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DECIPHERING *STREPTOCOCCUS MUTANS* FROM DENTAL CARIES: INTEGRATED EVALUATION OF PLANT-DERIVED COMPOUNDS, ESSENTIAL OILS AND BISMUTH OXIDE NANOPARTICLE AS ANTIVIRULENCE AGENTS

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ABSTRACT

Dental caries is a chronic biofilm-mediated oral disease in which *Streptococcus mutans* play a major role in disease progression through acid production and biofilm formation. Increasing antimicrobial resistance and the limitations of conventional antimicrobial agents have encouraged the exploration of plant-derived therapeutics and nanotechnology-based alternatives for oral healthcare applications. **Methods:** *Streptococcus mutans* was isolated from pediatric dental caries samples and evaluated for biofilm formation, antimicrobial susceptibility, and sensitivity toward selected medicinal plant extracts, essential oils, and phyto-fabricated bismuth oxide nanoparticles using standard microbiological methods, agar well diffusion, and MIC assays. Nanoparticles were characterized using UV–Visible spectroscopy, FTIR, and DLS analysis. **Results:** Among 60 dental caries samples, 42 (70%) were positive for *S. mutans*, of which 76.1% were biofilm producers. Antimicrobial susceptibility testing revealed higher resistance to methicillin (47.9%), ciprofloxacin (41.9%), gentamycin (32.6%), and penicillin (32.4%), while clindamycin showed the highest sensitivity (86.86%). Antibacterial activity was evaluated at concentrations of 100–400 µg/µL. Among the plant extracts, *Piper betle* (24.50 ± 2.64 mm) and *Nigella sativa* (24.25 ± 1.70 mm) showed the highest inhibition zones. Clove oil demonstrated the highest antibacterial activity among essential oils (25.75 ± 1.50 mm). Among the nanoparticles, aqueous-mediated *Piper betle* nanoparticles exhibited the highest activity (24.50 ± 1.29 mm). UV–Visible spectroscopy showed absorption peaks at 220–240 nm, while FTIR and DLS confirmed nanoparticle stabilization and nanoscale distribution. **Conclusion:** Essential oils, medicinal plant extracts, and phyto-mediated bismuth nanoparticles demonstrated significant antibacterial and antibiofilm activity against *S. mutans* and may serve as promising plant-based nanotherapeutic agents for pediatric dental caries management.

KEYWORDS: Dental caries, *Streptococcus mutans*, Biofilm, *Nigella sativa*, *Piper betle*, Bismuth oxide nanoparticles.

1. INTRODUCTION

Dental caries is a progressive, biofilm-mediated most commonly prevalent dental infections worldwide. Global epidemiological studies have reported that nearly 46–48% of children are affected by dental caries in primary tooth, while a similarly high prevalence has been observed in permanent teeth also. Among pediatric patients with active dental caries, *Streptococcus mutans* is one of the most frequently isolated cariogenic pathogens playing a major etiological role in disease progression. The organism possesses several virulence properties including acidogenicity, aciduricity, strong

adherence to tooth surfaces and extracellular polysaccharide production, which promote persistent biofilm formation and progressive enamel demineralization. Although fluoride therapy, chlorhexidine mouth rinses and antimicrobial agents are commonly used in caries management, prolonged use of these agents may result in tooth staining, altered taste sensation, mucosal irritation, and increasing antimicrobial resistance. In addition, biofilm-associated resistance significantly reduces the effectiveness of conventional therapeutic agents, thereby emphasizing the need for safer and more effective alternative treatment strategies.[1-3]

Medicinal plant extracts, essential oils, and nanomaterials have emerged as promising alternatives because of their broad-spectrum antimicrobial, antioxidant, anti-inflammatory, and antibiofilm properties. In the present study, essential oils, including clove, cinnamon, and *Nigella sativa* oil were evaluated due to their rich content of bioactive phytochemicals with known antimicrobial potential against oral pathogens. In addition, aqueous plant extracts of *Achyranthes aspera*, *Glycyrrhiza glabra*, *Nigella sativa*, and *Piper betle* were investigated for their inhibitory effects against *S. mutans*. Recent advances in nanotherapeutics shown the potential of plant-based antimicrobials. Therefore, fabricated bismuth nanoparticles using aqueous and ethanolic extracts of *N. sativa* and *Piper betle*were included in this study. These phyto-fabricated nanoparticles possess unique physicochemical properties including high surface area, enhanced antimicrobial reactivity, improved penetration into microbial biofilms, favorable biocompatibility, and relatively low toxicity. However, there remains a significant lacuna regarding the comparative antibacterial and antibiofilm efficacy of medicinal plants, seed oils, and plant-mediated bismuth nanoparticles against clinical isolates of *S. mutans* from dental plaque or caries patients.[4-6]

Therefore, the study aimed to isolate and characterize *S. mutans* and evaluate the antibacterial and antibiofilm activities of selected essential oils, medicinal plant extracts, and green-synthesized bismuth nanoparticles against the isolated strains. Furthermore, the fabricated nanoparticles were screened under visible UV spectroscopy, FTIR, and DLS analyses to confirm the phytochemical compounds, particle size distribution, functional group interactions, and stability. The study results may contribute to the advancement of plant-based nano therapeutic strategies and support the future development of safer and more effective antimicrobial mouth rinses, dental coatings, toothpastes, and targeted antibiofilm therapies for the treatment and management of caries.

2. MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

Dental caries samples were collected from pediatric outpatients attending Balaji Medical Hospital, Chennai, India, during the study period from November 2025 to March 2026. Before sample collection, the oral cavity was rinsed with sterile water to remove food debris. With the support of dental practitioners, dental caries specimens were collected aseptically using a sterile excavator and immediately transferred into screw-capped tubes containing BHI broth for transport and enrichment.

Ethical Clearance

This study was conducted in accordance by the approval of the research committee, Faculty of Dental Medicine, Bharaath Medical College and Hospital, with an ethical clearance sl No. BIEC -167-25 Informed consent from all patients was obtained.

Isolation and Identification of *Streptococcus mutans*

The collected clinical samples were inoculated into BHI broth and incubated at 37°C for 24 hours under anaerobic conditions using a candle jar. An aliquot of the cultured broth was streaked onto Mitis Salivarius Bacitracin (MSB) agar medium and further inoculated at 37°C for 48 hrs. Bacterial colony showing characteristic morphology were selected and sub cultured on blood agar plates for further identification. Bacterial colony were characterized using techniques like gram staining and biochemical characteristics.

Detection of Biofilm Formation

Congo Red Agar Method

Biofilm formation were evaluated using the CRA techniques. BHI agar of 2% supplemented sucrose with red dye were utilized for the preparation of CRA plates. Sterile concentrated congo red dded to the medium at 55°C. Bacterial isolates streaking on the prepared agar plates and at 37°C incubation for 24–48 hours. Dark Blackish colonies with features of crystalline structure were considered presence of positive biofilm *S.Mutans* organisms, whereas pink colonies indicated weak or non-biofilm producers. The assay was performed in triplicate.

Nigella sativa Seed Collection and Extract Preparation

Nigella sativa seeds were procured from a regional ayurvedic shop in Triplicane, Chennai, Tamil Nadu, India and authenticated by the Department of Plant Biology and Biotechnology, Presidency College. Sample seeds were de-contaminated and washed with deionize water to remove dust, impurities and shade-drying for 15 days. The dried seeds ground into powder form.

Twenty grams of seed powder were mixed with distilled water of 400 millilitre and heated to boiling temperature for 20 minutes with continuous stirring. *Nigella sativa* extract was cooled, taken for Whatmans 1 filtration, using a rotary evaporator to concentrate and lyophilized under freeze drying technique and stored at 4 degree celcius. For further analysis, 1:1 ratio of extract powder and deionized water with 1 mL methanol to make final concentration of 1 mg/mL.

Fabrication of Bismuth Nanoparticles

Bismuth nanoparticles were synthesized using *Nigella sativa* seed extract by green synthesis. Briefly, 2 g of bismuth nitrate pentahydrate titrated in 20 mL of demineralized water of 80 mL of *N. sativa* and continuously stir at 60°C for 3 hours.

Further, using centrifugation technique and repeat washing with demineralized water to produce the nanoparticles. Upon, drying at 105°C for 1 hour the bismuth oxide nanoparticles and reconstituted in dimethyl sulfoxide for further analysis.

Characterization of Bismuth Nanoparticles

Fabricated bismuth nanoparticles were analysed under visible UV spectroscopy, FTIR, and Dynamic Light Scattering (DLS) techniques. These analyses were performed to confirm nanoparticle formation, determine particle size distribution, and identification of functional groups involved in the reduction and stabilization of bismuth ions.

Agar Well Diffusion Assay

Antibacterial activity of *Nigella sativa* extract & fabricated BiNPs against *S. mutans* was assessed using agar well techniques. In sterile saline, the bacterial suspensions were prepared at 0.5 McFarland. Using sterile cotton swabs, bacterial suspension was evenly spread onto Mueller–Hinton agar plates. Using sterile cork, 6-8 mm wells were prepared and 100 µL of test samples were added into each well. The plates were incubated at 37°C for 24–48 hours, and the zones of inhibition were measured in millimeters. Ciprofloxacin was used as the positive control, while DMSO served as the negative control. Results were interpreted according to CLSI guidelines.

