Antimicrobial efficacy of *syzygium aromaticum* & *acacia nilotica* against isolated oral pathogens

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Abstract

Oral infections associated with pathogenic bacteria are commonly encountered and continue to be an area of active research in relation to antibacterial strategies. Plant-derived phytochemicals have become a topic of interest due to their reported antimicrobial activities. The present investigation focused on the isolation and identification of oral microorganisms and the assessment of plant-based phytochemicals for potential use in oral healthcare applications. Dental caries samples were collected at random from 50 pediatric patients, aged between 4 and 14 years, attending the Government Dental College and Hospital, Nagpur. The samples were cultured on Mitis Salivarius agar to isolate oral bacteria. A total of 26 different bacterial isolates were identified using morphological, cultural, and biochemical methods. The antibacterial properties of solvent extracts from selected plants were tested using the agar well diffusion technique by evaluating the inhibition zones.

Keywords: Antibacterial, Dental caries, Phytochemicals, Agar well diffusion

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I. Introduction

A significant portion of school-aged children—estimated between 60% and 90%—as well as many adults, are affected by dental caries globally. In more severe cases, periodontitis contributes to tooth loss in 5–20% of the adult population globally. Untreated dental caries is currently regarded as the most prevalent health condition, with an estimated impact on 3.5 billion individuals worldwide (Talathi et al., 2017). It is classified as a biofilm-associated disease, primarily influenced by specific cariogenic bacterial species. The progression of the disease results in irreversible enamel damage, highlighting the need for effective preventive strategies (Paray et al., 2018). Emerging research has also established associations between dental caries and systemic health conditions such as cardiovascular diseases and complications during pregnancy, reinforcing the importance of oral health maintenance (Talathi et al., 2017). The etiology of dental caries is explained by the four-factor theory, which includes the involvement of microorganisms, the oral environment, host susceptibility, and time. This model suggests that the intake of dietary sugars alters the microbial equilibrium within the oral cavity. Dental plaque—also termed microbial plaque, oral biofilm, or bacterial plaque biofilm—is a primary contributor to tooth decay and periodontal diseases.

Periodontal diseases and dental caries represent two of the most prevalent oral health conditions globally. These disorders are primarily attributed to the presence of plaque-forming bacteria and yeast within the oral environment (Paray et al., 2018). Effective management of such infections involves the reduction or elimination of microbial buildup in areas prone to retention, such as the occlusal surfaces and interproximal spaces, through consistent oral hygiene practices like daily brushing and routine professional dental cleaning or prophylaxis (Bansal et al., 2019). Antimicrobial agents, including antibiotics such as penicillin and tetracycline, have significantly contributed to improved global health outcomes (Bathija et al., 2019). In areas where access to formal dental care is limited, traditional medicine practices employing herbal preparations remain common. This study explores the antimicrobial potential of plant-based substances such as *Syzygium aromaticum* (clove) and *Acacia nilotica* (Babool), both historically recognized for their antibacterial properties. The continued use of these herbal remedies highlights the intersection of cultural traditions and accessible healthcare solutions in resource-constrained settings (Chandra Shekar et al., 2022). The current research supports the integration of traditional herbal practices with contemporary dental

healthcare strategies and underscores the need for further investigation into the effectiveness of plant-derived antimicrobial agents.

II. Methodology

1. Sample Collection

Dental caries samples were obtained from school-aged children (up to 15 years old) attending the Government Dental Hospital, Nagpur. Dental plaque was collected using a sterile spoon excavator and transferred into 2 ml of sterile saline solution. The samples were then stored at 4°C until further analysis.

2. Isolation of Oral Pathogenic Bacteria

Isolation of bacterial pathogens was performed using the spread plate technique on Mitis Salivarius Agar, a selective medium for oral microorganisms. Inoculated plates were incubated at 37°C for 24–48 hours. Bacterial colonies were selected based on morphological characteristics such as size, shape, and hemolytic activity. Identification was further confirmed through Gram staining and standard biochemical assays, including sugar fermentation tests.

3. Collection of Plant Material

Leaves of *Syzygium aromaticum* (clove) and *Acacia nilotica* (babul) were collected from the Godhan region of Nagpur. Botanical authentication of the plant specimens was carried out by the Department of Botany at a nearby college.

