ORIGINAL ARTICLE

Antimicrobial Activity of Selected Yoruba Chewing Sticks and Branded Toothpastes on Bacteria Isolated from Carious Teeth

Okunye OL[1], Kotun BC[2], Babalola CO[3], Daodu JO[4], Ayedun JS[2], Ibitoye SF[4], Iloka B[1], Olumuyiwa A[5], Oluwaseun EA[6], Igbokwe CO[1], V-Thompson EP[1], Ajayi PO[1], Coker ME*

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University

[2]Department of Biological Sciences & Biotechnology, College of Pure & Applied Sciences, Caleb University, Lagos

[3]Department of Pharmaceutics & Pharmaceutical Technology, Faculty of Pharmacy, Olabisi Onabanjo University

[4]Department of Pharmaceutical & Medicinal Chemistry, Faculty of Pharmacy, Olabisi Onabanjo University

[5]Department of Biotechnology, Faculty of Life Sciences, Midibo Adama University, Yola

[6]Department of Microbiology, University of Ilesa, Ilesa ,*University of Ibadan

Correspondence: Okunye OL[1]

Email:okunyelionel@oouagoiwoye.edu.ng

ABSTRACT

Objectives: Carious teeth cause pain and discomfort and are often linked to bacterial invasion. This study in vestigated the antimicrobial activities of methanol extracts from three selected Yoruba chewing sticks—*Sorindeia warneckei (Afunsese), Garcinia kola (Orogbo)* and *Vernonia amygdalina (Ewuro)* and aqueous extracts of three branded toothpastes (Naturacentials [NCT], Maclean [MCL], and Olive [OLV]) on bacterial isolates from carious teeth.

Methods: Thirty bacterial isolates (10 *Streptococcus oralis,* 10 *Lactobacillus spp.* and 10 *Streptococcus mutans)* from patients with tooth decay in Ibadan were culture on sheep blood agar and de Man Rogosa Sharpe agar, Gram stained and confirmed by biochemical tests. The isolates were challenged with methanol extracts of the chewing sticks and aqueous extracts of the toothpastes using standard broth dilution methods to determine the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). Chewing stick extracts were screened for phytochemical constituents. The data were analyzed using one ANOVA (Turkey's post hoc test) with Graph Pad prism version 8.1.

Results: Crude extract yields were 9.25% for *S. warneckei,* 6.8% for *G. kola*, and 5.7% for *V. amygdalina.* Alkaloids, anthraquinones, tannins, flavonoids, and saponins were detected in the chewing sticks examined. Methanol extracts exhibited stronger antimicrobial activity than toothpaste aqueous extracts. MIC values for chewing sticks ranged from 1.56–25 µg/mL, compared with 12.5–100 µg/mL for toothpastes. ANOVA confirmed significant inhibition by chewing stick extracts.

Conclusion: Methanol extracts of Yoruba chewing sticks demonstrated competitive antimicrobial activity compared with branded toothpastes. Their bioactive constituents hold promise for clinical application and potential incorporation into conventional toothpaste formulations.

Keywords: Yoruba chewing sticks, Branded toothpastes, Tooth decay

INTRODUCTION

Dental caries (tooth decay) is widespread, causing oral/dental pain and eventual tooth loss if left unattended. The term toothache is commonly used by the lay public to describe a wide variety of painful dental and non-dental conditions. These include acute pulpitis (often characterized by exacerbations when biting), acute periodontitis, acute abscesses with throbbing pain, pericoronitis, maxillary sinusitis, dry socket, acute ulcerative gingivitis, and idiopathic trigeminal neuralgia, which may present as sharp, excruciating spasms. Other causes include caries, gingival bleeding, and periodontal disease.[1] Decayed teeth may harbor diverse microorganisms, particularly in immunocompromised patients. Reported bacterial species include *Streptococcus oralis, Streptococcus mutans, Streptococcus salivarius, Streptococcus intermedius, Streptococcus mitis*, *Streptococcus anginosus, Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus fermentum, Bifidobacterium dentium, Actinomyces odontolyticus,* and *Veillonella spp.,* among others.[2]

The Yoruba people predominantly occupy southwestern Nigeria, with an estimated population of about 30 million (approximately 21% of the Nigerian population). Yoruba land is largely forested, interspersed with derived savannah, and is rich in medicinal flora and fauna. While Yoruba people are also found in Togo, Benin Republic, Cuba, Brazil, and the Caribbean, Ile-Ife in Nigeria is widely regarded as their ancestral origin.[3] Among the numerous plant species used traditionally as chewing sticks in Yoruba land are: *Anogeissus leiocarpus (Orin Odan), Lecaniodiscus cupanioides (Aika), Jatropha curcas (Lapalapa), Massularia acuminata (Pako Ijebu), Jatropha gossypiifolia (Ogegee), Napoleona vogelii (Boribori), Paullinia pinnata (Kakase nla), Sorindeia warneckei (Afunsese), Khaya senegalensis (Oganwo), Garcinia kola*

(Orogbo), Baphia nitida (Iyere osun), Terminalia glaucescens (Orin pupa), Vernonia amygdalina (Ewuro), and Nauclea latifolia (Egbesi).[3,4] Traditional healers and herbalists have long claimed that these plants contain bioactive chemical constituents with therapeutic potential, stimulating research into their antimicrobial properties.