Minimum Inhibitory Concentration (MIC)

MIC of BiNPs against *S. mutans* was assessed using the broth microdilution technique according to CLSI guidelines. Two-fold serial dilutions of BiNPs, with concentrations ranging from 0.40 to 1 µg/mL, were prepared using Mueller–Hinton broth. Equal volumes (100 µL) of a bacterial inoculum adjusted to 0.5 McFarland standard and the respective test solutions were added to each well. Chlorhexidine (2%) served as the positive control. Following incubation at 37°C, the MIC was determined as the lowest concentration at which no visible bacterial growth was observed.

Determination of Antibiofilm Activity

Antibiofilm activity was assessed by the microtiter plate method. Bacterial suspensions were prepared in Mueller–Hinton broth supplemented with 1% glucose and adjusted to a turbidity equivalent to the 0.5 McFarland standard. Sterile flat-bottom 96-well microplates were inoculated with the prepared broth culture and incubated at 37°C for 24 hours. Following incubation, non-adherent planktonic cells were carefully discarded, and the wells were rinsed three times with phosphate-buffered saline (PBS) to remove loosely attached bacteria. The remaining adherent biofilm was stained with safranine for 15 minutes. Subsequently, excess stain was removed, and the extent of biofilm formation was quantified using a microplate reader.

Preliminary Qualitative Phytochemical Analysis

The seed extract of N. sativa was subjected to preliminary phytochemical evaluation using established standard methods to identify the presence of major secondary metabolites and other constituents, including alkaloids, carbohydrates, amino acids, glycosides, phenolic compounds, tannins, phytosterols, proteins, saponins, gums, mucilage, fixed oils, fats, and volatile oils.

Quality Control

Ciprofloxacin served as the positive control during antibacterial susceptibility testing, while DMSO was used as the negative control. In the determination of minimum inhibitory concentration (MIC), 2% chlorhexidine was included as the reference standard. All assays were carried out in triplicate to maintain reproducibility, and standard aseptic precautions were strictly followed to minimize contamination during the experimental process.

Statistical Analysis

All collected data were systematically entered into Microsoft Excel for organization and analysis. Descriptive statistical methods were applied to summarize the findings, and the results were presented in terms of frequencies, percentages, means, and standard deviations.

3. RESULTS

In the present study, a total of 60 dental caries samples were collected from pediatric patients. The samples were analyzed to determine the prevalence, biofilm-forming potential of *Streptococcus mutans*, and the antibacterial efficacy of selected plant extracts and phyto-fabricated bismuth nanoparticles. An equal distribution of male and female participants was observed in the study population, with each group comprising 50% of the total samples ($p=0.49$). Among the 60 samples analyzed, 42 (70%) showed positivity for S. mutans, whereas 18 (30%) were negative, demonstrating a statistically significant difference ($p<0.001$). Identification of S. mutans was confirmed by the appearance of characteristic small, elevated, pale blue colonies on Mitis Salivarius agar (Figure 7). Among the positive isolates, 32 (76.1%) demonstrated biofilm-forming ability, whereas 10 (23.9%) were non-biofilm producers ($p=0.01$) (Table 1). With Congo Red agar *S. mutans* revealed dense, black, dry crystalline colonies in the majority of isolates, indicating strong biofilm production (Figure 8). These phenotypic features collectively reflect enhanced extracellular polysaccharide synthesis and adherence capacity, underscoring the biofilm-associated virulence of *S. mutans* in dental caries.

Table 1: Demographic and microbiological characteristics of *Streptococcus mutans.*

Characteristics	N (%)	P value
Male	30 (50)	**0.49**
Female	30 (50)	
Streptococcus mutans (Mitis Salivarus Agar)		
Yes	42 (70)	**0.001***
No	18 (30)	
Biofilm formation (Congo Red Method)		
Yes	32 (76.1)	**0.01***
No	10 (23.9)	