4. Preparation of Plant Extracts

Collected plant materials were shade-dried to avoid photodegradation and were then ground into a fine powder using a sterile mortar and pestle. Various extraction methods were employed as follows:

- **Hot Water Extract:** 10 g of powdered plant material was added to 100 ml of sterile distilled water and heated in a water bath until the volume reduced to approximately 25% (~75 ml). The extract was then filtered using Whatman No. 1 filter paper and evaporated to dryness.

- **Cold Methanolic Extract:** 100 mg of powdered material was soaked in methanol for 24 hours at room temperature. The solution was subsequently filtered through Whatman No. 1 filter paper.

- **Hot Methanolic Extract:** 20 g of dried flower powder was extracted in 250 ml of methanol using a Soxhlet apparatus over a period of 4 hours. The extract was concentrated by evaporation and stored in sterile, opaque bottles under refrigeration for future use.

5. Antibacterial Assay Using the Agar Well Diffusion Method

The antibacterial activity of the prepared plant extracts was evaluated using the agar well diffusion method. Mueller Hinton Agar (MHA) was used as the culture medium. The medium was sterilized by autoclaving at 121°C under 15 lbs pressure for 15 minutes. Once sterilized, the molten agar was allowed to cool to approximately 45°C and then poured into sterile Petri dishes (20 ml per plate) under aseptic conditions.

After solidification, a lawn of the test organisms was prepared by uniformly spreading the bacterial suspension over the surface of the agar using sterile cotton swabs. Wells of uniform diameter were created in the agar using a sterile cork borer. Each well was filled with a specific plant extract—hot water, cold methanolic, or hot methanolic extract—prepared from *Syzygium aromaticum* (clove) and *Acacia nilotica* (babul).

The plates were then incubated at 37°C for 24 hours. Following incubation, the diameter of the zone of inhibition around each well was measured using a transparent zone measuring scale. The size of the inhibition zone was recorded in millimeters and used as an indicator of the antibacterial effectiveness of the plant extracts against the test organisms.

Sample Distribution and Bacterial Isolation

A total of 100 dental plaque samples were collected from patients at the Government Dental Hospital, Nagpur. Of these, 26 bacterial isolates were successfully cultured and identified. The samples were inoculated on selective Mitis Salivarius Agar to promote the growth of oral pathogenic bacteria.

Gender-Based Distribution of Dental Caries

The collected samples were analyzed for gender-based prevalence. A pie chart representation indicated that although samples were collected equally from male and female participants, the occurrence of dental caries was higher in males (70%) compared to females (30%). This suggests a greater susceptibility to dental caries among the male population in the sampled group.

Table 1: Morphological & Cultural Characteristics of Bacterial Isolates.

ISOLATION AND IDENTIFICATION OF BACTERIAL STRAINS									
Sr. No.	**Isolates sample**	**Morphological Characteristics**	**Cultural Characteristics**						
			Shape	**Size**	**Margine**	**Elevation**	**Color**	**Texture**	**Opacity**
1	S1P4	*gram positive*	*round*	*small*	*Entire*	*Convex*	*grey*	*Slimy, mucoid & smooth*	*Translucent*
2	S2P1G	*gram positive*	*round*	*small*	*Entire*	*Convex*	*grey*	*Shiny, slim mucoid*	*Translucent*
3	S2P1B	*gram positive*	*round*	*small*	*Entire*	*Flat*	*black*	*Slimy, mucoid & smooth*	*opaque*
4	S2P4G	*Gram positive*	*round*	*medium*	*Entire*	*Convex*	*grey*	*Slimy, mucoid & smooth*	*translucent*
5	S2P4B	*Gram positive*	*round*	*small*	*Entire*	*Flat*	*black*	*Slimy, mucoid & smooth*	*opaque*
6	S2P8G	*Gram positive*	*round*	*small*	*Entire*	*Convex*	*grey*	*Slimy, mucoid & smooth*	*translucent*
7	S2P8B	*Gram positive*	*round*	*medium*	*Entire*	*Convex*	*black*	*Slimy, mucoid & smooth*	*Opaque*
8	S2P5LG	*gram positive*	*round*	*medium*	*entire*	*Convex*	*Light grey*	*Metallic Shiny, Slimy, Mucoid*	*translucent*
9	S2P5DG	*gram positive*	*round*	*medium*	*entire*	*Convex*	*Dark grey*	*Shiny, Slimy, Mucoid, Smooth*	*opaque*
10	S2P7	*gram positive*	*round*	*small*	*entire*	*Flat*	*black*	*Slimy, Mucoid, & smooth*	*Opaque*
11	S2P2GB	*gram positive*	*round*	*medium*	*entire*	*Convex*	*Greyish black*	*Slimy, mucoid, & smooth*	*opaque*

