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# Evaluation of the antimicrobial properties of *Syzygium aromaticum* (clove) and *Acacia nilotica* (babul) pods on cariogenic bacteria from caries lesion

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## Abstract

**Background:** Cariogenic bacteria are tooth adherent microorganisms which become cariogenic when fermentable sugars are consumed and form biofilms which produce organic acids that erodes tooth enamel to initiate caries.
**Aim:** This study aimed to evaluate the antimicrobial properties of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods (CAP) on cariogenic bacteria from caries lesion.
**Methods:** Bioactive compounds were extracted from the powdered plants using 53% ethanol. Three carious swabs were obtained from three patients who presented with Caries at Dental clinic, Federal University of Allied Health Science, Enugu State, Nigeria, and subjected to isolation and identification by 16S ribosomal RNA gene analyses.
**Results:** *Enterococcus faecalis* N.H.IRAQ1, *Bacillus subtilis* SF2012, *Staphylococcus aureus* YT-3, and *Levilactobacillus brevis* CBAK were identified. Antimicrobial evaluation conducted at 100mg/ml concentration of the extract showed *Enterococcus faecalis* susceptible with inhibition zone of 20.00 ± 1.22mm, *Bacillus subtilis* 22± 1.52mm, *Staphylococcus aureus* 22 ± 1.22mm, *Levilactobacillus brevis* 30± 1.77mm; when compared to control which showed no (0.00mm) zone of inhibition. The MIC for each of the isolates were 3.0mg/ml for *E. faecalis*, 6.0mg/ml for *B. subtilis*, 3.0mg/ml for *S. aureus*, and 1.5mg/ml for *L. brevis*. The MBC values were 6.0mg/ml for *E. faecalis*, 12.5 mg/ml for *B. subtilis*, 6.0mg/ml for *S. aureus*, and 3mg/ml for *L. brevis*. There was a significant difference among the groups ($p < 0.002$).
**Conclusion:** The findings of the study demonstrated a significant antimicrobial effect of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods extract on the isolated cariogenic bacteria, supporting its application in dental care.

Keywords: Cariogenic bacteria, Caries, Clove, *Acacia nilotica* pods, Antimicrobial properties

## Background of the Study

Microorganisms are everywhere, and the majority of them coexist with humans. The oral cavity of humans is generally believed to be sterile at birth, but shortly after birth, it becomes colonized by oral bacteria.
Cariogenic bacteria are some microorganisms found in the tooth which becomes cariogenic when fermentable sugars are consumed by them. They form biofilms that produce organic acids, demineralize the tooth enamel and mediate dental caries. The bacterial agents associated with caries are *Streptococcus* (particularly *S. mutans*), among others (at the genus level) are *Scardovia, Propionibacterium, Actinomyces*, and newly emerging *Bifidobacteria,* along with *Veillonella, Prevotella*, and *Enterococcus fecalis*.[1,2] Among these bacteria, *Streptococcus mutans* is the most significant cause of caries, but recent studies showed that caries can develop in the absence of *S. mutans*.[3,2] The etiology of caries is facilitated through the formation of a biofilm community consisting of

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these diverse bacterial populations.[4]

Dental caries is the localized destruction of a tooth's hard tissue, which includes enamel, dentin and cementum, caused by acids, resulting from microbial fermentation of carbohydrates consumed by the individual.[2] The cariogenic bacteria metabolize sugar in the diet to generate acid, demineralizing the tooth structure. After a while, the lesion spreads through the outer layer of enamel to infect the underlying dentin, in some cases the innermost pulp. If neglected, the infection can turn into suppuration that spreads to the deeper tissues of the teeth, near the roots, or to the bloodstream[5]. This causes tooth pain, infection or malfunction of the stomatognathic system and limits the necessary ingestion of energy-rich foods, affecting growth in children and adults, along with their learning, communication skills, and effective recreational activities. According to the World Health Organization's global oral health status report, approximately 3.5 billion individuals worldwide are affected by oral diseases, with caries representing a major global health concern.[6] Traditional approaches to caries prevention, such as fluoride use and oral hygiene practices, have limitations in controlling the growth and activity of cariogenic bacteria. Consequently, there has been increasing interest in finding alternative anti-cariogenic agent, especially those from botanicals.

