UNIVERSITI TEKNOLOGI MARA

MODULATORY EFFECTS OF BROMELAIN ON DUAL-SPECIES ORAL BIOFILM FORMATION AND QUORUM SENSING–ASSOCIATED INFLAMMATORY RESPONSES IN HUMAN CELL LINES

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ABSTRACT

Dental caries and periodontitis are driven by QS-regulated oral biofilms formed by *Streptococcus mutans* (*S. mutans*) and *Staphylococcus aureus* (*S. aureus*), which promote inflammation via NF-κB and TRPV1 signaling, Bromelain, a proteolytic enzyme derived from pineapple, has been reported to possess antibiofilm, QS-inhibitory, and anti-inflammatory properties, making it a promising natural therapeutic candidate. To date, no studies have examined the dual role of bromelain in disrupting oral biofilm formation and QS while simultaneously modulating TRPV1-mediated inflammatory responses in human cell models. Thus, this study aimed to evaluate the effects of ultrafiltered bromelain (UFB) on dual-species oral biofilm formation, QS regulation, and biofilm-induced inflammatory responses in human cell lines. Bromelain was extracted, purified, and characterized by SDS-PAGE, HPLC, Bradford assay and UV–Vis spectrophotometry. Dual-species biofilms of *S. mutans* and *S. aureus* were developed and validated using crystal violet staining, colony counting, and scanning electron microscopy (SEM). The co-culture of dual-species biofilms with HaCaT and HGF cells was established to examine cytotoxicity and inflammatory responses. Biofilm inhibition and disruption were assessed by crystal violet assays, microscopy, and SEM, while QS modulation was evaluated by qPCR targeting *comC, comDE,* and *gtfC* in *S. mutans*, and *agrD, agrA, agrC,* and *RNAIII* in *S. aureus*. Gene expression of IL-6, NF-κB, and TRPV1 in biofilm-induced HaCaT cells was also analyzed. Characterization confirmed the presence of bromelain with high purity and protein integrity, while model validation established successful development of dual-species biofilms and biofilm-cell co-culture system. Results demonstrated that UFB significantly inhibited mono- and dual-species biofilm formation in a dose-dependent manner and disrupted mature biofilm architecture. Treatment with UFB also significantly inhibit QS-related gene expression in both species, indicating interference with bacterial communication systems. Cytotoxicity assays confirmed that UFB exhibited no harmful effects on HGF and HaCaT cells at concentrations less than 250 µg/mL. Importantly, in the dual-species biofilm, UFB significantly downregulated *comC, comDE,* and *gtfC* in *S. mutans*, and *agrD, agrA, agrC,* and *RNAIII* in *S. aureus*, proving that UFB potentially supressed biofilm formation via the QS pathway. UFB also significantly downregulated IL-6 and TRPV1 expression in biofilm-induced cells, with TRPV1 suppression likely reducing calcium influx and thereby attenuating downstream NF-κB activation. Collectively, these findings highlight the dual antibiofilm and anti-inflammatory actions of bromelain, mediated through structural degradation of biofilms, suppression of QS-regulated virulence, and attenuation of host inflammatory signaling. This study also proposes that TRPV1 serves a dual of not only being a catalyst for inflammation but also as receptors for QS molecules. This study provides novel evidence supporting bromelain as a potential therapeutic agent for oral biofilm-associated diseases.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Periodontal disease is a largely preventable chronic condition that continues to affect a significant proportion of the global population. Globally, more than 50% of suffered from periodontitis (Relvas et al., 2022), with prevalence increasing markedly among older adult (Nazir et al., 2020). Key contributing factors include inadequate oral hygiene practices, insufficient government funding for oral health services, limited oral health promotion initiatives, and a lack of policies tailored to the needs of the senior population (Chávez et al., 2022). Additionally, aging contributes to the severity of periodontal diseases as it alters the quality of antibodies in our immune response (Ebersole et al., 2018). In Malaysia, the economic burden of managing non-surgical periodontal disease management is substantial and estimated at MYR 696 million, reflecting to both its high prevalence and cost of treatment (Anuwar et al., 2024).

Dental caries and periodontitis are two distinct diseases driven by the formation and persistence of microbial biofilms on tooth surfaces (Rösing et al., 2023). Dental caries is characterized by localized destruction of dental hard tissue caused by cariogenic bacteria, such as *Streptococcus mutans (S. mutans)* and *Staphylococcus aureus (S. aureus)*, in the presence of fermentable carbohydrates (Al-Shahrani, 2019). Periodontal disease encompasses inflammatory conditions of gingiva, periodontal ligament and alveolar bone that triggered by microbial colonization within subgingival plaque. While dental caries primarily results from acid-mediated demineralization, periodontal disease is largely driven by the host immune response to bacterial toxins, leading to chronic inflammation and tissue destruction (Mann et al., 2020). Both conditions impair oral function and are increasingly linked to systemic diseases, including diabetes and cardiovascular disorders. For instance, evidence from the NHANES-III analysis indicates that individuals with periodontitis had a 66% increased chance of getting diabetes than those without, with the likelihood rising further in severe cases of periodontitis (Mehriz et al., 2022).