12	S2P3BB	*gram positive*	*round*	*small*	*entire*	*Flat*	*black*	*Slimy, mucoid & smooth*	*opaque*
13	S3P1GB	*gram positive*	*round*	*small*	*entire*	*Convex*	*Greyish black*	*Shiny, Slimy, mucoid & smooth*	*opaque*
14	S3P3NB	*gram positive*	*round*	*small*	*entire*	*Convex*	*Navy blue*	*Shiny, Slimy, mucoid & smooth*	*opaque*
15	S3P2NB	*gram positive*	*round*	*small*	*entire*	*Convex*	*Navy blue*	*Slimy, mucoid & smooth*	*Opaque*
16	S3P4G	*gram positive*	*round*	*small*	*entire*	*Convex*	*grey*	*Slimy, mucoid & smooth*	*translucent*
17	S3P5G	*gram positive*	*round*	*medium*	*entire*	*Convex*	*grey*	*Shiny, Slimy, mucoid & smooth*	*translucent*
18	S3P5BB	*gram positive*	*round*	*small*	*entire*	*Flat*	*black*	*Slimy, mucoid & smooth*	*opaque*
19	S3P6LG	*gram positive*	*round*	*small*	*entire*	*Convex*	*Light grey*	*Slimy, mucoid & smooth*	*translucent*
20	S4P2GB	*gram positive*	*round*	*medium*	*entire*	*Convex*	*Greyish black*	*Slimy, mucoid & smooth*	*opaque*
21	S4P4G	*gram positive*	*round*	*medium*	*entire*	*Convex*	*grey*	*Slimy, mucoid & smooth*	*opaque*
22	S4P4B	*gram positive*	*round*	*small*	*entire*	*Flat*	*black*	*Slimy, mucoid & smooth*	*opaque*
23	S4P2B	*gram positive*	*round*	*small*	*entire*	*Flat*	*black*	*Slimy, mucoid & smooth*	*Opaque*
24	S4P1B	*gram positive*	*round*	*small*	*entire*	*Flat*	*black*	*Slimy, mucoid & smooth*	*opaque*
25	S4P6G	*gram positive*	*round*	*small*	*entire*	*Convex*	*grey*	*Slimy, mucoid & smooth*	*translucent*
26	S4P5B	*gram positive*	*round*	*small*	*entire*	*Flat*	*black*	*Slimy, mucoid & smooth*	*opaque*

Table 2: Biochemical Characteristics of Bacterial Isolates.

Sr. No.	Isolate ID	Indole	MR	VP	Citrate	Catalase	D (Dextrose)	S (Sucrose)	F (Fructose)	L (Lactose)
1	S1P4	*NEG*	*POS*	*POS*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*G*
2	S2P1G	*NEG*	*POS*	*POS*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
3	S2P1B	*NEG*	*POS*	*POS*	*POS*	*NEG*	*A+G*	*A+G*	*G*	*A+G*
4	S2P4G	*NEG*	*POS*	*POS*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
5	S2P4B	*NEG*	*POS*	*POS*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*G*
6	S2P8G	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*G*
7	S2P8B	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
8	S2P5LG	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
9	S2P5DG	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*G*	*A+G*
10	S2P7	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
11	S2P2GB	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
12	S2P3BB	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
13	S3P1GB	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
14	S3P3NB	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
15	S3P2NB	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*G*	*A+G*
16	S3P4G	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*G*
17	S3P5G	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*G*
18	S3P5BB	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
19	S3P6LG	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*G*	*G*	*G*	*A+G*
20	S4P2GB	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*G*	*A+G*

21	S4P4G	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*G*
22	S4P4B	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*G*
23	S4P2B	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*
24	S4P1B	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*G*	*G*	*G*	*A+G*
25	S4P6G	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*G*	*A+G*
26	S4P5B	*NEG*	*POS*	*NEG*	*POS*	*NEG*	*A+G*	*A+G*	*A+G*	*A+G*

