OPEN ACCESS

Online ISSN:2353-0391

Algerian Journal of Natural Products
www.univ-bejaia.dz/ajnp

Type of the Paper (Article)

Antibacterial Effects of Aqueous Extracts of Herbal Sticks on Oral Isolates

Bolanle Kudirat SALIU *, Abidemi Esther OLAOYE, Rahmat Folakemi ZAKARIYAH

Department of Microbiology, University of Ilorin P.M.B. 1515, Ilorin, Nigeria
*Author to whom correspondence should be addressed; Email: saliu.bk@unilorin.edu.ng
Telephone: 08023093294

Received: 17/08/2020 /Accepted: 10/05/2021

Abstract: This study compared the antibacterial effects of some herbal sticks commonly used for oral hygiene in Ilorin, Nigeria, on oral isolates. Aqueous extracts of the sticks were used to challenge bacteria isolated from various parts of the oral cavity. Stem extract of Distemonanthus benthamianus (Baill) was effective against all the isolates except Pseudomonas aeruginosa, with activity indices ranging from 0.59 to 1.35 compared to streptomycin. Micrococcus species was inhibited by all the extracts while the other isolates including Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtillis, Staphylococcus epidermidis, Streptococcus mutans, Micrococcus luteus, Lactobacillus bulgaricus and Staphylococcus saprophyticus showed various patterns of susceptibility. Inhibition zone ranged from 10.0 mm for extract of Spilanthes calva (DC) to 33.0 mm for that of D. benthamianus. This study showed that herbal sticks have antibacterial effects on oral organisms and their extracts are recommended as components of products used in maintaining oral hygiene.

Keywords: antibacterial; herbal sticks; oral hygiene

I. Introduction

The human oral cavity comprises of the teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and the tonsils, which provides suitable habitats for over 600 species of microorganisms including bacteria and fungi [1]. Members of the genera *Streptococcus, Neisseria, Actinomyces, Veillonella Corynobacterium and Lactobacillus* are among the dominant bacteria that inhabit the oral cavity [2]. Unhygienic attitude with the oral cavity may cause proliferation of these microorganisms with the consequence of dental plague which may eventually lead to dental caries, or any other orodental diseases. *Streptococcus mutans*, *S.*

sanguis, other *Streptococcus*, *Lactobacillus* and *Actinomycetes* species are important agents of oral diseases.

Herbal sticks are common means of maintaining oral hygiene in Nigeria, particularly in rural communities. Sticks are made from roots, stems and twigs of many plants with or without the bark. Teeth are cleaned by chewing the root or slim stem of the plants until they acquire brush-like end [3]. While chewing, the plant's phytochemicals which may include phenolic compounds, steroids, alkaloids, glycosides and a host of others are extracted into the mouth cavity. Most of the chemicals possess antimicrobial properties which contribute to maintenance of oral hygiene [4]. World Health Organization (WHO) recommends the use of herbal sticks and/or their extracts in the ethno-medical treatment of oral infections [5].

A variety of herbal sticks are used in Ilorin, Kwara State of Nigeria, with claims of one being more effective than others. "*Pako Ijebu*" is a very popular one made from a shrub/small tree known as *Massularia accumunata*. "*Orin emi gbegiri*" is produced from the shea butter tree, *Vitellaria paradoxa*; "orin ayin" is from (African birch) *Anogeissus leiocarpa* (DC.) Guill. & Perr.; and "orin ayan" from *Distemonanthus benthamianus* (Baill). Other plants used include *Fagara zantholoides*, "orin ata"; *Garcinia kola*, "orin orogbo"; *Terminalia glaucescens*; "Orin idi" and *Spilanthes calva* (DC) "orin sesedo". Each of these plants has reported antimicrobial activities which are being exploited in the treatment of various illnesses. In this study, the efficacy of herbal sticks from these plants in controlling the microbial population of the oral cavity was compared.

II. Materials and Methods

Herbal sticks

The herbal sticks used in this study were purchased from a retail market, Ilorin, Nigeria. The sticks were identified at the herbarium of the Department of Plant Biology, University of Ilorin. The details of the plants and parts used for chewing stick is presented in Table 1.

