CHAPTER I

INTRODUCTION

1.1 Background

Oral disease is a major public health concern covering chronic clinical conditions of the teeth and mouth. Oral problems tend to be left untreated due to high treatment costs, which may lead to pain, sepsis, and poor life quality (Peres *et al.*, 2019). The Global Burden of Diseases, Injuries, and Risk Factors Study 2017 reported that around 3.47 billion people in the world suffer from oral health problems, with dental caries being the most common condition.

Dental caries is defined as a biofilm-mediated disease that is often related with frequent consumption of sugar and low environmental pH (Bhagavathy *et al.*, 2019). Early biofilm development is facilitated by the binding of oral microorganisms to salivary pellicle layer on the enamel surface. Dental biofilm microorganisms will then metabolize fermentable sugars obtained from dietary intake into organic acids, mainly lactic acid, which lowers the biofilm pH and causes enamel demineralization. Early stage of caries can be arrested by the help of fluoride agents and buffering capacity of the saliva that promote remineralization process. However, in the case of poor oral hygiene, the structure of the biofilm may undergo maturation due to production of bacterial exopolysaccharides and accumulation of food-derived polysaccharides, leading to a more complex bacterial aggregation and greater acid production in the biofilm. Prolonged fall of oral biofilm pH will enhance the demineralization reaction resulting in a more porous and softer tooth surface which allow acids to penetrate and destroy deeper tooth

layers. Furthermore, dental caries will appear clinically as white spots after substantial loss of minerals, and it may progress into black stain, cavitation, infection, and even tooth loss if not properly treated (Pitts *et al.*, 2017).

Streptococcus mutans is the main causative bacterium for caries initiation and progression due its ability to integrate into dental tissues and metabolize fermentable carbohydrates within a low pH condition of 4.5 to 5.5 (Dianawati *et al.*, 2020; Suratri *et al.*, 2017). This is in accordance with Rivera-Quiroga *et al.* (2020) where acid-producing and acid-tolerant nature of *S. mutans* helps the pathogen to outcompete other oral microorganisms and dominate oral adhesion binding sites in early caries stage. Moreover, *S. mutans* synthesizes important enzyme and various surface proteins, such as glucan-binding protein C and antigen I/II, which promote dental plaque development and caries progression (Rivera-Quiroga *et al.*, 2020).

Management of dental caries is often proposed to involve the use of antimicrobial agents such as antiseptics and antibiotics. However, exposure to those synthetic compounds must be limited as the use of antiseptics, namely chlorhexidine, has been known to exhibit undesirable side effects including tongue staining, taste interference and burning sensation, while the use of antibiotics is reported to be ineffective over time due to the emergence of resistant bacterial strains (Deshpande and Kadam, 2013; Syahidah *et al.,* 2017). These drawbacks have drove a shift in interest towards discovery of anticariogenic compounds from natural sources. Natural anticariogenic agent is viewed as safe and eco-friendly, therefore it can be incorporated in healthy food products to help prevent caries initiation and progression.

Betel (*Piper betle* L.) is an herbal plant widely cultivated in Southeast Asia with various medicinal properties (Deshpande and Kadam, 2013). Consumption of betel leaves by chewing has become a traditional habit in Indonesia that is believed to prevent halitosis and improve dental care (Mahoklory *et al.*, 2019). Furthermore, betel leaves have been reported to contain secondary metabolites such as saponin, tannin, flavonoid, terpenes, polyphenols, and steroids which act as antimicrobials (Vifta *et al.*, 2017). Correspondingly, previous *in vitro* studies have demonstrated the inhibitory effects of betel leaves extracts on *S. mutans* growth and biofilm formation, hence indicating the potential of betel leaves as an alternative in dental caries treatment. However, up to now, the incorporation of betel leaves in food products as anticariogenic agent is still limited. This might be caused by the lack of knowledge regarding the mechanism of betel leaves as anticariogenic agent, specifically against *S. mutans*.

Molecular docking is an optimization study which could determine the most suitable orientation in binding of ligands and proteins. The method is widely used as it can predict the binding affinity and interaction between bioactive compounds and target proteins (Vijesh *et al.*, 2013). Furthermore, molecular docking is an *in silico* study that relies on computational technique hence efficient in terms of time, energy and cost compared to the conventional method (Suhud, 2015). Molecular docking study of active compounds from betel (*Piper betle* L.) leaves as inhibitors against *Streptococcus mutans* may provide better understanding on the mechanism of betel leaves as anticariogenic agent hence support the incorporation of betel leaves in food products.

1.2 Research Problem

Previous *in vitro* studies conducted on betel (*Piper betle* L.) leaves have demonstrated the inhibitory effect of betel leaves extracts against growth and biofilm formation by *S. mutans*. Betel leaves therefore exhibit potential as alternatives for dental caries prevention and treatment. However, the incorporation of betel leaves as anticariogenic agent in food product is still limited, which might be caused by the lack of understanding on the anticariogenic mechanism of betel leaves active compounds, specifically against *S. mutans*. Therefore, *in silico* study must be conducted through molecular docking analysis to provide further insights on the mechanism of betel leaves in inhibiting target proteins from *S. mutans*, which include the binding affinity and visualization of protein and ligand interaction.

1.3 Objectives

1.3.1 General Objective

The general objective of this research was to conduct *in silico* study of betel (*Piper betle* L.) leaves active compounds as inhibitors against *Streptococcus mutans*.

1.3.2 Specific Objectives

The specific objectives of this research were:

1. To determine the binding affinity of ligands from betel leaves ethanolic extract toward proteins from *S. mutans,* particularly glucan-binding protein C and antigen I/II.
2. To comprehend the mechanism of interaction of ligands and target proteins through visualization of the intermolecular structure.