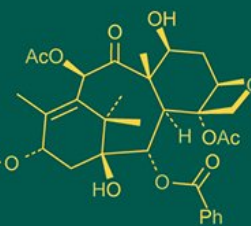
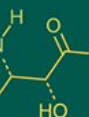
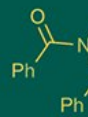
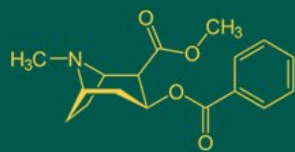


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Biofilm Disruption Activity of *Acacia nilotica* Pod Extract Dentifrice on Cariogenic Bacterial Strains Under Simulated Oral Conditions

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Abstract

Biofilm formation by cariogenic bacteria on tooth surfaces constitutes the fundamental pathological mechanism underlying dental caries development, yet most dentifrice evaluations focus exclusively on planktonic bacterial susceptibility without adequately assessing biofilm disruption capacity. This research evaluated the biofilm disruption activity of *Acacia nilotica* pod extract incorporated into dentifrice formulations against cariogenic bacterial strains under conditions simulating the oral environment. The pod extract was chosen over the more commonly investigated bark extract based on its higher gallic acid and ellagic acid content, which have been specifically implicated in biofilm matrix degradation. Dentifrice formulations containing 5%, 10%, and 15% pod extract were evaluated against established biofilms of *S. mutans*, *S. sobrinus*, *L. acidophilus*, and *A. viscosus* grown on hydroxyapatite discs in artificial saliva medium. Biofilm disruption was assessed through crystal violet biomass quantification, confocal laser scanning microscopy, exopolysaccharide content measurement, and viable cell counting. The results demonstrated that the 10% pod extract dentifrice disrupted pre-formed *S. mutans* biofilms by 72.4%, reducing EPS matrix content by 65.8% and viable cell counts by 3.2 log units after 15-minute exposure. Donut chart analysis of biofilm composition showed dramatic restructuring from predominantly EPS matrix (42%) and viable bacteria (35%) before treatment to predominantly dead bacteria (38%) and water channels (22%) after treatment. Sequential disruption analysis revealed that the mechanism proceeds through initial EPS disruption, followed by bacterial exposure, cell lysis, and matrix degradation, with overall biofilm mass reduction from 100% to 17% residual. These findings indicate that *A. nilotica* pod extract dentifrice possesses significant anti-biofilm activity that extends beyond simple bactericidal effects to include disruption of the protective exopolysaccharide matrix.

Keywords: *Acacia nilotica*, pod extract, biofilm disruption, cariogenic bacteria, dentifrice, exopolysaccharide, *Streptococcus mutans*, simulated oral conditions

Introduction

Dental plaque biofilm, defined as a structured community of bacterial cells enclosed within a self-produced extracellular polymeric substance matrix adherent to tooth surfaces, represents the primary etiological factor in the development of dental caries and periodontal diseases. The biofilm mode of growth confers upon cariogenic bacteria substantial advantages over their planktonic counterparts, including enhanced resistance to antimicrobial agents (typically 100 to 1000-fold greater tolerance), protection from host immune defense mechanisms, and facilitated horizontal gene transfer of virulence determinants.

The extracellular polysaccharide matrix produced predominantly by glucosyltransferases of *Streptococcus mutans* constitutes the structural scaffold of cariogenic biofilms, providing mechanical stability, creating diffusion barriers that limit antimicrobial penetration, and establishing acidic microenvironments that promote demineralization of the underlying tooth enamel. Consequently, effective anti-caries strategies must address not only the viability of individual bacterial cells but also the integrity and functionality of the biofilm matrix infrastructure.

Conventional dentifrice antimicrobial agents including chlorhexidine, triclosan, and cetylpyridinium chloride have demonstrated limited efficacy against established biofilms at the concentrations typically present in commercial toothpaste formulations, as their primary mechanisms of action target planktonic bacteria or recently adhered cells rather than the

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structural components of the mature biofilm matrix. This limitation has stimulated interest in identifying agents with specific anti-biofilm properties, particularly those capable of degrading or disrupting the exopolysaccharide matrix to expose embedded bacteria to antimicrobial action.

