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Antimicrobial activities of *Garcinia kola* on oral *Fusobacterium nucleatum* and biofilm

Francisca Nwaokorie[1]*, Akitoye Coker[1], Folasade Ogunsola[1], Elerson Gaetti-Jardim Jr.[2], Oyedele Gabriel[3], Ayanbadejo Patricia[4], Abdurrazaq Taiwo[5] and Umezudike Adesola[4]

[1]Department of Medical Microbiology and Parasitology, College of Medicine University of Lagos, Lagos, Nigeria.
[2]Anaerobe Laboratory, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo, SP, Brazil.
[3]Department of Pharmaceutics and Pharmaceutical Technology, College of Medicine, University of Lagos, Nigeria. [4]Department of Preventive Dentistry, College of Medicine, University of Lagos, Lagos, Nigeria.
[5]Department of Oral /maxillofacial surgery, College of Medicine, University of Lagos, Lagos, Nigeria.

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The extracts from the root, bark and seed of *Garcinia kola* are currently used in traditional medicine in Nigeria. The aim of this study was to evaluate the inhibitory activity of crude extracts of *G. kola* on *Fusobacterium nucleatum* isolated from the oral cavity. Methanol and aqueous extracts were prepared from the seed and the minimal inhibitory concentration was evaluated by the agar dilution method, using a Wilkins-Chalgren agar supplemented with horse blood (5%), hemin (5 µg/ml) and menadione (1 µg/ml). Antimicrobial activity of plant extracts on microbial biofilms was determined in microtiter plates. The seed of *G. kola* demonstrated significant inhibitory action on *F. nucleatum* isolates at a concentration of 1.25 and 12.5 mg/ml for amoxicillin resistant strain. It was able to inhibit the microbial biofilm formed by the association of *F. nucleatum* with *Porphyromonas gingivalis ATCC* 33277, *Aggregatibacter actinomycetemcomitans* ATCC 33384 and *Prevotella intermedia* ATCC 2564 at a concentration of 25 mg/ml. The in-vitro inhibitory effect of *G. kola* on *F. nucleatum* population suggests a potential role for its use in oral hygiene.

Key words: *Fusobacterium nucleatum, Garcinia kola*, oral disease, natural medicine, anaerobic bacteria, biofilm.

INTRODUCTION

Fusobacterium nucleatum is important in the formation and maturation of plaque because it provides anchoring sites for bacterial species responsible for the formation of biofilm and the maintenance of existing species on previously developed plaque (Iwaki et al., 2006; Kolenbrander et al., 2006; Jervoe-Storm et al., 2007). *Fusobacterium* species have been isolated from patients with Noma (cancrum oris) (Paster et al., 2002; Erickson et al., 2002) oro-facial infections (Egwari et al., 2001), dentoalveola abscesses (Akinwande et al., 1996), malnourished children (Falkler et al., 2000) and in women who had experienced preterm labour with low birth weight (Urban et al., 2006; Han et al., 2009). This organism often recovered in high numbers from periodontal poc-kets interacts with other periodontal pathogens such as *Aggregatibacter (* formerly *Actinobacillus) actinomycetemcometans, Porphyromonas gingivalis Prevotella* species and *Treponema denticola* to establish a complex ecological relationship that contributes to the initiation and progression of oro-facial infections (Kolenbrander et al., 2006; Jervoe-Storm et al., 2007). Any agent capable of inhibiting *F. nucleatum* is therefore likely to reduce the rate of plaque formation. Establishing an efficient

*Corresponding author. E-mail: franoby@yahoo.com. Tel: 234 1 5354760-74, Ext: 2138

and universal therapeutic approach to control the formation of dental biofilms is still to be developed.

Treatment of anaerobic infections is usually empirical but the universality of susceptibility of anaerobes to the usual drugs cannot always be assumed as reports of resistant strains have been growing in the literature from all over the world; New Zealand (Roberts et al., 2006), Finland (Nyfors et al., 2003) and Norway (Al-Haroni and Skaug, 2007; Kommedal et al., 2007). There is therefore a need for continuous search for an urgent alternative to synthetic drugs. In Africa, Asia, North and South America, medicinal plants were used for the treatment of infections before the introduction of antibiotics and other modern drugs (Haslam, 1989; Lee et al., 2004; Lima et al., 2006). Traditional chewing sticks are commonly used especially by rural dwellers in maintaining oral hygiene (Ndukwe et al., 2005) with about 80 - 90% of Nigerians using chewing sticks from a variety of plants (Soto and Wilson, 1995). The reasons can be attributed to culture, affordability, accessibility and the popular though misguided belief that natural medicines have no ill effects (Lima et al., 2006). A lot of work is therefore ongoing to validate the efficacy of these herbs and standardize the dosages (Ndukwe et al., 2005; Odugbemi, 2006; Ogbulie et al., 2007).