Chewing sticks are obtained from stems, roots, or stumps of plants. They are typically cut into short lengths, washed, and chewed for oral cleansing. In Yoruba land, chewing sticks are widely used not only for mechanical cleaning of teeth but also for their perceived therapeutic properties, breath freshening, and flavor. Their effectiveness is attributed to both the release of bioactive compounds and the frictional cleaning action of their fibers. Many have been reported to possess antimicrobial, anti-inflammatory, and anti-caries properties.[5] It is estimated that 80–90% of rural dwellers in Yoruba land use chewing sticks daily due to their affordability and accessibility.[6] Conversely, branded toothpastes—commonly fortified with fluoride—are widely used in urban areas. For this study, three toothpastes were selected, namely, NCT, MCL and OLV. NCT contains bee propolis, tea tree extract, myrrh, and aloe vera, in addition to vitamins and phytonutrients.[7,8,9] Given the continued reliance on chewing sticks in Nigeria and the widespread use of fluoride toothpastes, this study compared the antimicrobial activities of methanol extracts of selected Yoruba chewing sticks with aqueous extracts of branded toothpastes against bacteria isolated from carious teeth.

METHODS

Procurement of Toothpastes

Three branded toothpastes—NCT, MCL, and OLV—were procured from Agbeni Market in Ibadan, Nigeria.

Collection of Chewing Sticks

Twigs (roots, stems, and leaves) of Sorindeia warneckei, Garcinia kola, and Vernonia amygdalina were collected at the onset of the sun from forested areas behind the Cocoa Research Institute of Nigeria, Onigambari, along the Adebayo–Ago-Iwoye route in Ibadan (latitude 7.37750°N, longitude 3.94700°E) during the raining season. The specimens were authenticated at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, where voucher specimens 2025301GK, 2025201SW, 2025 541VA were archived.

Collection of Clinical Isolates

Thirty biochemically characterized isolates were collected from patients presenting with tooth decay at the Government Dental Clinic, Ibadan. These comprised 10 isolates each of *Streptococcus oralis*, *Lactobacillus spp*., and *Streptococcus mutans.*

Bacteriological Confirmation

Streptococcus species were subcultured on sheep blood agar fortified with serum, while *Lactobacillus spp.* were cultured on de Man Rogosa Sharpe (MRS) agar. Plates were incubated anaerobically at 37 °C for 24 hours. Isolates were confirmed by Gram staining and biochemical tests, including catalase, aesculin hydrolysis, citrate, indole, and carbohydrate fermentation (fructose, sucrose, arabinose, starch, lactose, salicin, galactose, trehalose, glucose, inulin, and mannitol). Additional tests such as urease and Voges–Proskauer were performed where relevant.

Preparation of Plant Extracts

Chewing sticks were thoroughly washed with sterile water, sliced into small pieces, sun-dried for 40 days, and milled. Twenty-five grams of each powdered specimen was macerated in 250 mL of 50% methanol (cold maceration) for four days. The extracts were filtered through Whatman No. 1 filter paper, concentrated with a rotary evaporator, and stored at 4 °C for further use.

Phytochemical Screening

Standard methods were used for preliminary phytochemical screening:

Alkaloids: Extracts were treated with dilute ammonia followed by Dragendorff's reagent; reddish-brown precipitate indicated a positive test.

Anthraquinones: Extracts were boiled with dilute H_2SO_4, filtered, and shaken with chloroform. The chloroform layer was treated with dilute ammonia; color change indicated a positive test.

Flavonoids: Extracts were treated with dilute ammonia followed by concentrated H_2SO_4; yellow coloration indicated flavonoids.

Saponins: Extracts were shaken vigorously with water; froth persistence and emulsion formation with olive oil indicated saponins.

Tannins: Extracts were boiled with water, filtered, and treated with ferric chloride; a blue-black or green coloration indicated tannins.

Preparation of Toothpaste Extracts

Two grams of each toothpaste sample was suspended in 10 mL sterile water, vortexed for 2 minutes, centrifuged at 10,000 × g for 15 minutes, and filtered to obtain stock solutions. A 0.2% chlorhexidine solution served as positive control, while methanol was used as negative control.

Determination of Minimum Inhibitory Concentration (MIC)

MICs were determined by broth microdilution. Serial concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 µg/mL) were prepared in tryptone soya broth. Test organisms adjusted to 0.5 McFarland standard were inoculated. After incubation at 37 °C for 24 hours under anaerobic conditions, resazurin dye was added to assess bacterial growth. The lowest concentration with no visible growth was recorded as the MIC.

Determination of Minimum Bactericidal Concentration (MBC)

Broth from MIC wells showing no growth was subcultured onto sterile blood agar plates and incubated anaerobically at 37 °C for 24 hours. The lowest concentration showing no bacterial growth on agar was recorded as the MBC. The experimental procedure was done in replicates.

Statistical Analysis

MIC and MBC results were expressed as mean ± SD and analyzed using GraphPad Prism (version 8.1). One-way ANOVA and Student's t-test were used where appropriate, with statistical significance set at $p < 0.05$.

Ethical Considerations

Ethical approval for this study was obtained from the Oyo State Ministry of Health, Department of Planning, Research and Statistics (Approval No. AD13/479/381C).

RESULTS

Extract Yields

The methanol extraction yields differed among the chewing sticks. *Sorindeia warneckei* had the highest yield (9.25%), followed by *Garcinia kola* (6.8%) and *Vernonia amygdalina* (5.7%) (Table 1).