Isolation of bacteria

Bacteria were isolated from the oral cavity of five human volunteers whose teeth appeared healthy. Sterile swab sticks were used to rub the teeth, tongue and palate of volunteers in the morning, before cleaning the mouth. The sticks were inserted back into their containers and taken to the laboratory for microbiological analyses within one hour of collection. Sample swab sticks were used to rub the surface of sterile Nutrient Agar plates and incubated at 37^{o}C. Plates were observed for growth after 24 and 48 hours of incubation. The colonial morphology of the organisms was described, individual isolates were transferred to fresh agar plates, and pure cultures maintained on agar slants. Isolates were further characterized by microscopic and biochemical techniques.

Preparation of extracts from herbal sticks

The sticks were first broken into coarse pieces using wooden mortar and pestle; and finally into fine particles in the laboratory ceramic mortar and pestle. Ten grams each of the crushed samples was measured into a 250ml Erlenmeyer's flask containing 50ml sterile distilled water to make 20% w/v suspension. The flask was covered with cotton plug which was in turn wrapped with aluminum foil; and placed on an orbital shaker at 150 rpm. Aqueous samples containing the

extract were withdrawn after 3 and 24 hours of shaking, by filtering through Whattman no 1 filter paper.

Antibacterial sensitivity tests of extracts on bacterial isolates

Fresh cultures of isolates were grown in nutrient broth for 18 hours at 37^{o}C and standardized to McFarland scale 0.5. Aliquots, 0.1 ml of these were used to inoculate Mueller-Hinton agar plates by spread plate technique. Wells measuring 4mm each were made on the seeded agar. Herbal stick extracts (0.1 ml), was transferred into appropriately labeled wells. Plates were incubated at 37^{o}C; and observed after 24 hours for growth. Clearance around loaded wells indicates inhibition of growth by the extract and hence antimicrobial activity. Sterile distilled water and 5% streptomycin (0.1 ml each) represented the negative and positive controls respectively.

Table 1: List of herbal sticks used in the study

Botanical name of plants	Indigenous name of herbal sticks	Plant part used	Phytochemical components
Massularia accuminata (G. Don)	"*Pako Ijebu*"	Stem	Flavonoids, anthocyanins, anthraquinones, tannins and phlobatanin [6]
Vitellaria paradoxa (Gaertn.F)	"*Orin emi gbegiri*"	Branches	Flavonoids, cardiac glycosides, anthraquinones, tannins, steroids, alkaloids and saponins [7]
Anogeissus leiocarpa (DC Guill & Perr)	"*Orin ayin*"	Stem	Flavonoids, tannins, steroids, alkaloids and phenols [8]
Distemonanthus benthamianus (Baill)	"*Orin ayan*"	Stem	Tannins, steroids, alkaloids and saponins [9]
Fagara zantholoides (Lam)	"*Orin ata*"	Roots	Tannins, flavonoids, alkaloids and saponins [10]
Garcinia kola (Heckel)	"*Orin orogbo*"	Bark	Flavonoids, alkaloids, glycosides, saponins, tannins, anthraquinones, and phlobatanin [11]
Terminalia glaucescens	"*Orin idi*"	Roots	Flavonoids, alkaloids, steroids, saponins, tannins, anthraquinones, and phlobatanin [12]
Spilanthes calva (DC)	"*Orin sesedo*"	Roots and branches	Hydrocarbons, terpenoids, esters, alcohols, alkamides aldehydes and ketones [13]

III. Results and Discussion

Bacterial isolates

Ten bacteria were isolated and characterized by morphology and biochemical tests as *Streptococcus pyogenes*, *S. mutans Staphylococcus aureus*, *S. epidermidis S. saprophyticus*, *Micrococcus species*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Lactobacillus bulgaricus* and *Bacillus subtillis* (Table 2). These bacteria are among the microbiota of human oral cavity [1] some of which have been implicated in oral health challenges such as carries, plagues and periodontal diseases, and were also isolated from healthy humans [14]. *Pseudomonas aeruginosa* and *Bacillus subtilis* were isolated as biofilm forming bacteria from oral cavity [15]. *B. subtilis* may serve beneficial role since it is an effective probiotic material and has been so proven in patients with periodontitis [16,17].

The most common bacteria that are found in the supragingival plaque include gram positive cocci belonging to the genera *Streptococcus* and *Staphylococcus* which together with some other gram positive and gram negative rods and cocci, are responsible for plaque formation as a result of their interactions with each other and with the tooth surface [18,19,20]. Lactobacilli are among the organisms that infest the human oral cavity during the first year of life due to affinity for milk, and play major roles in dental caries development [21].

