



Received on 26 May 2025; received in revised form, 20 June 2025; accepted, 25 June 2025; published 30 June 2025

A COMPARATIVE STUDY OF THE ANTI-CARIES ACTIVITY OF TWO COMMON CHEWING STICKS USED IN NIGERIA, *ZANTHOXYLUM ZANTHOXYLOIDES* S AND *NAUCLEA LATIFOLIA*

Chike-Nwankwo Obianuju Chioma and Ejiofor Innocentmary Ifedibaluchukwu *

Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka 420110, Anambra State, Nigeria.

Keywords:

Dental Caries, *Zanthoxylum zanthoxyloides*, *Nauclea latifolia*, Powder Microscopy

Correspondence to Author:

Ejiofor Innocentmary Ifedibaluchukwu

Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka 420110, Anambra State, Nigeria.

E-mail: ii.ejiofor@unizik.edu.ng

ABSTRACT: Dental caries, a widespread infection caused by cariogenic bacteria such as *Streptococcus mutans*, is traditionally managed in Nigeria using chewing sticks like *Zanthoxylum zanthoxyloides* and *Nauclea latifolia*. This study compares the anti-caries activity of these two plants and provides a scientific basis for their traditional use. The stems of *Z. zanthoxyloides* and *N. latifolia* were collected, identified, and subjected to powder microscopy, proximate analysis, phytochemical screening, and GC-MS analysis. The antimicrobial activity of the extracts was tested against *Staphylococcus aureus*, *Streptococcus spp.*, and *Candida albicans*. Both plants exhibited significant antibacterial activity, with *Z. zanthoxyloides* showing effectiveness against *S. aureus* even at low concentrations, while *N. latifolia* was broadly effective against all tested organisms. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and steroids in both extracts, with GC-MS analysis identifying key bioactive compounds. Incorporating *Z. zanthoxyloides* into a plain toothpaste enhanced its antimicrobial activity, suggesting its potential in modern preventive dental care. These findings validate the traditional use of *Z. zanthoxyloides* and *N. latifolia* as effective oral hygiene agents, offering a cost-effective alternative for managing dental caries in Nigeria. The study confirms the presence of bioactive compounds responsible for their antimicrobial properties and supports their integration into contemporary dental care practices.

INTRODUCTION: Dental caries, commonly known as tooth decay, is one of the most prevalent chronic diseases worldwide, affecting individuals across all age groups. It is a dynamic, multifactorial disease characterized by the demineralization and destruction of dental hard tissues, primarily caused by the activity of acid-producing bacteria within dental biofilms.

These bacteria metabolize fermentable carbohydrates, especially dietary sugars, leading to acid production that gradually erodes tooth enamel and dentin¹⁻³. Traditional oral hygiene practices in Nigeria, such as chewing sticks, are widespread and passed down through generations.

Among the most commonly used chewing sticks are *Zanthoxylum zanthoxyloides* and *Nauclea latifolia*, both valued for their purported medicinal properties. *Zanthoxylum zanthoxyloides* has a long history of ethnopharmacological use for treating dental caries, toothache, and mouthwash. Scientific investigations have demonstrated that aqueous methanol extracts of its root bark exhibit significant



antimicrobial activity against key oral pathogens implicated in dental caries, such as *Streptococcus mutans* and *Lactobacillus species*, as well as notable pain-relieving effects. These findings support its traditional use as a natural remedy for oral health issues in Nigeria ⁴. This study aims to evaluate the anti-dental caries activity of *Zanthoxylum zanthoxyloides* and *Nauclea latifolia*, two widely used chewing sticks in Nigeria, to provide scientific evidence for their effectiveness and potential role in oral healthcare.

MATERIALS AND METHODS:

Collection and Identification of Plant Material:

The stems of *Zanthoxylum zanthoxyloide* and *Nauclea latifolia* were collected from Akure in Ondo State and Amawbia in Anambra State, respectively. The plants were identified and authenticated in the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. The powdered samples were deposited in the Departmental herbarium with the voucher number NAU/PCG/ZZP/024/028 and NAU/PCG/NLP/024/029 for *Zanthoxylum zanthoxyloide* and *Nauclea latifolia* respectively

Reagents and Chemicals: Analytical grades of n-hexane, ethyl acetate, methanol, and butanol (JHD, England), tyrode solution, acetylcholine, atropine, castor oil, charcoal (Kasliwal Brothers, Indore India), nutrient agar, nutrient broth, Mueller Hinton agar (Oxoid Limited, England), dimethyl sulphoxide (DMSO), Mcfarland turbidity standard (prepared from barium chloride, sulfuric acid, and distilled water, (JHD, England) were used. All laboratory reagents were freshly prepared.

Equipment: Animal weighing balance (Scout Pro Spu 401. Ohaus corporation, Pine Brook, Nj USA: Model no: 7126140063), incubator (Genlab, UK), autoclave (EQUITRON Partially Automatic Autoclave, by balance (Ohaus Corp., USA), mechanical grinder, refrigerator, water bath, rotary evaporator (Model RE 300, by Barloworld Scientific Ltd, UK), incubator (Genlab, UK), autoclave (EQUITRON partially automatic autoclave, by Medica Instrument Manufacturing Co., India), hot air oven (Genlab, UK), electronic weighing balance (Ohaus Corp., USA) and mechanical grinder.

Powder Microscopy: The powder microscopy was carried out according to the method of Amponsah *et al.*, 2017 ⁵. The coarsely powdered stems of *Zanthoxylum zanthoxyloide* and *Nauclea latifolia* were studied under the microscope. Small quantities of the various plant parts were mounted on a slide using chloral hydrate, phloroglucinol in concentrated HCl and iodine solution. Photomicrographs of the different cellular structures and inclusions were taken.

Moisture Content: The moisture content determination was done using AOAC 1990 guidelines ⁶. Petri dishes were washed, dried in the oven, cooled, and weighed. Approximately 2g of each sample was weighed into different Petri dishes. The weight of the petri dish and sample was noted before drying. The Petri dish and sample were in the oven and heated at 105°C for 2hrs. The petri dish containing the samples was weighed, and the results were noted. The samples were heated for another 1 hour, cooled, and weighed. The drying procedure was continued until constant weights were obtained. The moisture content was calculated using the formula below.