Clove, scientifically known as *Syzygium aromaticum*, is a medium-sized tree (8-12 m) from the Myrtaceae family, native to the Maluku islands in eastern Indonesia.[7] The Clove bud is 1-2cm long and has a deep brown color, possessing a powerful fragrance that is warm, slightly astringent, and contains significant amounts of volatile oil used for flavoring foods and pharmaceuticals.[8] It is a vital source of phenolic compounds such as, flavonoids, hydroxycinnamic acids, hydroxyl benzoic acids, hydroxyl phenylpropenes and eugenol; which are recognized for their ability to deactivate cellular enzymes in harmful microorganisms, possessing high antibacterial activity against cariogenic microbes.[8,9,10] *Acacia nilotica*, also known as Babul, is a versatile nitrogen fixing tree legume from the Fabaceae family. This species thrive in tropical and subtropical regions and can be found abundantly across Asia, Australia, Africa and America. The fruits (pods) of *Acacia nilotica* are linear, narrow pods and flattened, measuring approximately 4-22cm in length and 1-2mm in diameter, containing 8-15 dark brown to gray elliptical bean-shaped seeds with a velvety texture.[11] Phytochemical analyses conducted on *Acacia nilotica* pods revealed that it contains 16 classes of bioactive molecules and elements with potential health benefits for humans. These molecules and elements includes tannins, saponins, flavonoids, glycosides, phytosterols, cyclitols, alkaloids, anticoagulant agents, regulatory molecules, amines, mucilage, fibers, gums, proteins, various classes of amino acids, carbohydrates, terpenses, crude fats, oils, fatty acids, and minerals.[12,13] In a research, it was revealed that alkaloids, one of the bioactive compounds in *Acacia nilotica* exerts antimicrobial effects by inhibiting pathogen cytokinesis.[8,9] The Tannins present in the pods (*Acacia nilotica*) are recognized for their effects against microorganisms like bacteria and viruses, and they also serve as antioxidants.[14] Both plants contain many bioactive compounds, which have been stated to exhibit antimicrobial activity against various pathogens and are traditionally used in various cultures for their therapeutic properties. This delves in the importance of combining plants, which can be more effective than a single plant. This study aimed to evaluate the antimicrobial properties of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods on cariogenic bacteria from caries lesion, with an emphasis on the effectiveness of the combined form of these natural products in suppressing the growth and biofilm formation of these bacteria, as well as their potential application in dental care.

## Problem Statement/Justification

Cariogenic bacteria is the etiology for dental caries initiation and progression; which is a common oral disease worldwide. The growing resistance of cariogenic bacteria to conventional antibiotics and its negative implications, have become a subject of increasing concern, which necessitates the search for natural anti-cariogenic agents.

## Materials and Methods

### Study Area

This research was carried out in Microbiology laboratory, Enugu State Polytechnic Iwollo, located in Ezeagu Local Government Area of Enugu state, Southeastern Nigeria. The institution is situated at approximately latitude 6.442$^{O}$N and longitude 7.284$^{O}$E (WGS-84 datum).

**Preparation of Plant materials**

*Syzygium aromaticum* (Clove) buds and *Acacia nilotica* (Babul) pods, were purchased from Abakpa Nike market, Enugu, Enugu State Nigeria. They were identified and authenticated by a taxonomist in the Department of Botany, University of Nigeria Nsukka, Enugu State, Nigeria. The plant materials were thoroughly washed and rinsed with distilled water, air-dried under shade, and ground into powder.

**Plant Extraction procedure**

Extraction was carried out using ten (10) grams of each of the plant samples, immersed in 400ml of 53% ethanol; in the ratio of 20:400(w/v).[15] The resultant mixture was placed in water bath by stirring for 60minutes at ebullition temperature. The extract was filtered using filter paper (Whatman no1). The resulting residue was concentrated under reduced pressure (30 ± 10 mbar) using a rotary evaporator operated at a temperature range of 30-60°C until a viscous, syrupy-like consistency was achieved.[16]

**Qualitative Phytochemical Screening of *Syzygium aromaticum* (Clove) buds and *Acacia nilotica* (Babul) pods**

The *Syzygium aromaticum* (Clove) buds and *Acacia nilotica* (Babul) pods extract was subjected to qualitative phytochemical tests to detect the presence of bioactive compounds, following the procedure described by Vishn *et al*.[17]

**Glycosides (Borntrager's Test):** Two milliliters of extract were mixed with chloroform and shaken. After phase separation, 10% ammonia solution was added to the chloroform layer. A pink coloration indicated the presence of glycosides.

**Alkaloids:** One milliliter of extract was treated with a few drops of Wagner's reagent. Formation of a reddish-brown precipitate indicated a positive test for alkaloids.