Susceptibility of isolates to the extracts

The bacterial isolates were challenged with crude aqueous extracts of the herbal sticks. The susceptibility pattern of the isolates to the extracts is presented in Table 3. The extracts produced varying antibacterial effects on the isolates with diameter zone of inhibition ranging from 10 to 33 mm (Table 4). Among the isolates, *P. aeruginosa* was not inhibited by any of the extracts; *S. mutans* by only two, *Distemonanthus benthamianus* and *Terminalia glaucescens*; while *Micrococcus species* and *Staphylococcus aureus* were susceptible to all. In general, extracts of *Distemonanthus benthamianus* produced the highest activity and together with *Terminalia glaucescens* was active against all the isolates except *P. aeruginosa*. The ability of *D. benthamianus* and *T. glaucescens* to inhibit a variety of oral isolates has been demonstrated earlier [22]. *Spilanthes calva* was the least active of the extract as it produced activity only against *Micrococcus species* and *Staphylococcus aureus*. *Streptococcus pyogenes* was not inhibited by extracts of *Massularia accumunata*, *Vitellaria paradoxa* and *Spilanthes calva*. Two of the isolates from the oral cavity were resistant to *Garcinia kola*, four to each of *Anogeissus leiocapus* and *Fagara zantholoides*; and five to *Massularia accumunata* and *Vitellaria paradoxa* (Table 4).

Table 2: Colonial, microscopic and biochemical characteristics of bacterial isolates

ISOLATES	A	B	C	D	E	F	G	H	I	J
Colony Morphology										
Margin	L	E	E	E	E	E	W	F	F	E
Size (mm)	7.0	10.0	3.0	2.0-3.0	1.5-2.0	1.5	3.0	25.0	20.0	4.0
Shape of colony	Ir	Rd	Rd	Rd	Rd	Rd	Ir	Rd	Ir	Rd
Elevation	Cvx	Flat	Flat	Cvx	Cvx	Cvx	Flat	Flat	Flat	Flat
Pigmentation	W	Crm	Y	O-Y	O/W	W	G-Y	Crm	C	O/W
Surface texture	Dry	Sm	Sm; Gl	Sm	Sm	Sm	Rgh	Dry	Dry	Rgh; Gl
Transparency	Opaq	Opaq	Opaq	Opaq	Opaq	Opaq	Opaq	Tran	Tran	Opaq
Cellular Morphology										
Gram reaction	+	+	+	+	+	+	-	+	+	+
Shape of cell	C	C	C	R	C	C	R	C	R	C
Arrangement	Cl	Ch	Cl	Sgl	Sgl	Ch	Cl	Cl	Cl	Sgl
Motility	-	-	-	+	-	-	+	-	-	-
Spore	-	-	-	+	-	-	-	-	-	-
Biochemical characteristics										
Catalase	+	-	-	+	+	-	+	+	+	+
Oxidase	+	-	-	+	-	+	+	+	-	+
Urease	+	-	+	-	+	+	-	+	+	+
Indole	+	+	-	-	-	+	-	-	-	-
Citrate	+	+	+	-	-	+	+	-	-	-
Methyl red	+	+	-	-	-	+	-	+	-	+
Voges poskauer	-	-	+	+	+	-	-	-	+	+
Oxygen relationship	FA	A	FA	A	FA	FA	FA	A	AN	FA
Starch hydrolysis	+	-	-	+	+	+	+	+	+	+
Growth on 6.5% NaCl	+	+	+	+	+	+	+	+	+	+
Growth on MacConkey	-	-	-	-	-	-	+	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	-	+	+	+	-	-	+	+
Lactose	+	+	+	-	+	+	-	-	+	+
Gas production	-	-	+	-	+	+	-	-	-	-
H_2S production	-	-	-	+	-	-	-	-	-	-

