CHAPTER I

INTRODUCTION

1.1 Introduction

Dental caries, also known as tooth decay, has become one of the most common diseases in both adults and children. It is a process that involves the balance of mineral loss in the teeth and its replacement over time as a reaction to the acid attack caused by food consumption. As time passes, changes occur in most aspects of life, including nutrition and in recent years, we can see a "craze" in modern food and drinks, particularly among young people. All these foods and beverages have a very high sugar content and are considered "excessive" by public health experts (Di Rienzi et al., 2020). We all know that toothache is triggered by the frequency and amount of carbohydrates and sugar consumed. It begins when *Streptococcus mutans*, the starter bacteria in the teeth, converts sugar to acid and forms dental plaque. Of course, the presence of pathogens has a negative impact on the host over time (McLaren & Callahan, 2020). This situation has become more difficult as commonly used antibiotics and chemotherapeutics, such as penicillin, cephalosporin, erythromycin and tetracycline, have become less effective at killing oral bacteria (González-Bello et al., 2019). Additionally, Ngangom et al. (2019) have shown how prolonged exposure to antibiotics might result in serious safety problems, such as the emergence of microbes that are resistant to them. As a result, these bacteria have the opportunity to survive, evolve and reproduce. Additionally, mouthwash, a commonly used item that rapidly destroys bacteria and refreshes the mouth, is likely to increase the incidence of oral cancer, particularly for smokers and drinkers (Ustrell-Borràs et al., 2020). Tobacco and alcohol have higher acetaldehyde concentrations. An extensive epidemiological study concluded that naturally occurring acetaldehyde, in addition to ethanol-derived acetaldehyde, poses a cancer risk for alcoholic beverage drinkers (Linderborg, 2020; Reddy et al., 2022). While the use of alcohol-based mouthwash causes an increase in salivary acetaldehyde levels immediately following ingestion. For this reason, researchers found that mouthwash combined with other risk factors like smoking or alcohol increases cancer risk. As a result, many researchers have begun to show a lot of interest in developing an organic medicine that is safer and offers effective plaque control with no or minimal side effects.

Acmella paniculata, also a synonym species to *Spilanthes Acmella* (National Parks, 2022) a common name used in most research has been taken as a traditional medicine for a variety of ailments including rheumatism, fever, diuretics, flu, toothaches, and periodontitis. According to previous research, this plant has analgesic, cytotoxic, antimicrobial, and antiplaque properties (Ali et al., 2015). The main constituents thought to be responsible for the pharmacological activity in *Acmella* species are alkyl ketones, alkamides, hydrocarbons, acetylenes, lactones, alkaloids, terpenoids, flavonoids, and coumarins (Kumar et al., 2021).

Despite being thought to be an anti-toothache plant, scientific evidence of its antimicrobial activity has yet to be reported. As a result, the aim of this study was to use an antimicrobial assay and transcriptomic analysis to investigate the biological activity of *A. paniculata* extracts against *S. mutans*.

1.2 Background of the Study

Dental caries, also called tooth decay is the most common chronic childhood disease and individuals will remain vulnerable to this disease throughout their lives (Peres et al., 2019). This disease is defined by the demineralization or weakening of the teeth, which leads to a caries lesion. An advanced caries lesion can progress to the point where the tooth surface forms a cavitation or a hole. This is the physical evidence of the tooth breakdown.

The tooth can be roughly divided into a few parts which are the root that sits within the alveolus, the neck which is the transition between the root portion covered by bone in the crown and the crown itself. The crown is the visible part of the tooth that protrudes from the gums and it is covered in enamel. Enamel has such a high mineral content, that 95% – 98% of it is calcium and phosphate ions that make up strong hydroxyapatite, which is considered to be the hardest substance in the human body (Akkus et al., 2016). Enamel mineralization is an important asset that positively correlates with the mechanical behaviour of other tissues such as bone and teeth (Hughes et al., 2019; Němec et al., 2019). Therefore, it is one of the important factors that affect bone strength and the healing of bone defects and fractures, which, if they occur, will cause the onset of dental problems. In fact, enamel serves as the protective layer for the inner sides of teeth against acids, bacteria and other harmful substances that would threaten the health of the tooth. Hence, it is important to maintain the mineral levels to ensure the enamel stays healthy (Neel et al., 2016; Gharibzahedi et al., 2017). Thus, there comes the saliva, which is secreted from several glands in the mouth and has bicarbonate ions that help neutralise acid in the mouth (Singh et al., 2017). Saliva also has calcium and phosphate which serve as replacement minerals to help remineralize the tooth surface (Liang et al., 2018).