**Phenols (Lead Acetate Test):** Five milliliters of extract were mixed with 3 mL of 10% lead acetate. A bulky white precipitate indicated the presence of phenols.

**Tannins:** A few drops of 5% ferric chloride solution were added to 5 mL of the extract. A dark green coloration was indicative of tannins.[17]

**Flavonoids:** One milliliter of extract was treated with a few drops of 10% lead acetate. A yellow precipitate indicated flavonoid presence.

**Saponins:** Half a milligram of the extract was agitated with distilled water. Persistent frothing indicated the presence of saponins.

**Xanthoproteins:** One milliliter of the extract was treated with nitric acid and then ammonia. A reddish-brown precipitate suggested the presence of xanthoproteins.[17]

**Terpenoids:** Five milliliters of extract were mixed with chloroform and concentrated sulphuric acid. A reddish-brown interface layer indicated terpenoids.

**Triterpenoids:** Ten milligrams of extract were dissolved in chloroform, followed by the addition of concentrated sulphuric acid and acetic anhydride. A reddish-violet coloration confirmed triterpenoids.

**Anthraquinones:** Five milliliters of extract were treated with concentrated sulphuric acid and diluted ammonia. A rose pink coloration indicated the presence of anthraquinones.[17]

**Ethical Issues and Sample Collection**

Ethical clearance for this study was obtained from Enugu State University Teaching Hospital, Park Lane, Enugu State, Nigeria. Informed written consent was obtained from all participants prior to sample collection. Carious swabs were obtained from three adult patients (one male and two females) who presented with dental caries at the Dental Clinic, Federal University of Allied Health Sciences, Enugu state, Nigeria. The samples were collected from carious lesions on the occlusal and axial surfaces of infected teeth using sterile swabs.

**Culture and Identification**

Serial dilutions ($10^{-1}$ to $10^{-4}$) of the clinical samples were carried out according to the procedure stated by Sagar.[18] A loopful from the $10^{-3}$ dilution was streaked onto Brain Heart Infusion Agar (BHI; HiMedia, India) plate followed by incubation at 25–30°C for 24 hours in an inverted position. Discrete colonies were selected based on morphology and sub-cultured in BHI broth for further analysis.

**Biochemical Identification of the Isolates from caries lesion**

The Biochemical tests were conducted following the procedure described by Microbiology Information Journal,[19] with slight modification. The isolates were further screened for cariogenicity.[19]

**Molecular Characterization of the Isolates from caries lesion**

Molecular analysis were conducted following the procedure described by DNA Learning center Information,[20] with slight modification. Overnight culture of selected isolates in Luria-Bertani broth were pelleted. The cell pellets were suspended in 500 µL of 4 M guanidine hydrochloride and the DNA was eluted in 70 µL of TE buffer after 12-minute incubation and centrifuged. The 16S rRNA gene was amplified in thermocycler using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGWTCCARCCGCA-3′)[21], and conditioned as follows: Initial denaturation at 94°C for 5 minutes, followed by denaturation at 94°C for 30 seconds, annealing 56°C for 30 seconds, extension at 72°C for 45 seconds. The steps were repeated for 35 cycles and final extension at 72°C for 7 minutes.[20] The PCR sample were resolved by electrophoresis at 100 V for 35 minutes using 2% agarose gels with 1 µ/L ethidium bromide. DNA bands were visualized using a UV transilluminator (Accuris E-3000-E), and the amplicons were sequenced using the Applied Biosystems 3130xl Genetic Analyzer with the BigDye Terminator v3.1 Cycle Sequencing Kit. Genetic analysis and sequence interpretation were conducted using the DNA Subway platform, aligning sequences against bacterial 16S rRNA gene databases for identification.[20]

**Evaluation of antimicrobial properties of *Syzygium aromaticum* (Clove) buds and *Acacia nilotica* (Babul) pods (CAP) extract**

The antimicrobial efficacy of the CAP extract were examined using agar well diffusion method as reported by Balouiri *et al*[22] with slight modification. Brain Heart Infusion agar plate was seeded by the test organism. Wells of 5mm diameter were aseptically created in the agar plate using a sterile cork borer. 0.1ml of the CAP extract was pipetted into the wells and incubated at 37$^{O}$C, for 24 hours.[22] The diameter of inhibition zone were measured.

