*	*C. albicans*	*C. tropicalis*	*C. krusei*
Zx	12.5	12.5	25	25	25
Pk	N.D	N.D	N.D	N.D	6.25

Zx = *Zanthoxylum xanthoxyloides*
Pk = Pseudocedrela kotschyi
N.D= Not determined
Results are average of duplicate experiments

Table 5. Minimum bactericidal concentration of *Pseudocedrela kotschyi* and *Zanthoxylum xanthoxyloides* extracts on the isolates

	Concentration (mg/mL)				
Sample	*S. mutans*	*S. aureus*	*C. albicans*	*C. tropicalis*	*C. krusei*
Zx	25	25	50	25	25
Pk	N.D	N.D	N.D	N.D	12.5

Zx = *Zanthoxylum xanthoxyloides*
Pk = Pseudocedrela kotschyi
N.D= Not determined

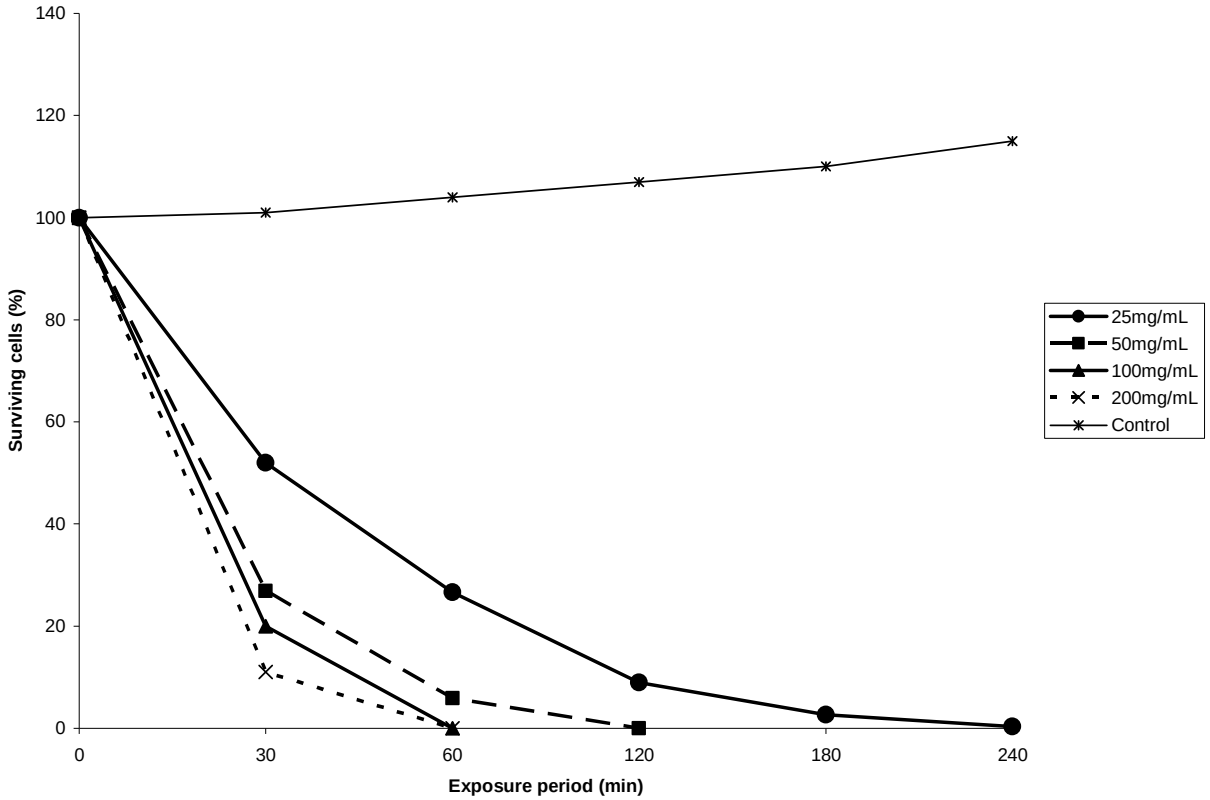

Fig.1. Kinetics of bactericidal activities of *Zanthoxylum xanthoxyloides* on *Candida albicans*