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Dry weight}}{\text{Initial weight}} \times 100$$

Crude Fiber Determination: The plant materials were powdered with a mechanical grinder to form a coarse powder, according to the AOAC guideline of 1990 ⁶. The powder was passed through sieve no. 40 and used for the extraction process. About 2g of ground material from each sample was extracted with petroleum ether to remove fat (Initial boiling temperature 35 - 38°C and final temperature 52°C). The samples were dried and boiled for 30 min in 60 % sulphuric acid, filtered through muslin, and washed thoroughly with boiling water. The samples were boiled with 200ml of sodium hydroxide solution (20 %) for 30min, filtered through muslin cloth again, and then washed with 25mL of boiling 1.25% H₂SO₄, three 50mL portions of water, and 25ml alcohol. The residues were removed and transferred to a dish. The residues were dried for 2h at 130 ±2°C, cooled in the dish in a desiccator, and weighed. The samples were ignited for 30 minutes at 600 ±15°C, cooled in a desiccator, and reweighed. The crude

fiber content was calculated using the formula below.

$$\% \text{ Crude fibre in ground sample} = \frac{\text{Loss in weight on ignition}}{\text{Weight of ground sample}} \times 100$$

Crude Fat:

Soxhlet Fat Extraction Method: This method is carried out by continuously extracting food with a non-polar organic solvent such as petroleum ether for about 1 hour or more, according to AOAC 1990 guidelines ⁶. A 500 ml clean boiling flask was washed and dried in an oven at 105 – 110°C for about 30 minutes. The flask was placed into a desiccator and allowed to cool. The flask was filled with 300 ml of petroleum ether. The Soxhlet was assembled with 200g of the sample and allowed to run for about 3 hours at 60°C. The extract was dried and concentrated in a water bath at 90°C in a pre-weighted beaker. After the drying, the sample was cooled in a desiccator and weighed.

$$\% \text{ fat} = \frac{\text{weight of beaker} + \text{dried extract} - \text{weight of beaker}}{\text{weight of the sample}} \times 100$$

Crude Proteins: This was done according to the AOAC 1990 guidelines ⁶. The method involves digesting a sample with hot, concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia, which is retained in the solution as ammonium sulphate. The solution is made alkaline and then distilled to release the ammonia. The ammonia is trapped in dilute acid and then titrated. Exactly 0.5g of sample was weighed into a 250 ml Kjehdal flask, stopped, and shaken. Then, 0.5g of the Kjehdahl catalyst mixture (potassium sulfate and copper sulfate) was added.

The mixture was heated cautiously until a clear solution was obtained. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling, distilled water was added up to 100 mL to prevent caking, and then 5ml was transferred to a distillation apparatus, followed by 50 ml of 40% sodium hydroxide. Into the distillate, the receiver added 5ml of 2 % boric acid and a mixture of Bromocresol blue (5 drops) and methylene blue (1 drop) as an indicator. After 50 drops of the distillate into the receiver flask, the solution was titrated to pink colour using 0.01N hydrochloric acid.

Calculations

$$\% \text{ Nitrogen} = \text{Titre value} \times 0.01 \times 14 \times 4$$

$$\% \text{ Protein} = \% \text{ Nitrogen} \times 6.25$$

Total Ash Value:

Total Ash: This was done according to the method of Bhargava *et al.*, 2023 ⁷. Accurately weighed 2 gm of the powdered drug was taken in a tarred silica dish, and it was incinerated at a temperature not exceeding 450°C until free from carbon. The sample was cooled and weighed. If a carbon-free ash cannot be obtained in this way, the charred mass is exhausted with hot water. The residue was collected on ash less filter paper and incinerated, and then the filtrate was evaporated to dryness and ignited at a temperature not exceeding 450°C. The percentage of ash was calculated concerning the air-dried drug.

Acid Insoluble Ash: This was done according to the method of Bhargava *et al.*, 2023 ⁷. A 0.30g of the ash obtained described as total ash was boiled for 5 min with 25 ml of dilute hydrochloric acid. The insoluble matter was collected on an ash-less filter paper washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated concerning the air-dried drug.

Water-soluble Ash: This was done according to the method of Bhargava *et al.*, 2023 ⁷. To 0.38g of the portion of the obtained total ash, 25 ml water was added and boiled for 5 minutes. The insoluble matter was collected on an ash-less filter paper, washed with hot water, and ignited in a crucible to a uniform weight. The weight of this residue was subtracted from the weight of the total ash. The content of water-soluble ash concerning dried drugs was calculated.

Carbohydrate Determination: Differential Method:

$$100 - (\% \text{ Protein} + \% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Fibre})$$

Extraction: The powdered samples of *Zanthoxylum zanthoxyloides* and *Nauclea latifolia*, each weighing 3.5 kg, were extracted with cold and hot extraction methods. For cold extraction, the samples were macerated in ethanol at room temperature for 72 hours and then filtered and concentrated in a rotary evaporator at 40°C.

Then, they were dried completely in a water bath at 50°C. The extracts were stored in a refrigerator until needed for use. For hot extraction, the plant materials were extracted using ethanol in a Soxhlet apparatus at 70 until complete extraction was achieved. The extracts were concentrated in a rotary evaporator at 40°C and then dried completely in a water bath at 50°C. The extracts were stored in a refrigerator until needed for use.

Phytochemical Analysis: The extracts were tested for the presence of various plant constituents like Alkaloids, Flavonoids, Reducing sugars, Saponins, Proteins, Tannins, Amino acids, Steroids, Triterpenoids and glycosides⁸⁻¹¹.

GCMS Analysis: The ethanol extracts of both the *Zanthoxylum zanthoxyloide* and *Nauclea latifolia* were analyzed for the presence of specific phytochemicals using GC-MS.

Test Organisms: The test organisms used in this work, which include *Staphylococcus aureus*, *Streptococcus spp.*, and *Candida albicans*, were collected from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University Awka. These organisms were further reconfirmed by subculturing and subjecting pure isolates to specific pure culture identification techniques.

Preparation of Extracts: Here, stock concentrations of each of *Nauclea latifolia* and *Zanthoxylum zanthoxyloide* extracts were made by weighing 400 and 200 mg, respectively, of each crude extract into sterile beakers. Then, 2 ml of Dimethyl sulfoxide (organic diluent) was added to each of the samples and reconstituted properly. This gives a stock concentration of 200 and 100 mg/mL of each extract, thereafter two-fold serial dilutions were made from each of the stock concentrations to get graded concentrations (100, 50, 25, 12.5 mg/mL) and (50, 25, 12.5 6.3 mg/mL) respectively of each of the crude extracts.

Preparation of Extracts and Plant Toothpaste Combination: The dried ethanol extracts of *Zanthoxylum zanthoxyloide* and *Nauclea latifolia* were individually combined with a plain, non-herbal toothpaste at a 1:1 ratio. The mixtures were solubilized in ethanol to ensure proper mixing.

The mixtures were then exposed to room temperature for complete dryness. The samples were tested against *Staphylococcus aureus*, *Streptococcus spp.*, and *Candida albicans* in comparison with herbal toothpaste.