Biochemical Test Abbreviations

- ***Indole*** *– Indole test,* ***MR*** *– Methyl Red,* ***VP*** *– Voges-Proskauer,* ***Citrate*** *– Citrate Utilization,* ***Catalase*** *– Catalase enzyme test,* ***D*** *– Dextrose fermentation,* ***S*** *– Sucrose fermentation,* ***F*** *– Fructose fermentation,* ***L*** *– Lactose fermentation*
- ***POS****– Positive,* ***NEG*** *– Negative,* ***A+G*** *– Acid and Gas produced,* ***G*** *– Gas produced*

Out of 100 collected samples, 26 were isolated. These 26 samples were further differentiated into 5 distinct bacterial strains based on their morphological, cultural, and biochemical characteristics, as shown in the table.

Table 3: Differentiate 26 isolated sample into 5 different bacterial strains.

Strain Name	Sample Codes
Lactococcus garvieae	S2P5DG, S2P7, S2P2GB, S2P3BB, S3P1GB, S3P3NB, S1P4, S3P5G
Streptococcus pasteuri	S4P5B, S2P1G, S4P6G, S3P6LG, S4P4B, S2P4G
Streptococcus salivarius	S4P1B, S2P1B, S2P8B, S2P5LG, S4P2GB
Streptococcus sobrinus	S3P2NB, S3P4G, S4P4G, S4P2B, S3P5BB, S2P8G

Observations

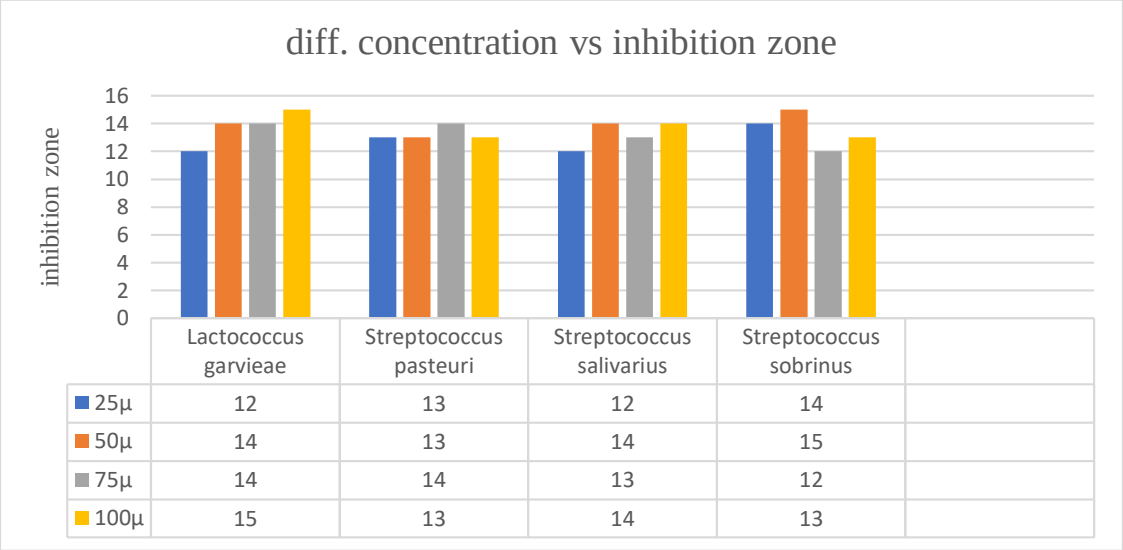


Figure 1: A. Antibacterial activity of Water Extracts of *Syzygium aromaticum* against isolated oral pathogen

