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# Evaluation of the Antimicrobial Effects of Common Oral Hygiene Products and Chewing Sticks on Selected Oral Pathogens

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**Abstract: Oral hygiene is critical for preventing dental caries and periodontal diseases, primarily caused by *Streptococcus mutans* and *Lactobacillus acidophilus*. Traditional chewing sticks and commercial oral hygiene products are widely used, yet comparative data on their antimicrobial efficacy are limited. This study evaluated the antibacterial activity of chewing stick extracts and commonly used dentifrice formulations against key oral pathogens and assessed their phytochemical composition. Methanolic extracts of *Zanthoxylum zanthoxyloides* and *Massularia acuminata* stems were prepared using Soxhlet extraction. Phytochemical screening was carried out using standard procedures. The antibacterial activity of extracts, toothpaste samples, mouthwash, dental powder, wooden coal, and table salt was tested against *S. mutans* and *L. acidophilus* using agar diffusion assays at different concentrations (500 mg/ml, 250 mg/ml, 125 mg/ml and 62.5 mg/ml). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined via microdilution. Extraction yields were 15.27% (*Z. zanthoxyloides*) and 16.27% (*M. acuminata*). Phytochemical evaluation indicated that neither reducing sugars nor carbohydrates were detected in the extracts. However, saponins, flavonoids, and tannins were present in both plant samples. In addition, cardiac glycosides were detected exclusively in *M. acuminata*. Toothpaste samples B, C and mouthwash showed the highest antibacterial activity with the lowest MIC and MBC (250 mg/mL) values against *S. mutans* while toothpaste sample B, *Z. zanthoxyloides*, *M. acuminata* and dental power demonstrated lower MICs (125 mg/mL each) with varying MBCs against *L. acidophilus* Chewing stick extracts displayed moderate antibacterial effects, while mouthwash and dental powder were effective at moderate concentrations. Traditional agents, including table salt and wooden charcoal, showed limited inhibitory activity and no bactericidal effect. Both chewing stick extracts and selected commercial oral hygiene products exhibit significant antibacterial activity against cariogenic bacteria. Toothpaste formulations and dental powders demonstrated superior efficacy at lower concentrations, whereas chewing sticks validate their traditional use. The findings support integrating plant-derived extracts with conventional oral hygiene products to enhance antimicrobial effectiveness and promote accessible oral health interventions.**

***Keywords:*** *Chewing Sticks, Oral Pathogens, Antibacterial, Dentifrice Formulations.*

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## I. INTRODUCTION

Poor oral hygiene significantly increases the likelihood of developing dental caries and periodontal diseases, conditions largely associated with *Streptococcus mutans* (WHO, 2022; 2025). Periodontal disease, commonly referred to as gum disease, is an inflammatory condition that initially affects the gums (gingivitis) and may advance to involve the supporting bone and connective tissues of the teeth, a stage known as periodontitis (Fang *et al*., 2024; Muzurel *et al*., 2025). Numerous bacterial species have been implicated in the development of periodontal diseases, including *Aggregatibacter actinomycetemcomitans*, *Bacteroides forsythus*, *Staphylococcus intermedius*, *Lactobacillus acidophilus*, *Porphyromonas gingivalis*, *Prevotella nigrescens*, and *Treponema denticola* (Spatafora *et al*., 2024; Fu *et al*., 2025). Collectively, these microorganisms play a critical role in the onset and progression of gingival inflammation and the subsequent destruction of periodontal tissues (Sharma *et al*., 2020; de Lima *et al*., 2021).

Maintaining optimal oral hygiene is fundamental to overall health and relies heavily on effective mechanical cleaning of the teeth (Ogbe *et al*., 2022). Good oral hygiene

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practices help limit dental plaque buildup, lower the risk of dental caries and periodontal diseases, and promote fresh breath and general well-being (Kalesinkas and Kacerguis, 2014; Joseph and Moses-Otutu, 2016). Tooth brushing with toothpaste is widely accepted as a routine daily practice and is often passed down through generations as a key component of oral health maintenance (Gberikon *et al*., 2015). Nevertheless, oral cleansing practices differ markedly across regions worldwide (Adesei-Mensah *et al*., 2011; Salzer *et al*., 2016). Although the toothbrush-and-toothpaste method is the most commonly recommended modern approach, several traditional methods that predate contemporary oral-care products are still widely practiced (Masadeha *et al*., 2013). These traditional techniques are particularly common in areas where access to commercial oral-care products is limited or where cultural beliefs strongly influence health behaviours (Shahoumi *et al*., 2023).

In such settings, oral hygiene may be maintained using chewing sticks or by manually applying natural substances such as plant bark, powdered herbs, dental powders, ash, charcoal, mouthwash, oils, or table salt directly onto the teeth and gums (Jauhari *et al*., 2017). The non-universal adoption of toothpaste and toothbrush use raises important questions regarding the comparative effectiveness of these alternative methods, many of which have not been sufficiently evaluated through scientific research (Ogbe *et al*., 2022).

