
1.0 INTRODUCTION

Oro-dental health has emerged as an integral part of overall health, significantly influencing systemic well-being and quality of life (Buckner et al. 2018). Dental caries, a pervasive issue across all age groups, results from an intricate balance of risk and protective factors, including environmental, genetic, and microbiological influences, which regulate the onset, progression, and severity. According to Centers for Disease Control and Prevention data (2024), 90% of adults develop cavities by age 34, 57% of adolescents (12-19 yrs), 52% of children (6-8 yrs), and 11% of young children (2-5 yrs) have untreated caries. Untreated cavities impact daily life by causing toothache, chewing difficulties, and disruptions, emphasizing the need for proactive oral hygiene maintenance. Excessive sugar consumption disrupts oral microbiota balance, promoting acidogenic bacteria, plaque formation, and dental biofilm harmony, leading to dental caries. The ecological plaque hypothesis links this imbalance to cariogenic bacterial dominance, acid production, and tooth demineralization (Pitts et al. 2017). Dental plaque, a complex microbial biofilm, is recognized as the primary factor in the development and progression of caries and periodontal diseases (Motallaei et al. 2021).

The oral cavity hosts a diverse mix of around 700-1000 bacterial species. Several microorganisms, including *Streptococcus sanguis*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, *Candida albicans*, *Actinomyces viscosus*, *Actinomyces naeslundii*, and *Enterococcus faecalis*, contribute to tooth decay. *Streptococcus mutans* is considered the primary pathogen responsible for dental caries due to its potent acid production and ability to adhere to tooth surfaces (Kara & Ipek, 2024; Brito et al. 2021; T. Chen et al. 2020). Dental caries is primarily attributed to *Streptococcus mutans*, a Gram-positive coccus that dominates the dental plaque's microbial community. *S. mutans* promotes disease progression through the colonization and metabolization of fermentable carbohydrates, ultimately leading to enamel degradation and cavitation (Hamad et al. 2023). *S. mutans* produces glucosyltransferases (GTFs), key enzymes in

dental caries development that break down sucrose into glucose and fructose, synthesizing water-soluble and insoluble glucans. Glucan-binding proteins (Gbp) facilitate biofilm formation and tooth surface adhesion, enabling *S. mutans* colonization. This synergistic action of *S. mutans* dissolves dental tissues, induces cavitation, and generates an acidic environment that allows tooth mineral and protein degradation, making it a primary culprit in dental caries formation (Jakubovics et al. 2021).

Damage to the oral mucosa enables the entry of oral pathogenic microorganisms into the bloodstream, increasing the risk of cardiovascular disease with elevated antibody levels (Mensch et al. 2019). Understanding the molecular mechanisms and virulence factors of *S. mutans* is crucial for developing novel antimicrobial therapies for oral infections, improving oral health, and preventing cardiovascular disease risk. The use of antibiotics such as ampicillin, chlorhexidine, erythromycin, spiramycin, and vancomycin effectively prevents dental caries. Still, their application is compromised by potential adverse effects, including antimicrobial resistance, gastrointestinal disturbances, and other unforeseen side effects (Jarvinen et al. 1993). Frequent administration of certain antibiotics fosters the evolution of multidrug-resistant (MDR) bacterial strains (Karam et al. 2016). Research on the antimicrobial properties of plant extracts has shown promising results in inhibiting biofilm formation by oral pathogens. The growing interest in herbal medicine has prompted scientists to explore plant extracts as therapeutic agents to control oral diseases, successfully isolating antimicrobial compounds from plants that inhibit harmful dental bacteria (Lobo et al. 2014; Rivero-Cruz et al. 2020). Traditional plants and their byproducts have been utilized for years to treat oral diseases, showing considerable effect against caries-causing microorganisms (Ernst, 2007; Kalemba & Kunicka, 2003). The effectiveness of the secondary metabolites of plants with antimicrobial properties depends on concentration and molecular interactions (Dzotam & Kuete, 2017).

Phytochemical research begins with screening herbs for antimicrobial potential against oral pathogens and identifying the natural antimicrobials. The effectiveness of these potential agents was assessed by inhibitory zone diameter,

minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and biofilm inhibitory concentration (BIC). These parameters collectively measure the antimicrobial activity, bacterial killing potential, and biofilm prevention capabilities, providing valuable insights into their therapeutic potential. Various plant extracts and phytochemicals exhibit antimicrobial activity against *S. mutans*, effectively inhibiting growth, and disrupting biofilm, acid production, and glucosyltransferase activity. They inhibit glucosyltransferases (GTFs), enzymes responsible for dental biofilm formation, bacterial adhesion, and accumulation. Research has consistently demonstrated that phytochemicals from plants such as *Camelia sinensis* (Xu et al. 2012), Cranberry juice (Yamanaka et al. 2004), *Verbascum speciosum* (Pourmoslemi et al. 2023), *Punica granatum* (Radmand et al. 2024), *Terminalia chebula*, *Psidium guajava*, *Azadirachta indica*, *Pongamia pinnata*, *Syzygium aromaticum*, and *Mentha piperita* (Mandava et al. 2019), *Ocimum sanctum*, *Tinospora cordifolia*, and *Glycyrrhiza glabra* (Yadav et al. 2020) exhibited anti-GTF activity. These plant-derived compounds disrupt glucan synthesis, biofilm formation, and GTF enzyme activity, preventing cariogenic bacteria adherence. Anti-GTF efficacy of medicinal plants, is correlated with polyphenolic, flavonoid, and terpenoid content, which interact with GTFs to reduce activity (Taheri et al. 2011). Thus, medicinal plants offer a promising natural strategy for preventing dental caries and promoting oral well-being, specifically targeting *S. mutans* related oral diseases.

Research has shown that dental plaque can be effectively controlled through physical removal and pharmaceutical formulations, including antimicrobial toothpastes and mouthwashes. Herbal formulations offer a promising alternative to synthetic oral care products, providing natural antimicrobial, anti-inflammatory, and antioxidant properties. These plant-based remedies inhibit bacterial growth, reduce plaque, and prevent enamel demineralization, thereby combating dental caries. Furthermore, herbal formulations are cost-effective, non-toxic, and eco-friendly, addressing concerns about chemical ingredients. By harnessing traditional knowledge and scientific validation, herbal formulations can provide an accessible, sustainable solution for oral health care, particularly in underserved communities. In an attempt to reduce costs and risks while enhancing drug

development, pharmaceutical industries increasingly rely on *in-silico* approaches. Molecular docking analysis predicts the binding interactions between a receptor molecule complex with binding energy expressed in ΔG indicating the affinity between the two molecules (Pinzi & Rastelli, 2019). Phytochemical analysis has identified various bioactive compounds with potential GTF inhibition. However, the molecular basis of phytochemical-enzyme interactions remains unclear. Integrating phytochemical analysis and molecular docking simulations facilitates the prediction of protein-ligand interactions enables the discovery of novel plant-derived GTF inhibitors, and elucidates the interaction mechanisms. This integrated approach provides a rational framework for developing natural, effective, and safe oral care products.

Dental caries complexity renders ineffective traditional pharmacological interventions, resulting in low drug development success rates. Systems, network medicine, and pharmacology are transforming the field by offering novel insights into disease mechanisms and enabling innovative diagnostic, therapeutic, and curative strategies (Nogales et al. 2022). Network pharmacology integrates pharmacology, bioinformatics, and molecular studies to analyze complex interactions between bioactive compounds and human biology, elucidating underlying mechanisms of action and relationships between compounds, proteins, and diseases (Nogales et al. 2022; Jiashuo et al. 2022). Although the anti-cariogenic effects of phytochemicals are well-documented, their underlying mechanisms remain unclear. The current research confirms the anti-cariogenic potential of the developed oral care product by identifying key bioactive compounds, predicting potential targets and pathways, and constructing a network model. By analyzing compound and biological process interactions, traditional plant-based oral remedies were scientifically validated, identifying potent inhibitors of *Streptococcus mutans* and designing a novel polyherbal dentifrice with evidenced-based anti-cariogenic properties. These findings pave the way for developing a natural, effective, and safe oral care product, providing a novel approach for validating its anti-cariogenic properties.

Hypothesis

The hypothesis set up for the present study is,

Null Hypothesis

The polyherbal dentifrice has no significant effect on the growth or viability of oral pathogens compared to a control group.

Alternative Hypothesis

The polyherbal dentifrice significantly inhibits the growth or viability of oral pathogens compared to a control group.

The following objectives were established to support the alternative hypothesis,

- To assess the phytochemical and antioxidant properties of traditionally used medicinal plants for dental caries.
- To evaluate the antimicrobial effectiveness of the selected plant extracts against clinical strains of dental plaque-causing pathogens.
- To identify the potential glucosyltransferase-C inhibitors from the plant extracts against *S. mutans* using molecular docking.
- To develop and evaluate a novel Polyherbal Dentifrice (PHDF) for dental caries prevention.
- To assess the *in vitro* antimicrobial efficacy of the formulated PHDF against microorganisms associated with dental caries.
- To validate the anti-cariogenic effectiveness of PHDF against *S. mutans* to determine targets and mechanisms through network pharmacology.