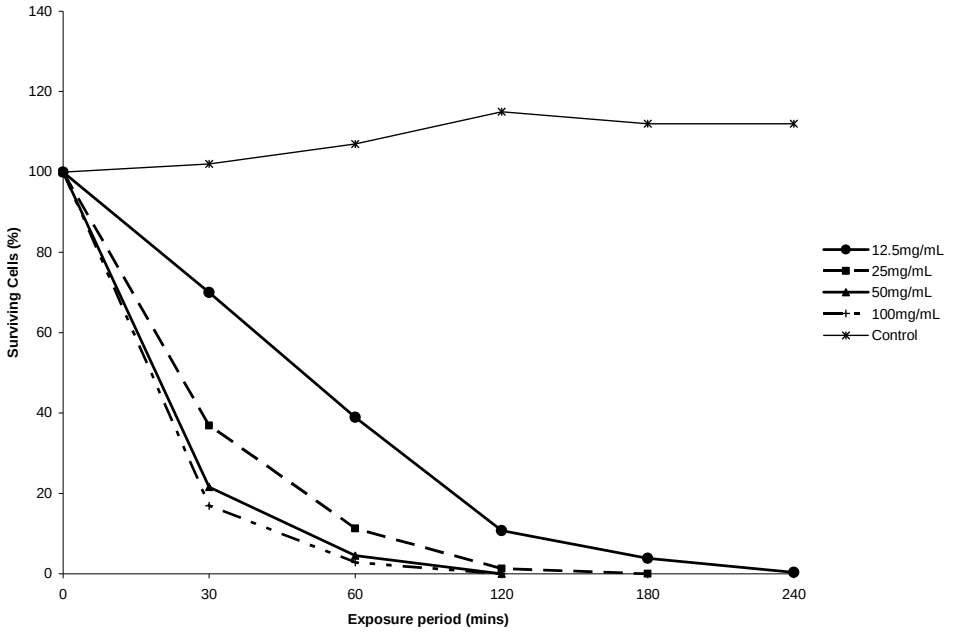

Fig.2. Kinetics of bactericidal activities of *Zanthoxylum xanthoxyloides* on *Streptococcus mutans*

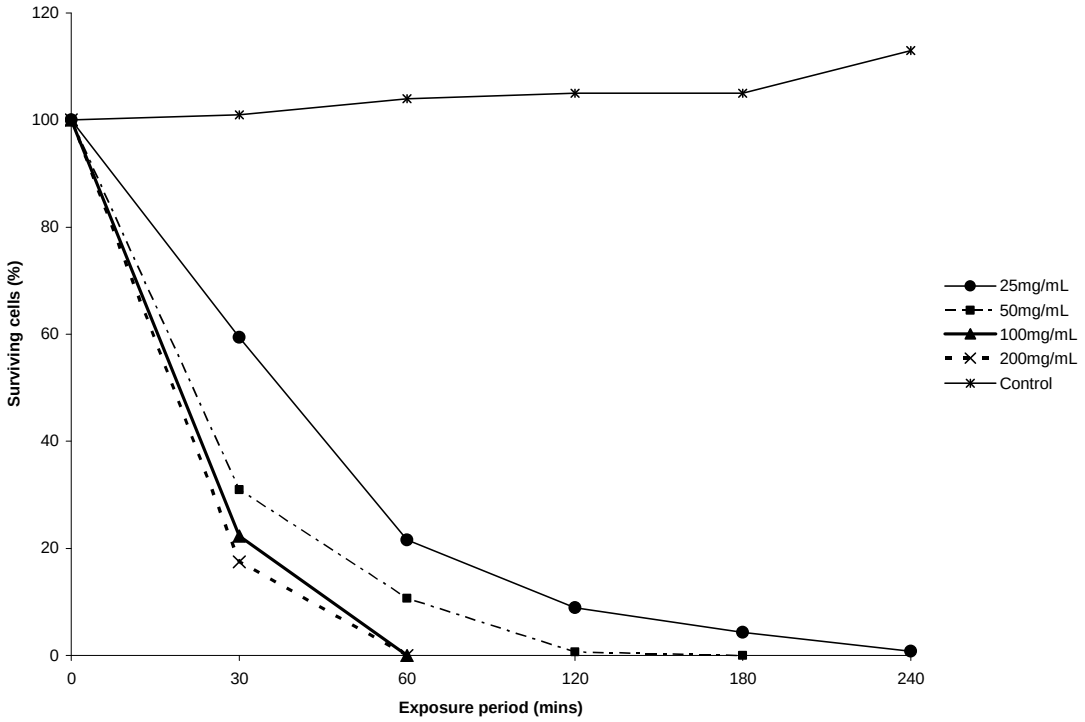

Fig. 3. Kinetics of bactericidal activities of *Zanthoxylum xanthoxyloides* on *Staphylococcus aureus*

DISCUSSION

From the result obtained in this study, it was observed that the assayed chewing sticks extracts demonstrated antimicrobial activities against the selected organisms. This was reflected in the varying zones of inhibition of the individual extracts on the oral pathogens *in- vitro.* This is in agreement with previous studies reporting the antimicrobial and inhibitory effects of chewing sticks on oral micro flora (Wolinsky and Sote, 1984; Rotimi *et al*., 1988; Taiwo *et al* 1999).

