The Antibacterial Activity of Aqueous Extract of *Syzygium Aromaticum* on Selected Pathogenic Bacteria Causing Dental Caries

Bashir Ismail Olawale [1], Hauwa Tahir [2], Irhekpono Grace Itohan [1], Sanusi Magaji [2], Oluwabunmi Olaitan Agarry [1]

[1] *The University of Abuja*
Main Campus Airport Road, Abuja, FCT, Nigeria

[2] *Abubakar Tatari Ali Polytechnic*
P. M. B. 0094, Bauchi, 740272, Nigeria

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Corresponding Author:
Bashir Ismail Olawale

Abstract. Oral health encompasses the condition of the mouth, teeth, and related facial structures, enabling vital activities such as eating, breathing, and speaking. It also includes psychological and social factors such as self-esteem, overall well-being, and the ability to interact and work without experiencing pain, discomfort, or embarrassment. The objective of this study was to evaluate the antibacterial effects of an aqueous extract of *Syzygium aromaticum* (clove) on certain bacteria known to cause dental caries. Among the tested bacteria, *Lactobacillus casei* showed the greatest sensitivity to the ethanolic clove extract, with inhibition zones ranging from 10 to 20 mm. The aqueous extract, however, showed lower inhibition, ranging from 8 to 14 mm. For *Streptococcus mutans*, the aqueous extract showed higher antibacterial activity, with inhibition zones of 4-8 mm. The minimum inhibitory concentration (MIC) of both aqueous and ethanolic extracts against *Lactobacillus casei* was 125 mg/mL. In the case of *Streptococcus mutans*, the aqueous extract exhibited a MIC of 62.5 mg/mL, whereas the ethanolic extract had a MIC of 250 mg/mL.

Regarding minimum bactericidal concentration (MBC), the aqueous and ethanolic extracts required concentrations of 250 mg/mL and 500 mg/mL, respectively, to kill *Lactobacillus casei*. The ethanolic extract also had an MBC of 500 mg/mL against *Streptococcus mutans*. These results suggest that extracts from *Syzygium aromaticum* possess significant antibacterial properties, particularly when extracted with water, emphasising their promise as alternative antibacterial agents for oral health treatment.

Keywords: Dental caries; Antibacterial; *Syzygium aromaticum;* Orofacial; Cloves.

INTRODUCTION

The condition of the mouth, teeth, and orofacial tissues that enables people to carry out daily tasks such as eating, breathing, and speaking is known as oral health. It also includes psychosocial functions like self-worth, well-being, and the capacity to interact with others and work without experiencing pain, discomfort, or embarrassment. From early childhood to old age, oral health changes throughout a person's life, contributes to overall health, and helps people reach their full potential and participate in society [1]. Dental caries, periodontal disease (gum disease), tooth loss, oral cancer, oro-dental trauma, and congenital deformities, including cleft lip and palate, are all considered oral disorders.

An estimated 3.5 billion individuals worldwide suffer from oral disorders, making them one of the most prevalent noncommunicable diseases. The overall burden of oral health disorders on services will continue to expand as a result of population expansion and ageing, even if the prevalence of these conditions is rising globally, especially in low- and middle-income nations [1]. The poorest groups are disproportionately affected by oral illnesses. Regardless of the nation's

income level, the burden of oral disease is higher among those with lower socioeconomic position, and this link persists throughout life, from childhood to old age [2].

Maintaining a healthy oral cavity is crucial to promoting overall well-being, as it serves as the body's primary entry point. Oral health impacts overall health, not just the ability to speak, eat, and socialise. Researchers have linked poor oral health to various chronic diseases, including heart disease, diabetes, and respiratory infections. The extensive effects of the oral cavity stem particularly from the size and diversity of the oral microbiome, with outsized effects on other parts of the body, such as the gut and respiratory system [2].

Dental caries is an infection caused by two sets of bacteria found in the mouth: Streptococcus mutans and Lactobacilli spp. predominantly colonise dental plaque. Dental caries causes inflammation of the dental pulp, periodontium, and gums. Dental caries, if left untreated, gradually leads to tooth loss, resulting in difficulty with mastication and aesthetic concerns [3]. Dental caries remains one of the most prevalent diseases of humankind, occurring quite often in epidemic numbers in developing countries, especially among people with low incomes.

Natural products such as chewing sticks and plant extracts have been used in oral hygiene and the treatment of dental caries due to their antibacterial activity and positive effects on the oral microbiota. *Syzygium aromaticum*, in the family Myrtaceae, has been used by humans therapeutically for over 2,020 years, chewed to ease toothache and widely used as an oral disinfectant, a root canal temporary filling, and an oral anaesthetic. It is a bioactive natural product with a broad array of antimicrobial activities against Gram-positive, Gram-negative, and acid-fast bacteria and fungi [4].