Research has shown that sodium chloride exhibits antimicrobial activity against several oral bacteria linked to dental caries and periodontal diseases. Salt solutions have been reported to suppress cariogenic organisms, including *Streptococcus mutans* and *Lactobacillus acidophilus* (Sälzer *et al*., 2016; Jauhari *et al*., 2017). Additionally, saltwater rinses have been associated with reduced counts of periodontal pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (Jauhari *et al*., 2017; Figuero *et al*., 2025).

Dental powders represent a low-cost oral-care option that can be easily formulated locally (Gberikon *et al*., 2016). These products are intended to fulfill multiple functions, including tooth cleaning, plaque and calculus removal, polishing of tooth surfaces, prevention of tooth decay, reduction of periodontal diseases, control of oral malodour, and breath freshening (Vohra *et al*., 2012). Because no single ingredient can effectively perform all these roles, tooth powders typically contain a blend of components such as abrasives, detergents or surfactants, sweeteners, flavoring agents, and colorants (Hannig *et al*., 2013; Simiyu *et al*., 2016). Herbal tooth powders, in particular, incorporate various crude plant materials to enhance oral cleanliness and promote dental health (Janan *et al*., 2015). Due to their fine particulate nature, the physicochemical properties of both herbal and conventional tooth powders are strongly influenced by particle size and micromeritic characteristics (Ogbe *et al*., 2022). For efficient cleansing, the powder must adequately disperse or suspend in oral fluids to generate foam, a process largely dependent on the interactions among its constituent ingredients (Mas-deha *et al*., 2013; Simiyu *et al*., 2016).

In Nigeria, especially in rural communities, there has been a gradual decline in the use of conventional fluoride-based toothpastes and mouthwashes, alongside an increasing reliance on traditional oral hygiene methods such as chewing sticks (Odelaye et al., 2016). Chewing sticks contain a variety of bioactive compounds, including volatile oils, tannins, sulfur-containing compounds, and sterols, which exhibit antiseptic, astringent, and bactericidal effects (Adusei-Mensah *et al*., 2011). These properties contribute to plaque control, prevention of dental caries, elimination of oral malodour, improvement of taste perception, and the management of certain systemic conditions (Itemire *et al*., 2013). Their continued popularity, particularly among Muslims, is influenced by religious and cultural values, while their widespread use in many developing countries is also attributed to affordability, accessibility, simplicity, and strong traditional acceptance (Oviasogie *et al*., 2015). Historically, chewing sticks were used for dental care by ancient civilizations, including the Arabs, Babylonians, Greeks, and Romans (Adebayo *et al*., 2012).

Numerous plant species have been identified for their beneficial effects on oral health, such as *Vernonia amygdalina*, *Terminalia glaucescens*, *Sorindeia warneckei*, *Vitex doniana*, *Fagara zanthoxyloides*, *Zanthoxylum zanthoxyloides*, *Massularia acuminata*, *Pseudocedrela kotschyi*, *Anogeissus leiocarpus*, *Azadirachta indica*, and *Anogeissus schimperi* (Olugbuyiro Afolayan, 2012; Visentin *et al*., 2023). Pharmacological studies have further confirmed the strong antibacterial activity of these plants against oral pathogens, thereby validating their long-standing use as natural agents for maintaining oral hygiene (Yapi *et al*., 2017).

Consequently, the present study was designed to evaluate the antimicrobial effectiveness of commonly used oral hygiene products and traditional chewing sticks against key oral pathogens, specifically *Streptococcus mutans* and *Lactobacillus acidophilus*, in order to provide scientific evidence supporting the efficacy of these oral hygiene practices.

## II. MATERIALS AND METHODS

- *Source of Materials*

Dental powder, table salt, wooden coal, and chewing sticks prepared from *Zanthoxylum zanthoxyloides* and *Massularia acuminata* were obtained from Watt Market, Calabar, Cross River State. Sticks of *Z. zanthoxyloides* and *M. acuminata*, obtained from stem cuttings, were subsequently identified and authenticated at the Department of Plant Science and Biotechnology, University of Cross River State, Calabar. Reference strains of odontopathogenic bacteria — *Streptococcus mutans* ATCC 35179 and *Lactobacillus acidophilus* ATCC 412TM — were sourced from VSR International Limited, Lagos, Nigeria. The strains were supplied in lyophilized Kwik-Stick form and stored under refrigeration until use, in accordance with the manufacturer's instructions.

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➢ *Preparation and Extraction Plant Material*

The chewing sticks were cut into smaller pieces of varying sizes and oven-dried at 50 °C in a well-ventilated oven, following the method described by Chidi *et al*. (2022). The dried materials were then ground into fine powder using a British milling machine (Gallanhap). Each powdered sample was properly labeled and stored separately in sterile, dry, screw-capped bottles, which were kept in a cool, dry environment.