Zanthoxylum xanthoxyloides extract investigated in this study displayed a good inhibitory effect on the investigated bacteria (*S. mutans and S. aureus).* It also showed a broad spectrum of activity by inhibiting the investigated *Candida* species, although with lower zones of inhibition compared to that which was obtained for *S. mutans* and *S. aureus*. The lower zones of inhibition recorded for *Z. xanthoxyloides* on *C. krusei* and C. *tropicalis* could be due to the facts that *Z. xanthoxyloides* possess more of antibacterial property than its antifungal property.

Zanthoxylum xanthoxyloides has been reported to be effective against oral isolates (Adesina, 2005). Lower zones of inhibition obtained for the *Candida* in this study means that higher doses of the plant extract would be needed in treatment of infections caused by fungi such as Oral thrush and Candidiasis or could be used in synergy with any other active plant with known potency against microorganism

Pseudocedrela kotschyi shows no appreciable effect on *S. mutans* and *S. aureus (*Table 3). There were no zones of inhibition at all concentrations tested. This is also in corroboration with the reports of Ogundiya *et al.* (2007). *Pseudocedrela kotschyi* was able to display some antifungal activity against *C. krusei* alone. The result of this finding thus suggests *P. kotschyi* contains some antifungal activity which was demonstrated on C. *krusei.* The inability of *P. kotschyi* to efficiently inhibit the rest organisms could be due to incomplete extraction of its active principles especially tannins and saponins which was revealed in its phytochemical screening and was also reported to be present in the plant by Ogundiya *et al.* (2007). It could therefore be deduce that the level of the secondary metabolites detected in the extract of *P. kotschyi* may be too low to demonstrate antimicrobial activities against the isolates. *Streptococcus mutans* is known to be the chief pathogen responsible for the formation of dental plaque which normally results to caries (Loesche 1986), its susceptibility to *Z. xanthoxyloides* in this study confirms the use of this plant as a chewing stick locally in removing dental plaque. Studies have shown that frequent users of chewing sticks show less carious lesion than people who use toothbrush (Almas and Al-zeid 2004). *Staphylococcus aureus* was also susceptible to *Z. xanthoxyloides* in this study, thus confirming the potency of the plant as it is been used among the traditional medicine in treatment of various ailments and infections.(Abbiw 1990; Adesina, 2005). The antimicrobial activity displayed by *Z. xanthoxyloides* could be due to the presence of secondary metabolites revealed in its phytochemical screening. The presence of alkaloids, tannins, saponins have been associated with antimicrobial activities of plants (Adeniyi *et al.,* 1996, Edeoga, 2005). The observed rate of kill displayed by *Z. xanthoxyloides* against *S. aureus, S. mutans* and *C. albicans* correlate with its bactericidal activity. The destruction of the organism

Destruction of these oral floras in the kinetic study could be due to the interactions of the plant active metabolites with the organism's cytoplasmic membrane leading to the leakage of intracellular components and precipitation of cytoplasmic contents. The cytoplasmic membrane of the organism contains certain amino acids and other cellular contents which are responsible for cell growth and development; increase in permeability of the cytoplasmic membrane leads to the loss of these cellular matters consequently the cell dies. The bactericidal activity of the plant extract could also be due to the inhibition of macromolecular synthesis, especially of DNA at higher concentrations (Adeniyi *et al*., 2000). The facts in this study confirms the use and potency of *Z. xanthoxyloides* as an effective medicinal plant whose active principles could serve as a potential chemotherapeutic agent. Further studies are hereby advised on the antimicrobial activities of *P. kotschyi* using a different approach and methodology.