The increasing scourge of antibiotic resistance among oral pathogens has highlighted the need for new, nature-based antimicrobial compounds. Because of their complex composition and diverse modes of action, natural products such as clove extracts offer a viable alternative, reducing the risk of resistance development. Furthermore, the World Health Organisation (WHO) has acknowledged the value of conventional medicine and has promoted research to examine its benefits and uses [1].

The global prevalence of oral diseases such as dental caries, gingivitis, and periodontal disease, and the role of the oral microbiome in causing these conditions, underscores the need for effective and accessible oral hygiene methods. The socioeconomic situation in Nigeria makes orthodox dental care products unaffordable for many, which explains the persistence of chewing sticks in dental care and the need to investigate their health benefits.

This *study aims* to address the knowledge gap by evaluating the antimicrobial activity of different chewing sticks against microorganisms isolated from the oral cavity. This research will identify the type of chewing stick that is most effective at inhibiting or eradicating harmful oral bacteria. Understanding the properties and antimicrobial activity of chewing sticks will provide a scientific basis for their importance in managing oral health, especially in the resource-constrained environment we currently experience.

METHODS

Study Area. Gwagwalada, one of the five Local Government Area Councils in Nigeria's Federal Capital Territory (FCT), served as the study area. It lies between latitudes 8°25′N and 7°45′E and covers approximately 1,069.589 km^2. The area experiences a semi-seasonal equatorial climate with distinct dry and rainy seasons [5]. With daytime highs of 28 to 30 degrees Celsius and nighttime lows of 22 to 23 degrees Celsius, the rainy season normally begins in April and lasts until October. On the other hand, afternoon lows of 12°C are possible during the dry season. Annual rainfall averages 1,400 mm, with the rainy season from March to November [6].

Preparation and Sterilisation of Media. Nutrient agar and Mueller-Hinton agar were prepared following the manufacturers' protocols. All glassware used for media preparation and culture work was sterilised at 160 °C for 2 hours in a hot-air oven, as per standard microbiological practices [7].

Isolation and Subculturing of Streptococcus mutans and Lactobacillus casei. Samples for bacterial isolation were obtained by swabbing the gingival surface with sterile cotton swabs. These swabs were then streaked onto nutrient agar plates and incubated at 37°C for 24 hours [8]. In a separate method, 0.2 g of fermented ogi was mixed with 10 ml of distilled water and diluted in six test

tubes, each containing 9 ml of distilled water. The researcher evenly plated 0.2 mL from the 10^{-6} dilution onto nutrient agar. After incubation, colonies that appeared similar to S. mutans were isolated and subcultured for further analysis [9].

Processing of Syzygium aromaticum (Clove). Clove samples (*Syzygium aromaticum*) were sourced from Gwagwalada market and dried at room temperature for 2 days. The researchers pulverised the dried material using a sterilised grinder and stored it in airtight containers to maintain its stability [10].

Extraction of Bioactive Compounds. The researchers divided 50 g of clove powder into two equal parts for aqueous and ethanol extraction. They mixed the aqueous portion with 250 mL of sterile distilled water and the ethanol portion with 250 mL of ethanol. Both mixtures were allowed to extract for 2–3 days at room temperature, with occasional stirring [11].

Determination of Antimicrobial Activity. The researchers prepared serial dilutions of both extracts using the doubling dilution technique to obtain concentrations of 500, 250, 125, and 62.5 mg/mL. They prepared separate aqueous and ethanol extracts. They suspended colonies of *S. mutans* and *L. casei* in 1 mL of distilled water. They then used the agar well diffusion method by punching wells into Mueller-Hinton and nutrient agar plates and adding different concentrations of the extracts into the wells. The plates were then incubated at 37 °C for 24 hours, after which antimicrobial activity was assessed by measuring the zones of inhibition [12, 13].

Minimum Inhibitory Concentration (MIC) Determination. The researchers added 2 mL of nutrient broth to 16 test tubes, with 8 tubes for each extract type, to confirm the MIC. They used the doubling dilution technique to test each concentration against the bacterial suspensions. They introduced a measured amount of the standardised inoculum and incubated the tubes at 37 °C for 24 hours. They determined the MIC as the lowest extract concentration that prevented visible bacterial growth [14].

Minimum Bactericidal Concentration (MBC) Determination. The contents from the MIC test tubes that showed no visible bacterial growth were transferred onto nutrient agar plates using the spread plate technique. After removing any excess liquid, the plates were incubated for 24 hours. The MBC was determined as the lowest concentration at which no bacterial colonies appeared, demonstrating bactericidal effect [12].