Determination of Antimicrobial Activity: The antibacterial assay for the crude extracts was carried out using the agar well diffusion assay as described by Okezie *et al.*, 2022¹², with slight modifications. The antimicrobial activity of the extracts of the plants under study was tested against two standard human pathogenic bacterial species, namely *Staphylococcus aureus* and *Streptococcus spp.*, and one fungus, *Candida albicans*.

The bacterial and fungal suspensions were adjusted to 0.5 and 0.8 McFarland turbidity standards respectively and inoculated onto previously sterilized Mueller-Hinton Agar plates (diameter: 90 mm) while the standardized fungi cultures were inoculated onto Sabouraud Dextrose plates. A sterile cork borer was used to make five wells (8 mm in diameter) on each of the MHA and MEA plates. Aliquots of 80 µl of each extract dilution were applied in each of the wells in the culture plates previously seeded with the test organisms. Ciprofloxacin (25 µg/mL) and Fluconazole 35 µg/mL served as the positive controls against the bacterial and fungal test organisms respectively. The cultures were incubated at 37 °C and 28 °C for 24 and 48 h for the bacterial and fungal plates respectively. The antimicrobial potential for each extract was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each of the crude extract, three replicates were conducted against each organism. Each of the samples was tested against all the test isolates.

Determination of Minimum Inhibitory Concentrations (MICs) of Extracts by Broth Dilution: The method described by the European Committee for Antimicrobial Susceptibility Testing (EUCAST)¹³ was adopted with slight modifications. Here a stock concentration of 400 and 200mg/mL of each extract was made in sterile test tubes using DMSO. Then, a two-fold serial dilution of each stock was done using sterile Nutrient broth. A volume (1 ml) of each test suspension previously standardized to Mcfarland

standard was added into each tube containing the diluted extract; the tubes were capped and then incubated at 28 and 37 °C for 24 and 48 h for the bacteria and fungi, respectively. After incubation, aliquots of 20 µl/mL of each of the reaction mixtures were plated on sterile nutrient/sabouraud dextrose agar and then incubated appropriately. The minimum inhibitory concentration of each extract against the test organism was obtained by checking for visible colonies on the surface of the agar plate after incubation. Then, the plate (concentration) with no growth was taken as the MIC.

Determination of Minimum Inhibitory Concentrations (MICs) of Extracts I Combination with Toothpaste by Broth Dilution: Ethanol extracts from both plants were

mixed with plain toothpaste at a 1:1 ratio and tested for antimicrobial efficacy.

Statistical Analysis: The results from the proximate analysis and antimicrobial studies are presented as the Mean±SD of triplicate determinations, respectively.

RESULTS AND DISCUSSION:

Powder Microscopy with Chloral Hydrate: Powder microscopy of *Zanthoxylum zanthoxyloide* showed the presence of starch grains, rosette and prismatic crystals of calcium oxalate and brown resins. *Nauclea latifolia* showed the presence of starch grains, fibers, Tracheids, and vessels along with medullary rays, Brown resin and rosette, and prismatic crystals of calcium oxalate, as shown in Fig. 1.

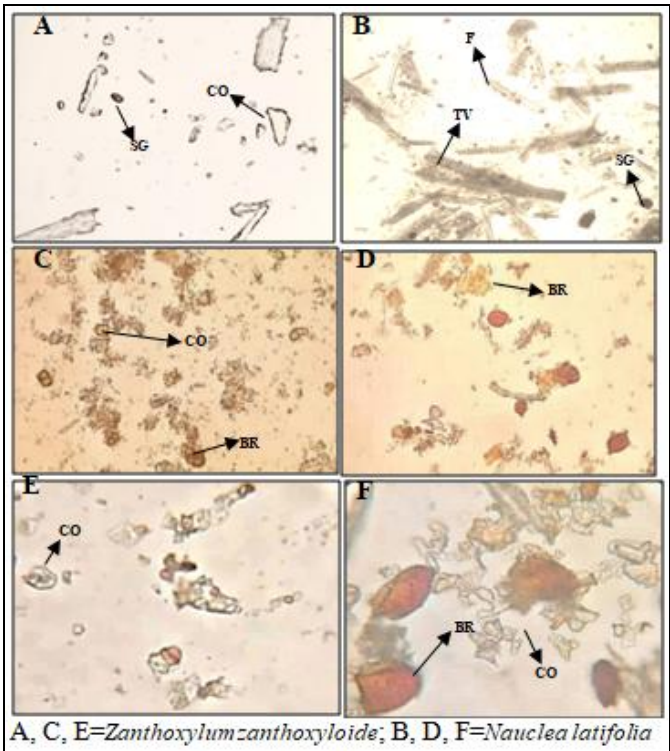


FIG. 1: POWDER MICROSCOPY SHOWING STARCH GRAINS (SG), CALCIUM OXALATE (CO), BROWN RESINS (BR), FIBERS (F), AND TRACHEIDS AND VESSELS ALONG WITH MEDULLARY RAYS (TV)

Moisture Content: The result presented in Table 1 below represents the moisture content values in percentage obtained from the moisture content

determination for the *Zanthoxylum zanthoxyloides* and *Nauclea latifolia* powder.

TABLE 1: MOISTURE CONTENT DETERMINATION FOR THE SAMPLES

Sample	Weight of sample (g)	Weight of crucible (g)	% weight of crucible + sample before drying (g)	Weight of crucible + sample after drying(g)	% moisture
<i>Zanthoxylum zanthoxyloides</i>	2.034	33.333	35.367	35.254	5.556
<i>Nauclea latifolia</i>	2.011	34.150	36.161	36.035	6.266

Moisture content is a critical parameter in the quality and stability of herbal materials and pharmaceutical products. It influences not only the efficacy of the active ingredients but also the safety and shelf life of these products. Excess moisture can lead to the degradation of active compounds, promoting microbial growth and affecting the overall stability of the product. For instance, a study revealed that 50% of herbal samples exceeded the 8% moisture limit set by regulatory bodies, which could lead to contamination by pathogenic bacteria ¹⁴. Maintaining appropriate moisture levels in herbal materials and

pharmaceuticals is vital for ensuring product quality, safety, and efficacy. The drying process must be carefully controlled to meet regulatory standards while preserving the beneficial properties of the herbs. Understanding moisture's role can help improve formulation practices and enhance the stability of herbal medicines and drugs.

Crude Fibre: The result presented in **Table 2** below represents the crude fiber values in percentage obtained from the crude fiber determination for the *Zanthoxylum zanthoxyloides* and *Nauclea latifolia* powder.