Extraction was carried out using a Soxhlet apparatus with 99.9% methanol as the solvent. In line with the procedure of Mustapha *et al*. (2015), 100 g of each powdered chewing stick sample was extracted separately using 0.9 L of absolute methanol. Each sample was placed in a porous cellulose thimble, which was positioned in the extraction chamber above a flask containing the methanol and fitted with a condenser. The solvent was heated to evaporate, condensed back into liquid form, and allowed to percolate through the sample repeatedly. The extraction process lasted approximately four hours.

At the completion of extraction, the solvent–extract mixtures were removed and concentrated to a paste-like consistency by heating at 98 °C in crucibles placed on a water bath. The concentrated extracts were transferred into well-labeled universal bottles and stored under refrigeration until further use. Phytochemical screening of the stem-cutting extracts was carried out according to the method described by Khalid *et al*. (2018).

The percentage yield of the fractions obtained from *J. tanjorensis* was determined using the formula below:

Percentage (%) recovery of fraction = (Wa / Wb) × 100

Where Wa represents the weight of the recovered extract, and Wb denotes the weight of the plant material initially used for extraction.

➢ *Microbial-Free and Sterility Testing of Crude Extracts of Chewing Sticks*

The sterility of the fractions was initially assessed by observing turbidity using the Millipore membrane filtration method. In this procedure, 2 mL of each fraction was aseptically introduced into 10 mL of sterile Mueller–Hinton broth and incubated at 37 °C for 24 hours. After incubation, the broth was examined for turbidity. The absence of visible turbidity, indicated by a clear broth, was interpreted as evidence that the fraction was free from microbial contamination (Sule & Agbabiaka, 2008).

To further confirm sterility and eliminate the possibility of residual microbial contamination, the fraction was reconstituted in absolute ethanol. Test concentrations suitable for bioassay evaluation were subsequently prepared using sterile deionized distilled water and sterilized by filtration through a 0.5 µm Millipore membrane filter. Thereafter, 1 mL of each prepared concentration was aseptically plated onto nutrient agar and incubated at 30 °C for 24 hours. The absence of microbial growth following incubation confirmed the sterility of the fraction (Ashish *et al*., 2016).

➢ *Preparation of Different Concentrations of Chewing Stick Extracts, Dental Powders, Wooden Coal, Table Salt, Mouthwash and Toothpaste Solutions*

To prepare the chewing stick extract solutions, 5 g of the extract was dissolved in 10 mL of 70% dimethyl sulfoxide (DMSO) to obtain a stock concentration of 500 mg/mL. This stock solution was then serially diluted to produce additional concentrations of 250, 125, and 62.5 mg/mL.

Similarly, 5 g of each dental powder sample was mixed with 10 mL of 70% dimethyl sulfoxide (DMSO) to yield a stock solution of 500 mg/mL. The resulting solutions were subsequently diluted to obtain concentrations of 250, 125, and 62.5 mg/mL.

For the toothpaste preparations, 5 g of each toothpaste sample was suspended in 10 mL of sterile distilled water to achieve a concentration of 500 mg/mL. Serial dilutions of this stock solution were then carried out to obtain final concentrations of 250.00, 125.00, and 62.5 mg/mL. The same procedure was repeated for table salt, wooden coal and mouthwash.

➢ *Preparation of McFarland Sstandard*

A 0.5 McFarland turbidity standard was prepared by combining 0.05 mL of barium chloride dihydrate with 9.95 mL of 1% sulfuric acid (Cockerill and Franklin, 2012). The resulting suspension was gently mixed to ensure uniform turbidity and was used as a reference standard. Bacterial suspensions prepared in sterile saline were visually compared against the McFarland standard, and their turbidity was adjusted accordingly to achieve the desired bacterial concentration.

➢ *Preparation of Toothpastes, Dental Powder, Table Salt, Wooden Coal, Mouthwash and Chewing Sticks Samples for Antibacterial Assay*

Samples each of toothpastes (A to D), dental powders, table salt, mouthwash, wooden coal, and selected chewing sticks were evaluated for their antibacterial activity against *Streptococcus mutans* and *Lactobacillus acidophilus*. Serial two-fold dilutions of each test material were prepared in four sterile cryogenic tubes with a capacity of 1.8 mL. Sterile distilled water was used as the diluent and dispensed into all sets of tubes using a calibrated micropipette.

➢ *Antibacterial Activity of Toothpastes, Dental Powder, Wooden Coal, Table Salt, Mouthwash and Chewing Sticks*

The antibacterial activity of the toothpastes, wooden coal, mouthwash, dental powders, table salt, and chewing stick extracts was evaluated using the Kirby–Bauer disc diffusion technique (Bauer *et al*., 1966). Sterile filter paper discs measuring 6 mm in diameter were prepared using a two-hole office puncher. The discs were wrapped in aluminum foil and sterilized in an autoclave at 121 °C for 25 minutes under a pressure of 15 psi.

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Using sterile forceps, the discs were aseptically immersed in the various concentrations of the toothpastes, wooden coal, table salt, dental powders, mouthwash, and chewing stick extracts. The discs were allowed to soak for 3 hours to ensure adequate absorption of the test substances. Thereafter, the impregnated discs were carefully transferred onto Mueller–Hinton agar plates that had been previously inoculated with standardized bacterial suspensions equivalent to $10^8$ cells, as adjusted using the McFarland standard.

