5.0 SUMMARY AND CONCLUSION

Research on oral microbial flora colonization indicates that certain microbiome species decompose dietary polysaccharides, generating organic acids that irreversibly solubilize enamel and dentin minerals. This process leads to the formation of bacterial biofilms and dental plaque, which ultimately causes tooth decay due to an imbalance between acid production and salivary buffering capacities. Tooth decay is a widespread oral health issue, affecting approximately 2.3 billion adults and 530 million children worldwide. If left untreated, it progresses into cavities, causing pain and sensitivity to sweets, heat, and cold. Although the plaque contains diverse microbial species, *Streptococcus mutans*, a Gram-positive primary bacterium responsible for dental caries, rapidly metabolizes and produces large amounts of glucosyltransferases (GTFs), greatly aiding adhesion to tooth surfaces. Among the Gtfs inhibitors, natural products have been recognized as a valuable source of therapeutic medicines.

Fluoride and chlorhexidine are widely recognized as the most effective antiplaque agents, inhibiting cariogenic activity by disrupting the endoplasmic membranes of various microorganisms, including bacteria and fungi. However, their use can be accompanied by additional complications such as altered taste, tartar development, mucosal irritation, tooth damage, disruption of the oral microbiome, genotoxicity, and cell apoptosis. Despite natural products promising to prevent dental caries, their development is hindered by several challenges that include a lack of standardized procedures, isolation of pure chemical compounds, elucidation of modes of action, and unknown interactions between compounds and Gtfs active sites. Recent advancements in technology and methodology have facilitated the development of novel anticaries therapies targeting glucosyltransferases, offering a promising solution to overcome the limitations of traditional antiplaque agents. Medicinal plants with anti-cariogenic qualities offer a safe and low-risk alternative with negligible adverse effects compared to artificial chemicals. By inhibiting bacterial growth and biofilm formation, plant-derived compounds can fight against antimicrobial resistance. These compounds not only prevent dental cavities but also support oral health by managing conditions like gingivitis and periodontitis through their anti-inflammatory and antioxidant qualities to protect oral tissues. In addition to inhibiting biofilm growth and plaque formation, they also help the remineralization of tooth enamel. They exhibit various mechanisms to suppress *S. mutans*, including antimicrobial, anti-adhesive, biofilm disruption, and enzyme inhibition activities. Furthermore, certain benign antimicrobial substances have demonstrated efficacy in controlling the growth of *S. mutans* by fostering the emergence of symbiotic bacteria, potentially contributing to the long-term management of dental caries. These substances exhibit strong antimicrobial properties, excellent selectivity, and low toxicity, as reported in recent studies.

In resource-constrained communities, where access to synthetic antimicrobials is hard to come by, the incorporation of medicinal plants into oral healthcare presents a viable, affordable, and approachable solution to prevent dental caries. Herbal remedies are easily available, particularly in rural areas, and are simple to integrate into daily oral care routines. Additionally, they support eco-friendly dental care, making them an attractive option. However, further research is required to standardize herbal extracts and formulations, elucidate the synergistic effects of phytochemicals, and establish their safety and efficacy through clinical trials. Thus, we can create sustainable, natural, and effective dental healthcare solutions that benefit human and environmental well-being. Thus, the present study on **'*Unveiling the Anticariogenic Properties of Medicinal Plants and Development of a Polyherbal Dentifrice*'** aimed to scientifically validate traditional plant-based oral remedies, identify potent inhibitors of *Streptococcus mutans,* and create a novel polyherbal dentifrice with evidence-based anti-cariogenic properties.

The study was carried out in five phases. In the first phase, based on the ethnobotanical information gathered on the traditional medicinal plants used by the Urali tribal people from Thottakombai Hill in the Anthiyur region of Erode district, Tamil Nadu, to treat dental caries, the following plant materials such as *Achyranthes aspera* root (AAR), *Acalypha indica* leaf (AIL), *Azadirachta indica* leaf (AZL), *Abrus precatorius* leaf (APL), *Barleria cuspidata* leaf (BCL), *Euphorbia* *hirta* leaf (EHL), *Ficus benghalensis* prop root (FBP), *Piper betle* leaf (PBL), *Psidium guajava* leaf (PGL), *Pongamia pinnata* leaf (PPL), *Tridax procumbens* leaf (TPL), and *Solanum virginianum* fruit (SVF) were subjected to methanol extraction and evaluated for their potential anticariogenic effects. Phytochemical analysis of the plant extracts revealed a rich and diverse profile, comprising a diverse range of phytochemicals. The phytochemical results showed that EHL, PBL, and PGL were rich in alkaloids, flavonoids, tannins, terpenoids, phenolics, and vitamin C. Quantitative estimation also demonstrated the highest concentrations of alkaloids and terpenoids in TPL, ascorbic acid in EHL, TPL, and BCL, phenolic compounds in PGL, AAR, PPL, and SVF, and flavonoid contents in PBL, PGL, and SVF. Additionally, the plant extracts PGL, SVF, BCL, FBP, and EHL exhibited the strongest DPPH radical scavenging activity, with IC_{50} values of 6.628±6.601, 14.09±5.08, 38.94±0.785, 47.14±20.02, and 55.23±2.210µg/mL, respectively.