Medicinal plants such as *Vernonia amygdalina* (Ewuro in Yoruba), *Terminalia glaucesens* (Ida-odan) *Nauclea latifolia, Serindeia warneckei,* and *Garcina kola (orogbo)* are capable of inhibiting the activities of some anaerobic pathogens (Ugorji et al., 2000*).* In addition, extracts of the *Garcinia* genus, particularly *Garcinia mangostana* and *Garcinia kola*, exhibit diverse antimicrobial activities and are used in the treatment of cough and sore throat (Madubunyi, 1995; Okunji et al*.*, 1995; Adefule-Ositelu et al., 2004). Its root and seed possesses anti-inflammatory activities (Braide, 1990), are hepatoprotective (Iwu et al., 1990), and exhibit anti-oxidative (Olatunde et al., 2004) and antiviral properties (Hong-xi and Song, 2001).

Garcinia kola belongs to a family of tropical plants known as *Guttiferae* (Plowden, 1972). The seed is generally known as Bitter kola and in Nigeria it is commonly called "*Namiji goro*" in *Hausa,* " *Agbilu*" in *Igbo* (Esemonu et al., 2005) and *"orogbo"* in Yoruba (Ndukwe et al., 2005). The edible seed is valued in Nigerian houses as a substitute for the true kola nuts (*Cola nitidais*). Generally, the mechanical cleansing effect and antimicrobial substances in the seed are seen as major beneficial effects of chewing this nut (Han et al., 2005).

Phytochemical analysis of extracts from both root, stem and seed of *Garcinia kola* and other members of the genus show that they contain reasonable amounts of phenolic compounds including biflavonoids (GB-1,GB-2), xanthones and benzophenones (Onunkwo et al., 2004; Okunji et al., 2007; Okoko, 2009).

Their antibacterial activities are due to flavonoids especially biflavonoid type GB1 (Hong-xi and Song, 2001) and this has been demonstrated using methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin- resistant *enterococci* (VRE) (Han et al., 2005), *Lactobacillus* spp. (Owoseni and Ogunnusi, 2006) and *Streptococcus pyogenese* (Ogbulie et al*.,* 2007). Similar study by Afolabi et al. (2008) has shown its antibacterial effects on *Streptococcus mutans* another important organism involved in plaque formation. However, few reports (Rotimi et al., 1988, Ugorji et al., 2000., Ndukwe et al., 2005) are available on the antibacterial activities of *G. kola* on oral anaerobic species.

This study was therefore aimed to assess the antimicrobial properties of crude extract of *Garcinia kola* on *F. nucleatum* isolates from the oral cavity.

MATERIALS AND METHODS

The bacteria used in this study were *Porphyromonas gingivalis* ATCC 33277, *Prevotella intermedia* ATCC 2564, *Aggregatibacter actinomycetemcomitans* ATCC 33384 available at the Anaerobic Laboratories, University of Sao Paulo, Brazil and 5 clinical isolates of *F. nucleatum* each obtained by culture from periodontal pockets of different patients with chronic periodontitis. Sub-gingival biofilms were collected aseptically by inserting two sterile paper points (No. 30, UnoDent, England), into the periodontal pockets to a depth of 5 mm for 60 s and placed into a dental transport medium (Anaerobe systems, USA). Isolates were grown anaerobically in 90% N_2 and 10% CO_2 on Fastidious Anaerobe agar FAA (Lab M, Bury, United Kingdom) supplemented with hemin (5µg/ml) menadione (1 µg/ml) and incubated at 37°C for 72 h. Isolates were identified using standard microbiological techniques for anaerobes as recommended by Summanen et al. (1993). Briefly, growths on plates were examined macroscopically and each white or gray speckled colony that was dry, irregular, crumb-like circular with an entire edge measuring 0.5 - 2 mm in diameter was Gram-stained and sub cultured onto FAA, as well as Brucella blood agar containing vancomycin (5 µg/ml), colistin (30 µ g/ml) and Kanamycin (10 µg/ml). Isolates that were gram-negative, spindle shaped with pointed ends were presumed to be *F. nucleatum.* Biochemical identification was carried out using API 20A (bioMérieux SA, Mercy-l'Etoile, France) and Indole/nitrate reagent (Anaerobe systems). All isolates that fluoresced yellow-green under ultra violet light at a wavelength of 366 nm, caused greening of blood agar on exposure to air, were indole positive (both on API 20A and Indole/nitrate reagent (Anaerobe systems), nitrate, lipase and esculine negative, ferment galactose and fructose inhibited by 20% bile, colistin (10µg) and Kanamycin (30 µg) but grew in the presence of vancomycin (50 µg) were stored as *F. nucleatum* in 10% skimmed milk at - 80°C in ultra low freezer (Nuaire, USA) and used for the study. Identities of the species were confirmed by DNA amplification using *F. nucleatum* species specific primer as described by Avila-Campos et al. (1999).