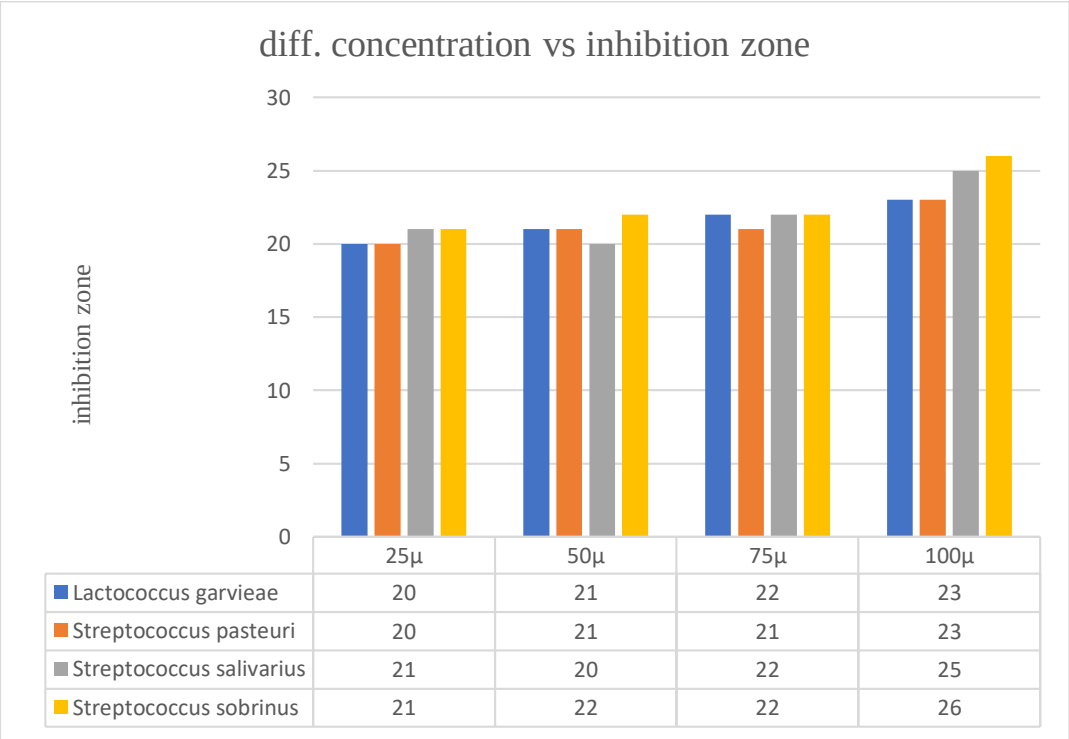
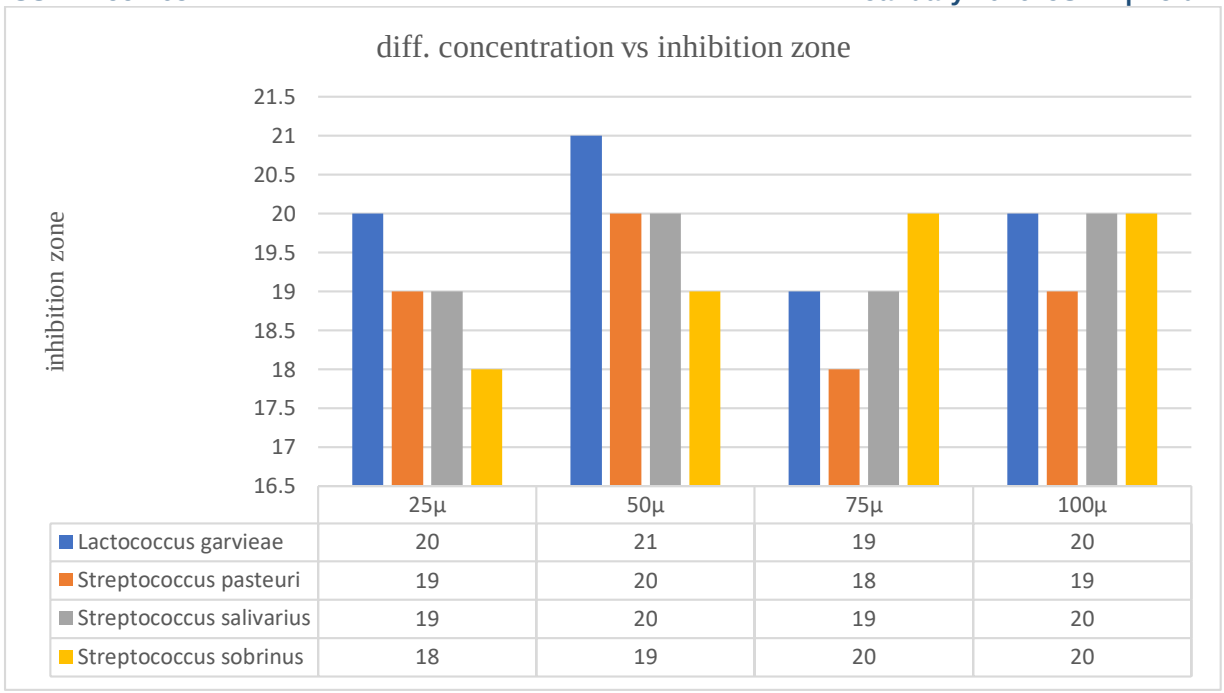