** Significant at the 5% level; associations between categorical variables were analyzed using the Chi-square test.*

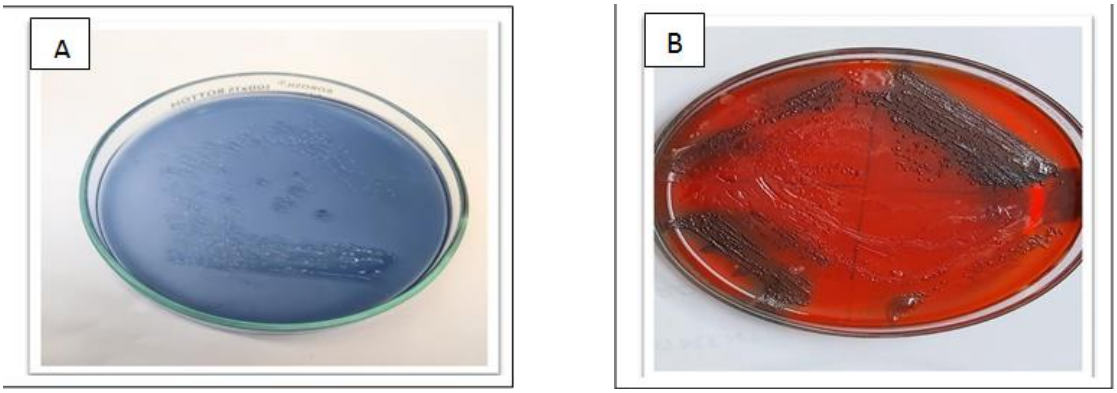


Figure 1A: Characteristic blue colonies in Mitis Salivarius agar of *Streptococcus mutans* on. 1B: Biofilm-producing *Streptococcus mutans* on Congo Red agar.

The aqueous extracts of medicinal plants exhibited distinct visual characteristics, with *Nigella sativa* extract appearing as a dark brown homogeneous solution, while *Glycyrrhiza glabra* and *Achyranthes aspera* extracts showed variations in colour intensity and clarity, reflecting differences in phytochemical composition (Fig. 2 & 3).

Fig.2 Aqueous extract of *Nigella sativa.*

Fig. 3 Aqueous extract of *G. glabra & A. aspera.*

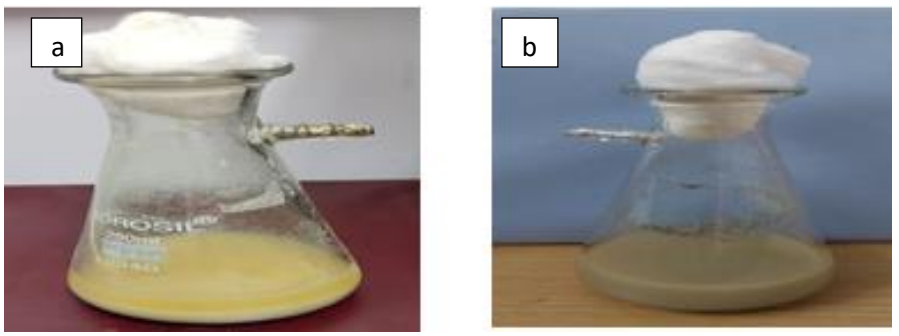


Figure.4 Synthesized Bismuth oxide NP: a) Before b) After 3 hours of shaking @ 60C

c) Bismuth oxide NP of *Nigella sativa* and *Piper betle.*

The synthesis of bismuth oxide nanoparticles was visually confirmed by a marked transition in the reaction mixture from a lighter suspension to a denser, turbid system following incubation, indicating nanoparticle formation (Fig. 4a &4b). The formation of fine nanoparticle precipitates further supported successful phyto-fabrication (Fig. 4c).

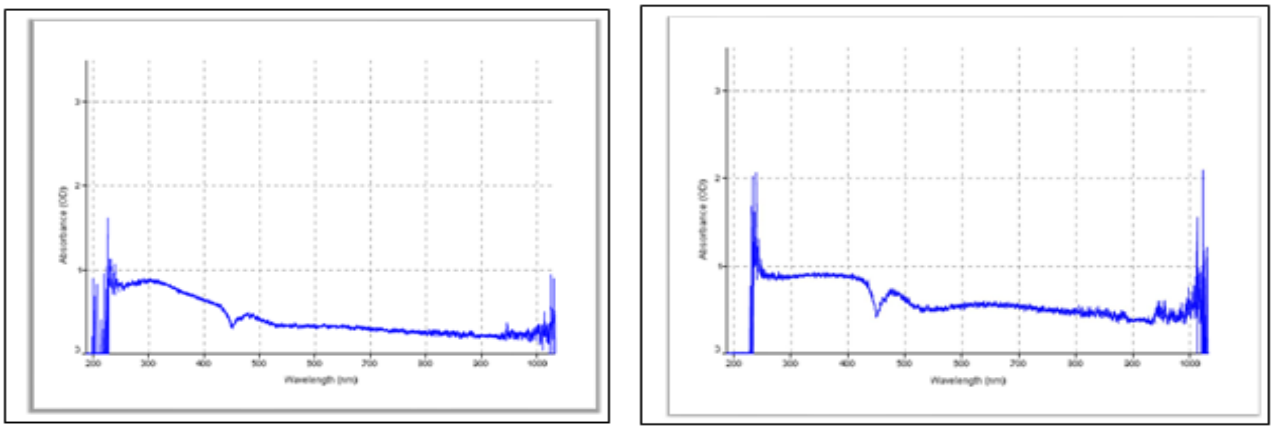


Figure 6. UV–Visible spectroscopic analysis. *Piper betle* mediated Bismuth Oxide Nanoparticles. B: *Nigella sativa* mediated Bismuth Oxide Nanoparticle.

