CHAPTER II

LITERATURE REVIEW

2.1 Dental Caries

Dental caries and dental plaque are common diseases that happen to humans and involve a relatively high cost of treatment. It is considered to be a major problem in life, although it is rarely life-threatening. Iheozor-Ejiofor et al. (2015) stated that dental caries has affected almost 60–90% of youngsters and most adults from all over the world. Caries is a microbial disease that affects the teeth and causes demineralization of the inorganic part of the tooth as well as dissolution of the organic part of the tooth. The presence of dental plaque is the most important factor that plays a role in the development of dental caries. Plaque is a deposit or biofilm that can appear as a yellowish-white or white substance and forms on the teeth or any other hard structure in the oral cavity. It is made up of a bacterial community that is predominately present in a matrix that is exfoliated with cells, salivary proteins, polysaccharides, lipids, calcium, and phosphates.

The formation of dental plaque begins with the formation of acquired pellicle, a glycoprotein film that coats the surface of the tooth enamel and forms immediately after brushing the teeth. This salivary pellicle coating aids bacterial adhesion to the tooth via a receptor that recognises the bacteria's adhesin molecule (Chawhuaveang et al., 2021). These initial bacteria, also known as primary colonisers in dental plaque, will then secrete extra-cellular polysaccharides that will help the adhesion of more bacteria to the existing bacteria in the plaque. Afterwards, the second coloniser fits in and bacteria

continue to stick to each other. Bacteria, along with metabolites and other proteins, accumulate and together form the plaque mass, which continues to grow and mature.

It has been suggested that *Streptococcus mutans* and various species of Lactobacillus are responsible for dental caries (Caufield et al., 2015; Sounah & Madfa, 2020). In order to satisfy its requirements for energy, *S. mutans* metabolises most of the sucrose and produces lactic acid. In addition, some of the sucrose is utilised in the synthesis of extra-polysaccharides like glucans and fructans. Fructans are soluble and have been used as reservoirs for further energy needs, whereas glucans are insoluble and contribute to the further accumulation of more streptococci that cause caries. As for fructans, they have been used as reservoirs for further energy needs. (Al-Shahrani, 2019; Alejandra et al., 2020). Glucans contain glucan-binding proteins, which help in their adhesion. Because of this, the increase in lactic acid will result in a decrease in the pH of the surrounding environment, making it much more acidic. Those bacteria that cause caries can thrive and proliferate in such an environment and start to increase in population. Besides striving, they also produce more acids and this ecological shift to an acidic environment increases the cariogenic bacteria population and decreases the non-cariogenic bacteria, altering the balance or homeostasis of the bacterial community (Chen et al., 2021).

When there is an increase in acidic conditions, the pH is usually neutralised by dilution of the saliva into the plaque interface, and this causes the saliva to begin to lack in minerals (Pitts et al., 2017; Anderson, 2019). Then, to meet the mutual demand of the saliva, hydroxyapatite crystals from the tooth start to disintegrate and move to the saliva. Once the acids have been neutralized, the saturated minerals in the saliva will then go back to the enamel to remineralize it (Valente et al., 2018). However, the problem arises when the pH drops below 5.5 and the minerals from the saliva keep

moving to the tooth plaque interface in order to buffer the acidic environment. As for that, the saliva begins to lack minerals, and the hydroxyapatite crystals continue to disintegrate. Finally forming dental caries and cavities on the tooth surface.

2.2 Saliva

Saliva is a mouth fluid that contains almost 90% water, electrolytes and enzymes that are produced by a group of cells called salivary glands (des Gachons & Breslin, 2016). Saliva is a complex fluid that facilitates many necessary functions such as lubricating the mouth, moistening the food, assisting in digestion, protecting the teeth and even defending the mouth from microorganisms. There are four kinds of salivary glands: (1) the parotid gland, which secretes a thin liquid rich in antibacterial proteins and compounds that remineralize our teeth; (2) the submandibular glands, which produce a more viscous fluid that can lubricate the throat and mouth; (3) the sublingual gland, which produces similar lubricating secretions; and (4) the minor salivary gland, which covers the tongue and the lining of our mouths with hundreds of tiny glands and functions to protect the mouth against infections (Ghannam & Singh, 2019; Thimmappa et al., 2021).

Saliva contains calcium (Ca) and phosphates (PO4), which are also the building blocks of the teeth and help keep the enamel strong and healthy (Vasudevan et al., 2017). However, bacteria, acids and sugar from foods found in your mouth are the major components that cause plaque formation by a process called demineralization. This process occurs when the acidic conditions are at a higher level and attract the mouth microbes to continue propagating in the mouth. These oral microbes can withstand acid, but not the teeth. At this point, saliva will remineralize the tooth by keeping Ca and PO4

circulating throughout the mouth to strengthen the enamel. Furthermore, this process helps to keep the mouth's pH between 6.2 and 7.4, reducing the survival of cariogenic bacteria (Ramsay et al., 2019).