The plates were incubated at 37 °C for 18–24 hours. After incubation, the diameters of the zones of inhibition surrounding each disc were measured in millimetres using a meter rule. To minimize parallax error and improve measurement accuracy, multiple readings were taken for each sample and the average value was recorded.

- *Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration*

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts, wooden coal, table salt, mouthwash, dental powder, and toothpastes were determined using the method described by Vinothkumar *et al*. (2010), with slight modifications. The lowest concentration that produced a visible zone of inhibition in the diffusion assay was selected and subjected to two-fold serial dilutions in Muella-Hinton Broth (Oxoid).

An equal volume of standardized bacterial suspension, adjusted to approximately $10^8$ cells using the McFarland standard, was added to each tube. The inoculated broths were incubated at 37 °C for 24 hours. Control tubes containing only the bacterial suspension without test samples were prepared simultaneously.

Following incubation, a loopful from each MIC tube was streaked onto Mueller–Hinton agar plates and incubated for an additional 24 hours at 37 °C. The MIC was defined as the lowest concentration that showed no visible turbidity in the broth, while the MBC was determined as the lowest concentration that showed no observable bacterial growth on the agar plates after incubation.

- *Statistical Analysis*

Data obtained from the study were analyzed using IBM® SPSS® Statistics version 21. One-way analysis of variance (ANOVA) was employed to compare the mean values of the outcome variables across different groups. Post hoc tests were conducted to identify specific group differences. A significance level of $P \leq 0.05$ was used to determine statistical significance.

## III. RESULTS

- *Percentage (%) Yield of Methanolic Extracts of Chewing Sticks*

The percentage yield of the plant materials obtained after Soxhlet extraction is presented in Table 1. Methanolic extraction of the chewing stick samples produced varying yields. The stem extract of *Zanthoxylum zanthoxyloides* (ZZ) recorded a percentage yield of 15.27%. Similarly, extraction of the *Massularia acuminata* (MA) stem resulted in a yield of 16.27%, representing the quantity of extract recovered from the round-bottom flask following the extraction process.

- *Microbial Purity of the Plant Extracts and Other Materials*

Table 2 presents the microbial purity assessment of the crude chewing sticks extracts and other oral hygiene materials used in this study. Following incubation on agar plates for 72 hours, no microbial growth was observed, indicating the absence of culturable bacteria after three days of incubation.

- *Qualitative Phytochemical Screening of Z. zanthoxyloides and M. acuminata Stem Extracts*

Table 3 summarizes the qualitative phytochemical composition of the stem extracts of *Z. zanthoxyloides* and *M. acuminata*. Neither reducing sugars nor carbohydrates were detected in the extracts. However, saponins, flavonoids, and tannins were present in both plant samples. In addition, cardiac glycosides were detected exclusively in *M. acuminata*.

- *Antibacterial Efficacy of Chewing Sticks Extracts and Other Oral Hygiene Materials Against S. mutans and L. acidophilus*

Table 4 presents the antibacterial activity of the dentifrice formulations against *Streptococcus mutans*. Among the toothpastes samples, sample B demonstrated the highest inhibitory effect, producing a zone of inhibition of 18.00 ± 0.10 mm at 500 mg/mL concentration. At the lowest concentration (62.50 mg/mL), the same sample showed a reduced inhibition zone of 8.00 ± 0.00 mm. In contrast, toothpaste sample D exhibited the weakest activity against the isolate, with an inhibition zone of 14.00 ± 0.00 mm at 500 mg/mL concentration.

The antibacterial activity of the dentifrice formulations against *Lactobacillus acidophilus* is also summarized in Table 4. Similarly, toothpaste sample B recorded the highest zone of inhibition (21.00 ± 0.87 mm) at 500 mg/mL concentration, while at 62.50 mg/mL concentration, the inhibition zone decreased to 8.00 ± 0.83 mm. Toothpaste sample D again showed the least antibacterial effect, with an inhibition diameter of 8.00 ± 0.00 mm.

Statistical analysis revealed no significant difference ($P > 0.05$) between the antibacterial activities of toothpaste samples C and D against *L. acidophilus*. However, sample B exhibited significantly greater activity compared to samples C and D ($P < 0.05$). Toothpaste sample A also showed a significant antibacterial effect when compared with samples C and D ($P < 0.05$), but its activity was not significantly different from that of sample B.

- *Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Chewing Stick Extracts and Other Oral Hygiene Formulations Against S. mutans*

Table 5 summarizes the MIC and MBC of the chewing stick extracts and other test materials evaluated against *S. mutans*. Based on the microdilution assay, toothpaste samples

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B and C showed MIC and MBC values of 1:1, equivalent to a concentration of 250 mg/mL. The plant extracts exhibited higher MIC and MBC values of 500 mg/mL. In comparison, the mouthwash and dental powder demonstrated antibacterial activity at lower concentrations, with MIC values of 250 mg/mL, whereas wooden coal and table salt showed higher MIC values of 500 mg/mL. Notably, no MBC was observed for wooden coal and table salt.