+ denotes positive reaction, while – denotes negativity; Margin (E = Entire, L = Lobate, F = Filamentous, W = Wavy); Shape of colony (Ir = Irregular, Rd = Round); Elevation (Cvx = convex); Pigmentation (W = White, Crm = Cream, Y = Yellow O = Orange, G = Green); Surface texture (Sm = Smooth, Gl = Glistening, Rgh = Rough); Transparency (Opaq = Opaque, Tran = Transparent); Shape of cell (C = Cocci, R = Rod); Arrangement (Sgl = Single, Cl = Cluster, Ch = Chain); Oxygen relationship (A = Aerobic, An = Anaerobic, FA = Facultative); Isolates were identified as A to J - Micrococcus species, Streptococcus pyogenes, Staphylococcus aureus, Bacillus subtillis, Staphylococcus epidermidis, Streptococcus mutans, Pseudomonas aeruginosa, Micrococcus luteus, Lactobacillus bulgaricus and Staphylococcus saprophyticus respectively.

Table 3: Susceptibility pattern of oral isolates to aqueous extracts of the studied herbal sticks

Plant	Antibacterial susceptibility									
	A	B	C	D	E	F	G	H	I	J
Massularia accumunata	++	-	++	++	-	++	-	+	-	-
Vitelleria paradoxa	++	-	++	-	++	-	-	++	-	++
Spilanthes calva	++	-	-	++	-	-	-	-	-	-
Fagara zantholoides	++	++	++	-	+	-	-	++	+	-
Anogeisus leiocarpus	++	+	+	++	-	-	-	-	++	++
Distemonanthus benthamianus	++	++	++	++	+	+	-	++	++	++
Terminalia glaucescens	++	++	++	++	++	+	-	++	+	++
Garcinia kola	++	+	+	++	++	-	-	++	++	++

+ denotes static inhibition, ++ denotes cidal effects, - denotes no inhibition. Acronyms A to J represent bacterial isolates which were characterized as presented on Table 2.

Table 4: Antibacterial effects of aqueous extracts of the oral isolates

Plants	Inhibition Zone (mm)									
	A	B	C	D	E	F	G	H	I	J
Massularia accumunata	15.3±0.6	0.0±0.0	15.7±0.6	17.3±1.2	0.0±.0.0	16.3±0.6	0.0±0.0	12.3±1.5	0.0±0.0	0.0±0.0
Vitelleria paradoxa	12.7±1.5	0.0±0.0	15.3±0.6	0.0±0.0	11.3±0.6	0.0±0.0	0.0±0.0	18.3±0.6	0.0±0.0	22.3±0.6
Spilanthes calva	10.3±0.6	0.0±0.0	0.0±0.0	14.7±1.5	0.0±0.0	00.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Fagara zantholoides	17.7±0.6	22.3±0.6	17.7±1.5	0.0±0.0	17.7±0.6	0.0±0.0	0.0±0.0	18.3±1.5	10.3±1.5	0.0±0.0
Anogeisus leiocarpus	18.0±1.0	12.3±0.6	15.3±0.6	17.7±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	12.7±0.6	12.3±1.5
Distemonanthus benthamianus	33.3±1.5	31.0±1.0	24.0±1.0	23.3±0.6	17.7±0.6	15.3±0.6	0.0±0.0	26.0±2.0	15.7±1.5	23.3±1.5
Terminalia glaucescens	26.7±1.5	21.3±0.6	22.3±0.6	15.3±0.6	19.3±1.2	16.3±0.6	0.0±0.0	18.3±0.6	12.3±0.6	22.7±0.6
Garcinia kola	20.3±1.5	18.3±0.6	11.7±0.6	15.7±0.6	16.3±0.6	0.0±0.0	0.0±0.0	16.3±0.6	24.3±2.5	16.3±1.5

Values represent mean of 3 independent readings ± standard deviation. Acronyms A to J represent bacterial isolates which were characterized as presented on Table 2.

Unlike most earlier reported researches on the susceptibility of *P. aeruginosa* to herbal stick extract, Ayankunle *et al.* (2012) [23] agreed with findings in this study that the bacterium, along with *Streptococcus pyogenes* are resistant to stem bark extract of *Vitellaria paradoxa*. Stem bark of *Anogeissus leiocarpus* contains tannins, flavonoids, terpenes and saponins [24,25] and this may account for its antibacterial activity against six of the isolates. It has also been shown to inhibit *S. aureus*, *Streptococcus pyogenes*, *B. subtilis*, and *P. aeruginosa* [26].