TABLE 2: CRUDE FIBER DETERMINATION FOR THE SAMPLES

Sample	Weight of sample (g)	Weight of crucible and sample before ignition (g)	Weight of crucible and sample after ignition (g)	% Crude fiber (g)
<i>Zanthoxylum zanthoxyloides</i>	2.010	29.031	29.105	3.682
<i>Nauclea latifolia</i>	2.025	27.271	27.356	4.198

In herbal medicine, crude fiber content serves as a criterion for assessing the purity and quality of herbal drugs. High levels of crude fiber can indicate the presence of woody or fibrous material that may not contribute to the therapeutic effects of the herb ¹⁵.

Crude fiber content is a vital aspect of herbal materials and drugs that influences their health benefits and quality. Understanding the levels of crude fiber in various plants can aid in selecting

appropriate herbs for medicinal use and ensuring their effectiveness in promoting health.

Crude Fat: The study results of crude fat determination presented in **Table 3** below indicate that *Zanthoxylum zanthoxyloides* contains 2.042% crude fat, while *Nauclea latifolia* has a crude fiber content of 0.645%. These findings provide insights into the nutritional and pharmacological properties of these herbal materials, which are significant in their medicinal applications.

TABLE 3: CRUDE FAT DETERMINATION FOR THE SAMPLES

Sample	Weight of sample (g)	Weight of beaker and sample (g)	Weight of beaker and dried sample(g)	% Crude Fat
<i>Zanthoxylum zanthoxyloides</i>	2.057	51.101	51.143	2.042
<i>Nauclea latifolia</i>	2.017	51.917	51.930	0.645

The crude fat content of 2.042% in *Zanthoxylum zanthoxyloides* is noteworthy, especially considering the beneficial properties associated with its fatty acid profile. Research shows that this species contains many unsaturated fatty acids, including oleic acid, linoleic acid, and linolenic acid, known for their health benefits, such as anti-inflammatory effects and cardiovascular protection ¹⁶. These essential fatty acids can enhance the therapeutic efficacy of herbal formulations derived from this plant, making it valuable in traditional medicine for various ailments. Also, the oil extracted from *Zanthoxylum zanthoxyloides* has been highlighted for its potential in sustainable conservation efforts, as the seeds can be utilized

instead of over-harvesting the roots ¹⁶. On the other hand, the crude fiber content reported for *Nauclea latifolia* is 0.645%, which is relatively low compared to other herbal materials. However, this plant is recognized for its significant nutritional value and medicinal properties. It has traditionally been used to treat conditions such as malaria, hypertension, and skin diseases ¹⁷. The low fiber content suggests that while it may not contribute significantly to dietary fiber intake, it can still provide other essential nutrients and bioactive compounds.

Crude Protein: Based on the study results presented in **Table 4**, *Zanthoxylum zanthoxyloides*

contains 5.60% crude protein, while *Nauclea latifolia* has a crude protein content of 6.65%. This indicates that *Nauclea latifolia* has a slightly higher crude protein content compared to *Zanthoxylum zanthoxyloides* in the studied samples.

TABLE 4: CRUDE PROTEIN DETERMINATION FOR THE SAMPLES

Sample	Titre value	% Nitrogen	% Protein
<i>Zanthoxylum zanthoxyloides</i>	1.6	0.896	5.60
<i>Nauclea latifolia</i>	1.9	1.064	6.65

Carbohydrate Determination: The results presented in Table 5 below indicate that *Zanthoxylum zanthoxyloides* contain 79.387% carbohydrates, while *Nauclea latifolia* has 76.564% carbohydrates.

TABLE 5: CARBOHYDRATE DETERMINATION FOR THE SAMPLES

Sample	% Carbohydrate
<i>Zanthoxylum zanthoxyloides</i>	79.387
<i>Nauclea latifolia</i>	76.564

The carbohydrate content of 79.387% in *Zanthoxylum zanthoxyloides* is notably high, suggesting that this plant may serve as a substantial source of energy. Carbohydrates are essential macronutrients that play a critical role in human nutrition, providing energy for metabolic processes.

The high carbohydrate content can be attributed to the presence of soluble and insoluble fibers, which contribute to the overall dietary fiber profile. Research has indicated that the carbohydrates

found in *Zanthoxylum zanthoxyloides* include various forms, such as simple sugars and complex polysaccharides, which can have beneficial effects on digestion and gut health. The presence of dietary fiber can aid in regulating blood sugar levels, improving satiety, and supporting overall gastrointestinal health. Additionally, the high carbohydrate content may enhance the palatability and acceptability of herbal formulations derived from this plant¹⁸.

Nauclea latifolia exhibits a carbohydrate content of 76.564%, which is also significant but slightly lower than that of *Zanthoxylum zanthoxyloides*. This moderate carbohydrate level indicates that *Nauclea latifolia* can still contribute meaningfully to dietary intake, particularly in regions where it is commonly consumed.

Total ash Determination: The results of the total ash determination are presented in Table 6 below:

TABLE 6: TOTAL ASH DETERMINATION FOR THE SAMPLES

Sample	Weight of sample (g)	Weight of crucible (g)	Weight of sample and crucible after incineration(g)	% Total ash
<i>Zanthoxylum zanthoxyloides</i>	2.036	31.737	31.813	3.733
<i>Nauclea latifolia</i>	2.008	28.254	28.368	5.677

The total ash content of 3.733% in *Zanthoxylum zanthoxyloides* indicates a relatively low level of inorganic residue. Total ash measures the mineral content in the plant material, including physiological ash (derived from the plant itself) and non-physiological ash (which may originate from environmental contaminants such as soil and sand)¹⁹.

A lower total ash value can suggest that the plant material is relatively pure and less contaminated by external substances. This can be particularly important for herbal medicines, as high ash levels may indicate adulteration or contamination, which can compromise safety and efficacy²⁰.

The mineral content in *Zanthoxylum zanthoxyloides* could also be beneficial, contributing to its therapeutic properties, as minerals play essential roles in various biological functions. In contrast, the total ash content of 5.677% in *Nauclea latifolia* is significantly higher. This elevated level may suggest a greater presence of inorganic materials, which could be attributed to either higher mineral accumulation or potential contamination during harvesting and processing¹⁹.