The formation of phyto-mediated bismuth oxide nanoparticles was confirmed through spectral and particle size characterization. UV–Visible spectral analysis of both *Piper betle*and *Nigella sativa*-mediated nanoparticles demonstrated strong absorption in the ultraviolet region, with a prominent peak observed around ~220–240 nm (Figure 3 & Figure 4). The spectra showed a gradual decline in absorbance across the visible range (300–800 nm), followed by minor fluctuations at higher wavelengths, indicating stable nanoparticle formation and consistent optical behavior associated with nanoscale materials.

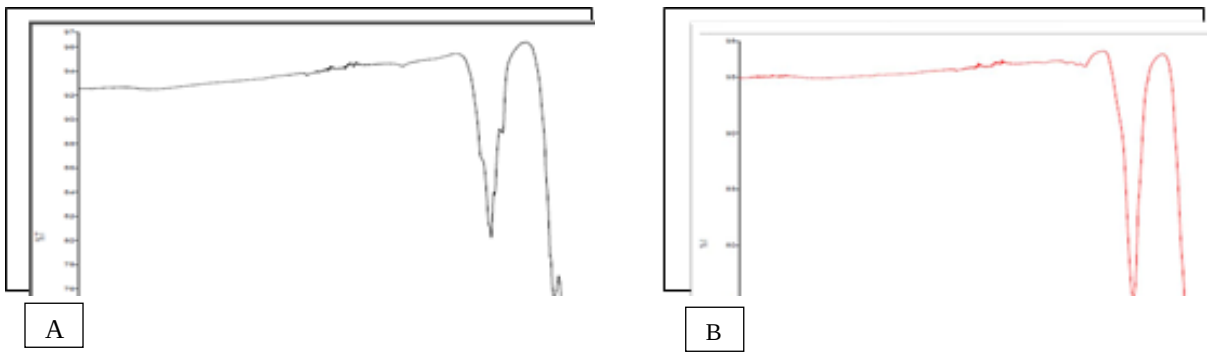
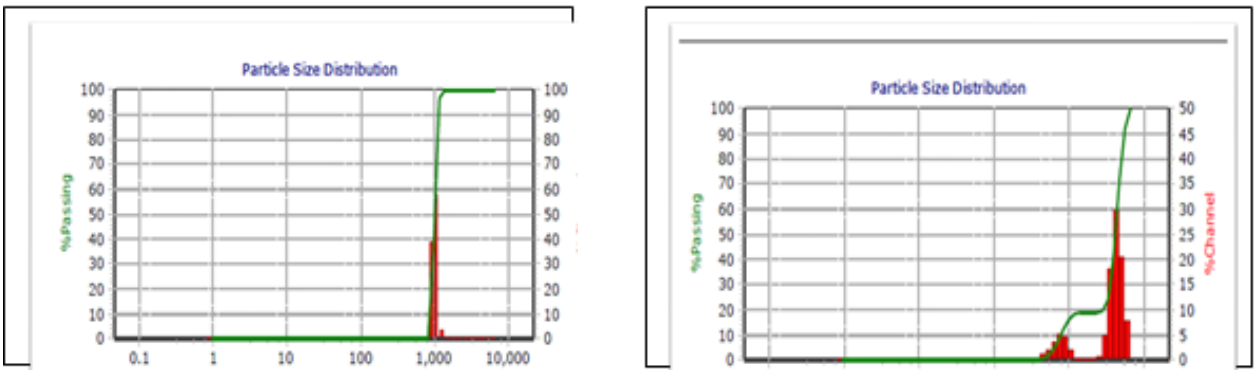


Figure 7. FTIR spectrum analysis. A: *Piper betle* mediated bismuth oxide nanoparticles; B: *Nigella sativa* mediated bismuth oxide nanoparticles.



A B

Figure 9. Particle size analysis by dynamic light scattering. A: Piper betle mediated bismuth oxide nanoparticles; B: Nigella sativa mediated bismuth oxide nanoparticles.

FTIR spectral analysis revealed distinct absorption bands corresponding to functional groups involved in nanoparticle stabilization. In Piper betle-mediated nanoparticles, prominent peaks were observed in the region (~1000–1100 cm^{-1}), indicating C–O and C–C stretching vibrations (Fig.7A). Similarly, *Nigella sativa*-mediated nanoparticles exhibited strong absorption bands in comparable regions, along with additional peaks suggestive of hydroxyl and carbonyl functional groups (Figure 7B). These findings indicate the involvement of phytoconstituents such as phenolics and flavonoids in the reduction and capping of nanoparticles.