Table 1: Yield of Crude Extract of Selected Chewing Sticks

Extract	Color	Weight of the Extracts		
		Initial weight (g)	Extract Weight (g)	% Yield
Sorindeia warneckei	Dark brownish	200	18.50	9.25
Garcinia kola	Brownish	200	13.60	6.8
Veronia amgdalina	Dark greenish	200	11.40	5.7

Phytochemical Constituents

Qualitative screening revealed alkaloids, anthraquinones, tannins, flavonoids, and saponins in all three chewing sticks. Alkaloids and anthraquinones were stronger in S. warneckei and *G. kola,* tannins and flavonoids were prominent in *S. warneckei* and *V. amygdalina* and saponins were particularly high in *G. kola* (Table 2).

Table 2: Bioactive constituents of Selected Chewing sticks Extracts

Bioactive constituents	*Sorindeia warneckei*	*Garcinia Kola*	*Veronia amygdalina*
Alkaloids	+	+	+
Anthraquinones	+	+	+
Tanins	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+

Antimicrobial Activity of Chewing-Stick Extracts

Methanol extracts of the chewing sticks inhibited all three test organisms in a concentration-dependent manner. For *Streptococcus oralis, S. warneckei* showed the lowest MIC (1.56 µg/mL) and MBC (3.12 µg/mL), followed by G. kola (MIC 6.25; MBC 12.5) and *V. amygdalina* (MIC 12.5; MBC 25). For *S. mutans,* MIC values were 3.12, 6.25, and 12.5 µg/mL for *S. warneckei, G. kola,* and *V. amygdalina* respectively. For *Lactobacillus spp.* MIC values were 6.25 µg/mL (*S. warneckei*), 12.5 µg/mL *(G. kola)* and 25 µg/mL (*V. amygdalina*).(Table 3).

Table 3: Minimum Inhibitory Concentration (µg/mL) of Aqueous and Methanol extract of Chewing stick specimen and Factory made Tooth paste

S/N	ISOLATES CODE	CHEWING STICKS EXTRACTS						FACTORY MADE TOOTH PASTE				
		SW		*GK*		*VA*		NCT	MCL	OLV	Positive Control	Negative Control
		AE	ME	AE	ME	AE	ME	AE NCT	AE MCL	AE OLV		
1	*S. oralis01*	100	3.125	100	6.25	100	25	25	25	100	1.25	0
2	*S. oralis 02*	-	1.56	50	6.25	100	25	-	-	50	1.25	0
3	*S. oralis 03*	100	25	50	6.25	50	-	25	25	100	2.5	0
4	*S. oralis 04*	25	1.56	100	3.125	50	-	12.5	25	50	2.5	0
5	*S. oralis 05*	12.56	-	100	6.25	100	12.56	12.5	12.5	50	1.25	0
6	*S. oralis 06*	-	12.56	100	12.56	50	25	50	100	100	1.25	0
7	*S. oralis 07*	-	12.56	-	-	-	-	-	-	50	5	0
8	*S. oralis 08*	100	3.125	100	6.25	100	25	25	25	100	5	0
9	*S. oralis 09*	12.5	-	100	6.25	100	12.56	12.56	12.5	50	2.5	0
10	*S. oralis 10*	-	1.56	50	6.25	100	25	12.56	50	50	1.25	0
11	*Lactobacilli spp11*	100	12.56	50	3.125	100	12.5	12.5	50	50	2.5	0
12	*Lactobacilli spp 12*	100	25	50	25	50	12.56	6.25	50	100	1.25	0
13	*Lactobacilli spp 13*	50	3.125	50	12.56	50	3.125	50	100	100	1.25	0
14	*Lactobacilli spp 14*	100	50	50	25	50	12.56	50	50	100	5	0
15	*Lactobacilli spp 15*	100	12.56	50	3.125	100	12.5	12.5	50	50	2.5	0
16	*Lactobacilli spp 16*	-	-	-	-	100	50	6.25	12.5	50	5	0
17	*Lactobacilli spp 17*	50	3.125	50	12.56	50	3.125	50	100	100	1.25	0
18	*Lactobacilli spp 18*	100	25	50	25	50	12.56	6.25	50	100	0.625	0
19	*Lactobacilli spp 19*	50	3.125	50	6.25	25	-	12.56	50	50	2.5	0
20	*Lactobacilli spp 20*	100	12.56	50	3.125	100	12.5	12.5	50	50	5	0
21	*S.mutans 21*	100	3.125	100	6.25	100	25	25	25	100	2.5	0
22	*S.mutans 22*	12.5	-	100	6.25	100	12.56	12.56	12.5	50	5	0
23	*S.mutans 23*	-	1.56	50	6.25	100	25	12.56	50	50	5	0
24	*S.mutans 24*	25	1.56	100	3.125	50	-	12.5	25	50	2.5	0
25	*S.mutans 25*	12.56	-	100	6.25	100	12.56	12.5	12.5	50	5	0
26	*S.mutans 26*	-	12.56	100	12.56	50	25	50	100	100	5	0
27	*S.mutans 27*	-	12.56	-	-	-	-	-	-	50	2.5	0
28	*S.mutans 28*	100	3.125	100	6.25	100	25	25	25	100	1.25	0
29	*S.mutans 29*	-	1.56	50	6.25	100	25	-	-	50	5	0
30	*S.mutans 30*	100	25	50	6.25	50	25	25	25	100	1.25	0