Acid Insoluble Ash: The results of the acid insoluble ash determination are presented in the Table 7 below:

TABLE 7: ACID INSOLUBLE ASH DETERMINATION FOR THE SAMPLES

Sample	Weight of sample	Weight of crucible	Weight of crucible and sample after drying	% Acid insoluble Ash
<i>Zanthoxylum zanthoxyloides</i>	2.066	31.685	31.698	0.629
<i>Nauclea latifolia</i>	2.016	18.096	18.099	0.149

The acid insoluble ash content of 0.629% in *Zanthoxylum zanthoxyloides* suggests a moderate level of inorganic residues that remain undissolved after treatment with dilute hydrochloric acid. This measurement is crucial as it indicates the presence of non-soluble minerals, silicates, and potential contaminants within the plant material. A higher acid-insoluble ash value can reflect the presence of impurities or adulterants, which may compromise the quality and safety of herbal preparations. In this case, the relatively moderate acid insoluble ash content may imply that *Zanthoxylum zanthoxyloides* is relatively pure, with minimal contamination from external sources such as soil or sand. This is significant for its application in herbal medicine, where purity directly correlates with efficacy and safety. Certain minerals may also enhance its therapeutic properties, contributing to its traditional uses for various ailments²⁰. Conversely, the acid-insoluble ash content in

Nauclea latifolia at 0.149% is notably low. This low value suggests a minimal presence of non-soluble inorganic materials, indicating a high degree of purity and potentially less contamination from environmental sources. Such a low level is advantageous as it implies that the herbal material is likely free from significant adulteration or impurities.

The low acid insoluble ash content can benefit formulations aimed at health benefits since it suggests that the active compounds present are not masked or compromised by unwanted inorganic materials. This characteristic supports its use in traditional medicine, where purity is paramount for effective therapeutic outcomes²¹.

Water Soluble Ash: The results of the water-soluble ash determination are presented in **Table 8** below

TABLE 8: WATER-SOLUBLE ASH DETERMINATION FOR THE SAMPLES

Sample	Weight of sample	Weight of crucible	Weight of crucible and sample after drying	% water soluble
<i>Zanthoxylum zanthoxyloides</i>	2.101	32.071	32.088	0.809
<i>Nauclea latifolia</i>	2.048	29.879	29.897	0.879

The water-soluble ash content of 0.809% in *Zanthoxylum zanthoxyloides* indicates the proportion of inorganic matter that dissolves in water. This measurement reflects the presence of water-soluble minerals such as potassium, sodium, and magnesium, which are essential for various physiological functions. A moderate level of water-soluble ash suggests that this plant contains bioavailable minerals that can contribute to its therapeutic efficacy. The relatively lower value compared to *Nauclea latifolia* may indicate differences in the mineral composition or solubility of the inorganic substances present in the plant. These minerals could play a role in the traditional uses of *Zanthoxylum zanthoxyloides*, such as its application for pain relief, antimicrobial activity, and other medicinal purposes²². In comparison, *Nauclea latifolia* exhibits a slightly higher water-soluble ash content of 0.879%, which suggests a greater presence of water-soluble minerals. This

higher value may enhance its nutritional and therapeutic potential, as these minerals are easily absorbed by the body and contribute to various biological processes. The higher water-soluble ash content could also indicate a better capacity for mineral extraction during preparation, making *Nauclea latifolia* potentially more effective in formulations aimed at providing mineral supplementation or addressing deficiencies. This characteristic aligns with its traditional use in treating conditions such as fever, malaria, and gastrointestinal disorders²².

Qualitative Phytochemical Tests: The results of the qualitative phytochemical analysis of the samples are presented in **Table 9** below. The result shows the presence (indicated with + sign) and absence (indicated with – sign) of the phytochemical classes tested for.

TABLE 9: QUALITATIVE PHYTOCHEMICAL TEST RESULT

Phytochemical class	Test	Zanthoxylum zanthoxyloides		Nauclea latifolia	
		Cold	Hot	Cold	Hot
Alkaloids	Mayer's	+	-	-	-
	Dragendorf's	+	+	+	+
	Wagner's	+	+	-	-
	Hager's	+	-	-	-
Flavonoids	Lead acetate test	+	+	+	+
	Alkaline reagent test	+	+	+	+
Reducing Sugars	Benedict's test	+	+	+	+
	Fehling's test	+	+	+	+
Saponins	Frothing test	+	+	+	+
Proteins	Millon's test	+	-	+	-
Tannins	Ferric Chloride test	+	+	+	-
Amino Acids	Ninhydrin test	+	-	-	-
Steroids	Salkowski test	+	+	+	+
Triterpenoids	Salkowski test	+	+	+	+
Glycosides	General test	+	-	+	-

The phytochemical screening indicates the presence of various bioactive compounds in both *Zanthoxylum zanthoxyloides* and *Nauclea latifolia*. The presence of alkaloids, flavonoids, tannins, and steroids suggests potential medicinal and pharmacological applications. Notably, *Zanthoxylum zanthoxyloides* exhibited a broader range of phytochemicals compared to *Nauclea latifolia*, especially in cold extraction conditions. This suggests that temperature influences the

presence of some phytochemicals. Due to the above result, further studies in this project were undertaken using cold extracts.

GC-MS Analysis: The GC-MS analysis of the extracts is presented in Fig. 2 and 3, and Table 10 and 11 showing the spectra and the phytochemicals present in the extracts of *Zanthoxylum zanthoxyloides* and *Nauclea latifolia* respectively.

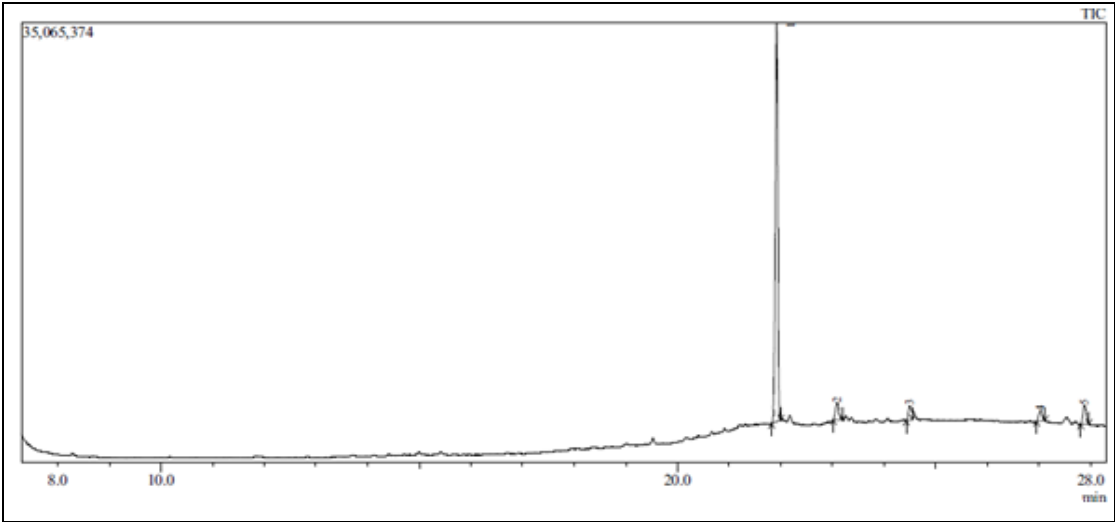


FIG. 2: GC-MS SPECTRA OF ZANTHOXYLUM ZANTHOXYLOIDES

TABLE 10: GC-MS OF ZANTHOXYLUM ZANTHOXYLOIDES EXTRACT

Peak#	R. Time	Area	Area%	Height	Height%	A/H	Name
1	21.919	107397884	83.3	31779261	86.87	3.38	Naphthalene, decahydro-1,1-dimethyl-
2	23.087	6197745	4.81	1370087	3.75	4.52	2-Propenenitrile, 3-(3,4-dimethoxyphenyl)-
3	24.495	5072116	3.93	1158325	3.17	4.38	1H-Indene, 2-butyl-4-hexyloctahydro-
4	27.026	3685468	2.86	770473	2.11	4.78	2-Hydroxy-3,4-tetramethylene-6-methoxy quin
5	27.878	6574225	5.1	1505442	4.12	4.37	5-Nonadecen-1-ol
		128927438	100	36583588	100		