- *Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Chewing Stick Extracts and Other Oral Hygiene Materials Against L. acidophilus*

Table 6 presents the MIC and MBC of the chewing sticks extracts and other test materials against *L. acidophilus.* Toothpaste C showed the strongest antibacterial activity, with the lowest MIC and MBC values (50 mg/mL each), meaning it inhibits and kills bacteria at lower concentrations. Toothpaste B also showed good activity, with an MIC of 125 mg/mL and MBC of 250 mg/mL. Toothpastes A and D only showed inhibitory activity (MIC) but no bactericidal activity (MBC not observed). *Z. zanthoxyloides* showed moderate activity (MIC 125 mg/mL, MBC 500 mg/mL), meaning higher concentrations are needed to completely kill bacteria. *M. acuminata* was slightly more effective, with MIC 125 mg/mL and MBC 250 mg/mL. Mouthwash and dental powder showed antibacterial activity at moderate concentrations (MIC 250 mg/mL for mouthwash and 125 mg/mL for dental powder) and bactericidal activity for dental powder (250 mg/mL), indicating they are effective against oral pathogens. Table salt and wooden charcoal only showed inhibitory activity at higher concentrations (MIC 500 mg/mL) with no bactericidal effect, suggesting limited antibacterial potential.

Table 1 Percentage (%) Yield of Methanolic Stem Extracts of Plants

| Plant Extract | Before Extraction (g) | After Extraction (g) | Percentage Yield (%) |
|---|---|---|---|
| *Z. zanthoxyloides* | 107.76 | 16.46 | 15.27 |
| *M. acuminata* | 121.97 | 19.84 | 16.27 |

G = Gram, % = Percentage

Table 2 Microbial Purity of the Plants Extracts and Other Materials

| | Presence of Colonies (+)/Incubation Period (Hours) | | |
|---|---|---|---|
| **Extracts** | **1** | **2** | **3** |
| *zanthoxyloides* | - | - | - |
| *M. acuminata* | - | - | - |
| Mouthwash | - | - | - |
| Table Salt | - | - | - |
| Wooden coal | - | - | - |
| Dental powder | - | - | - |

+ = Presence of Colonies, - = Absence of Colonies

Table 3 Qualitative Phytochemical Screening of *Z. zanthoxyloides* and *M. acuminata* Stem Extracts

| | Plant Extracts | |
|---|---|---|
| **Phytochemicals** | *Zanthoxylum zanthoxyloides* | *Massularia acuminata* |
| Alkaloid | + | + |
| Carbohydrate | - | - |
| Reducing sugar | - | - |
| Saponins | + | + |
| Tannin | + | + |
| Flavonoid | + | + |
| Cardiac glycosides | - | + |

+ = Detected, - = Not Detected

Table 4 Antibacterial Efficacy of Plants' Extracts and Other Materials Against *S. mutans* and *L. acidophilus*

| Materials/test organisms | Zone of inhibition (mm) ± S. D Concentration in percentage (mg/mL) | | | |
|---|---|---|---|---|
| | **500.00** | **250.00** | **125.00** | **62.50** |
| *Streptococcus mutans* | | | | |
| Toothpaste A | 14.00±0.87[a] | 10.00±0.59[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| Toothpaste B | 18.00±0.10[a] | 14.00±0.35a | 12.00±0.49[a] | 8.00±0.00[a] |
| Toothpaste C | 16.00±0.15[a] | 12.00±0.05[a] | 18.00±0.00[a] | 0.00±0.00[a] |
| Toothpaste D | 14.00±0.00[a] | 8.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| Mouthwash | 26.00±0.20[a] | 18.00±0.00[a] | 12.00±0.00[a] | 8.00±0.00[a] |
| Table salt | 10.00±0.10[b] | 8.00±0.15[b] | 0.00±0.00[b] | 0.00±0.00[b] |
| Wooden coal | 10.00±0.15[b] | 8.00±0.00[b] | 0.00±0.00[b] | 0.00±0.00[b] |