Key: *SW: Sorindeia warneckei GK: Garcinia Kola, VA: Veronia amygdalina*
NCT: Naturalcentials, MCL:Maclean, OLV: Olive, AE: Aqueous Exract, ME: Methanol Extract .
Positive control: chlorhexidine Negative control : Methanol

Antimicrobial Activity of Toothpaste Extracts

Aqueous extracts of toothpastes were also active, but at higher MIC/MBC values than chewing sticks For *S. oralis,* MIC values were 25 µg/mL for NCT, 50 µg/mL for MCL, and 100 µg/mL for OLV. For *S. mutans,* MICs were 12.5 µg/mL (NCT), 25 µg/mL (MCL), and 50 µg/mL (OLV). For *Lactobacillus spp.,* MICs ranged 25–100 µg/mL. Corresponding MBC values were about twice the MICs.(Table 4; Figure 2).

Table 4: Minimum Bactericidal Concentration (µg/mL) of Aqueous and Methanol extract of Chewing stick specimen and branded Tooth paste

S/N	ISOLATES CODE	CHEWING STICKS EXTRACTS						FACTORY MADE TOOTH PASTE				
		SW		*GK*		*VA*		NCT	MCL	OLV	+ve control	-ve control
		AE	ME	AE	ME	AE	ME	AE	AE	AE		
1	*S. oralis01*	-	25	-	50	-	100	50	50	-	10	0
2	*S. oralis 02*	-	-	100	12.56	-	50	-	-	100	10	0
3	*S. oralis 03*	100	50	100	25.6	100	-	50	50	100	2.5	0
4	*S. oralis 04*	50	-	100	-	100	-	50	50	100	2.5	0
5	*S. oralis 05*	50	-	-	50	-	50	50	50	100	10	0
6	*S. oralis 06*	-	50	100	50	100	50	-	100	-	2.5	0
7	*S. oralis 07*	-	25	-	-	-	-	-	-	100	5	0
8	*S. oralis 08*	-	12.56	-	50	-	50	-	-	-	10	0
9	*S. oralis 09*	50	-	100	25	-	-	-	50	100	10	0
10	*S. oralis 10*	-	-	100	12.56	-	50	50	100	100	5	0
11	*Lactobacilli spp11*	-	50	100	12.56	-	-	-	100	100	10	0
12	*Lactobacilli spp 12*	-	50	100	50	-	-	-	-	-	10	0
13	*Lactobacilli spp 13*	100	-	100	25	100	-	-	-	100	10	0
14	*Lactobacilli spp 14*	-	100	100	-	100	50	-	100	-	10	0
15	*Lactobacilli spp 15*	-	50	100	-	100	25	25	100	100	2.5	0
16	*Lactobacilli spp 16*	-	-	-	-	-	-	-	-	-	10	0
17	*Lactobacilli spp 17*	-	-	100	50	100	25	100	100	100	10	0
18	*Lactobacilli spp 18*	100	50	100	-	-	-	25	100	100	10	0
19	*Lactobacilli spp 19*	100	12.56	100	25	-	-	-	100	100	5	0
20	*Lactobacilli spp 20*	-	50	100	25	-	25	25	100	100	10	0
21	*S.mutans 21*	-	12.56	-	25	-	100	-	50	100	1.25	0
22	*S.mutans 22*	100	-	-	100	-	50	50	100	100	10	0
23	*S.mutans 23*	-	50	100	25	-	-	-	-		10	0
24	*S.mutans 24*	100	-	100	25	100	-	50	100	100	5	0
25	*S.mutans 25*	50	-	-	50	100	25	-	50	50	5	0
26	*S.mutans 26*	-	-	-	-	-	-	-	-	-	10	0
27	*S.mutans 27*	-	50	-	-	-	-	-	-	100	10	0
28	*S.mutans 28*	100	6.25	-	50	-	50	-	50	100	10	0
29	*S.mutans 29*	-	12.56	50	50	-	50	50	50	100	10	0
30	*S.mutans 30*	-	50	100	50	100	-	50	100	100	5	0

Key: *SW: Sorindeia warneckei GK: Garcinia Kola, VA: Veronia amygdalina;* NCT, MCL, OLV, AE: Aqueous Extract, ME: Methanol ExtractPositive control:chlorhexidine Negative control : Methan

Comparative Analysis

A consolidated analysis showed that methanol extracts of chewing sticks were significantly more effective than toothpaste extracts ($p < 0.05$, one-way ANOVA).*Sorindeia warneckei* consistently produced the lowest MIC/MBC values among chewing sticks, whereas NCT was the most active toothpaste. (Table 5)

Table 5: Comparisons of mean minimum inhibitory concentration and minimum bactericidal concentration for *Streptococcus oralis* isolates (graphical data)

	MIC		**MBC**	
	MEAN	SD ±	MEAN	SD ±
SW Chewing Stick (Methanol Extract)	58.34	45.86	62.5	25
SW Chewing Stick (Aqueous Extract)	7.631	8.46	32.51	16.75
GK Chewing Stick (Methanol Extract)	83.33	25	100	0
GK Chewing Stick (Aqueous Extract)	15.94	29.63	34.47	17.29
V A Chewing Stick (Methanol Extract)	83.33	25	100	0
V A Chewing Stick (Aqueous Extract)	21.45	6.07	58.33	20.41
NCT Toothpaste Aqueous Extract	21.89	12.93	50	0
MCL Toothpaste Aqueous Extract	34.38	28.93	64.29	24.40
OLV Toothpaste Aqueous Extract	70	25.82	100	0

Omnibus ANOVA Tests

Toothpastes (NCT, MCL, OLV): One-way ANOVA revealed significant variation among the three extracts (F value significant, $p < 0.05$), indicating they were not equally effective (Table 6).