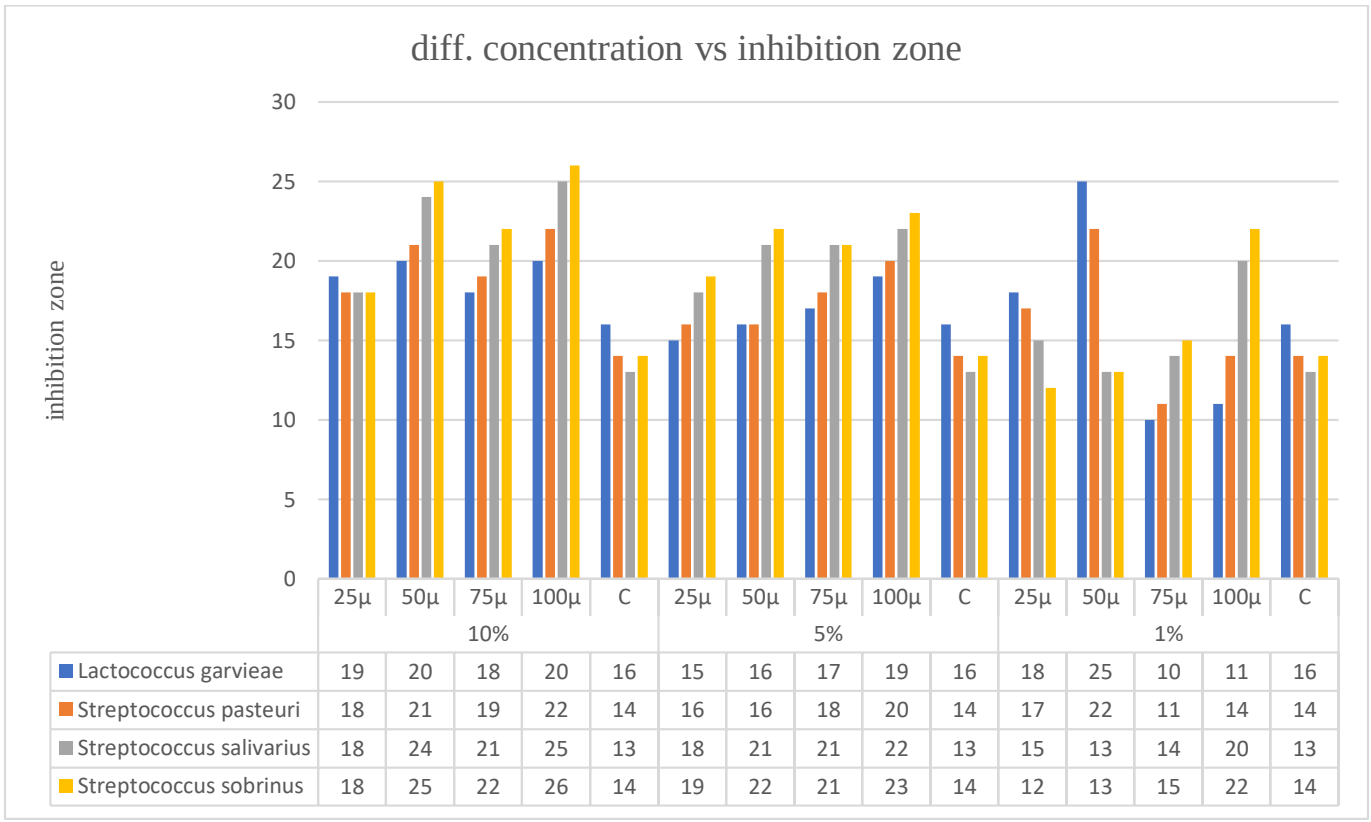