Antimicrobial susceptibility testing

The antimicrobial susceptibility of the five clinical strains of *F. nucleatum* on amoxicillin (Glaxo SmithKline, Philadelphia, PA, USA) was performed as recommended by Clinical Laboratory Standard Institute (CLSI) (2007).

Antimicrobial agent was reconstituted according to the manufacturers' instructions and serial two-fold dilutions (ranging from 0.06 - 64 µg/ml) were prepared on the day of the test and added to Brucella blood agar supplemented with hemin (5 µg/ml), vitamin K (1 µ g/ml) and 5% horse blood. Plates were inoculated with 10^5 cfu/ml of *F. nucleatum* using a Steers' replicator (Cefar Ltd, São Paulo, SP, Brazil). Control plates without amoxicillin were

inoculated before and after each set of drug- containing plates. Plates were then incubated at 37°C for 48 h in an anaerobic atmosphere. Reference strains *F. nucleatum* ATCC 25586 and *F. nucleatum* ATCC 10953 were included as controls. The MIC was defined as the lowest concentration of the antibiotic that yielded no bacterial growth.

Beta-lactamase production

Amoxicillin-resistant strains were evaluated to verify -lactamase production using the nitrocefin method (Oxoid Ltd, Sao Paulo, SP, Brazil). A pure colony from a blood agar plate was dissolved in a drop of nitrocefin. After Incubation at room temperature for 30 min, -lactamase activity was observed by the production of a characteristic red color. A -lactamase producing strain *Bacteroides fragilis* ATCC 43858 was used as control.

Preparation of plant extract

Garcinia kola seeds purchased from a local market were identified and authenticated at the Department of Pharmacognocy, College of Medicine, University of Lagos, Idi Araba. Extracts were obtained using the methods of Iwaki et al. (2006). The *Garcinia kola* seeds were sliced and dried over a period of 5-7 days at 37°C in an oven (GallenKamp) and ground into a powder. Methanol extracts were prepared by adding 25 g of seed powder to 125 ml of 80% methanol. The mixture was loaded into a Soxhlet extractor and extracted at 60°C for 24 h. Aqueous extracts were prepared by mixing 25g of the plant powder in 125 ml of de- ionized water. The mixture was boiled at 100°C for 5 min at 55°C for 1 h and finally kept at room temperature for 3 days to extract the water soluble active principles. The extracts were filter-sterilized using 0.22 µm (pore size) cellulose membrane (Millipore). Both alcoholic and aqueous extracts were freeze- dried using Edwards high vacuum freeze-drier (Oakville, Ontario, Canada). Products were prepared immediately by dissolving 5 g in 100 ml of 50 mm sodium phosphate buffer (pH 7.0) to give a concentration of 50 mg/ml before use and to avoid oxidation. All extracts were stored at -40°C.

Determination of minimum inhibitory concentration (MIC)

The MICs of the extracts on the five *F. nucleatum* isolates were determined by CLSI method (2007) using Wilkins-Chalgren agar supplemented with horse blood (5%) hemin (5 µg/ml) and menadione (1 µg/ml). The inocula were prepared by picking three to five colonies of the test organism and inoculating them into 5 ml of Brain heart infusion broth supplemented with menadione (1 µg/ml), and hemin (5 µg/ml). The broth cultures were incubated for 48 h at 37°C and used to prepare an organism suspension in pre-reduced brain heart infusion broth equivalent in density to a 0.5 McFarland standard.

Media containing two-fold serial dilutions of the plant extract ranging from 0.125 - 64 mg/ml were inoculated with a Steers replicator which delivered a final inoculum of approximately 1.5 x 10^8 cfu/spot. Sterile tubes containing brain heart infusion broth having similar dilutions were also inoculated for the determination of the minimum bactericidal concentration (MBC). Both the plates and tubes were incubated anaerobically at 37°C for 48 h. All experiments were performed in duplicate while the MIC was defined as the lowest concentration of plant extract inhibiting bacterial growth.