Table 6: Comparisons of (mean) minimum inhibitory concentration and minimum bactericidal concentration for *lactobacilli spp.* Isolates (graphical data)

	MIC		**MBC**	
	MEAN	SD ±	MEAN	SD ±
SW Chewing Stick (Methanol Extract)	83.33	25	100	0
SW Chewing Stick (Aqueous Extract)	16.34	15.21	51.79	25.43
GK Chewing Stick (Methanol Extract)	50	0	100	0
GK Chewing Stick (Aqueous Extract)	12.86	9.81	31.26	15.29
V A Chewing Stick (Methanol Extract)	67.5	28.99	100	0
V A Chewing Stick (Aqueous Extract)	14.6	13.88	31.25	12.5
NCT Toothpaste Aqueous Extract	21.88	19.60	44.75	37.5
MCL Toothpaste Aqueous Extract	56.25	25.85	100	0
OLV Toothpaste Aqueous Extract	75	26.35	100	0

Chewing sticks (SW, GK, VA): ANOVA likewise showed overall differences among extracts ($p < 0.05$), supporting post-hoc pairwise comparisons. Toothpastes: Tukey's multiple comparison test confirmed that NCT(AE) was significantly more active than OLV(AE) (mean diff. −52.96, 95% CI −64.89 to −41.02, $p < 0.0001$). MCL(AE) was also significantly superior to OLV(AE) (mean diff. −38.13, 95% CI −65.86 to −10.39, $p = 0.0118$). In contrast, NCT vs MCL did not differ significantly ($p = 0.1792$). (Table 7, 8).

Table 7: Comparisons of (mean) minimum inhibitory concentration and minimum bactericidal concentration on *Streptococcus mutans* isolates(graphical data)

	MIC		**MBC**	
	MEAN	SD ±	MEAN	SD ±
SW Chewing Stick (Methanol Extract)	58.34	45.86	87.5	25
SW Chewing Stick (Aqueous Extract)	7.631	8.46	30.23	21.78
GK Chewing Stick (Methanol Extract)	83.33	25	87.5	25
GK Chewing Stick (Aqueous Extract)	6.604	2.46	46.88	24.78
VA Chewing Stick (Methanol Extract)	83.33	25	100	0
VA Chewing Stick (Aqueous Extract)	21.45	6.07	55	27.39
NCT Toothpaste Aqueous Extract	21.89	12.93	50	0
MCL Toothpaste Aqueous Extract	34.38	28.93	71.43	26.73
OLV Toothpaste Aqueous Extract	70	25.82	93.75	17.68

Table 8: Post-hoc Tukey Comparisons

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**DF**	**Adjusted P Value**
NCT(AE) vs. MCL(AE)	-14.83	-36.53 to 6.866	No	7	0.1792
NCT(AE) vs. OLV(AE)	-52.96	-64.89 to -41.02	Yes	7	<0.0001
MCL(AE) vs. OLV(AE)	-38.13	-65.86 to -10.39	Yes	7	0.0118

Chewing sticks: Tukey's analysis demonstrated significant potency differences between methanol and aqueous extracts. For example, GK(ME) was significantly more potent than GK(AE) (mean diff. +64.75, 95% CI 47.87–81.64, $p < 0.0001$) and VA(ME) was significantly more active than VA(AE) (mean diff. +55.05, 95% CI 29.03–81.06, $p = 0.0012$). Similarly, SW(AE) was significantly less effective than GK(ME) (mean diff. −77.4, 95% CI −104.9 to −49.87, $p = 0.0002$) and VA(ME) ($p = 0.0009$). Not all pairwise comparisons reached significance, but the general pattern favored methanol extracts (Table 9).

Table 9: Tukey's multiple comparisons test

Tukey's multiple comparisons test	**Mean Diff.**	**95.00% CI of diff.**	**Significant?**	**DF**	**Adjusted P Value**
SW(ME) vs. SW(AE)	52.4	-17.11 to 121.9	No	3	0.1043
SW(ME) vs. GK(ME)	-25	-92.38 to 42.39	No	5	0.6393
SW(ME) vs. GK(AE)	39.76	-14.10 to 93.62	No	5	0.1439
SW(ME) vs. VA(ME)	-25	-87.51 to 37.52	No	5	0.5794
SW(ME) vs. VA(AE)	30.05	-29.67 to 89.77	No	3	0.2623
SW(AE) vs. GK(ME)	-77.4	-104.9 to -49.87	Yes	6	0.0002
SW(AE) vs. GK(AE)	-12.65	-50.66 to 25.37	No	7	0.7968
SW(AE) vs. VA(ME)	-77.4	-112.3 to -42.45	Yes	6	0.0009
SW(AE) vs. VA(AE)	-22.35	-54.39 to 9.688	No	4	0.1504
GK(ME) vs. GK(AE)	64.75	47.87 to 81.64	Yes	8	<0.0001
GK(ME) vs. VA(ME)	0	-21.53 to 21.53	No	8	>0.9999
GK(ME) vs. VA(AE)	55.05	27.45 to 82.64	Yes	6	0.0017
GK(AE) vs. VA(ME)	-64.75	-85.31 to -44.20	Yes	8	<0.0001
GK(AE) vs. VA(AE)	-9.706	-31.66 to 12.24	No	6	0.5457
VA(ME) vs. VA(AE)	55.05	29.03 to 81.06	Yes	6	0.0012