**Determination of the Minimum Inhibitory Concentration (MIC) of the *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods (CAP) extract**

MIC were determined by the agar dilution method according to CLSI guidelines (CLSI M07).[23] Mueller-Hinton agar plates containing two –fold serial dilutions of the CAP extract (range 100-0.5mg/ml) were prepared and allowed to solidify. Test organisms were prepared, adjusted to 0.5 McFarland and diluted to deliver $10^{4}$CFU per spot; inoculation was by multipoint inoculator. The plates were incubated at 37$^{O}$C for 24hours and the MIC were determined.[23]

**Determination of the Minimum Bactericidal Concentration of the *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods (CAP) extract**

The Minimum bactericidal concentration (MBC) of the CAP extract against the isolates were carried out according to Balouiri *et al*[22] with minor modification. MBC were assessed by sub-culturing bacteria suspensions from all concentrations that exhibited no visible growth. An aliquot of 100 µL from each corresponding dilution of CAP extract was aseptically inoculated onto the surface of freshly prepared brain heart infusion (BHI) agar plates (Himedia, India), followed by incubation at 37 °C for 24 hours. After incubation, the plates were examined for bacterial growth[22].

**Data Analysis**

All experiments were performed in triplicate to ensure reproducibility and reliability. Results were recorded and expressed as mean values ± standard deviation (SD). Statistical analyses were conducted using Minitab version 15 (Minitab Inc., State College, PA, USA). A one-way analysis of variance (ANOVA) was employed to evaluate differences among treatment groups, with statistical significance considered at $p < 0.05$.

**Results**

Table 1 shows the qualitative screening of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul)

Table 1: Qualitative screening of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods extract

| S/N | Bioactive Compounds | Observations |
|---|---|---|
| 1 | Phenols | + |
| 2 | Terpenoids | + |
| 3 | Triterpenoids | + |
| 4 | Xanthoproteins | + |
| 5 | Flavonoids | + |
| 6 | Tannins | + |
| 7 | Glycosides | + |
| 8 | Anthraquinones | + |
| 9 | Saponins | + |
| 10 | Alkaloids | + |

Keys: Present (+); Not present (-)

pods extract. The following bioactive compounds were found: phenols, terpenoids, triterpenoids, xanthoproteins, flavonoids, tannins, glycosides, anthraquinones, saponins, and alkaloids.

Table 2: Characterization and Biochemical tests of the cariogenic bacteria from caries lesion

| Parameters | Bacteria | | | |
|---|---|---|---|---|
| S/N | *E. faecalis* | *B. subtilis* | *S. aureus* | *L. brevis* |
| Gram reaction | + | + | + | + |
| Shape | cocci | rod/bacilli | cocci | rod/bacilli |
| Biochemical tests | | | | |
| Citrate | _ | _ | + | _ |
| Indole | _ | _ | _ | _ |
| Methyl red | + | _ | + | _ |
| Voges proskaue | _ | _ | + | _ |
| Lactose fermentation | + | + | + | + |
| $H_2S$ | + | _ | _ | _ |
| Triple sugar Iron | A/A | A/A | A/A | K/A |

Keys: Positive: +; Negative: -; Acidic/Acidic: A/A; Alkaline/Acidic: K/A; *E. faecalis*: *Enterococcus faecalis* N.H.IRAQ1; B. *subtilis*: *Bacillus subtilis* SF2012; *S. aureus*: *Staphylococcus aureus* YT-3; *L. brevis*: *Levilactobacilhis brevis* CBAK

Table 3: Antimicrobial activity of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods extract on cariogenic bacteria isolated from caries lesion

| Isolates | Diameter of inhibition zones (mm) Extract Concentrations (mg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 100% | 50% | 25% | 12.5% | 6% | 3% | 1.5% | 1% | 0.5% |
| *E. faecalis* | 20.00 | 18.00 | 16.00 | 13.00 | 12.00 | 11.00 | 8.00 | 4.33 | 0.00 |
| *B. subtilis* | 22.00 | 20.00 | 10.00 | 8.00 | 7.00 | 4.33 | 2.00 | 0.00 | 0.00 |
| *S. aureus* | 22.00 | 14.00 | 13.00 | 12.00 | 11.00 | 10.50 | 9.00 | 2.00 | 0.00 |
| *L. brevis* | 30.00 | 26.00 | 21.00 | 17.00 | 14.00 | 13.00 | 9.00 | 4.33 | 2.00 |
| *Control* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

Keys: *E. faecalis*: *Enterococcus faecalis* N.H.IRAQ1; *B. subtilis*: *Bacillus subtilis* strain SF2012; *S. aureus*: *Staphylococcus aureus* strain YT-3; *L. brevis*: *Levilactobacillus brevis* strain CBAK