Dynamic light scattering analysis demonstrated particle size distribution within the nanometer range. Piper betle-mediated nanoparticles showed a relatively narrow distribution with a dominant peak around ~500–600 nm, indicating moderate uniformity (Fig.7). In contrast, *Nigella sativa*-mediated nanoparticles exhibited a broader size distribution with multiple peaks extending toward higher nanometer ranges (~1000–10,000 nm), suggesting polydispersity and possible aggregation (Figure 8). Microscopic examination further demonstrated characteristic Gram-positive coccoid cells arranged predominantly in short chains and clusters, confirming morphological features consistent with *Streptococcus mutans* (**Fig. 9**).

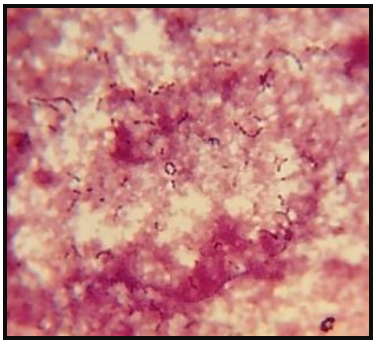


Figure 10: Microscopic morphology of *Streptococcus mutans.*

The AST pattern of *Streptococcus mutans* isolates is presented in **Table 2**. The isolates demonstrated variable resistance and sensitivity profiles against the tested antibiotics. Among the antimicrobial agents evaluated, methicillin exhibited the highest resistance rate (47.9%), followed by ciprofloxacin (41.9%), gentamycin (32.6%), and penicillin (32.4%). Moderate resistance was observed for streptomycin (20.6%) and azithromycin (17.6%), whereas lower resistance rates were noted for rifampicin (14.39%), amikacin (9.5%), erythromycin (9.2%), clindamycin (8.4%), chloramphenicol (7.8%), and bacitracin (6.8%). Sensitivity analysis revealed that clindamycin showed the highest sensitivity (86.86%), followed by streptomycin (68.84%), azithromycin (65.3%), chloramphenicol (62.22%), methicillin (53.6%), and ciprofloxacin (48.26%). These findings indicate heterogeneous antimicrobial susceptibility patterns among the cariogenic isolates. A subset of isolates exhibited resistance to multiple antimicrobial agents, indicating the presence of multidrug-resistant phenotypes. Based on the antibiogram profile 66% of the resistant isolates were categorized as MDR strains due to three or more classes of antibiotics resistance. Microscopic examination revealed characteristic Gram-positive coccoid morphology of *S. mutans*, predominantly arranged in short chains and clusters (**Figure 9**). Overall, the antimicrobial susceptibility findings provide important insights into the resistance

characteristics of *S. mutans* isolates associated with dental caries.

Table 2: Antibacterial sensitivity testing pattern of *Streptococcus mutans.*

S. no.	Antibiotic disc	Disk – potency µg/ml	No of resistant isolates (%)	No of sensitive isolates (%)
1.	Amikacin	10	9.5	28.2
2.	Azithromycin	10	17.6	65.3
3.	Bacitracin	30	6.8	15.45
4.	Chloramphenicol	10	7.8	62.22
5.	Ciprofloxacin	15	41.9	48.26
6.	Clindamycin	30	8.4	86.86
7.	Erythromycin	10	9.2	13.73
8.	Gentamycin	10	32.6	26.9
9.	Methicillin	30	47.9	53.6
10.	Penicillin	30	32.4	7.09
11.	Rifampicin	30	14.39	10.1
12.	Streptomycin	30	20.6	68.84

The antibacterial activity of plant extracts, essential oils, and phyto-fabricated bismuth nanoparticles against *Streptococcus mutans* was evaluated at concentrations of 100, 200, 300, and 400 µg/µL (Table 3). Among the plant extracts, *Piper betle* (24.50 ± 2.64 mm) and Nigella sativa (24.25 ± 1.70 mm) showed the highest mean zones of inhibition, followed by *Achyranthes aspera* (19.00 ± 1.83 mm) and *Glycyrrhiza glabra* (12.75 ± 2.22 mm) ($p < 0.01$). Among the essential oils, clove oil demonstrated the highest antibacterial activity (25.75 ± 1.50 mm), followed by cinnamon oil (23.75 ± 2.50 mm) and *Nigella sativa* oil (18.50 ± 2.38 mm) ($p = 0.003$). Among the phyto-fabricated bismuth nanoparticles, aqueous-mediated *Piper betle* nanoparticles exhibited the highest antibacterial activity (24.50 ± 1.29 mm), followed by aqueous-mediated *Nigella sativa* nanoparticles (20.00 ± 3.56 mm). In contrast, ethanolic nanoparticle formulations showed lower activity (14.25–14.75 mm) ($p = 0.001$). Antibacterial activity increased with increasing concentration in all tested samples. Chlorhexidine showed the highest inhibitory activity, while no inhibition was observed in the negative control.