Paired t-Tests (MIC vs MBC Within Extracts)
Paired t-tests compared MICs to MBCs for each extract:
For S. oralis, significant MIC–MBC differences were observed for OLV(AE) (+42.86, p = 0.001), MCL(AE) (+28.57, p = 0.0029), NCT(AE) (+32.49, p = 0.0004), VA(AE) (+35.41, p = 0.0075), GK(AE) (+27.43, p = 0.0023), and SW(AE) (+21.24, p = 0.0129). GK(ME) (+25, p = 0.0756) and SW(ME) (+24.99, p = 0.0662) did not reach significance (Table 10).

Table 10: T-Test comparisons of effect on *S. oralis*

T test	Mean Diff.	95.00% CI of diff.	Significant?	DF	Adjusted P Value
MBC OLV(AE) vs. MIC OLV(AE)	42.86	25.38 to 60.34	Yes	6	0.001
MBC MCL(AE) vs. MIC MCL(AE)	28.57	14.08 to 43.06	Yes	6	0.0029
MBC NCT(AE) vs. MIC NCT(AE)	32.49	24.00 to 40.98	Yes	4	0.0004
MBC VA(AE) vs. MIC VA(AE)	35.41	14.39 to 56.42	Yes	5	0.0075
MBC VA(ME) vs. MIC VA(ME)					
MBC GK(AE) vs. MIC GK(AE)	27.43	13.54 to 41.32	Yes	7	0.0023
MBC GK(ME) vs. MIC GK(ME)	25	-3.740 to 53.74	No	5	0.0756
MBC SW(AE) vs. MIC SW(AE)	21.24	7.442 to 35.03	Yes	4	0.0129
MBC SW(ME) vs. MIC SW(ME)	24.99	-3.122 to 53.09	No	3	0.0662

For Lactobacillus spp., significant MIC–MBC differences were seen for OLV(AE) (+28.57, p = 0.03), MCL(AE) (+42.86, p = 0.001), VA(AE) (+21.08, p = 0.0372), GK(AE) (+20.82, p = 0.0038), and SW(AE) (+31.68, p = 0.0007). Non-significant results included NCT(AE) (p = 0.0796), VA(ME) (p = 0.0577), GK(ME) (not reported), and SW(ME) (p = 0.1835) (Table 11).

Table 11: T-Test comparisons of effect on *Lactobacilli spp.*

T test	Mean Diff.	95.00% CI of diff.	Significant?	DF	Adjusted P Value
MBC OLV(AE) vs. MIC OLV(AE)	28.57	3.854 to 53.29	Yes	6	0.03
MBC MCL(AE) vs. MIC MCL(AE)	42.86	25.38 to 60.34	Yes	6	0.001
MBC NCT(AE) vs. MIC NCT(AE)	23.44	-5.128 to 52.00	No	3	0.0796
MBC VA(AE) vs. MIC VA(AE)	21.08	2.352 to 39.81	Yes	3	0.0372
MBC VA(ME) vs. MIC VA(ME)	37.5	-2.281 to 77.28	No	3	0.0577
MBC GK(AE) vs. MIC GK(AE)	20.82	10.34 to 31.31	Yes	5	0.0038
MBC GK(ME) vs. MIC GK(ME)					
MBC SW(AE) vs. MIC SW(AE)	31.68	19.63 to 43.73	Yes	6	0.0007
MBC SW(ME) vs. MIC SW(ME)	33.33	-38.38 to 105.0	No	2	0.1835

For S. mutans, significant differences were found in OLV(AE) (+25, p = 0.0331), MCL(AE) (+54.17, p = 0.0053), NCT(AE) (+33.31, p = 0.0152), VA(AE) (+34.98, p = 0.0313), GK(AE) (+41.02, p = 0.0021), and SW(AE) (+22.41, p = 0.0273). Non-significant results included VA(ME) (p = 0.1835), GK(ME) (p = 0.1817), and SW(ME) (p = 0.0855) (Table 12).

Table 12:T-Test comparisons of effect on *S. mutans*

T test	Mean Diff.	95.00% CI of diff.	Significant?	DF	Adjusted P Value
MBC OLV(AE) vs. MIC OLV(AE)	25	2.656 to 47.34	Yes	7	0.0331
MBC MCL(AE) vs. MIC MCL(AE)	54.17	24.64 to 83.69	Yes	5	0.0053
MBC NCT(AE) vs. MIC NCT(AE)	33.31	15.43 to 51.20	Yes	2	0.0152
MBC VA(AE) vs. MIC VA(AE)	34.98	5.106 to 64.85	Yes	4	0.0313
MBC VA(ME) vs. MIC VA(ME)	33.33	-38.38 to 105.0	No	2	0.1835
MBC GK(AE) vs. MIC GK(AE)	41.02	20.61 to 61.42	Yes	7	0.0021
MBC GK(ME) vs. MIC GK(ME)	25	-20.93 to 70.93	No	3	0.1817
MBC SW(AE) vs. MIC SW(AE)	22.41	3.748 to 41.07	Yes	5	0.0273
MBC SW(ME) vs. MIC SW(ME)	49.99	-12.92 to 112.9	No	3	0.0855

Controls: The positive control (0.2% chlorhexidine) completely inhibited all isolates at the lowest concentration tested (<0.78 µg/mL), validating the assays. The methanol negative control showed no inhibition.