Determination of minimum bactericidal concentration (MBC)

Aliquots of 100 µl from broths showing no growth were plated onto Wilkins-Chalgren agar supplemented with horse blood (5%), hemin (5 µg/ml) and menadione (1 µg/ml). The plates were incubated at 37°C for 48 h and the minimum bactericidal concentration (MBC) was determined. The MBC was defined as the highest dilution from which no bacterial growth was recorded. In all tests, sterile phosphate buffer saline and broth without plant extract were used as controls.

Inhibitory activity of plant extract on biofilms and Determination of death rate

Bacterial associations of a clinical isolate of *F. nucleatum* with *Porphyromonas gingivalis ATCC* 33277, *Actinobacillus actinomycetemcomitans* ATCC 33384 and *Prevotella intermedia* ATCC 2564 were cultivated. All tested strains were grown individually in Brain heart infusion broth supplemented with hemin (5 µg/ml) menadione (1 µg/ml) and glucose (1%). For the combined growth (biofilm) a final bacterial inocula of 10^6 cfu of each strain was used. Initially, 200 µl of a pure clinical isolate of *F. nucleatum* suspension was transferred into sterile 6-well polystyrene microtitre plates and incubated anaerobically at 37°C for 2 h. Thereafter, 200µl each of the other bacterial isolates as well as 2ml of supplemented BHI broth were added to each well and incubated at 37°C for 7 days. Another six wells containing similar isolates were set up and used to determine the MICs and MBCs of the aqueous and ethanol extracts on biofilm as described above.

To obtain the biofilms, cells growing as a film on the surface of the broth (Planktonic cells) were removed daily by discarding the supernatant, and supplemented BHI broth was added to the remaining broth to replace nutrients. After 7 days of incubation, a thin membrane containing the bacterial biofilm was observed in the wells. In order to determine the death rate, dehydrated plant extract was mixed with 1500 µl of supplemented BHI broth (to give a final concentration of 100 mg/ml and added to the microplates to cover up all microbial growth. The biofilm was incubated anaerobically at 37°C for 5 10, 20, 40, 60, 120, minutes and 24 h respectively. The medium from each well was removed and bacterial biofilm were harvested using a spatula.

The cells were vigorously homogenized for 15 s in 3 ml of VMG 1 solution (Möller, 1966) and submitted to 10 fold serial dilution in VMG 1. Thereafter, 0.1ml of each dilution was plated on blood agar and incubated anaerobically at 37°C for 3 days after which a viable count was done. Inhibitory activity was considered to be 90% reduction in the viable count when compared to the controls (phosphate buffer saline PBS). Tests were performed in triplicate and the results were expressed as the time required to reduce the microbial population of the biofilm by 90%. Clinical Isolate; *F. nucleatum* 015^b was also evaluated.

RESULTS

The clinical isolates of *F. nucleatum* were identified based on their unique spindle shape with tapered ends, indole reaction, susceptibility to kanamycin and colistin, resistance to vancomycin and confirmed by polymerase chain reaction (PCR) assay using species -specific primers. The antibacterial activity of seeds of *G. kola* on five clinical isolates of *F. nucleatum* obtained by culture from the periodontal pockets of five different patients with chronic periodontitis and on bacterial biofilm formed by a strain of *F. nucleatum* in association with standard ATCC strains of four periodontal pathogens was determined. Of the five clinical isolates tested one (*F. nucleatum* 015) was -lactamase producing and resistant to amoxicillin

Table 1. Antimicrobial susceptibility of Amoxicillin on oral *Fusobacterium nucleatum* isolates obtained from Nigerian patients with chronic periodontitis

Test strains production	B-lactamase	Range tested (µg/ml) Breakpoint* 8 µg/ml	MIC obtained (µg/ml)
F. nucleatum 05	-	≤ 0.06 - 32	0.06
F. nucleatum 015	+	≤ 0.06 - 32	32.0
F. nucleatum 035	-	≤ 0.06 - 32	0.06
F. nucleatum 037	-	≤ 0.06 - 32	0.06
F. nucleatum 050	-	≤ 0.06 - 32	1.25

*Breakpoint used in accordance with CLSI.

Table 2. Antimicrobial activity of Methanol and Aqueous plant extracts against clinical isolates of *Fusobacterium nucleatum* and Biofilm.