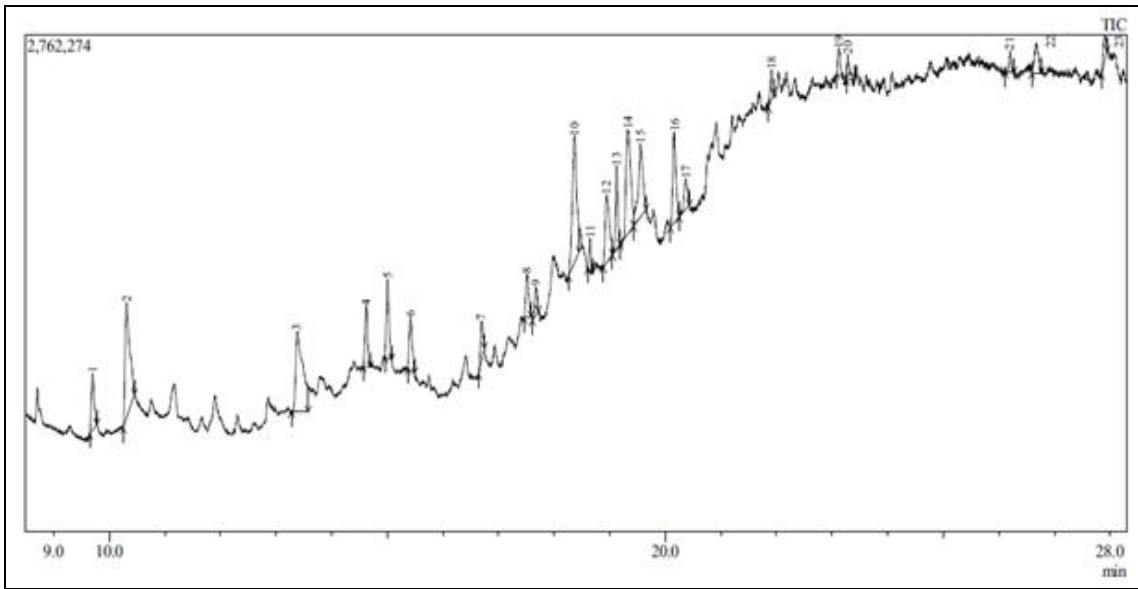


FIG. 3: GC-MS SPECTRA OF NAUCLEA LATIFOLIA

TABLE 11: GC-MS OF NAUCLEA LATIFOLIA EXTRACT

Peak#	R. Time	Area	Area%	Height	Height%	A/H	Name
1	9.702	1125385	3.33	315102	4.28	3.57	Cyclohexanone
2	10.315	3778041	11.19	637577	8.65	5.93	Glycerin
3	13.38	4063621	12.04	447747	6.08	9.08	Catechol
4	14.624	1091740	3.23	339004	4.6	3.22	2H-Pyran-2-one, 5,6-dihydro-6-pentyl-
5	15.004	1449434	4.29	439622	5.97	3.3	2-Methoxy-4-vinylphenol
6	15.418	1125799	3.34	293351	3.98	3.84	Phenol, 2,6-dimethoxy-
7	16.698	730711	2.16	219804	2.98	3.32	Phenol, 2-methoxy-4-(1-propenyl)-
8	17.518	892281	2.64	245152	3.33	3.64	2H-Pyran-2-one, tetrahydro-4-hydroxy-6-pent
9	17.675	474281	1.41	160154	2.17	2.96	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)
10	18.373	3858888	11.43	700431	9.51	5.51	(-)-Mellein
11	18.649	406697	1.2	182779	2.48	2.23	1-Hexadecanol
12	18.945	1991421	5.9	378343	5.14	5.26	n-Butyric acid 2-ethylhexyl ester
13	19.127	1362671	4.04	463467	6.29	2.94	Ethanol, 2-(dodecyloxy)-
14	19.326	3424823	10.15	580832	7.88	5.9	3-O-Methyl-d-glucose
15	19.554	2301102	6.82	407658	5.53	5.64	2H-Pyran-2-one, 5-ethylidenetetrahydro-4-(2-
16	20.164	2014644	5.97	506146	6.87	3.98	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphe
17	20.373	722845	2.14	170116	2.31	4.25	2-Propenal, 3-(4-hydroxy-3-methoxyphenyl)-
18	21.91	503481	1.49	166778	2.26	3.02	Hexadecanoic acid, ethyl ester
19	23.115	420144	1.24	144243	1.96	2.91	1-Heneicosyl formate
20	23.285	309655	0.92	136839	1.86	2.26	6-Octadecenoic acid, methyl ester, (Z)-
21	26.205	345579	1.02	122246	1.66	2.83	Octadecanoic acid, 2-hydroxy-1,3-propanediyl
22	26.678	823663	2.44	168710	2.29	4.88	Tridecanal
23	27.905	534407	1.58	141346	1.92	3.78	9-Octadecenamide, (Z)-
		33751313	100	7367447	100		

Antimicrobial Effect of *Zanthoxylum zanthoxyloides* and *Nauclea latifolia* Extracts:
 The results of the antimicrobial effect of the plain extracts of *Zanthoxylum zanthoxyloides* and *Nauclea latifolia* are presented in **Table 12** and **13**.

The extracts were also individually incorporated in a toothpaste formula and tested against the same microbes. The results obtained are presented in **Table 14** and **15**.

TABLE 12: ANTIMICROBIAL ACTIVITIES OF ZANTHOXYLUM ZANTHOXYLOIDES AGAINST THE TEST ORGANISMS

Concentration (mg/mL)	Test organisms / Inhibition Zone Diameter		
	<i>S. aureus</i>	<i>Streptococcus spp</i>	<i>C. albicans</i>
100	9.5±0.6	4±0	5±0
50	8.5±0.6	3.5±0	3±0
25	7.5±0.6	0±0	0±0
12.5	5.5±1.2	0±0	0±0
6.3	2.5±0.6	0±0	0±0
Positive control	12.5	14.5	0

Key: Ciprofloxacin 25µg (Positive control against *Streptococcus spp* and *S. aureus*); Miconazole 35µg (control against *C. albicans*); *S. aureus* (*Staphylococcus aureus*); *Streptococcus spp*, *C. albicans* (*Candida albicans*).