Ethanol extracts of *Fagara zanthoxyloides* and *Massularia acuminata* showed anti-caries activity [27]. *Massularia accumunata* phytochemicals include alkaloids, saponins, polyphenolics, tannins, anthraquinones and phenolics [28] which were attributed for its antimicrobial activities [29] while Alkaloids, Tannins, Saponins, Flavonoids, Phenolic compounds, and Phytosterols were found in *Fagara zanthoxyloides* [30].

Antimicrobial activity of *Terminalia glaucescens* and *Fagara* zanthoxyloides against *Pseudomonas aerugenosa*, *Streptococcus mutans*, *Streptococcus pyogenes*, and *Staphylococcus aureus* was also reported [31]. Susceptibility of *Streptococcus mutans, Staphylococcus* sp and *Lactobacillus salivarius* to *Vitellaria paradoxa*, *Distermonanthus benthamianus* (Baill), *Anogeisus leiocaspus* (DC Guill & Perr) and *Terminalia schimperianah* (Flanch) has also been demonstrated [22,32]. *Spilanthes calva* DC was described as an important herb for oral health care due to its antibacterial and antifungal activities [33].

Compared to streptomycin, higher activity was obtained from *D. benthamianus* against *Streptococcus pyogenes*, *Bacillus subtilis*, and *Micrococcus luteus* indicating that extracts of the plants may serve better value in controlling the population of the organisms in the oral cavity. This further supports claims of its potency in the treatment of gingivitis [34]; which can be attributed to its content of flavonoids, phenol, steroid and tannin as active principle [35]. Higher activity index was also obtained for extract of *Garcinia kola* against *Lactobacillus bulgaricus*. Fifty three percent of the test produced activity indices greater than 0.50, of which 29% were greater than 0.75 (Table 5).

Table 5: Activity index of extracts against reference drug, streptomycin

Plants	Activity index									
	A	B	C	D	E	F	G	H	I	J
Massularia accumunata	0.43	0.00	0.39	0.78	0.00	0.53	0.00	0.50	0.00	0.00
Vitelleria paradoxa	0.37	0.00	0.37	0.00	0.55	0.00	0.00	0.75	0.00	0.58
Spilanthes calva	0.29	0.00	0.00	0.65	0.00	0.00	0.00	0.00	0.00	0.00
Fagara zantholoides	0.51	0.96	0.44	0.00	0.90	0.00	0.00	0.75	0.56	0.00
Anogeisus leiocarpus	0.51	0.52	0.37	0.78	0.00	0.00	0.00	0.00	0.72	0.32
Distemonanthus benthamianus	0.94	1.35	0.59	1.00	0.90	0.50	0.00	1.08	0.89	0.61
Terminalia glaucescens	0.77	0.91	0.54	0.65	0.95	0.53	0.00	0.75	0.67	0.58
Garcinia kola	0.57	0.78	0.29	0.70	0.75	0.00	0.00	0.67	1.33	0.42

Legend: Acronyms A to J represent bacterial isolates which were characterized as presented on Table 2. Activity index was determined as the mean inhibition zone of crude extract of herbal sticks divided by the mean inhibition zone of streptomycin (100 µl of 10% solution)

IV. Conclusion Overall, most of the extracts showed potentials in controlling the microbial population of the oral cavity thereby supporting claims in folk medicine about the use of herbal sticks to prevent some common oral diseases. Extracts from the herbal sticks particularly that of *D. benthamianus* are hereby recommended as herbal components of mouth care formulas such as tooth paste and mouth wash.

V. References

[1] Dewhirst F.E., Chen, T., Izard, J., Paster, B.J., Tanner, A.C.R. Yu, W.H., Lakshmanan, A. and. Wade, W.G. (2010). The Human Oral Microbiome. *Journal of Bacteriology* 192 (19): 5002 – 5017.

[2] Palmer, R.J. (2014). Composition and development of oral bacterial communities. *Periodontal 2000* 64(1).

[3] El-Said, Fadulu, S.O., Kuye, J. O., and Sofowora, E. A. (1970). Native cures in Nigeria. The antimicrobial properties of buffered extract of chewing sticks. *Journal of Pharmaceutical Sciences* 34(2):172 - 174.