MIC/MBC* (mg/ml)				
	Aqueous extract		Methanol extract	
Test organisms	MIC	MBC	MIC	MBC
Biofim[a]	NG	NG	25.00	25.00
F. nucleatum 05[b]	NG	NG	1.25	7.50
F. nucleatum 015[b]	12.50	12.50	12.50	12.50
F. nucleatum 035[b]	NG	NG	1.25	5.00
F. nucleatum 037[b]	5.0	12.50	1.25	5.00
F. nucleatum 050[b]	N G	NG	1.25	7.50

*MIC: Minimum inhibitory concentration, MBC Minimum bactericidal concentration.

NG: No growth after incubation at 37^0C for 3 days in anaerobiosis.

a b Biofilm: Combined growth of *F. nucleatum* 015 *A. actinomycetemcomitans* ATCC 33384, *P. gingivalis* ATCC 33277, *P.*

b clinical isolate.

(MIC = 32 µg/ml) as shown on Table 1. The methanol extract showed more activity than the aqueous extracts in both the clinical strains and on biofilm (Table 2). Three clinical strains of *F. nucleatum* and the biofilm tested against aqueous extract did not show any growth after anaerobic incubation. For *F. nucleatum,* the MIC of the methanol extract against antibiotic-sensitive strains was 1.25 mg/ml, the MIC against antibiotic-resistant, (-lactamase producing) strains was 12.5 mg/ml, while the MBC ranged between 5.0 mg/ml to 12.5 mg/ml as shown in Table 2. The MIC was lower than the MBC except for the amoxicillin resistance strain. The antimicrobial activity of the methanol extracts were different against the biofilm produced by the association of *F. nucleatum* isolates, *A. actinomycetemcomitans* , *P. gingivalis and P. intermedia* because the MIC and MBC were obtained at a concentration of 25mg/ml. In biofilm assays, the log bacterial reduction was achieved within 60min after contact with methanol extracts (Figure 1). This result was also observed when clinical isolates of *F. nucleatum* was evaluated.

DISCUSSION

The activity of *G kola* was studied against both individual isolates of *F. nucleatum* and biofilms of *F.nucleatum* and A*. actinomycetemcomitans, P. gingivalis* and *P. intermedia*. Extracts obtained from the seed lead to a significant decrease in *F. nucleatum* counts when compared with the control. This result relatively corresponds with the findings of Afolabi et al. (2003) who studied their effects on *P. gingivalis* and *P. intermedia* in Lagos. The extract also exhibited strong antibacterial activity against the only -lactamase producing strain resistant to amoxycillin at a higher MIC of 12.5 mg/ml. which suggests that the mechanism of antibiotic resistance may affect the uptake or interaction of *G. kola* and *F. nucleatum. Garcinia kola* contains phenolic compound recognized to have antibacterial activities. These phenolic compounds when present at a high concentration acts as a protoplasmic poison by penetrating and disrupting bacterial cell wall in addition to precipitating the cell proteins (O'Connor and Rubino, 1991). In lower concentrations, it inactivates the cellular enzyme system causing leakage of essential metabolites from the cell (Widmer and Frei, 2003).

The methanol extract showed better activity than aqueous extract when subsequently tested at the same concentrations in both the clinical strains and on biofilms. This has been attributed to a better solubility of the active agents; xanthones, benzophenones, and flavonoids especially biflavonoid type GB1 (Hong-xi and Song, 2001, Han et al*.,* 2005) in organic solvents such as alcohol, than in water (Taiwo et al., 1999; Obi and Onuoha, 2000; Ogueke et al*.,* 2006: Ogbulie et al., 2007) This study is similar to that of Owoseni and Ogunnus (2006) in Iwo and Ibadan using *Lactobacillus* species obtained from the oral cavity. It was observed that three clinical strains of *F. nucleatum* and the biofilm tested against the aqueous extract did not show any growth after anaerobic incubation unfortunately the reason for this was not determined. Using the results obtained from the plates with viable growth the MIC was lower than the MBC except for the amoxicillin resistance strain indicating that the extracts are bacteriostatic at lower concentra-tions and bactericidal at higher concentrations.