RESULTS AND DISCUSSION

Table 1 – Zone Diameter of Inhibition (ZDI) in millimetres of Aqueous and Ethanolic *Syzygium aromaticum* Extracts against *Lactobacillus casei* and *Streptococcus mutans*

Extracts	Concentration in mg/mL			
	62.5	125	250	500
Lactobacillus casei				
Aqueous	8	14	10	8
Ethanolic	No zone	10	14	20
Streptococcus mutans				
Aqueous	4	6	6	8
Ethanolic	No zone	4	4	6

Table 2 – Minimum Inhibitory Concentration of Aqueous and Ethanolic Extracts of *Syzygium aromaticum* against *Lactobacillus casei* and *Streptococcus mutans*

Extracts	Concentration in mg/mL			
	62.5	125	250	500
Lactobacillus casei				
Aqueous	+	MIC	-	-
Ethanolic	+	MIC	-	-
Streptococcus mutans				
Aqueous	MIC	-	-	-
Ethanolic	+	+	MIC	-

Notes: + =Present, - =Absent.

Table 3 – Minimum Bactericidal Concentration of Aqueous and Ethanolic Extracts of *Syzygium aromaticum* against *Lactobacillus casei* and *Streptococcus mutans*

Extracts	Concentration in mg/mL			
	62.5	125	250	500
Lactobacillus casei				
Aqueous	+	+	MBC	-
Ethanolic	+	+	+	MBC
Streptococcus mutans				
Aqueous	+	+	+	+
Ethanolic	+	+	+	MBC

Notes: + =Present, - =Absent.

The state of the mouth, teeth, and surrounding face structures that enable a person to perform essential functions like speaking, breathing, and

eating is referred to as oral health. Self-esteem, general well-being, and the ability to socialise and work effectively without pain, discomfort, or shame are among its psychological and social components. The purpose of the study was to evaluate the antibacterial activity of *Syzygium aromaticum* aqueous extract against certain pathogenic microorganisms that cause dental caries.

Lactobacillus casei showed the highest susceptibility to the ethanolic extract of Clove buds, with a zone of inhibition ranging from 10 to 20 mm. In comparison, the aqueous extract showed the lowest susceptibility, with a zone of inhibition ranging from 8 to 14 mm. *Streptococcus mutans* showed the highest susceptibility to the aqueous extract of Clove buds, with a zone of inhibition ranging from 4 to 8 mm. In comparison, the ethanolic extract showed the lowest susceptibility, with a zone of inhibition ranging from 4 to 6 mm. The results showed that both extracts demonstrated significant antibacterial activity, with the aqueous extracts exhibiting larger inhibition zones than the ethanolic extracts; this suggests that water is a more effective solvent for extracting bioactive compounds, likely due to its ability to solubilise a broader range of them. These findings agree with those of authors [15, 16], who reported similarly high antibacterial activity of Syzygium aromaticum buds against *S. mutans, E. coli, P. aeruginosa, S. pneumonia, S. aureus, and S. epidermidis.*

Aqueous and ethanolic extracts of *Syzygium aromaticum* buds had MICs of 125 mg/mL against *Lactobacillus casei*. In contrast, the aqueous and ethanolic extracts showed MICs of 62.5 and 250 mg/mL, respectively, against *Streptococcus mutans*. The aqueous and ethanolic extracts of *Syzygium aromaticum* buds had MBCs of 250 mg/mL and 500 mg/mL, respectively, against *Lactobacillus casei. In contrast,* the ethanolic extract of *Syzygium aromaticum* buds had an MBC of 500 mg/ml against *Streptococcus mutans*. This result corroborates those of authors [15, 17], who reported similar inhibitory and bactericidal concentrations for Syzygium aromaticum buds, highlighting the efficacy of clove extracts due to their high eugenol content, a compound known for its potent antibacterial properties. The relatively higher MBC values for aqueous extracts indicate that the active components may be less concentrated or less effective when water is used as the extraction solvent.

Overall, the findings suggest that *S. aromaticum* extracts exhibit strong antibacterial activity, particularly when water is used as the extraction medium. The observed activity supports the traditional use of clove in oral care. It highlights its potential as an alternative antibacterial agent, especially against dental pathogens such as *L. casei* and *S. mutans*. However, the disparity in MIC and MBC values suggests that further refinement of extraction and purification techniques could enhance the potency of these extracts.

CONCLUSIONS

This study demonstrates that *Syzygium aromaticum* extracts, particularly aqueous ones, exhibit significant antibacterial activity against *Lactobacillus casei* and *Streptococcus mutans*. The findings support clove's potential as a natural antimicrobial agent for managing dental caries, with eugenol likely playing a key role in its efficacy.

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Conflict of interests

The authors declared no conflicting interests.

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