Figure 2: B. Antibacterial activity of water extracts of *Acacia nilotica* against isolated oral pathogens



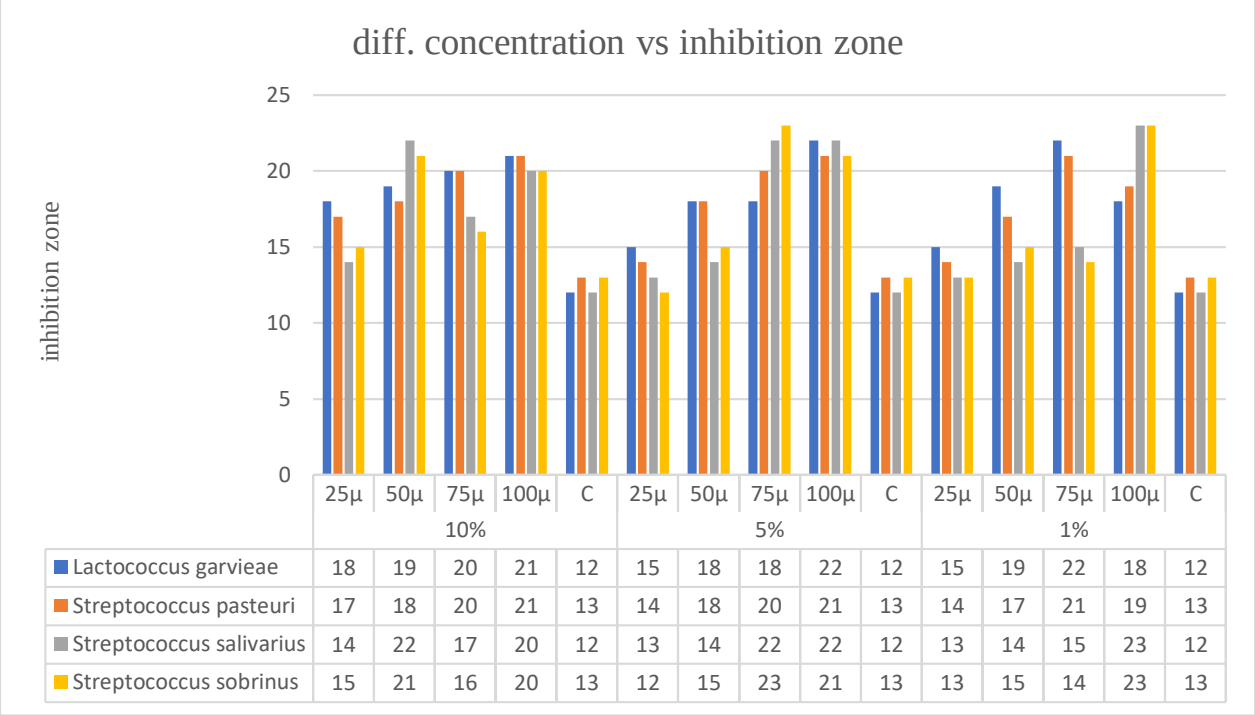
	25µ	50µ	75µ	100µ
Lactococcus garvieae	20	21	19	20
Streptococcus pasteuri	19	20	18	19
Streptococcus salivarius	19	20	19	20
Streptococcus sobrinus	18	19	20	20

Figure 3: C Antibacterial activity of Hot methanolic extracts of *Syzygium aromaticum* against isolated oral pathogens



	10%					5%					1%				
	25µ	50µ	75µ	100µ	C	25µ	50µ	75µ	100µ	C	25µ	50µ	75µ	100µ	C
Lactococcus garvieae	19	20	18	20	16	15	16	17	19	16	18	25	10	11	16
Streptococcus pasteuri	18	21	19	22	14	16	16	18	20	14	17	22	11	14	14
Streptococcus salivarius	18	24	21	25	13	18	21	21	22	13	15	13	14	20	13
Streptococcus sobrinus	18	25	22	26	14	19	22	21	23	14	12	13	15	22	14

Figure 4: D. Antibacterial activity of cold methanolic Extracts of *Syzygium aromaticum* against isolated oral pathogens.