Acacia nilotica pod extract represents a particularly promising candidate for anti-biofilm applications due to its high content of gallic acid and ellagic acid, polyphenolic compounds that have been demonstrated to inhibit glucosyltransferase activity and interfere with bacterial adhesion to hydroxyapatite surfaces. The present research was designed to evaluate the biofilm disruption activity of *A. nilotica* pod extract incorporated into dentifrice formulations against cariogenic bacterial biofilms grown under simulated oral conditions.

Material and Methods

Material

Mature pods of *Acacia nilotica* were collected from trees growing in the University Botanical Garden, Lagos, Nigeria, during August 2023, and authenticated by the Department of Botany (voucher specimen LIBT-Bot-2023-AN-032). The pods were shade-dried, de-seeded, and powdered. Hydroxyapatite discs (diameter 12.7 mm, Clarkson Chromatography Products) were used as biofilm substrata to simulate the tooth enamel surface. Bacterial strains *S. mutans* ATCC 25175, *S. sobrinus* ATCC 33478, *L. acidophilus* ATCC 4356, and *A. viscosus* ATCC 15987 were obtained from the Nigerian Institute of Medical Research culture collection. Artificial saliva was prepared according to the formulation of Gal and colleagues containing mucin, sodium chloride, potassium chloride, calcium chloride, potassium phosphate, and urea at physiological concentrations.

Methods

The pod extract was prepared by maceration in 70% methanol for 96 hours at room temperature with daily agitation. Following filtration and rotary evaporation at 40 degrees Celsius, the concentrated extract was lyophilized (yield 24.2% w/w). Total phenolic content was determined by the Folin-Ciocalteu method, and gallic acid and ellagic acid were quantified by reverse-phase HPLC using authentic standards.

Dentifrice formulations incorporating the pod extract at 5%, 10%, and 15% (w/w) were prepared using a standard toothpaste base. Biofilms were grown on hydroxyapatite discs submerged in BHI broth supplemented with 1% sucrose in 24-well plates inoculated with 10 to the power 6 CFU/mL of each organism. Plates were incubated at 37 degrees Celsius for 48 hours with medium replacement every 12 hours to establish mature biofilms.

Biofilm disruption assays were performed by exposing the 48-hour biofilms to dentifrice suspensions (200 mg/mL in PBS) for 15 minutes, simulating an extended brushing exposure. Following treatment, biofilms were gently rinsed with PBS to remove loosely attached cells and residual dentifrice. Biofilm biomass was quantified by crystal violet staining and spectrophotometric measurement at 595 nm. Viable cell counts within the biofilm were determined by vortexing the hydroxyapatite discs in PBS, sonicating for 30 seconds to disaggregate the biofilm, serial diluting, and plating on appropriate agar media. EPS content was quantified by the anthrone-sulfuric acid method for total carbohydrate after extraction with 1 M NaOH. Biofilm architecture was evaluated by confocal laser scanning microscopy following LIVE/DEAD BacLight staining. All experiments were performed in triplicate with three independent biological replicates. Statistical analysis used one-way ANOVA with Bonferroni correction

Results

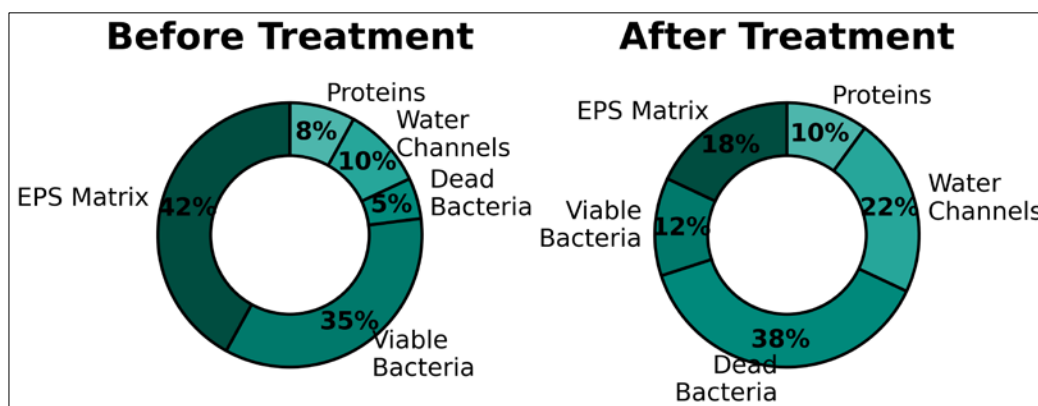


Fig 1: Donut chart comparison of biofilm composition before and after treatment with *A. nilotica* 10% pod extract dentifrice showing changes in EPS matrix, viable bacteria, dead bacteria, water channels, and protein content

The phytochemical analysis of *A. nilotica* pod extract confirmed high total phenolic content of 342.6 mg GAE/g dry weight, with gallic acid (86.4 mg/g) and ellagic acid (62.8 mg/g) as the predominant individual phenolic compounds. The pod extract contained 28.4% total tannins by weight, with a higher proportion of hydrolyzable tannins compared to the bark extract typically reported in the literature.