[4] Gupta, P. and Shetty, H. (2018) Use of natural hygiene maintenance: revisiting traditional medicine. *Journal of Complementary and Interactive Medicine* 15(3)

[5] Ndukwe K.C., Okeke, I.N., Akinwande, J.A., Aboderin A.O. and Lamikanra, A. (2004). Bacteriology and antimicrobial susceptibility profile of orofacial infections in Nigerians. *African Journal of Clinical and Experimental Microbiology* 5(3): 272 – 277. doi:10.4314/ajcem.v5i3.7390

[6] Bankole, P.O., Adedotun, A.A., Oyede, R.T. and Faparusi, F. (2012) Antimicrobial activities and phytochemical screening of two Tropical Nigerian chewing sticks. *International Journal of Applied Science and Technology* 2(6):131 -138.

[7] Akoma, O.A, Nma, N.Y., Musa, S.A. and Salihu, A.B. (2018) Nutritional and phytochemical composition of *Vitellaria paradoxa. International Journal of Biochemical Research and Reviews* 22(1) 1 – 7.

[8] Ahmad I.M. and Wudil, A.M. (2013) Phytochemical screening and toxicological of aqueous stem bark extracts of *Anogeissus leicocarpus* in rats. *Asian Journal of Scientific Research* 6(4): 781 – 788.

[9] Aiyegoro, O.A.,Akinpelu, D.A., Afolayan, A.J. and Okoh, A.I. (2008) Antibacterial activities of crude stem bark extracts of *Distemonanthus benthamianus*. *Journal of Biological Sciences* 8: 356 – 361.

[10] Adefisoye, M.A. Ako-Nai, K.A. and Bisi-Johnson, A.A. (2012) Phytochemical and antibacterial activities of the extracts of *Fagara xanthoxyloides* on selected caryogenic and enteric bacterial isolates. *Journal of Intercultural Ethnopharmacology* 1(1): 1 – 6. doi:10.5455/jice.55-1333123486

[11] Ibedu, C.L., Ihetu, C.C., Okoro, O.S. and Obeagu, E.I. (2018) Phytochemical composition and antimicrobial properties of *Garcinia kola* (Bitter kola). *International Journal of Current Research in Chemical and Pharmaceutical Sciences* 5(11): 8- 12.

[12] Adebayo E.A. and Ishola, O.R. (2009) Phytochemical and antimicrobial screening of crude extracts from the roots, stem barks and leaves of *Terminalia glaucescens. African Journal of Pharmacy and Pharmacology* 3(5): 217 – 221.

[13] Lavanya, D.K., Shivanna, M.B. and Ganeshan, S. (2016) Chemical variation of *Spilanthes* species (Asteraceae) a medicinal herb in Peninsula India revealed by GCMS-MS. *International Journal of Pharmacy and Biological Sciences* 6(3): 86 – 97.

[14] Tedwins, E.J., Benjamin, O.U.U., Ayobola, E.D., Goodies, M.E. and Oghenesuvwe, E.E. (2016) A comparative study on the effect of *Massularia acuminata* and mouthwash against isolates from the oral cavity. *Journal of Research Dentistry* 4:64 – 68.

[15] Jain, K., Parida, S., Mangwani, N., Dash, H.R. and Das, S. (2013) Isolation and characterization of biofilm-forming bacteria and associated extracellular polymeric substances from oral cavity. *Annals of Microbiology* 63(4): 1553 -1562.

[16] Tsubura, S., Mizunuma, H., Ishikawa, S. et al. (2009) The effect of *Bacillus subtilis* mouth rinsing in patients with periodontitis *Eur J Clin Microbiol Infect Dis* 28: 1353.

[17] Tsubura S., Waki Y. and Tsubura T. (2012) Probiotic effect of *Bacillus subtilis* tablets on periodontopathic oral bacteria. *Microbiology Research* 3(23): 94 – 98.

[18] Sbordone, L. and Bortolaia, C. (2003) Oral microbial biofilms and plaque-related diseases: microbial communities and their role in the shift from oral health to disease. *Clin Oral Invest*. 7:181 – 188.

[19] Rozkiewicz D, Daniluk , T., Zaremba , M.L., Cylwik-Rokicka , D ., Luczaj-Cepowicz , E., Milewska , R., Marczuk-Kolada , G. and Stokowska W. (2006) Bacterial composition in the supragingival plaques of children with and without dental caries. *Advances in Medical Sciences*. 51 (1): 182 – 186.