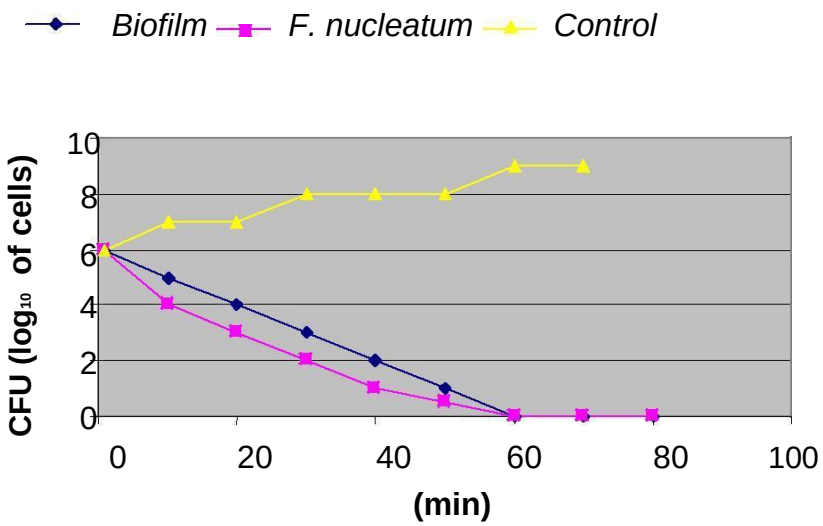

Figure 1. Inhibitory activities of *Garcinia kola* on *F. nucleatum* and biofilm.

Although *G. kola* extract showed good activity against this gram -negative anaerobe, it was important to detect the effect when the organism was associated with other organisms in a biofilm as it occurs *in- vivo.* A general view concerning the formation of dental biofilm suggests that *F. nucleatum* initially colonizes the tooth and gingival surfaces to form the substrate onto which later colonizers, such as periodontopathogens can adhere before migrating to deeper periodontal pockets (Sharma et al., 2005). This synergistic relationship between Fusobacteria and these Gram-negative rods could interfere with their susceptibility to antimicrobial drugs and the expression of virulence genes (Kolenbrander et al., 2006). In this study, individual strains of gram-negative bacteria produce a robust biofilm with *F. nucleatum*. For a new drug to be considered for use as a therapeutic agent for oral infections it must be active against biofilms (Bakri and Douglas, 2005). In the present study, the methanol extract of *Garcinia kola* was able to inhibit the biofilm formed by the periodontal bacteria tested *in vitro* although at a higher MIC when compared with the clinical isolates. It was interesting to note that the killing rate for the methanol extract on *F. nucleatum* and biofilm was 1 h showing that the extract was able to kill all the cells after 1 h of exposure. On the other hand, the MIC virtually corresponded with the MBC for the amoxicillin resistant strain and biofilm showing that the extract was bactericidal at that concentration. The inhibition of experimental biofilm at such concentration suggests that more of the bitter kola needs to be chewed to achieve an antibacterial effect in the oral ecology. In addition, the concentration at which the crude extract inhibited the organism and biofilm is high especially when compared with standard antibiotic used in therapy (Roberts, 2006). However, lower MIC may be achieved if the active agent is identified, synthe - sized and the appropriate dose determined. When this is achieved it can then be incorporated into gels, toothpaste or used for topical applications at a non lethal dosage. There is a need therefore for more studies on the efficacy of *G. kola in-vivo* and on toxicity.

Conclusion

This study demonstrated that extracts obtained from *Garcinia kola* displays a good activity against clinical isolates of *F. nucleatum* and its association with periodontal pathogens. The reduction of *F. nucleatum* population during the experiment can be related to the inhibitory activity produced by the extract thus the extract may be an alternative for maintaining oral hygiene.

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REFERENCES

Adefule-Ositelu AO, Adefule AK, Oosa BO, Onyenefa PC (2004). Antifungal activity of *Garcinia kola* nut extract as an ocular bacterial isolates in Lagos. Niger Qt. J. Hosp. Med. 14: 112-114.

Afolabi OC, Ogunsola FT, Coker T (2003). Susceptibility of Periodontal Anaerobesn to Extracts of Some Local Plants http://iadr.confex.com/iadr/2003SanAnton/techprogram/abstract_23659.htm.

Akinwande JA, Coker AO, Egwari LO, Ladeinde C (1996). A clinical and bacteriological study of dentoaveolar abscess in two specialist hospitals in Lagos, Nigeria. The Niger. Postgr. Med. J. 3: 98-104.

Al-Haroni M, Skaug N (2007). Incidence of antibiotic prescribing in dental in Norway and its contribution to national consumption. J.