When it comes to dental caries, there is a conflict between the pathogenic factors that promote tooth demineralization and the protective factors that promote tooth remineralization. That includes the individuals that have had a prior or current carries lesion (Al-Shahrani & Research, 2019), have problems with saliva production (Pedersen et al., 2018), have specific abnormal levels of acid-forming bacteria in their mouths (such as *Streptococcus mutans* and *Lactobacillus*) and those that have poor dietary habits like frequent snacking as well as a high sugar diet (Punitha et al., 2015). However, those problems eventually contribute to the response of existing live bacteria around the tooth area, especially *S. mutans* which is known as the starter bacteria that initiate the formation of dental caries. To address this issue, most treatment is based on the level of risk, or, more precisely, on reducing pathogenic risk factors like bacteria with antibacterial rinses and increasing protective factors like those with saliva-like products. In addition, brushing and flossing are also useful because they physically remove dental plaque, deliver fluoride to the teeth to help with remineralization (Zhao et al., 2020) and also kill the bacteria present (Myers et al., 2019; Pradiptama et al., 2019).

Nevertheless, as mentioned earlier, prolonged application of an antimicrobial agent may lead to the presence of resistant bacteria. For a decade, fluoride has been used extensively in many oral care products as an anti-cavity agent. However, most living things have the ability to adapt to their environment and evolve (Rillig & Antonovics, 2019). Thus, to overcome the toxic effect of fluorides, the oral bacteria are able to develop resistance to fluoride through phenotypic adaptation or genotype changes (Bottner, 2018). These fluoride-resistant bacteria have been discovered in several clinical studies, in which fluoride-resistant *S. mutans* colonies have been found in patients recovering from Xerostomia patients who had been treated with gels containing a high dose of NaF concentration (Liao et al., 2017, 2018). Up to date, a research study done by Majeed et al. (2021) concludes that fluoride export confers fluoride resistance to oral *S. mutans* where the sequencing analysis explains that genetic conversion in chromosomes leads to a fluoride-resistant strain. In fact, in order to understand the mechanisms of microbial fluoride resistance, the researchers have created fluoride-resistant strains in the laboratory by selecting colonies that could grow in the presence of 400–600 ppm fluoride (Liao et al., 2017). Until now, fluoride-resistant strains have been created for several streptococci, including *S. mutans* (Liao et al., 2015; Cai et al., 2017; Nassar & Gregory, 2017). Those resistance strains demonstrated an obvious phenotypic transformation in growth, adherence and metabolic activity compared to those that are sensitive. However, excessive fluoride use can have negative effects on human health, such as the development of dental fluorosis in children and skeletal fluorosis in both children and adults (Zuo et al., 2018; Tepec & Ponikvar-Svet, 2019). Therefore, it may become necessary for researchers to find other alternative medicines to help reduce tooth decay problems, especially by reducing the growth of *S. mutans* in the tooth area.

Alongside the rise in public acceptance, there has been a noticeable increase in people's interest in traditional and herbal treatments. This is likely due to the widespread perception that these types of treatments are significantly less hazardous to both people and the environment. Based on historical evidence, it also shows that medicinal plants have been used as traditional treatments for various diseases for thousands of years around the world (Jain et al., 2019; Giannenas et al., 2020). Furthermore, as a believer, Allah SWT mentions plants in the Quran in Surah Luqman ayah 10: "And We send down water from the sky, and We cause (plants) of every goodly kind to grow therein." In terms of science, ‘natural products’ derived from plants, herbs, spices and fruits are rich in secondary metabolites or phytochemicals (Ashraf et al., 2018; Hussein & El-Anssary, 2019; Mandava et al., 2019). To address the issue of multidrug resistance, the demand for bioactive compounds derived from plants is increasing, and many researchers are now focusing on the study of plant extracts as a replacement for existing drugs.