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| | | | | |
|---|---|---|---|---|
| Dental powder | 16.00±0.30 [ab] | 12.00±0.65 [ab] | 8.00±0.00 [ab] | 0.00±0.00 [ab] |
| *Zanthoxylum zanthoxyloides* | 14.00±0.20 [a] | 8.00±0.00 [a] | 0.00±0.00 [a] | 0.00±0.00 [a] |
| *Massularia acuminata* *Lactobacillus acidophilus* | 16.00±0.30 [a] | 10.00±0.65 [a] | 8.00±0.00 [a] | 0.00±0.00 [a] |
| Toothpaste A | 12.00±0.10 [a] | 10.00±0.35 [a] | 8.00±0.49 [a] | 0.00±0.00 [a] |
| Toothpaste B | 21.00±0.87[a] | 16.00±0.59 [a] | 12.00±0.71 [a] | 8.00±0.83 [a] |
| Toothpaste C | 12.00±0.15 [b] | 8.00±0.05 [b] | 0.00±0.00 [b] | 0.00±0.00 [b] |
| Toothpaste D | 8.00±0.00 [b] | 0.00±0.00 [b] | 0.00±0.00 [b] | 0.00±0.00 [b] |
| Mouthwash | 24.00±0.95 [a] | 14.00±0.15 [a] | 10.00±0.00 [a] | 0.00±0.00 [a] |
| Table salt | 10.00±0.00 [a] | 8.00±0.00 [a] | 0.00±0.00 [a] | 0.00±0.00 [a] |
| Wooden coal | 12.00±0.00 [a] | 8.00±0.00 [a] | 0.00±0.00 [a] | 0.00±0.00 [a] |
| Dental powder | 16.00±0.35 [a] | 10.00±0.13 [a] | 8.00±0.00 [a] | 0.00±0.00 [a] |
| *Zanthoxylum zanthoxyloides* | 12.00±0.55 [a] | 8.00±0.10 [a] | 0.00±0.00 [a] | 0.00±0.00 [a] |
| *Massularia acuminata* | 14.00±0.25 [a] | 10.00±0.20 [a] | 0.00±0.00 [a] | 0.00±0.00 [a] |

Values are mean ±SE of triplicates, A-D = are different toothpaste samples, similar alphabets (a & b) along each column indicate no significant difference.

Table 5 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Chewing Stick Extracts and Other Hygiene Materials Against *S. mutans*

| Sample code | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|
| Toothpaste A | - | - |
| Toothpaste B | 250 | 250 |
| Toothpaste C | 250 | 250 |
| Toothpaste D | - | - |
| *Z. zanthoxyloides* | 500 | 500 |
| *acuminata* | 500 | 500 |
| Mouthwash | 250 | 250 |
| Table salt | 500 | - |
| Wooden coal | 500 | - |
| Dental powder | 250 | 500 |

Mg/mL - Microgram Per Milliliter, - Indicates No Bacteriostatic/Bactericidal Activity Recorded

Table 6 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Toothpastes, Chewing Sticks Extracts and Other Materials Against *L. acidophilus*

| Sample code | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|
| Toothpaste A | 250 | - |
| Toothpaste B | 125 | 250 |
| Toothpaste C | 250 | 250 |
| Toothpaste D | 250 | - |
| *Z. zanthoxyloides* | 125 | 500 |
| *acuminata* | 125 | 250 |
| Mouthwash | 250 | - |
| Table salt | 500 | - |
| Wooden coal | 500 | - |
| Dental powder | 125 | 250 |

Mg/mL - Microgram Per Millimeter, - Indicates No Bactericidal Activity Recorded

## IV. DISCUSSION

The percentage yield of extracts obtained through Soxhlet extraction provides a foundational insight into how much material is recoverable from the raw plant sample after exhaustive solvent extraction. In this study, the methanolic extraction of chewing sticks samples yielded 15.27% from *Zanthoxylum zanthoxyloides* (ZZ) stem and 16.27% from *Massularia acuminata* (MA) stem, indicating moderate efficiency of methanol in retrieving plant constituents under the conditions used. These yield values are consistent with typical outcomes seen in phytochemical extraction studies, where methanol often produces moderate to high yields due to its polarity and ability to dissolve a broad range of secondary metabolites (Tambunan *et al.*, 2017). The efficiency of extraction is influenced by factors such as solvent choice, plant matrix characteristics, particle size, temperature, and duration of extraction, all of which determine the mass transfer of compounds from the plant material into the solvent phase. In essence, a higher extraction yield generally reflects a more effective interaction between solvent and plant components, resulting in more extractable

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compounds recovered as crude material after evaporation of the solvent (Dhanani *et al*., 2017).

Comparatively, other studies have demonstrated variation in yield depending on plant species and extraction conditions. For example, research on *Zanthoxylum schinifolium* reported that stems yielded relatively low percentages (about 2.3–4.4%) with methanol extraction, whereas leaves could produce higher yields depending on solvent strength and extraction parameters used in different studies (Mohammed, 2024). Though direct comparisons are limited by differences in species and methodologies, these trends highlight that the anatomical part of the plant and its biochemical composition critically influence the amount of material that can be solubilized during extraction (Banwo and Calixo, 2022; Mohammed, 2024).

The yields observed in this study (15–16%) fall within a reasonable range for Soxhlet methanolic extraction of plant stems, given that many medicinal plant extractions reported in the literature frequently show yield ranges from low single digits up to the low 20% range when methanol is used as the extraction solvent (e.g., some leaf or stem extractions yielding ~11–23% depending on solvent and processing conditions) (Getahun *et al*., 2019; Otu *et al*., 2025). These variations reflect not only differences in plant chemistry but also methodological differences such as solvent polarity and extraction duration.