Table 3: Antibacterial activity of seed oils, plant extracts, and bismuth nanoparticles against *Streptococcus mutans* (ATCC 25175) by Agar Well Diffusion Method.

Characteristics	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	P value
				Lower Bound	Upper Bound			
Essential Oils								
Clove oil	15	25.75	1.50	23.36	28.14	24	27	0.003*
Cinnamon oil	15	23.75	2.50	19.77	27.73	21	27	
Nigella sativa oil	15	18.50	2.38	14.71	22.29	16	21	
Clove oil	15	25.75	1.50	23.36	28.14	24	27	
Plant Extract								
Achyranthens aspera	20	19.00	1.826	.913	16.09	21.91	17	<0.01*
Glycyrrhiza glabra	20	12.75	2.217	1.109	9.22	16.28	10	
Nigella sativa	20	24.25	1.708	.854	21.53	26.97	22	
Piper betle	20	24.50	2.646	1.323	20.29	28.71	22	
Fabricated Nanoparticles Against *Streptococcus mutans*								
Nigella sativa Aqueous NP	15	20.00	3.559	1.780	14.34	25.66	17	0.001*
Nigella sativa Ethanol NP	15	14.25	2.986	1.493	9.50	19.00	11	
Piper betle Aqueous NP	15	24.50	1.291	.645	22.45	26.55	23	
Piper betle Ethanol NP	15	14.75	3.096	1.548	9.82	19.68	12	

**Significant at the 5% level ($p < 0.05$); Differences between mean zones of inhibition were analyzed using ANOVA.*

MIC profile of the tested extracts and nanoparticles is summarized in **Table 4**. Among the essential oils, clove and cinnamon oils demonstrated the lowest MIC values (80 µg/mL), whereas *Nigella sativa* oil exhibited an MIC of 160 µg/mL. Among the aqueous plant extracts, *Nigella sativa* showed the lowest MIC value (160 µg/mL), followed by *Achyranthes aspera* and *Piper betle* (320 µg/mL), while *Glycyrrhiza glabra* exhibited the

highest MIC value (640 µg/mL). Notably, aqueous-mediated *Nigella sativa* bismuth nanoparticles demonstrated the lowest MIC value (80 µg/mL) among the phyto-fabricated nanoparticles, followed by aqueous-mediated *Piper betle* nanoparticles (160 µg/mL). Ethanolic nanoparticle formulations demonstrated comparatively higher MIC values, indicating reduced antibacterial efficacy (**Table 4**).

Table 4: MIC determination of aqueous plant extract µg/mL against *S. mutans*) by Broth Dilution Method.

	MIC value(µg/ml)
Oils (a)	
Clove oil	80
Cinnamon oil	80
*Nigella sativa*oil	160
Plant extract (b)	
Achyranthensaspera	320
Glycyrrhizaglabra	640
Nigella sativa	160
Piper betle	320
Fabricated Nanoparticle (c)	
Nigella sativa (Aqueous)	80
Nigella sativa (Ethanol)	320
*Piper betle (*Aqueous)	160
Piper betle (Ethanol)	640

The qualitative phytochemical analysis of aqueous extracts from Achyranthes aspera, Glycyrrhiza glabra, Nigella sativa, and Piper betle revealed the secondary phytometabolites (**Table 5**). Alkaloids, tannins, glycosides, saponins, and flavonoids were detected across all the investigated plant extracts, indicating a common distribution of bioactive constituents. Carbohydrates were identified in *Achyranthes aspera* and *Nigella sativa*, while terpenoids and steroids were observed in *Glycyrrhiza glabra*, *Nigella sativa*, and *Piper betle*, but were absent in *Achyranthes aspera*. Anthraquinones were detected only in *Glycyrrhiza glabra*. The varied phytochemical composition observed among the plant extracts may contribute to their differential antimicrobial properties against *Streptococcus mutans*.

Table 5: Qualitative Phytochemical screening of selected aqueous plant extracts.