[20] Kuramitsu, H.K., He, X., Lux, R., Anderson. M.H. and Shi, W. (2007) Interspecies Interactions within Oral Microbial Communities. *Microbiology and Molecular Biology Reviews*. 653 - 670.

[21] Badet, C. and Thebaud, N.B. (2008) Ecology of Lactobacilli in the Oral Cavity: A Review of Literature. *Open Microbiol. Journal* 2: 38 – 48.
[22] David O.M., Famurewa O. and Olawale A.K. (2010) In vitro assessment of aqueous and ethanolic extracts of some Nigerian chewing sticks on bacteria associated with dental infections. *African Journal of Microbiology Research* 4(19): 1949 – 1953.
[23] Ayankunle, A.A., Kolawole, O.T., Adesokan A.A. and Akiibinu, M.O. (2012). Antibacterial Activity and Sub-chronic Toxicity Studies of *Vitellaria paradoxa* Stem Bark Extract. *Journal of Pharmacology and Toxicology* 7: 298 - 304.
[24] Elegami A.A. (2002) Antimicrobial activity of some species of the family Combretaceae. *Phytother Research* 16(6):555 - 561.
[25] Salau AK, Yakubu MT and Oladiji AT. (2013) Cytotoxic activity of aqueous extracts of *Anogeissus leiocarpus* and *Terminalia avicennioides* root barks against Ehrlich Ascites Carcinoma cells. *Indian Journal of Pharmacology*. 2013;45(4):381-385.
[26] Abdullahi M. (2012), Evaluation of antimicrobial activity of *Anogeissus leiocarpus* and *Terminalia avicennioides* against infectious diseases prevalent in hospital environments in Nigeria. *Journal of Microbiology Research* 2(1): 6-10
[27] Odeleye O.F., Okunye O.L., Kesi C.and Abatan T.O. (2016) A Study of the Anticaries Activity of Three Common Chewing Sticks and Two Brands of Toothpaste in South West Nigeria. British *Journal of Pharmaceutical Research* 11(5): 1-7
[28] Yakubu, M.T., Akanji, M.A., Oladiji, A.T. and Adesokan A.A. (2008)Androgenic potentials of aqeous extracts of *Massularia acuminate* (G Don) Bullock ex Hoyl. Stem in male Wistar rats. Journal of Ethnopharmacology 118(3): 508 – 513.
[29] Bankole, P.O., Adekunle, A.A., Oyede, R.T., Faparusi, F. and Adewole, A. (2012). Antimicrobial Activities and Phytochemical Screening of two Tropical Nigerian Chewing Sticks. *International Journal of Applied Science and Technology* 2(6): 131 - 138.
[30] Itemire O. A., Ogbimi O.A., MacDonald I. (2013) Phytochemistry and antimicrobial activity of *Zanthoxylum zanthoxyloides* root used as chewing stick in Nigeria. *The Journal of Phytopharmacology* 2(6): 1 – 7.
[31] Ayepola O.O (2009) Evaluation of the antimicrobial activity of root and leaf extracts of *Terminalia glaucescens*. *Advances in Natural and Applied Sciences* 3(2): 188 – 191.
[32] Olaleye O.O., Adetunji C.O., Kolawole O.M. (2015) Identification of phytochemical constituents of the methanolic extract of *Vitellaria paradoxa* responsible for antimicrobial activity against selected pathogenic organisms. *SMU Medical Journal* 2(1): 177 – 190.
[33] Dube LM, Kumari M, Pandya M, Dube S, Trivedi P. (2013). Comparative evaluation of antibacterial efficacy of *Spilanthes calva* DC root extract, sodium hypochlorite, chlorhexidine and doxycycline at different concentrations on enterococcus faecalis- An in-vitro study. *Endodontology* 25: 63 - 72.
[34] Aderinokun G.A, Lawoyin J.O, and Onyeaso C.O. (1999) Effect of Two Common Nigerian Chewing Sticks on Gingival Health and Oral Hygiene. *Odonto-Stomatologie Tropicale* 87: 13 – 18.
[35] Kehinde, T.K., Alli, S.O., Atayese, A.O., Ezeh, A.R. and Alaga, T.O. (2012). Antibacterial effect of *Distemonanthus benthamianus* extract against some oral pathogens. *International Journal of Applied Science and Technology* 2 (2): 114