Generally, the moderate yields obtained suggest that both *Z. zanthoxyloides* and *M. acuminata* stems contain appreciable quantities of compounds that are soluble in methanol. This outcome supports their potential for further phytochemical and bioactivity investigations, as a meaningful proportion of plant constituents was successfully recovered using conventional Soxhlet extraction (Chidi *et al*., 2022; Riyadi *et al*., 2023).

Verifying the microbial purity of crude extracts before conducting antibacterial assays is an essential preparatory step in antimicrobial research. In this study, no microbial growth was observed on agar plates after 72 hours of incubation, demonstrating that the crude chewing sticks extracts and other materials were free from culturable bacterial contamination prior to testing. This outcome confirms that the extraction and handling procedures maintained sample sterility, which is critical for ensuring that subsequent antibacterial activity results are attributable to the extracts themselves rather than to external contaminants (Oyetayo, 2007; Ahiabor *et al*., 2024).

Establishing the sterility of plant extracts before bioassays prevents confounding results. If extracts were contaminated with environmental microorganisms, those microbes could interfere with or mask the activity of the test organisms, making it difficult to attribute observed effects accurately. For example, sterile control plates are recommended in antimicrobial studies to confirm that growth inhibition is due to the test extract and not to pre-existing contaminants present in the sample or introduced during handling (Alharbi, 2024). This practice ensures the reliability of antibacterial screening methods such as agar diffusion, broth microdilution, or other susceptibility assays, which are widely used in phytochemical research to assess antimicrobial properties (Kneifel *et al*., 2002).

The antibacterial screening of dentifrice formulations against *Streptococcus mutans* revealed notable differences in efficacy among the toothpaste samples tested. Toothpaste sample B exhibited the strongest inhibitory effect, with a zone of inhibition measuring 18.00 ± 0.10 mm at 500 mg/mL. At the lowest concentration tested (62.50 mg/mL), the same formulation still produced measurable inhibition (8.00 ± 0.00 mm), highlighting its dose-dependent antibacterial activity. In contrast, toothpaste sample D showed the weakest activity against *S. mutans*, with an inhibition zone of 14.00 ± 0.00 mm at the highest concentration, suggesting comparatively lower potency.

The high inhibitory effect of toothpaste sample B aligns with findings from other studies that have demonstrated variation in antimicrobial efficacy across commercial toothpaste brands and formulations. Antibacterial activity in dentifrices is often linked to the presence and concentration of active agents such as fluoride compounds, triclosan, herbal extracts, essential oils, or other antimicrobial additives that target cariogenic bacteria like *S. mutans* (Oluwapelumi *et al*., 2021). Formulations with enhanced antimicrobial components tend to produce larger zones of inhibition in agar diffusion assays, particularly at higher concentrations (Ahiabor *et al*., 2024).

Dose-dependent responses, such as the progressive reduction in inhibition zones observed with decreasing concentration of toothpaste B, are commonly reported in susceptibility assays. This trend reflects the principle that higher concentrations of antimicrobial agents can more effectively penetrate bacterial cell walls, disrupt metabolic processes, and inhibit growth. Similar patterns have been described in studies where dentifrices exhibited concentration-dependent inhibition against *S. mutans* and other oral pathogens (Oluwapelumi *et al*., 2021; Chidi *et al*., 2022).

The comparatively weaker inhibition exerted by toothpaste sample D may be attributable to differences in formulation, including lower levels of antimicrobial compounds or absence of synergistic herbal components. Previous research has documented that toothpaste formulations lacking strong antibacterial agents or containing only mild abrasives may show limited activity against cariogenic bacteria i*n vitro* (Adeleye *et al*., 2021; Chidi *et al*., 2022). For example, studies comparing herbal and conventional toothpastes often find that certain herbal components (e.g., neem, miswak, clove) enhance antimicrobial effects, while formulations with basic abrasive bases alone show reduced inhibition zones (Oviasogie *et al*., 2015).

The antibacterial screening against *Lactobacillus acidophilus* revealed a pattern similar to that observed with *Streptococcus mutans*. Toothpaste sample B demonstrated

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the highest zone of inhibition (21.00 ± 0.87 mm) at the highest concentration (500 mg/mL), while the inhibitory effect decreased to 8.00 ± 0.83 mm at 62.50 mg/mL. Sample D again showed the least antibacterial effect, with an inhibition zone of 8.00 ± 0.00 mm at its tested concentration.

Statistical analysis further clarified these differences: there was no significant difference between the antibacterial activities of toothpaste samples C and D against *L. acidophilus* ($P > 0.05$), suggesting similar and limited efficacy between those two formulations. However, sample B showed significantly greater activity compared to both C and D ($P < 0.05$), and toothpaste A also exhibited significantly stronger activity than samples C and D ($P < 0.05$), though not significantly different from sample B. These distinctions highlight meaningful variability in antibacterial potency among the toothpaste formulations against *L. acidophilus.*

These findings are consistent with broader observations in the literature that dentifrice formulations vary in their antibacterial efficacy depending on their composition. The enhanced activity of toothpaste B and A against *L. acidophilus* may be attributed to higher concentrations or more effective combinations of antimicrobial agents — such as fluoride, triclosan, essential oils, or plant-based antibacterials — which are known to disrupt bacterial cell membranes or inhibit metabolic processes in cariogenic organisms (Montero *et al*., 2017; Screenivasan *et al*., 2020). Conversely, toothpaste formulations lacking such potent active ingredients often show reduced zones of inhibition in agar diffusion assays.