Antimicrob. Chemother. 59: 1161–1166.
Avila-Campos MJ, Sacchi CT, Whitney AM, Steigerwalt AG, Mayer LW (1999). Arbitrarily primed-polymerase chain reaction for identification and epidemiologic subtyping of oral isolates of *Fusobacterium nucleatum.* J. Periodontol. 70: 155-159.
Bakri IM, Douglas CWI (2005). Inhibitory effect of garlic extract on oral bacteria. Arch. Oral Biol. 50: 645-51.
Braide VB (1990). Pharmacological effects of chronic ingestion of *Garcinia kola* seeds in rats. Phytother. Res. 4: 39-41.
Clinical and Laboratory Standards Institute (2007). Methods for antimicrobial susceptibility testing of anaerobic bacteria: approved standard; CLSI document M 11-A7. Clinical and Laboratory Standards Institute, Wayne, PA. 2007.
Egwari LO, Adeleye IA, Obisesan B (2001). Bacteriology of oral-facial infections in Lagos Nigeria. J. Int. Med. 4: 1-5.
Esemonu UG, El-taalu B, Anuka A, Ndodo D, Salim A, Atiku K (2005). Effect of ingestion of ethanol extract of garcinia kola seed on erythrocytes in Wistar rats Niger. J. Physiol. Sci. 20: 30-32.
Falkler WA, Enwonwu CO, Ewell AJ, Idigbe EO (2000). Isolation of Fusobacteria from the oral cavities of malnourished Nigerian children living in agricultural and herding villages. Oral Dis. 6: 102-105.
Jervoe-Storm PM, Al Ahdab H, Semaan E, Fimmers R, Jepsen I (2007). Microbiological outcomes of quadrant versus full-mouth root planning as monitored by real-time PCR. J. Clin. Periodontol. 34: 156–63.
Han YW, Ikegami A, Rajanna C, Kawsar H, Zhou Y, Li M, Sojar HT, Genco R, Kuramitsu H, Deng C (2005). Identification and characterization of novel adhesin unique to oral Fusobacteria. J. Bacteriol. 187: 5330-5340.
Han QB, Lee S, Qiao CF, He ZD, Song JZ, Sun HD, Xu HX (2005). Complete NMR Assignments of the Antibacterial Biflavonoid GB1 from *Garcinia kola.* Chem. Pharm. Bull. 53: 1034-1036.
Han, YW, Shen T, Chung P, Buhimschi IA, Buhimschi CS (2009). Uncultivated Bacteria as Etiologic Agents of Intra-Amniotic Inflammation Leading to Preterm Birth. J. Clin. Microbiol. 4: 38–47.
Haslam E, Lilley TH, Ya Cai R, Martin R, Magnolato D (1989). Traditional herbal medicine. The role of polyphenols. Planta Med. 55: 1-8.