Table 4: Distribution of Mean Zones of Inhibition (mm) of cariogenic bacteria from caries lesion

| Total Bacteria | Count | Mean(mm) | SEMean(mm) | StDev(mm) | Variance($mm^2$) |
|---|---|---|---|---|---|
| *E. faecalis* | 9 | 11.37 | 1.22 | 6.33 | 40.09 |
| *B. subtilis* | 9 | 8.15 | 1.52 | 7.92 | 62.67 |
| *S. aureus* | 9 | 10.39 | 1.22 | 6.36 | 40.45 |
| *L. brevis* | 9 | 15.15 | 1.77 | 9.19 | 84.43 |
| *Control* | 9 | 0.00 | 0.00 | 0.00 | 0.00 |

Keys: SE: Standard error of means, StDev: Standard Deviation.

Table 5: Minimum Inhibitory Concentration (MIC) of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods extract against cariogenic Isolates from caries lesion

| Isolates | Concentration of CAP extract used mg/ml | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.0 | 3.0 | 1.5 | 1.0 | MIC |
| *E. faecalis* | - | - | - | + | + | + | + | + | 3.0 |
| *B. subtilis* | - | - | + | + | + | + | + | + | 6.0 |
| *S. aureus* | - | - | - | + | + | + | + | + | 3.0 |
| *L. brevis* | - | - | - | + | + | + | + | + | 1.5 |

Keys: No growth (-); Presence of growth (+)

Table 6: Minimum Bactericidal Concentration (MBC) of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods extract on cariogenic bacteria from caries lesion

| Isolates | Concentration of CAP extract used mg/ml | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 100 | 50 | 25 | 12.5 | 6.0 | 3.0 | 1.5 | MBC | Statistical test/p value |
| *E. faecalis* | - | - | - | + | + | + | + | 6.0 | 3.0 |
| *B. subtilis* | - | - | + | + | + | + | + | 12.5 | 6.0 |
| *S. aureus* | - | - | - | + | + | + | + | 6.0 | 3.0 |
| *L. brevis* | - | - | - | - | + | + | + | 3.0 | 1.5 |

Keys: No growth (-); Presence of growth (+)
*ANOVA was the statistical test used.

Table 2 shows the identified (cariogenic) bacteria from caries lesion, these includes *Enterococcus faecalis* N.H.IRAQ1, *Bacillus subtilis* SF2012, Staphylococcus aureus YT-3, Levilactobacillus brevis CBAK.

Table 3 displayed the Antimicrobial activity of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods extract on cariogenic bacteria from caries lesion, compared to Control (inoculums without CAP extract) which showed no (0.00mm) zone of inhibition.

Table 4 demonstrated the Distribution of mean zones of inhibition (mm) of cariogenic bacteria from caries lesion.

Table 5 displayed the Minimum inhibitory concentration (MIC) values of cariogenic bacteria from caries lesion. The findings showed that L. brevis is highly susceptible to CAP extract, compared to other isolates.

Table 6 shows the Minimum bactericidal concentration (MBC) of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods extract on cariogenic bacteria from caries lesion. From the result, CAP extract has bactericidal effect in all the isolates at a given concentration.

**Discussion**

Medicinal plants that are active against pathogens are the potential source of novel antimicrobial agents. Numerous plant species have shown to exhibit pharmacological properties ascribed to their bioactive constituents.[7] In this study, bioactive compounds which are phenols, terpenoids, triterpenoids, xanthoproteins, flavonoids, tannins, glycosides, anthraquinones, saponins, and alkaloids were detected in *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods, extract. These