The antibacterial screening of the polyherbal crude extracts was performed against various oral pathogens, including *Streptococcus mutans* (SMU), *Streptococcus salivarius* (SSA), *Streptococcus oralis* (SOS), *Streptococcus parasanguinis* (SPSA), *Klebsiella pneumoniae* (KP), *Pseudomonas aeruginosa* (PA), *Acinetobacter baumannii* (AB), and *Candida albicans* (CA). The study revealed that extracts AAR, EHL, PGL, SVF, BCL, FBP, and PBL exhibited statistically significant ($p<0.0001$) inhibitory activity against *S. mutans*. The extracts demonstrated varying degrees of susceptibility against Gram-positive and Gram-negative bacteria and fungi, with notable biofilm inhibition by EHL, SVF, and AAR (90-94% against Gram-positive bacteria) and BCL, EHL, FBP, and PGL (86.2-93.6% against Gram-negative bacteria). Additionally, EHL and BCL revealed promising inhibition of 84.5% and 81.2%, significantly ($p<0.001$) against CA.

In the second phase, the docking studies and molecular dynamics simulations (MDS) were conducted to evaluate the potential of major bioactive compounds from six potential plants (AAR, BCL, EHL, FBP, PGL, SVF) in inhibiting gtfC protein, associated with SMU in dental caries. Initially, 508 bioactive compounds were retrieved and 78 compounds were selected based on their drug-likeness (DL) score for docking studies. The docking analysis revealed that PGL compounds, specifically guavin A, stachyurin, and guavin C, exhibited strong binding affinity to GtfC, with binding scores of -14.534, -13.44, and -13.388Kcal/mol, respectively, indicating potential inhibitory activity against *S. mutans*. Other notable compounds with significant binding affinity to GtfC, included guavin D, amritoside, casuaritcin, so strictinin, guavin B, procyanidin B3, tellimagrandin-1, quercetin-3-O-gentiobioside, procyanidin B1, strictinin, procyanidin B2, leucocyanidin, and goreishic acid. Guavin A from PGL exhibited stability for 80ns during a 100ns MD simulation, indicating a strong and stable binding interaction. Guavin A formed ionic bonds, hydrogen bonds, and hydrophobic interactions with critical amino acids in the GtfC, indicating its strong potential to inhibit SMU and prevent dental caries. These findings identified guavin A as a promising candidate for further exploration in dental caries prevention through GtfC inhibition.

In the third phase, a randomized factorial design using MODDE software successfully identified optimal combinations of six plant extracts (AAR, BCL, EHL, FBP, PGL, and SVF) with high antimicrobial efficacy. The PLS model was constructed with an R^2 value of 0.945 indicating a strong fit between the data and the model. The optimized combinations exhibited low MIC values (0.3125 mg/ml) against SMU and showed a notable synergistic effect, enhancing the collective antimicrobial efficacy of the plant extracts and used in the development of PHDF formulation. The formulated PHDF, toothpowder tablets exhibited good mechanical strength, and minimal hardness and were found to be safe, and free from heavy metals like arsenic, cadmium, lead, and mercury. Atomic Absorption Spectrometric analysis revealed the presence of essential minerals like calcium, potassium, and magnesium contributing to oral hygiene maintenance. Additionally, toxicity tests indicated relatively less toxic, with a 17% mortality at 1.5mg/ml concentration. The tablets were designed to release active ingredients upon chewing, providing an effective way to maintain oral hygiene through brushing.

The fourth phase of the study demonstrated the anti-cariogenic efficacy of PHDF, revealing significant inhibition zones against the tested clinical isolates, indicating strong antibacterial activity. PHDF achieved substantial inhibition zones against Gram-positive, and Gram-negative isolates as well as the fungal pathogen *C. albicans*. The MIC and MBC/MFC values ranged from 0.3125 to 1.25 mg/mL, and 0.625 to 2.5 mg/mL, respectively, demonstrating effective prevention of bacterial growth. Furthermore, the PHDF significantly decreased the hydrophobicity index of *S. mutans,* hindered acid production, and significantly inhibited biofilm formation. The biofilm morphological changes observed by SEM analysis revealed significant biofilm morphological changes, with the control sample showing biofilm aggregation, whereas treatment with NaF and PHDF resulted in disruption of bacterial biofilms, indicating effective antibiofilm activity. Overall, the formulated polyherbal toothpowder tablets emerged as a better alternative to toothpaste, demonstrating greater efficacy against major cariogenic microbes compared to marketed products, indicating superior performance in preventing dental caries.

To further investigate the mechanisms underlying this efficacy, a network pharmacology approach was employed in the final phase. The analysis identified 9 key targets related to dental caries and revealed a complex network of interactions between 78 active compounds and these targets. The results also highlighted the synergistic interactions between compounds, such as oleanolic acid, maslinic acid, asiatic acid, pyridoxine, procyanidin B2, quercetin, catechin, kaempferol, ergosterol acetate, and putative targets of SMU. Thus, these findings provided valuable insights into the molecular mechanisms underlying the polyherbal tooth powder tablets antimicrobial efficacy, highlighting their potential as an effective treatment for dental caries.

This research highlighted the importance of integrating traditional knowledge with modern scientific approaches to develop effective, natural, and environment friendly solutions for oral health care. The medicinal plants utilized in this research exhibited a rich phytochemical profile, demonstrating strong effectiveness against cavity-causing microbes. The polyherbal tooth powder tablets employed a multifaceted approach, targeting multiple pathways and mechanisms involved in dental caries and likely to provide substantial protection against cavities and related conditions.

Recommendations for further studies

- *In vitro* studies, including enzyme inhibition assays, cell line studies, and gene expression profiling, can be conducted to assess the interactions between PHDF's bioactive compounds and targets associated with various diseases beyond dental caries.
- Preclinical trials can be assessed to evaluate the efficacy and safety of the PHDF in animal models for diseases other than dental caries.
- Clinical trials can be undertaken to evaluate the therapeutic potential, efficacy, and safety of PHDF in human subjects for diseases beyond dental caries.
- Development of different dentifrice formulations based on the proposed PHDF formula, investigating variations in composition and delivery methods, textures, and sensory characteristics.