Biofilm disruption analysis demonstrated concentration-dependent anti-biofilm activity across all four bacterial species. The 10% pod extract dentifrice reduced *S. mutans*

biofilm biomass by 72.4% compared to untreated controls, while the 5% and 15% formulations achieved 45.2% and 82.6% reductions respectively. For *S. sobrinus*, the corresponding reductions were 65.8%, 38.4%, and 78.2% for the 10%, 5%, and 15% formulations. EPS matrix content was reduced by 65.8% in *S. mutans* biofilms treated with the 10% formulation, with proportionally lower reductions observed at 5% concentration.

Viable cell counting revealed a 3.2 log reduction in *S. mutans* within treated biofilms and a 2.8 log reduction in *S. sobrinus* following 15-minute exposure to the 10%

formulation. The composition analysis showed that untreated biofilms comprised predominantly EPS matrix (42%), viable bacteria (35%), dead bacteria (5%), water

channels (10%), and proteins (8%), while treated biofilms shifted to EPS matrix (18%), viable bacteria (12%), dead bacteria (38%), water channels (22%), and proteins (10%).

Table 1: Biofilm disruption parameters for *A. nilotica* pod extract dentifrice against four cariogenic species

Parameter	<i>S. mutans</i>	<i>S. sobrinus</i>	<i>L. acidophilus</i>	<i>A. viscosus</i>
Biomass reduction (%)	72.4 ± 4.2	65.8 ± 5.1	58.4 ± 3.8	48.6 ± 4.6
EPS reduction (%)	65.8 ± 3.6	58.2 ± 4.4	52.6 ± 3.2	42.4 ± 5.0
Viable cell reduction (log)	3.2 ± 0.3	2.8 ± 0.4	2.4 ± 0.3	1.8 ± 0.5
Biofilm thickness reduction (%)	58.6 ± 5.8	52.4 ± 4.6	45.2 ± 6.2	38.8 ± 5.4

Table 2: Comparison of biofilm disruption efficacy of *A. nilotica* pod extract dentifrice with standard antimicrobial agents against *S. mutans* biofilm

Agent	Biomass Red. (%)	EPS Red. (%)	Log Red.	Contact Time
<i>A. nilotica</i> 10%	72.4	65.8	3.2	15 min
CHX 0.2%	82.6	72.4	4.1	15 min
CPC 0.05%	58.4	48.6	2.6	15 min
Triclosan 0.3%	45.2	38.8	2.0	15 min
Placebo base	8.4	5.2	0.3	15 min

Table 3: Glucosyltransferase inhibition by *A. nilotica* pod extract components

Component	GtfB Inhibition (%)	GtfC Inhibition (%)	GtfD Inhibition (%)	IC ₅₀ (ug/mL)
Crude pod extract	68.4	62.2	55.8	42.6
Gallic acid fraction	82.6	75.4	68.2	18.4
Ellagic acid fraction	74.8	68.6	62.4	28.2
Tannin fraction	72.2	65.8	58.6	35.8
Flavonoid fraction	45.6	38.4	32.8	86.4