REFERENCES

Abbiw, D. 1990. Useful plants of Ghana. Intermediate Technology Publishing Ltd U. K. Summerfield and A. H. Bunting. Kew Royal Botanical Gardens. Pp 114-164

Adeniyi, B. A. Odelola, H. A. and Oso, B. A. (1996). Antimicrobial potential of *Diospyros mespiliformis. African Journal of Medicine and. Medical Sciences* Vol. 25(2): 179- 184.

Adeniyi, B. A., Fong, H. H. S., Pezzuto, J. M., Luyengi, L. and Odelola, H. A.. (2000). Antibacterial activity of diospyrin, isodiospyrin, and bisisodiospyrin from *Diospyros piscatoria*(Gurke) [Ebenaceae] .*Phytotherapy Research* .Vol 14 :112-117.

Adeniyi, B.A., Odufowoke, R.O and Olaleye, S.B. (2006). Antibacterial and Gastro-protective properties of *Eucalyptus torelliana* (Myrtaceae) crude extracts. *International Journal of Pharmacol*. **2** (3) 362-365

Adesina, S. K., 2005 The Nigerian *Zanthoxylum*; Chemical and Biological values *Afr. J. Trad. CAM* **2** (3): Pp 282 – 301

Aibinu, I., Adenipekun, .T., Adelowotan, .T., Ogunsanya, T., Odugbemi, T. 2007. Evaluation of the Antimicrobial Property of different pats of Citrus aurantifoloa (Lime fruit) as used locally. Afr.J.Trad. CAM, **4** (2) Pp 185-195

Almas K, Al-Zeid Z. The immediate antimicrobial effect of a toothbrush and miswak on cariogenic bacteria: a clinical study. J Contemp Dent Pract 2004; 5:105-14.

Buada, C.V. and Boakye-Yiadom, K. (1973). The antibacterial activity of some Ghanian chewing sticks. *Ghana pharmaceutical Journal* 1:150-151

Clark, J.K. 1924 one the bacterial factor in the aetiology of dental caries. British J Exp Pathol; **5**:141-147

Edeoga, H.O., Okwu, D.E and Mbaebie, B.O 2005. Phytochemical constituents of some Nigerian medicinal plant. Afri.Journal of Biotech**. 4** (7) 685-688

Fudulu, S.O. 1975. Antimicrobial properties of the buffer extracts of chewing sticks used in Nigeria. Planta medica; 27: 123-126.

Harbone J.B (1998). Phytochemical Methods. A Guide to Modern Techniques of plant Analysis. 3rd edition. Chapman and Hall, Newyork. Pp. 1-198.

Jeevarathan, J., Deepti, A., Muthu, MS., Rathna Prabhu V., Chamundeeswari G.S. 2007 Effect of fluoride varnish on *Streptococcus mutans* counts in plaque of caries-free children using dentocult SM strip mutans test: A randomized controlled triple blind study. J Indian Soc Pedod Prev Dent;.**25**:157-63

Loesche, W.J. 1986. Role of Streptococcus mutans in human dental decay. Microbiol Rev 50: 353-380

Ogundiya, M.O., Okunade, M.B., Kolapo, A.L. 2007 Antimicrobial Activities of some Nigerian chewing sticks . *EthnoBotanical Leaflets* http://www.siu/- ebl/leaflets

Okeke I.N, Ogundaini A, Ogungbamila F. A. (1999a) Antimicrobial spectrum of *Alchornea cordifolia* leaf extract. Phytotherapy Res; **13:** 67-69.

Rotimi, V.O., Laughon, B.E., Bartlett, J.G. and Mosadomi, H.A. (1988). Activities of Nigerian chewing sticks extracts against bacteriodes- gingivalis and bacteriodes-melaninogenicus. *Antimicrobiological Agents and Chemotherapy* 32(4), 598-600.

Taiwo O, Xu H, Lee S (1999);. Antibacterial activities of extracts from Nigerian chewing sticks. Phytotherapy Res 13:675-679.

Takada .k., Hirasawa. M. (2005). A novel selective medium of isolation of *Streptococcus mutans*. J. Microbiol methods 60 :Pp 189-193

Trease, G. and Evans, W. (1972). Pharmacognosy, Univ. Press, Aberdeen, Great Britain. p. 161-163

Van Vuuren S.F., Viljoen A.M. (2006): The *in vitro* antimicrobial activity of toothbrush sticks used in Ethiopia South African Journal of Botany **72,** (4), Pp 646-648

Wolinsky LE, Sote EO (1984). Isolation of natural plaque-inhibiting substances from Nigerian chewing sticks. Caries Res; 18:216-225.