The lack of significant difference between samples C and D suggests that those formulations may contain similar antimicrobial profiles or lower concentrations of active agents, resulting in limited inhibitory effects against *L. acidophilus*. This pattern highlights the importance of formulation design in achieving optimal antibacterial outcomes in oral care products.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) data provide deeper insight into the inhibitory and killing potential of chewing sticks extracts and other materials against *S. mutans*. In this study, toothpaste samples B and C exhibited MIC and MBC values of 250 mg/mL, indicating that at this concentration, both formulations effectively prevented bacterial growth and exerted bactericidal effects.

In contrast, plant extracts demonstrated higher MIC and MBC values (500 mg/mL), suggesting that greater concentrations are required to inhibit and kill *S. mutans*. Although these values are higher than those of some toothpaste formulations, they still indicate measurable antibacterial activity. Other studies on chewing sticks extracts have reported similar trends, where crude plant extracts require higher concentrations compared to well-formulated commercial toothpastes to achieve bacteriostatic and bactericidal effects (Umar *et al*., 2020). The presence of bioactive phytochemicals such as flavonoids, tannins, and saponins — which were detected in the extracts used in this study — may partly explain these antibacterial properties, though they may act less potently on their own than optimized constituents in toothpaste formulations (Chidi *et al*., 2022).

Interestingly, mouthwash and dental powder showed activity at MIC values of 250 mg/mL, comparable to toothpastes B and C, indicating that these products contain antimicrobial agents capable of inhibiting *S. mutans* at moderate concentrations. Mouthwash formulations often include antiseptic agents such as chlorhexidine, essential oils, or alcohol-based compounds that disrupt bacterial cell walls and biofilms, which can explain their relatively strong activity (van Strydonck *et al*., 2012). Dental powders, depending on their composition, may also include abrasive and antimicrobial ingredients that contribute to their inhibitory effects.

In contrast, wooden coal and table salt showed higher MIC values of 500 mg/mL and no observable MBC, indicating that at the tested concentrations these materials inhibited bacterial growth but did not demonstrate bactericidal activity. This absence of an MBC suggests that while these traditional oral hygiene agents may exert some inhibitory effect on bacterial proliferation, they are not potent enough under the conditions tested to kill *S. mutans* and *L. acidophilus*. Literature examining traditional substances like table salt and wooden coal often reports modest inhibitory effects with limited bactericidal potential, supportive of their role as mechanical or adjunctive cleaners rather than primary antimicrobial agents in oral hygiene (Schillinger and Lucke, 1989; de Lima *et a*l., 2020).

Overall, the MIC/MBC profile from this study highlights the superior antibacterial efficiency of certain toothpaste formulations and formulated mouthwash products, while also supporting the potential role of chewing sticks extracts as natural antibacterial agents, albeit at higher concentrations. The limited activity of wooden coal and table salt further underscores the need for caution when relying on traditional agents alone for managing cariogenic bacteria.

The findings from this study underscore the importance of formulation chemistry in determining dentifrice antibacterial activity. Effective inhibition of *S. mutans* is particularly relevant to oral health, as this bacterium plays a central role in dental plaque formation and caries development. The observed inhibition by toothpaste sample B suggests its potential usefulness in reducing cariogenic bacterial load when used as part of daily oral hygiene, while the relatively lower activity of toothpaste sample D may warrant further review of its composition or inclusion of additional active ingredients.

## V. CONCLUSION

This study demonstrates that both traditional chewing sticks extracts (*Zanthoxylum zanthoxyloides* and *Massularia acuminata*) and commercial oral hygiene products exhibit significant antibacterial activity against key oral pathogens, *Streptococcus mutans* and *Lactobacillus acidophilus*. Methanolic extraction of plant stems yielded moderate

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amounts of crude extracts, which contained bioactive phytochemicals such as saponins, flavonoids, tannins, and cardiac glycosides. These compounds likely contributed to the observed antibacterial effects. Among the dentifrice formulations tested, toothpaste samples B and C showed the highest antibacterial efficacy at lower concentrations, while mouthwash and dental powder also demonstrated notable activity. Traditional agents such as table salt and wooden coal displayed limited antimicrobial potency, requiring higher concentrations for inhibitory effects and showing no bactericidal activity.

The findings highlight the potential of integrating plant-derived extracts with conventional oral hygiene formulations to enhance antimicrobial effectiveness against cariogenic bacteria. The study supports the ethnomedicinal use of chewing sticks and underscores the importance of formulation and concentration in achieving optimal antibacterial activity. Further research focusing on the isolation of specific active compounds and formulation optimization could provide novel, effective, and accessible alternatives for oral health management.

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