In summary:

- Methanolic maceration yields were: *Sorindeia warneckei* (9.25%), Garcinia kola (6.8%), and Vernonia amygdalina (5.7%).
- All three chewing sticks contained alkaloids, anthraquinones, tannins, flavonoids, and saponins.
- Alkaloids and anthraquinones were strongest in *S. warneckei* and G. kola.
- Tannins and flavonoids were most prominent in *S. warneckei* and V. amygdalina.
- Saponins were most abundant in G. kola.
- The methanol extracts inhibited all bacterial isolates in a concentration-dependent manner.
- Against all organisms, *S. warneckei* was the most effective chewing stick.
- Chewing-stick methanol extracts consistently outperformed toothpaste aqueous extracts against all organisms.
- MBC values were generally higher than MICs, confirming a higher concentration was needed to kill bacteria than to just inhibit growth.
- The positive control (Chlorhexidine 0.2%) was highly effective, and the negative control (Methanol) had no effect, validating the assay's sensitivity.

DISCUSSION

Findings: The percentage yield of the extracts of the three selected chewing sticks varied for each specimen. *Sorindeia warneckei* yielded 9.25%, while *Garcinia kola* and *Vernonia amygdalina* yielded 6.8% and 5.7%, respectively, from an initial weight of 200 g. The variation observed in yield may be attributed to solvent choice, particle size, temperature, or pH, in agreement with Ogundipe et al.[9]

The presence of alkaloids, anthraquinones, tannins, flavonoids, and saponins in *S. warneckei*, *G. kola* and *V. amygdalina* could explain their antibacterial effects, corroborating Osho and Adelani[10], who reported antimicrobial activity of Nigerian chewing sticks against Candida species. Antibacterial activity of plant extracts is routinely assessed by agar dilution, agar diffusion, and MIC testing. Serial dilution for MIC is particularly useful in determining the lowest concentration that inhibits microbial growth.[11]

Sodeinda wanecki on Streptococcus oralis, Lactobacillus spp.,* and *Streptococcus mutans

The MIC of aqueous extract against *S. oralis* ranged from 12.56 µg/mL (isolates 05 and 09) to 100 µg/mL (isolates 01, 03, 08). Methanol extracts showed superior activity, ranging from 1.56 µg/mL (isolates 02 and 04) to 25 µg/mL (isolate 03), with MBC values generally twice the MIC.

For *Lactobacillus spp* , aqueous extracts ranged from 50 µg/mL (isolates 13, 17, 19) to 100 µg/mL (isolates 11, 12, 14, 15, 20), while methanol extracts ranged from 3.125–12.56 µg/mL. Against S. mutans,

aqueous extracts gave MICs of 12.56–100 µg/mL, while methanol extracts inhibited at 3.125–25 µg/mL. Methanol extracts thus had greater efficacy, attributable to solvent polarity, which is consistent with previous phytochemical studies.[11]

Garcinia kola on Streptococcus oralis, Lactobacillus spp.,* and *Streptococcus mutans

The MIC of aqueous extract against *S. oralis* ranged from 50 µg/mL (isolates 02, 03) to 100 µg/mL (isolates 04–06, 09, 10). Methanol extracts ranged between 3.125 µg/mL (isolate 04) and 12.56 µg/mL (isolate 06).

For *Lactobacillus spp* ., aqueous extracts inhibited at 50–100 µg/mL, while methanol extracts ranged from 3.125–12.56 µg/mL. Against S. mutans, aqueous extracts ranged from 50–100 µg/mL (isolates 23–30), whereas methanol extracts showed MICs of 3.125–12.56 µg/mL (isolates 24 and 26). These results highlight the potent antimicrobial activity of G. kola, as previously reported by Antwi-Boasiako and Abubakari.[6]

Veronia amygdalina on Streptococcus oralis, Lactobacillus spp.* and *Streptococcus mutans

For *S. oralis*, aqueous extracts showed MICs of 50 µg/mL (isolates 03, 04, 06) and 100 µg/mL (isolates 01, 02, 05, 08–10). Methanol extracts were more effective, ranging from 12.56 µg/mL (isolates 04, 05, 09) to 25 µg/mL (isolates 01, 02, 06, 08, 10).

For *Lactobacillus spp* ., aqueous extracts inhibited at 25–100 µg/mL, while methanol extracts showed activity at 3.125–12.56 µg/mL. Against S. mutans, aqueous extracts ranged from 50–100 µg/mL, whereas methanol extracts were effective at 12.56–25 µg/mL. These findings corroborate Ugoji et al.[12], who emphasized solvent polarity in determining extract potency.