Hong Xl, Song FL (2001). Activity of plant Flavonoids Against Antibiotic-Resistant Bacteria. Phythother. Res. 15: 39-43.
Iwaki K, Koya-Miyata S, Kohno K, Ushio S, Fukud S (2006). Antimicrobial activity of *Polygonum tinctorium* Lour extract against oral pathogenic bacteria. J. Nat. Med. 60: 121-5.
Iwu MM, Igboko OA, Okunji CO, Tempesta MS (1990). Antidiabetic and aldose reductase activities of Biflavanones of *Garcinia kola.* J. Pharm. Pharmacol. 42: 290-292.
Kolenbrander PE, Palmer RJ, Rickard AH, Jakubovics N, Chalmers NI, Diaz PI (2006). Bacterial interactions and successions during plaque development. Periodontology 2000. 42: 47-79.
Kommedal O, Nystad TW, Bolstad B, Digranes A (2007). Antibiotic susceptibility of blood culture isolates of anaerobic bacteria at a Norwegian university hospital. APMIS. 115: 956–61.
Lee SS, Zhang W, Li Y (2004) .The antimicrobial potential of 14 natural herbal centifrices. Results of an *in vitro* diffusion method study. J. Am. Dent. Assoc. 135: 1133-41.
Lima MRF, Luna JS, Santos AF, Andrade MCC, Sant'Ana AEG, Genet JP, Marquez B, Neuville L, Moreau N (2006). Anti-bacterial activity of some Brazillian medicinal plants. J. Ethnopharmacol. 105: 137-147.
Madubunyi II (1995). Antimicrobial activities of the constituents of *Garcinia kola* seeds. Int. J Pharmacog. 33: 232-237.
Möller AJ (1966). Microbial examination of root canals and periapical tissues of human teeth: methodological studies. Odontol Tidskr. 74: 1-138
Ndukwe KC, Okeke IN, Lamikanra A, Adesina SK, Aboderin OJ (2005). Antibacterial activities of aqueous extracts of selected chewing sticks. J. Contemp. Dent. Pract. 3: 86-94.
Nyfors S, Kononen E, Syrjanen AR, Komulainen E, Jousimies–Somer H (2003). Emergence of penicillin resistance among *Fusobacterium nucleatum* of commensal oral flora during early childhood. J. Antimicrob. Chemother. 51: 107-112.
Obi VI, Onuoha C (2000). Extraction and Characterization methods of plants and plant products. In: Biol and Agricultural Techniques. Ogbulie JN, Ojiako OA. (eds.), Websmedia Publishers, Owerri. pp. 271-286.
O'Connor DO, Rubino JR (1991). Phenolic compounds. *In* S.S. Block (ed), Disinfection, sterilization and preservation. Lea and Febiger, Philadelphia, Pa. pp. 204-224,
Odugbemi T (2006). Introduction. *In:* Outlines and pictures of medicinal plants from Nigeria. Ed. Odugbemi, T. University Press, Lagos p: 1-4.
Ogbulie JN, Ogueke CC, Nwanebu FC (2007). Antibacterial properties of *Uvaria chamae, Congronema latifolium, Garcinia kola, Vemonia amygdalina* and *Aframomium melegueta.* Afr. J. Biotech. 6: 1549-1553.
Ogueke CC, Ogbulie JN, joku HO (2006). Antimicrobial properties and preliminary Phytochemical analysis of ethanolic extracts of Alstonia bonnie. Niger. J. Microbiol. 20: 896-899.
Okoko T (2009). *In vitro* antioxidant and free radical scavenging activities of Garcinia kola seeds. Food and Chemical Toxicology Article in Press, Available online 25 July 2009. Corrected Proof - doi:10.1016/j.fct.07.023.
Okunji C, Komarnytsky S, Fear G, Poulev A, Ribnicky DM, Awachie PI, Raskinn I (2007). Preparative isolation and identification of tyrosinase inhibitors fro the seeds of *Garcinia kola* by high-speed counter-current chromatography. J. Chromatog. 1151: 45-50.
Olatunde FE, Hansen M, Rain-Haren P, Dragsted LO (2004). Commonly Consumed and Naturally Occuring Dietary Substances affect Bio Makers of Oxidative Stress and DNA damage in Healthy Rats. Food Chem. Toxicol. 42: 1315-1322.
Onunkwo GC, Egeonu HC, Adikwu MU, Ojile JE, Olowosu AK (2004). Some Physical Properties of Tabletted Seed of *Garcinia kola* (HECKEL) Chem. Pharm. Bull. 52: 649-653.
Owoseni A, Ogunnusi T (2006). Antibacterial effects of three chewing stick extracts on *Lactobacillus* species. Int. J. Trop. Med. 3: 103-106.
Paster BJ, Falkler WA, Enwonwu CO, Idigbe EO, Salvage KO, Levanos VA, Tamer MA, Ericson RL, Lau CN, Dewhirst FE (2002). Prevalent bacterial species and novel phytotypes in advanced Noma lesions. J Clin. Microbiol. 40: 2187-2191.
Plowden CC (1972). A manual of plants names. 3rd ed. London. George Ltd. p. 239.
Roberts SA, Shore KP, Paviour SD, Holland D, Morris AJ (2006). Antimicrobial susceptibility of anaerobic bacteria in New Zealand. J. Antimicrob. Chemother. 57: 992–998.
Rotimi VO, Laughon BE, Bartlett JG, Mosadomi HA (1988). Activities of Nigerian chewing sticks extracts against *Bacteriodes gingivalis* and *Bacteriodes melaninogenicus*. Antimicrob. Agents Chemother. 32: 598-600.
Sharma A, Inagaki S, Sigurdson W, Kuramitsu HK (2005). Synergy between *Tannerella forsythia* and *Fusobacterium nucleatum* in biofilm formation. Oral Microbiol. Immunol. 20: 39–42.
Soto EO, Wilson M (1995). *In vitro* antimicrobial effects of extracts of Nigerian tooth cleaning sticks on periodopathic bacteria. Afr. Dent J. 9: 15-19.
Taiwo O, Xu H, Lee SF (1999). Antibacterial Activities of Extracts from Nigerian Chewing Sticks. Phytother. Res. 13: 675–679.
Ugoji E, Egwari LO, Obisesan B (2000). Antibacterial activities of aqueous extracts of ten African chewing sticks on oral pathogens. Niger. J. Int. Med. 3: 7-11.

Urban E, Radnai M, Novak T, Gorzo I, Pal A, Nagy E (2006). Distribution of anaerobic bacteria among pregnant periodontitis patients who experience preterm delivery. Anaerobe 12: 52-57.
Widmer AF, Frei R (2003). Decontamination, Disinfection, and sterilization. *In*: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (eds). Manual of Clinical Microbiology. 8th edition.