TABLE 13: ANTIMICROBIAL ACTIVITIES OF NAUCLEA LATIFOLIA AGAINST THE TEST ORGANISMS

Concentration (mg/mL)	Test organisms / Inhibition Zone Diameter		
	<i>S. aureus</i>	<i>Streptococcus spp</i>	<i>C. albicans</i>
200	4±0	5±0	8±0
100	2±0	4±0	7.5±0.7
50	0±0	0±0	0±0
25	0±0	0±0	0±0
12.5	0±0	0±0	0±0
Positive control	12.5	14.5	0

Key: Ciprofloxacin 25µg (Positive control against *Streptococcus spp* and *S. aureus*); Miconazole 35µg (control against *C. albicans*); *S. aureus* (*Staphylococcus aureus*); *Streptococcus spp*, *C. albicans* (*Candida albicans*).

TABLE 14: ANTIMICROBIAL ACTIVITIES OF ZANTHOXYLUM ZANTHOXYLOIDES EXTRACT WITH A PLAIN TOOTHPASTE

Concentration (mg/mL)	Test organisms / Inhibition Zone Diameter					
	<i>S. aureus</i>		<i>Streptococcus spp</i>		<i>C. albicans</i>	
	Herbal toothpaste	Sample	Herbal toothpaste	Sample		
100	4±0	9.9±0	6±0	5±0	3±0	5.5±0
50	2±0	8±0	4±0	4±0	2±0.2	4±0.2
25	2±0	7.8±0.6	3±0	2±0	1±0.1	1±0.1
12.5	0±0	6.0±1.5	1±0	2±0	0±0	0±0
6.3	0±0	3.3±0.0	0±0	0±0	0±0	0±0
Positive control	12.5		14.5		0	

TABLE 15: ANTIMICROBIAL EFFECT OF NAUCLEA LATIFOLIA EXTRACT WITH PLAIN TOOTHPASTE

Concentration (mg/mL)	Test organisms / Inhibition Zone Diameter					
	<i>Streptococcus spp</i>		<i>S. aureus</i>		<i>C. albicans</i>	
	Herbal toothpaste	Sample	Herbal toothpaste	Sample	Herbal toothpaste	Sample
200	5±0	0±0	6±0	0±0	3±0	0±0
100	4±0	0±0	4±0	0±0	2±0.2	0±0
50	2±0	0±0	3±0	0±0	1±0.1	0±0
25	2±0	0±0	1±0	0±0	0±0	0±0
12.5	0±0	0±0	0±0	0±0	0±0	0±0
Positive control	12.5		14.5		0	

Zanthoxylum zanthoxyloides extract demonstrated broad-spectrum antimicrobial activity, showing inhibition against *S. aureus*, *Streptococcus spp*, and *C. albicans*. Its effectiveness generally increased with higher concentrations. Compared to the positive controls, *Zanthoxylum zanthoxyloides* showed good activity, particularly against *S. aureus* where its highest concentration (9.5±0.6 mm) was notable against the control's 12.5 mm. *Nauclea latifolia* extract also exhibited antimicrobial

properties, though generally with smaller inhibition zones and requiring higher concentrations compared to *Zanthoxylum zanthoxyloides* for similar effects. It was effective against all three tested organisms, with the strongest inhibition against *C. albicans* at its highest concentration (8±0 mm). When incorporated into toothpaste, the *Zanthoxylum zanthoxyloides* extract maintained its antimicrobial activity, and in some cases, it appeared to have an enhanced effect compared to

the "Herbal toothpaste," which might represent the plain toothpaste without the extract or a different formulation. For instance, against *S. aureus*, the sample had a significantly larger inhibition zone (9.9 ± 0 mm at 100 mg/mL) than a commercial herbal toothpaste (4 ± 0 mm) at the same concentration. This suggests that *Zanthoxylum zanthoxyloides* could be a beneficial additive to toothpaste for antimicrobial purposes. Conversely, the *Nauclea latifolia* seemed to lose its antimicrobial efficacy almost entirely when incorporated into the toothpaste formula, as indicated by the 0 mm inhibition zones across all concentrations for all tested organisms. This is a significant finding, suggesting that the toothpaste matrix or other ingredients might interfere with the active compounds of *Nauclea latifolia* or its stability, rendering it ineffective in this formulation. The "Herbal toothpaste" (plain toothpaste) itself showed some baseline inhibition, which might be due to its inherent ingredients, but the addition of *Nauclea latifolia* extract did not enhance this effect. Previous studies have shown that *Zanthoxylum zanthoxyloides* root extracts and their toothpaste formulations demonstrated significant antibacterial activity against oral pathogens, with effectiveness comparable to or exceeding commercial toothpaste at higher concentrations. The activity was particularly notable against *Staphylococcus aureus* and *Streptococcus spp*²³⁻²⁶. *Zanthoxylum zanthoxyloides* extracts showed broad-spectrum antimicrobial activity, including inhibition of *S. aureus*, *Streptococcus spp.*, and *Candida albicans*, and maintained or enhanced this activity when incorporated into toothpaste formulations²³⁻²⁶.

Nauclea latifolia extracts exhibited antimicrobial and antioxidant activities in extract and nanoparticle cream formulations, but there is no direct evidence on whether these effects are retained when incorporated into toothpaste; some studies suggest reduced efficacy in complex formulations^{27, 28}.

CONCLUSION: This study investigated the phytochemical, antimicrobial, and physicochemical properties of *Zanthoxylum zanthoxyloides* and *Nauclea latifolia*. The results demonstrated that both plants contain significant bioactive compounds, including alkaloids, flavonoids,

tannins, saponins, and steroids, contributing to their medicinal properties. The antimicrobial analysis showed that *Zanthoxylum zanthoxyloides* exhibited more potent inhibitory activity against *Staphylococcus aureus*, *Streptococcus spp.*, and *Candida albicans* than *Nauclea latifolia*. Combining the extracts with plain toothpaste enhanced antimicrobial effects, particularly for *Zanthoxylum zanthoxyloides*.