Branded toothpaste on *Streptococcus oralis, Lactobacillus spp.,* and *Streptococcus mutans*

The MIC of NCT ranged between 12.56–25 µg/mL for *S. oralis*, 6.25–50 µg/mL for Lactobacillus spp., and 12.56–50 µg/mL for S. mutans. MCL exhibited MICs of 12.56–100 µg/mL across isolates, while OLV showed the least antimicrobial activity (50–100 µg/mL).

Statistical analysis (ANOVA and Tukey's test) revealed no significant difference between NCT and MCL but significant differences between NCT and OLV, and between MCL and OLV. The higher efficacy of NCT may be attributed to its phytochemical fortification (bee propolis, aloe vera, tea tree extract, myrrh).[2]

Implications: These findings indicate that Nigerian chewing sticks could provide affordable and effective alternatives to branded toothpastes, particularly for rural populations. Positive is usually expressed at the expense of negative, some users may develop allergy to some selected chewing sticks, while through excessive brushing traumatic lesion and clinical attachment loss may occur. Nevertheless, their bioactive compounds, if isolated, could be incorporated into commercial formulations, thereby enhancing the therapeutic efficacy of toothpastes against bacterial dental infections. [3,13]

Trade-Offs (Limitations): In-vitro activity does not always translate directly into in-vivo efficacy due to host immune responses, oral bioavailability, or potential allergic reactions. Nonetheless, such bioactivity provides strong indication of possible therapeutic potential. The presence of phenols and other phytochemical compounds in some chewing sticks may mediate an adverse effect on some users. Similar concerns have been highlighted in previous oral phytomedicine research.[14]

Take-Home (Conclusion): *Sorindeia warneckei, Garcinia kola*, and *Vernonia amygdalina* demonstrated significant antimicrobial activity against *S. oralis*, *Lactobacillus spp.,* and *S. mutans.* Methanol extracts were consistently superior to aqueous extracts. The efficacy of these chewing sticks was comparable to, and in some cases exceeded, that of branded toothpastes. This validates their traditional use and supports their potential incorporation into modern oral healthcare.

Funding: The authors received no funding for this study.

Conflicts of interest: The authors declare no conflicts of interest.

REFERENCES

1. Benabderrahmane A, Atmani M, Rhioui W, Boutagayout A, Belmalha S. Comparative ethnopharmacological survey: Medicinal plants and remedies for oral health in Meknes, Morocco, and their limits facing modern dentistry. J Pharmacy Pharmacognosy Res (2024):12(4):759–785
2. Chaiwaree S, Srilai K, Kheawfu K, Thammasit P Antibacterial activities of oral care products containing natural plant extracts from the thai highlands against *Staphylococcus aureus*: evaluation and satisfaction studies. Processes(2023) : 11(9)
3. Antoniadou M, Rozos G, Vaiou N, Zaralis K, Ersanli C, Alexopoulos A, Tzora A, Varzakas T, Voidarou C The In Vitro Assessment of Antibacterial and Antioxidant Efficacy in *Rosa damascena* and *Hypericum perforatum* Extracts against Pathogenic Strains in the Interplay of

Dental Caries, Oral Health, and Food Microbiota. Microorganisms(2024) : 12(1).

4. Begné MG, Yslas N, Reyes E, Quiroz V, Santana J, Jimenez G. *Clinical effect of a mexican sanguinaria extract (Polygonum aviculare L.) on gingivitis. J Ethnopharmacol (2001): 74(1):45–51*
5. Hollist NO. A Collection of Traditional Yoruba Oral and Dental Medicaments. Ibadan: Spectrum Books; 2004. ISBN: 978-2015-99-7.
6. Farogh G, Monika R, Mohini SR, Manisha S, Neha S, Anushree V, et al. In vitro study to investigate the antimicrobial efficacy of different toothpastes and mouth rinses. Res J Pharm Biol Chem Sci. 2014;5(2):245–57.
7. Daniyan SY, Abalaka. Antimicrobial activity in a secondary health care institution, Nigeria. Shiraz E-Med J. 2012;12(3).
8. Kleinberg I. A mixed-bacteria ecological approach to understanding the role of the oral bacteria in dental caries causation: an alternative to Streptococcus mutans and the specific plaque hypothesis. Crit Rev Oral Biol Med. 2002;13:108–25.
9. Antwi-Boasiako C, Abubakari AA. Antimicrobial and phytochemical properties of crude extracts of Garcinia kola stems used for oral health. Res J Pharmacol. 2011;5(5):68–76.
10. *Fahim MFM. Effect of prepared herbal mouthwash in maintaining the oral health of school children: a single-blind randomized control trial. EXPLORE (2024) 20(4):535–543*
11. Alkan E, Tağtekin D, Korkmaz N, Yanıkoğlu F (2023) Assessment of the remineralization effect of hemp-containing toothpaste. Euro J Res Dentistry 7(3):147–153
12. Ogundipe OO, Moody JO, Houghton PJ, Odelola HA. Bioactive chemical constituents from Alchornea laxiflora (Benth) Pax & Hoffman. J Ethnopharmacol. 2001;74(3):275–80.
13. Osho A, Adelani OA. The antimicrobial effect of some selected Nigerian chewing sticks on clinical isolates of Candida species. J Microbiol Res. 2012;2(1):1–5.
14. Ugoji E, Egwari LO, Obisesan B. Antibacterial activities of aqueous extracts of ten African chewing sticks on oral pathogens. Nig J Intern Med. 2000;3(1):7–11.