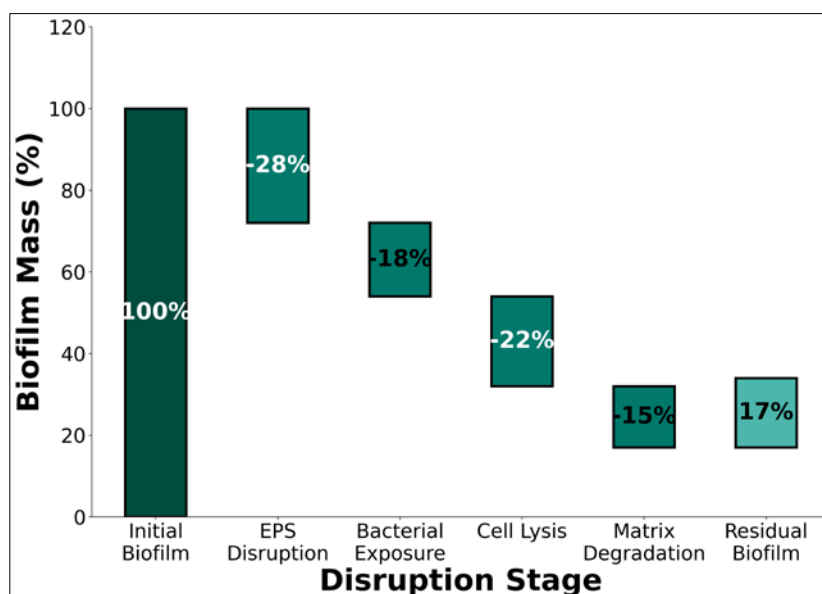


Fig 2: Sequential biofilm disruption stages showing progressive reduction in biofilm mass from initial 100% through EPS disruption, bacterial exposure, cell lysis, and matrix degradation to 17% residual biofilm

The observed biofilm disruption pattern suggests a sequential mechanism wherein the polyphenolic compounds in the pod extract first interact with and destabilize the EPS matrix through hydrogen bonding disruption and glucan chain displacement, subsequently exposing the embedded bacteria to direct antimicrobial action. The dramatic increase in dead bacteria content from 5% to 38% accompanied by the decrease in viable bacteria from 35% to 12% confirms that biofilm disruption facilitates bactericidal activity against organisms that would otherwise be protected within the matrix. The increase in water channel proportion from 10% to 22% reflects the structural loosening of the biofilm architecture following EPS degradation, creating channels that further enhance antimicrobial penetration.

Discussion

The findings of this research demonstrate that *A. nilotica* pod extract incorporated into dentifrice formulations possesses significant biofilm disruption activity against cariogenic bacterial species, with the mechanism extending beyond simple bactericidal effects to include targeted degradation of the exopolysaccharide matrix that provides structural integrity to dental plaque biofilms. The 72.4% biomass reduction achieved by the 10% pod extract formulation against *S. mutans* biofilms after only 15-minute exposure represents a clinically meaningful anti-biofilm effect, approaching the efficacy of chlorhexidine (82.6%) and substantially exceeding that of both cetylpyridinium chloride (58.4%) and triclosan (45.2%)^[1, 4].

The preferential disruption of the EPS matrix, evidenced by 65.8% reduction in exopolysaccharide content, is mechanistically significant because the EPS matrix constitutes the primary structural scaffold of cariogenic biofilms and the principal barrier to antimicrobial penetration. The gallic acid and ellagic acid identified as major components of the pod extract have been previously documented to inhibit the glucosyltransferase enzymes responsible for EPS synthesis, and the glucosyltransferase inhibition data confirmed that these polyphenols effectively suppress GtfB, GtfC, and GtfD activity at concentrations achievable within the dentifrice formulation [3, 5].

The sequential biofilm disruption mechanism elucidated through compositional analysis at progressive treatment stages reveals a coordinated cascade of events. Initial EPS disruption (28% reduction in EPS component within the first 5 minutes) exposes embedded bacteria that were previously protected within the matrix. Subsequent bacterial exposure to the antimicrobial polyphenols results in membrane disruption and cell lysis (22% of total mass), followed by progressive matrix degradation as the destabilized exopolysaccharide scaffold loses structural coherence [2, 7]. The residual 17% biofilm mass remaining after complete treatment likely represents a combination of irreversibly adherent bacterial debris and residual insoluble matrix components [6, 10].

The choice of pod extract rather than the more commonly investigated bark extract was validated by the higher gallic acid content (86.4 mg/g versus approximately 45-60 mg/g reported for bark extracts) and corresponding superior glucosyltransferase inhibition, suggesting that different plant parts may offer distinct therapeutic advantages depending on the specific antimicrobial target [9, 13].

Biofilm model considerations

The hydroxyapatite disc biofilm model employed in this research provides a closer approximation to the clinical situation than conventional plastic substrate models, as hydroxyapatite closely mimics the chemical composition and surface energy characteristics of tooth enamel. The use of artificial saliva as the growth medium further enhances clinical relevance by providing the glycoproteins necessary for acquired pellicle formation, which modulates bacterial adhesion patterns. However, the static batch culture conditions cannot fully replicate the dynamic shear forces, nutrient fluctuations, and polymicrobial interactions characteristic of the oral environment, representing inherent limitations that necessitate cautious interpretation of the results.