ACKNOWLEDGEMENT: The authors acknowledge the support of Dr. Ugochukwu Okezie of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

CONFLICT OF INTEREST: The authors declare no conflict of interest

REFERENCES:

1. Pitts N, Zero D, Marsh P, Ekstrand K, Weintraub J, Ramos-Gomez F, Tagami J, Twetman S, Tsakos G and Ismail A: Dental caries. Nature Reviews Disease Primers 2017; 3.
2. He X: Research on causes, treatment, and prevention of dental caries. Theoretical and Natural Science 2024.
3. Sheiham A and James W: Diet and Dental Caries. Journal of Dental Research 2015; 94: 1341-1347.
4. Nwankwo O: Aqueous methanol extract of the root bark of *Zanthoxylum zanthoxyloides* provides a natural remedy for dental caries and toothache. World Journal of Pharmaceutical Research 2017; 6: 336-349.
5. Amponsah IK, Mensah AY, Otoo A, Mensah MLK and Jonathan J: Pharmacognostic standardisation of *Hillieria latifolia* (Lam.) H. Walt. (Phytolaccaceae). Asian Pacific Journal of Tropical Biomedicine 2014; 4(12): 941-946.
6. AOAC: Official methods of analysis of the AOAC, 15th ed. Methods 932.06, 925.09, 985.29, 923.03. Association of Official Analytical Chemists 1990; Arlington, VA, USA.
7. Bhargava VV, Saluja AK and Dholwani KK: Detection of Heavy Metal Contents and Proximate Analysis of Roots of *Anogeissus latifolia*. J Pharmacogn Phytochem 2013; 1(6): 61-65.
8. Plummer DI: An Introduction to Practical Biochemistry, 2nd Ed. Tata Magraw-Hill Publishing Co. Ltd., New Delhi 1985; 136: 143.
9. Kokate C: Practical Pharmacognosy, 4th ed. Vallabh Prakashan, New Delhi 1994; 112-120.
10. Harborne JB: Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis. 5th Ed. Chapman and Hall Ltd, London 1998; 21-72.
11. Khandelwal KR: Practical Pharmacognosy. 16th ed. Nirali Prakashan, Pune 2007; 149-156.
12. Okezie UM, Eze PM, Okoye FBC and Esimone CO: Orthosporin, a major component of the fermentation product of *Lasiodiplodia theobromae* an endophytic fungus of *Musa paradisiaca* as a potential antimicrobial agent. Notulae Scientia Biologicae 2022; 14(2).

13. EUCAST: Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. The European Committee for Antimicrobial Susceptibility Testing 2003.
14. Abba D, Inabo HI, Yakubu SE and Olonitola OS: Contamination of herbal medicinal products marketed in Kaduna metropolis with selected pathogenic bacteria. *African Journal of Traditional, Complementary, and Alternative Medicines* 2008; 6(1): 70–77.
15. Okhale SE, Omoregie H, Comfort OE and Folashade O: Phytochemical and proximate analyses and thin layer chromatography fingerprinting of the aerial part of *Chenopodium ambrosioides* Linn. (Chenopodiaceae). *Journal of Medicinal Plants Research* 2012; 6: 2289–2294.
16. Li S, Xu Y, Guo W, Chen F, Zhang C, Tan HY, Wang N and Feng Y: The impacts of herbal medicines and natural products on regulating hepatic lipid metabolism. *Frontiers in Pharmacology* 2020; 11: 351.
17. Petersen KS, Maki KC, Calder PC, Belury MA, Messina M, Kirkpatrick CF and Harris WS: Perspective on the health effects of unsaturated fatty acids and commonly consumed plant oils high in unsaturated fat. *British Journal of Nutrition* 2024; 132(8): 1039–1050.
18. Olushola-Siedoks AA, Igbo UE, Asieba G, Damola IA and Igwe CC: Elemental analysis and phytochemical characterization of *Zanthoxylum zanthoxyloides* (Lam.) Zepern. and Timler stem bark. *Journal of Pharmacognosy and Phytochemistry* 2020; 9(5): 41–46.
19. Momin RK and Kadam VB: Determination of ash values of some medicinal plants of genus *Sesbania* of the Marathwada region in Maharashtra. *Journal of Phytology* 2011; 3(12): 52–54.
20. Rao Y and Xiang B: Determination of total ash and acid-insoluble ash of Chinese herbal medicine *Prunellae spica* by near infrared spectroscopy. *Yakugaku Zasshi* 2009; 129(7): 881–886.
21. Wang H, Chen Y, Wang L, Liu Q, Yang S and Wang C: Advancing herbal medicine: Enhancing product quality and safety through robust quality control practices. *Frontiers in Pharmacology* 2023; 14: 1265178.
22. Adegbolagun OM, Ibukun OD and Aleb O: Comparative physicochemical and antioxidant activities of eight commercially available antidiabetic polyherbal products. *Nigerian Journal of Pharmaceutical Research* 2021; 17(2): 189–200.
23. Orafidiya L, Akinkunmi E, Oginni F and Oluwamakin A: Effectiveness of extracts of the root of *Zanthoxylum zanthoxyloides* (Linn.) Waterman (Rutaceae) formulated as toothpastes. *Nigerian Journal of Natural Products and Medicine* 2012; 14: 21–26.
24. Adeniyi C, Odumosu B, Aiyelaagbe O and Kolude B: *In-vitro* antimicrobial activities of methanol extracts of *Zanthoxylum xanthoxyloides* and *Pseudocedrela kotschy*. *African Journal of Biomedical Research* 2010; 13: 61–68.
25. Adebisi A, Koekemoer T, Adebisi A, Smith N, Baxter E, Naudé R and Venter M: Antimicrobial and antioxidant activities of crude extracts of two Nigerian chewing sticks. *Pharmaceutical Biology* 2009; 47: 320–327.
26. Glory M and Kevin M: The antimicrobial properties of *Terminalia glaucescens* and *Zanthoxylum zanthoxyloides* extracts and the spectrum of their activity 2015.
27. Odeniyi M, Okumah V, Adebayo-Tayo B and Odeniyi O: Green synthesis and cream formulations of silver nanoparticles of *Nauclea latifolia* (African peach) fruit extracts and evaluation of antimicrobial and antioxidant activities. *Sustainable Chemistry and Pharmacy* 2020; 15: 100197.
28. Ajayi O, Aderogba M, Akinkunmi E, Obuotor E and Majinda R: Bioactive compounds from *Nauclea latifolia* leaf extracts. *Journal of King Saud University – Science* 2020; 32: 2419–2425.

How to cite this article:

Chioma CO and Ifedibaluchukwu EI: A comparative study of the anti-caries activity of two common chewing sticks used in Nigeria, *Zanthoxylum zanthoxyloides* and *Nauclea latifolia*. *Int J Pharmacognosy* 2025; 12(6): 509-21. doi link: [http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.12\(6\).509-21](http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.12(6).509-21).

This Journal licensed under a Creative Commons Attribution-Non-commercial-Share Alike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)