	10%					5%					1%				
	25µ	50µ	75µ	100µ	C	25µ	50µ	75µ	100µ	C	25µ	50µ	75µ	100µ	C
Lactococcus garvieae	18	19	20	21	12	15	18	18	22	12	15	19	22	18	12
Streptococcus pasteuri	17	18	20	21	13	14	18	20	21	13	14	17	21	19	13
Streptococcus salivarius	14	22	17	20	12	13	14	22	22	12	13	14	15	23	12
Streptococcus sobrinus	15	21	16	20	13	12	15	23	21	13	13	15	14	23	13

Figure 5: E. Antibacterial activity of cold methanolic Extracts of *Acacia nilotica* against isolated oral pathogens

Discussion

The absence of oral health education programs was significantly associated with a higher prevalence of dental caries. Similar observations have been reported in studies from Nepal and Bangladesh, where children lacking exposure to oral health awareness were found to have a higher incidence of caries. This may be attributed to the fact that children who receive oral health education are better informed about the etiological factors of dental caries and effective preventive measures. In the present investigation, another important factor contributing to the development of dental caries was the lack of parental encouragement and supervision regarding their children's oral hygiene practices. Parental neglect in this area may lead to poor oral habits among children, such as infrequent tooth brushing and excessive intake of sugary foods—both of which are strongly linked to dental caries. This study also assessed the antibacterial efficacy of crude leaf extracts from *Syzygium aromaticum* and *Acacia nilotica* using methanol and water as solvents, targeting common oral pathogens. The antibacterial activity was determined through zone of inhibition measurements (Table 4). Notably, methanolic and cold-water extracts of *Syzygium aromaticum* exhibited promising potential as sources of bioactive compounds for caries management, suggesting that plant-based antimicrobials could serve as effective alternatives in the development of novel therapeutics.

Among the tested solvents, methanol extracts demonstrated superior efficacy against oral pathogens. Preliminary phytochemical screening (Table 4) confirmed the presence of tannins, alkaloids, and flavonoids in both methanolic and aqueous extracts. Specifically, the methanolic extracts of *Syzygium aromaticum* and *Acacia nilotica* tested positive for both alkaloids and tannins, whereas the aqueous extract also indicated the presence of similar phytochemicals. Furthermore, both methanolic and aqueous extracts of *Acacia nilotica* effectively inhibited the growth of oral pathogens, aligning with previous research that demonstrated its antimicrobial potential. For instance, crude extracts of *Acacia nilotica* produced inhibition zones ranging from 6 mm to 22 mm against a variety of clinical isolates.

In this study, 100 dental plaque samples were collected from children aged 4–14 years at a government hospital in Nagpur. Of these, 26 microbial isolates were consistently recovered and identified based on cultural, morphological, and biochemical characteristics. The major isolates included *Lactococcus garvieae*, *Streptococcus pasteuri*, *Streptococcus salivarius*, and *Streptococcus sobrinus*.

Gender-wise analysis revealed that male children were more susceptible to dental caries, with 63% of the positive samples collected from males and 27% from females, indicating a significant difference in caries prevalence between genders.

Previous studies support the current findings regarding the antimicrobial potential of *Acacia nilotica*. Gurjinder Kaur (2016) reported that its flower extract exhibited strong antibacterial activity, producing inhibition zones of 19–21 mm at 40 mg/mL against *Streptococcus mutans* and *Lactobacillus acidophilus*, exceeding the activity of chlorhexidine. Similarly, Abhilasha S. B. (2023) found maximum inhibition—78% and 91% against *S. mutans* and *L. acidophilus*, respectively—at 600 nm OD, with statistically significant minimum bactericidal concentration (MBC) values, particularly for *L. acidophilus*.

III. Conclusion

The present study demonstrated that leaf extracts of *Syzygium aromaticum* (clove) and *Acacia nilotica* (Babool) possess notable antimicrobial activity, thereby validating their traditional use in the management of dental caries. Among the tested extracts, the methanolic extracts exhibited the highest inhibitory effects against isolated oral pathogens, particularly at a 5% concentration, with *Syzygium aromaticum* showing superior activity followed by *Acacia nilotica*. Although water-based extracts also showed antibacterial potential, their efficacy was comparatively lower.

The findings suggest that these plant extracts, especially in methanolic form, could be explored as natural alternatives for preventing dental plaque formation and managing early-stage caries, particularly in high-risk individuals. Their incorporation into oral hygiene products such as mouthwashes holds promise for clinical application. However, further research, particularly in vivo studies, is essential to evaluate their efficacy under oral conditions, including their effects on salivary pH, dental enamel, and the integrity of oral mucosal tissues. Additionally, assessments of potential cytotoxicity and long-term use must be conducted to ensure safety and effectiveness. Such investigations could pave the way for the development of novel, plant-based chemical plaque control agents.

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