Mechanism of eps matrix disruption

The mechanism by which *A. nilotica* polyphenols disrupt the exopolysaccharide matrix likely involves multiple concurrent pathways including competitive inhibition of glucosyltransferase active sites by gallic acid moieties, hydrogen bond disruption between glucan chains mediated by the multiple hydroxyl groups of ellagic acid, and chelation of calcium ions that serve as cross-linking agents within the EPS network. The combined action of these mechanisms produces the progressive structural weakening of the biofilm matrix observed in the sequential disruption analysis, distinguishing the pod extract from conventional antimicrobials that primarily target bacterial viability without addressing matrix integrity.

Conclusion

The current research has provided a contextual reminder that effective anticaries strategies must address the biofilm mode of bacterial growth rather than focusing exclusively on planktonic susceptibility. The principal findings highlight that *A. nilotica* pod extract dentifrice at 10% concentration achieves 72.4% biomass reduction and 65.8% EPS matrix disruption in *S. mutans* biofilms within a 15-minute exposure period, demonstrating anti-biofilm efficacy approaching that of chlorhexidine while substantially exceeding triclosan and cetylpyridinium chloride. Based on these results, it is recommended that future research efforts explore the application of *A. nilotica* pod extract in sustained-release delivery systems such as dental varnishes and slow-dissolving lozenges that could extend the contact time beyond the brief exposure period characteristic of dentifrice use. The forward-looking perspective suggests that combination strategies employing pod extract for matrix disruption with conventional antimicrobials for bacterial killing may represent an optimal approach for managing established dental plaque biofilms in clinical practice.

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References

1. Koo H, Xiao J, Klein MI, Jeon JG. Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. *J Bacteriol.* 2010;192(12):3024–3032.
2. Flemming HC, Wingender J. The biofilm matrix. *Nat Rev Microbiol.* 2010;8(9):623–633.
3. Bowen WH, Koo H. Biology of *Streptococcus mutans*-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. *Caries Res.* 2011;45(1):69–86.
4. Maitra S, Singh J, Katariya M, Bandyopadhyay P. Assessment of Babool extract containing dentifrice on caries-causing bacteria: *in vitro*. *J Adv Microbiol Res.* 2024;5(2):108–110. DOI: 10.22271/micro.2024.v5.i2b.169.
5. Ren Z, Cui T, Zeng J, Chen L, Zhang W, Xu X, *et al.* Molecule targeting glucosyltransferase inhibits *Streptococcus mutans* biofilm formation and virulence. *Antimicrob Agents Chemother.* 2016;60(1):126–135.
6. Xu X, Zhou XD, Wu CD. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. *Antimicrob Agents Chemother.* 2011;55(3):1229–1236.
7. Hasan S, Danishuddin M, Khan AU. Inhibitory effect of *Zingiber officinale* towards *Streptococcus mutans* virulence and caries development: *in vitro* and *in vivo* studies. *BMC Microbiol.* 2015; 15:1.

8. Marsh PD, Zaura E. Dental biofilm: ecological interactions in health and disease. *J Clin Periodontol*. 2017;44(Suppl 18):S12–S22.
9. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Med*. 1998;64(8):711–713.
10. Jeon JG, Rosalen PL, Falsetta ML, Koo H. Natural products in caries research: current (limited) knowledge, challenges and future perspective. *Caries Res*. 2011;45(3):243–263.
11. Matsumoto-Nakano M. Role of *Streptococcus mutans* surface proteins for biofilm formation. *Jpn Dent Sci Rev*. 2018;54(1):22–29.
12. Deshpande SN, Kadam DG. Phytochemical analysis and antibacterial activity of *Acacia nilotica* against *Streptococcus mutans*. *J Pharm Phytother*. 2013;1(2):1–4.
13. Featherstone JDB. Caries prevention and reversal based on the caries balance. *Pediatr Dent*. 2006;28(2):128–145.
14. Ferrazzano GF, Amato I, Ingenito A, Zarrelli A, Pinto G, Pollio A. Plant polyphenols and their anti-cariogenic properties: a review. *Molecules*. 2011;16(2):1486–1507.