Phytochemical	***Achyranthes Aspera***	***Glycyrrhiza Glabra***	***Nigella sativa***	***Piper betel***
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Carbohydrates	+	-	+	NA
Flavonoids	+	+	+	+
Terpenoids	-	+	+	+
Steroids	-	+	+	+
Anthraquinones	NA	+	NA	NA

DISCUSSION

The present study provides significant insights into the prevalence, biofilm-forming ability, antimicrobial resistance, and comparative antibacterial efficacy of medicinal plant extracts, seed oils, and phyto-fabricated bismuth nanoparticles against *Streptococcus mutans.*[7] A significant *Streptococcus mutans* was isolated from 70% of dental caries samples, supporting previous epidemiological studies identifying *S. mutans* as a predominant cariogenic pathogen in children. Among the isolates, 76.1% demonstrated biofilm-forming ability on Congo Red agar, indicating enhanced extracellular polysaccharide production and adherence capacity. Biofilm formation is a critical virulence determinant that facilitates bacterial persistence, acid retention, and resistance to antimicrobial agents within the oral cavity. The antimicrobial susceptibility profile revealed heterogeneous resistance patterns, with higher resistance observed against methicillin (47.9%), ciprofloxacin (41.9%), gentamycin (32.6%), and penicillin (32.4%). In contrast, clindamycin showed the highest sensitivity (86.86%), followed by streptomycin (68.84%) and azithromycin (65.3%). The detection of multidrug-resistant phenotypes among certain isolates further emphasizes the growing therapeutic challenge posed by resistant oral pathogens.[8-12]

Among the aqueous plant extracts, *Nigella sativa* and *Piper betle* demonstrated the highest antibacterial activity, exhibiting inhibition zones of 22–28 mm,

whereas *Glycyrrhiza glabra* showed comparatively lower activity (10–15 mm). The concentration-dependent antibacterial effect may be attributed to phytochemicals such as alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids identified during phytochemical screening (13-14). These compounds posses potential to disrupt cell membranes\ of bacterial pathogens, inhibit glucosyltransferase, and interfere with biofilm formation. Among the essential oils, clove oil demonstrated the highest antibacterial activity (24–27mm; MIC 80 µg/mL), followed by cinnamon oil (21–27 mm; MIC 80µg/mL) and *Nigella sativa* oil (16–21 mm; MIC 160 µg/mL). These findings correlate with previous reports demonstrating potent anti-*S. mutans* activity of eugenol- and cinnamaldehyde-rich essential oils.[15]

A major finding of our study showed the enhanced therapeutic efficacy of phyto-fabricated bismuth oxide nanoparticles compared to several crude plant extracts. Among the nanoparticle formulations, aqueous-mediated *Piper betle* nanoparticles of 23–26 mm inhibition zones, while aqueous-mediated *Nigella sativa* nanoparticles exhibited inhibition zones of 17–25 mm with the lowest MIC value (80 µg/mL). Ethanolic nanoparticle formulations demonstrated comparatively lower activity. The superior antibacterial efficacy of these nanoparticles may be attributed to increased surface area, enhanced penetration into microbial biofilms, prolonged interaction with bacterial membranes, and oxidative stress-mediated cellular damage. Characterization studies confirmed successful nanoparticle synthesis and stabilization. UV–Visible spectroscopy demonstrated characteristic absorption peaks around 220–240 nm, while FTIR analysis revealed hydroxyl, carbonyl, and C–O functional groups, indicating the involvement of phenolics and flavonoids in nanoparticle reduction and capping. Dynamic Light Scattering analysis confirmed nanoparticle formation within the nanometer range, although partial aggregation was observed in certain formulations.[16-18]

Overall, the findings suggest that essential oils and phyto-fabricated bismuth nanoparticles possess superior antibacterial and antibiofilm activity against *S. mutans* compared to several crude extracts and certain conventional antibiotics against resistant strains.[19] These results highlight the therapeutic potential of plant-based nanomaterials for future oral healthcare applications, including antimicrobial mouth rinses, dental varnishes, toothpastes, and targeted antibiofilm formulations. However, further studies confirms therapeutic mechanism of action studies, toxicity evaluation, and in vivo clinical investigations are required to validate their long-term safety and clinical applicability.[20]

CONCLUSION

The present study demonstrated that Streptococcus mutans isolated from pediatric dental caries showed substantial biofilm-forming ability and antimicrobial resistance. Among the tested agents, clove oil, cinnamon oil, *Piper betle*, and *Nigella sativa* exhibited strong antibacterial activity, while aqueous-mediated phyto-fabricated bismuth oxide nanoparticles showed enhanced inhibitory effects against *S. mutans* compared to several crude extracts. Nanoparticle characterization by UV–Visible spectroscopy, FTIR, and DLS confirmed successful synthesis and stabilization. The findings highlight the potential of plant-derived compounds and green-synthesized bismuth nanoparticles as promising alternative antibacterial and antibiofilm agents for dental caries management. Further in vivo studies and clinical investigations are warranted to evaluate their safety, efficacy, and potential application in oral healthcare formulations.

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