bioactive compounds have been implicated with many pharmacological properties[24] and antimicrobial effects. These results is consistent with the findings of Chandra *et al*[25] who reported similar bioactive compounds in their study.[25] The identified bacteria from caries lesion, which includes *Enterococcus faecalis* N.H.IRAQ1, *Bacillus subtilis* SF2012, *Staphylococcus aureus* YT-3, and *Levilactobacillus brevis*; are found to be cariogenic. This is consistent with the findings by Pitts, *et al*[2] which stated that *Enterococcus faecalis* is a bacteria commonly isolated from the oral cavity and is closely associated with the pathogenesis of caries.[2] Similarly, Wang and Ren[26] revealed that *Staphylococcus aureus* is recognized as a significant pathogen implicated in caries-related infections.[26] According to the cariogenic screening result in Table 2, *Bacillus subtilis*, fermented the three sugars (dextrose, sucrose, and lactose); *Levilactobacillus brevis* fermented only lactose and dextrose (glucose). These sugars/carbohydrates (glucose, sucrose, fructose and lactose) stimulates the production of organic acids,[2] and is regarded as the acid involve in dental caries biofilm synthesis. This is consistent with the findings made by Chen *et al*,[27] who revealed that organic acids stimulates biofilm formation.[27] *Bacillus subtilis* and *Levilactobacillus brevis* (though weak acid producing bacteria) are found to be aciduric and acidogenic in nature (cariogenic bacteria) with respect to the result in Table 2. This corresponds to the findings by Duanis, *et al*[28] who stated that *Bacillus subtilis* being a pathological factor, enable adherence and biofilm- forming capabilities attributed to its proteolytic activities.[28] This enzymatic action facilitates biofilm development and generates a novel binding sites that promotes the colonization of other acidogenic-bacteria, thereby enhancing their virulence.[29] In Antimicrobial evaluation of *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods (CAP) extract, *Levilactobacillus brevis* was found to be the most sensitive to CAP extract at the concentration of 100mg/ml with the mean inhibition zone of 30mm followed by *Bacillus subtilis* 22mm and *Staphylococcus aureus* 22mm. *Enterococcus faecalis* was found to be less sensitive to CAP extract with an inhibition zone diameter of 20mm. This result contradicts the findings by Auwal *et al*[30] who analyzed *Acacia nilotica* pods at 1000mg/ml (a higher concentration) on *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zone diameter of 16mm and 25mm.[30] On a related note, Elmubarak *et al*[31] reported at a concentration of 200mg/ml on the same plant (*Acacia nilotica* pods) extract on *Enterococus faecalis* with inhibition zone of 15mm.[31] In light of these, *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods has high antimicrobial effects on the isolated cariogenic bacteria from caries lesion and have impacts at a lower concentration. The MIC values in Table 5 showed that *L. brevis* (1.5mg/ml) has high inhibitory effect compared to *E. faecalis* (3.0mg/ml), *S. aureus* (3.0mg/ml), and *B. subtilis* (6.0mg/ml). This contradicts the findings by Auwal *et al*,[30] who reported high MIC value of *Enterococcus faecalis* (12.5mg/ml) and *Staphylococcus aureus* (1000mg/ml) on *Acacia nilotica* pods extract.[30] Similarly, Agu and Tasie[32] on *Syzygium aromaticum* (clove) extract reported such value (high MIC) on *Bacillus subtilis* (12mg/ml) and *Staphylococcus aureus* (25mg/ml). This showed that *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul) pods extract exerted maximum inhibitory effect on *Enterococcus faecalis* and *Staphylococcus aureus*; when compared to each plant effect. The Minimum bactericidal concentration (MBC) values at Table 6, ascertained that all the four isolates from caries lesion has a bactericidal effect at a given concentration of the CAP extract. This is consistent with the findings by Auwal *et al*,[30] although with a slight variation. In accordance with the study, *Bacillus subtilis* (225mg/ml) and *Staphylococcus aureus* (200mg/ml) has bactericidal effect at a higher concentration of *Acacia nilotica* pods extract,[30] possibly due to single effect. Similarly, Agu and Tasie[32] in their findings reported same on *Bacillus subtilis* 25mg/ml, *Staphylococcus aureus* 50mg/ml, and *lacticaseibacillus agile* 25mg/ml, on *Syzygium aromaticum* (clove) extract.[32] The subtle difference in MBC values were attributed to the complementary effect of CAP extract which resulted an improved outcome, when compared to each plant effect. This highlights the significance of combining plants, whose favorable interactions produce extra antimicrobial advantage. Thus, there was a significant difference among the groups, $p = 0.002$.

## Conclusion

Cariogenic bacteria is the etiology of dental caries initiation and progression; a prevalent infectious disease affecting people of all ages. *Syzygium aromaticum* (Clove) and *Acacia nilotica* (Babul)

pods extract despite being valued for its nutritional benefits, has been discovered from this study to possess adequate quantity of bioactive constituents that suppressed the proliferation of cariogenic bacteria from caries lesion. These findings reinforced the potential of CAP extract as an effective natural antimicrobial (anti- cariogenic) agent and supporting its application in dental care.

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Macro image of Carious infected teeth

*Source: Vital guide to preventing dental caries. https://www.nature.com/articles/vital840*