In dentistry, there are many reports of the use of traditional plants and natural products for the treatment of tooth disease, such as reducing swelling, inhibiting the growth of mouth pathogens and acting as antiseptics, antioxidants or analgesics (Oluwafemi et al., 2020; Farag et al., 2020). The *A. paniculata* plant is one of them. Due to the condition of plants from the genus Acmella Rich (Asteraceae) which are easy to grow and easily found in tropical and subtropical regions including Malaysia. The leaves can be eaten raw or as a vegetable and it is normally known as the "Subang nenek" plant in Malaysia but is commercially referred to as the "toothache plant" (Joseph et al., 2017; Purushothaman et al., 2018; Rani et al., 2019). The flowers and leaves have a pungent taste and when consumed, cause tingling and numbness, which acts as an anaesthetic and relieves tooth pain. Due to its growing use, even by raw consumption, many researchers have started to prove that the Acmella plants can be the best remedial agent for toothache (in accordance with what is being commercialized), sore throat and gum infections; it is even believed to be helpful in cases of tuberculosis and some others (Purushothaman et al., 2018; Apoorva et al., 2021). Therefore, this research was designed to determine the anti-plaque activity of a bioactive compound from *A. paniculata* leaves against *S. mutans* and its mechanism.

1.3 Problem of Statement

Plaque development will lead to dental caries. Because *S. mutans* produces acid, its presence on the tooth surface may result in tooth demineralization. Commercial antiseptics, antibacterial agents and antioxidants used for the prevention and treatment of plaque development were reported to exhibit toxicity, teeth staining and oral cancer. Furthermore, *S. mutans* has been found resistant to antibiotics such as penicillin, amoxicillin and chloramphenicol, as the target of the antibiotic may be modified by genetic modification (mutation), which reduces the drug's affinity for its substrate (Gerits et al., 2017; Loyola-Rodriguez et al., 2018; Priya et al., 2021; Nam & Hwang, 2021). Therefore, extracts from natural products need to be tested as an alternative to treat and prevent plaque formation. Many people, including researchers, have reported that the *A. paniculata* plant has been used orally to treat a variety of diseases, including diuretics, flu, toothaches, and periodontitis (Vishwanathan et al., 2021). However, scientific evidence for the bioactive compounds of *A. paniculata* against *S. mutans* plaque development is still lacking.

1.4 Research Questions

1. Do *A. paniculata* extracts show antibacterial activity effects?
2. Is there any morphological changes of *S. mutans* cell wall when treated with *A. paniculata* extracts?
3. What is phytochemistry of *A. paniculata* extract?
4. Is there any gene expression occur when *S. mutans* exposed to *A. paniculata* extract.

1.5 Objectives of the Study

The general objective of this study is to determine antibacterial activity of *A. paniculata* extracts against *S. mutans*.

The specific objectives are:

1. To determine anti-bacterial activities of *A. paniculata* extracts against *S. mutans* by using disc diffusion assay, minimum inhibitory concentration, minimum bactericidal concentration, and anti-biofilm assay.
2. To investigate morphological changes of *S. mutans* cell wall when treated with *A. paniculata* extracts.
3. To investigate phytochemical of *A. paniculata* extracts by using gas chromatography - mass spectrometry.
4. To determine *S. mutans* gene regulation when expose with *A. paniculata* extracts.

1.6 The Significance of the Study

The pharmaceutical industry in Malaysia would develop antibacterial drugs using *A. paniculata* as a new drug for treatment and this is in alignment with the National Policy on Biological Diversity, which encourages the use of biodiversity as the basis of drug development.