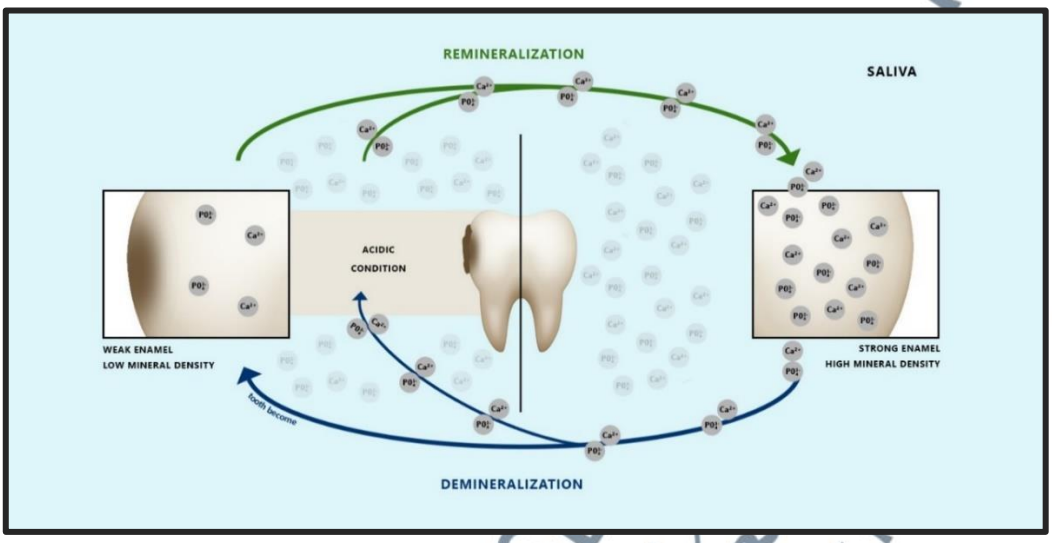

Figure 2.1: The Demineralization and Remineralization Equilibrium of the Plaque-Enamel Interface. Saliva Acts as a Buffer System Consist of Mineral and Fluoride Ions.

2.3 Cariogenic Bacteria Cause Dental Caries

Dental caries, also known as cavities, are caused by bacterial infections and lead to the destruction of tooth structure. It is estimated that our mouths are home to over 700 distinct species of bacteria, each of which serves a specific purpose (Apoorva, 2020). People are born with these bacteria because they are usually passed from the mother to the baby soon after birth, especially when the baby is breastfed. Some other common ways in which the bacteria are passed are when the baby is kissed near the mouth. When a person's teeth first emerge from their gums, they already have a head start on the process of naturally harbouring communities of bacteria. Cavities are a dental disease caused by an overpopulation of certain microbes, which can occur when a person consumes an abnormally high amount of sugar (Bowen et al., 2018; Jayadevan

et al., 2019; Bilbilova, 2020; Nakai & Mori-Suzuki, 2022). This tooth decay is caused by the metabolic activity of bacteria that live in the mouth and on the teeth. When our teeth are exposed to saliva and food, a layer of proteins and carbohydrates forms on the surface of the teeth (Pedersen & Belstrøm, 2019). As a result, at this stage, *S. mutans* and lactobacilli strains are more attracted to foods high in sugar, which eventually leads to plaque formation (Esberg et al., 2020; Butera et al., 2022).

2.3.1 *Streptococcus mutans*

It is widely believed that *Streptococcus mutans* is a major causative agent of dental caries. It is a member of the Gram-positive bacteria family, which can be found naturally in the oral flora of virtually every human being. Because it can reproduce either in the absence or presence of oxygen, it thrives in environments that are facultatively anaerobic. *S. mutans*, just like other streptococci, are referred to as such because they are in the shape of cocci and line up like chains (in Greek, streptos = necklace).

As previously stated, *S. mutans* has received the most attention as the most important species that causes oral cavities (Alexa et al., 2019; Nomura et al., 2020). Unlike other types of Streptococcus bacteria, *S. sobrinus* has only been found in places where caries is happening. *S. mutans*, on the other hand, has been discovered in both active and non-carious sites (Sahal, 2021). This is why scientists state that *S. mutans* is the originating bacteria for dental caries, as it is much easier to grow on teeth. Parents are usually responsible for making the first contact with *S. mutans* in infants, for example, by sharing a spoon with the baby or putting a pacifier in the mouth, where bacteria can be found in abundance (Damle et al., 2016).

However, the presence of *S. mutans* does not continue to cause the development of caries. These bacteria must first be given the right conditions for them to react (Lemos et al., 2019). For example, consuming sugary foods and drinks will help the bacteria create an acidic environment that causes demineralization of the tooth enamel. Based on the Stephan curve, it takes about 30–40 minutes for the saliva pH to get back to the safe zone for the remineralization process to occur. Therefore, the longer you snack, the longer it takes for your saliva pH to recover (Hariyani et al., 2019). This may result in an increase in the number of bacteria in the saliva, which may increase the likelihood of caries occurring. However, Kim (2021) did mention that the excessive reliance on the Stephan Curve leads to describing food as "safe" when there is no drop in pH below the so-called "critical pH". Acid production is just one of the many other biological processes that occur in plaque when exposed to sugar. There are also other methods that help in the production of acids and even alkalis that could produce other research evidence.

Sucrose is a sugar that can be easily converted into acid. Therefore, the presence of *S. mutans* causes sucrose to be converted to lactic acid, resulting in a rapid drop in pH (Matsumoto-Nakano, 2018). Apart from that, *S. mutans* is also able to convert sucrose into extracellular polysaccharides. Polysaccharides, as we know, are made up of monosaccharide chains, which are simple sugars like glucose and fructose. Thus, in other conditions, the enzyme invertase of *S. mutans* can hydrolyze the sucrose to both glucose and fructose.

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2.3.1.1 Biofilm Formation by *S. mutans*

Dental plaque is a type of biofilm that forms specifically on teeth and is primarily composed of bacteria and their extracellular products. Normally, there is a layer called the pellicle that covers the tooth and bacteria in the mouth, for example the viridans group of streptococci settle on that pellicle layer. These bacteria are called early colonizers and they attach to surfaces using bacterial surface proteins called adhesin (Kreve & Dos Reis, 2021). As the *S. mutans* are on the pellicle of the teeth, they come into contact with sucrose, a sugar found in various foods and drinks. In *S. mutans*, sugars are utilised both intracellularly and extracellularly.

In the cell, sucrose is primarily used for glycolysis, which leads to the presence of lactic acid, a major virulence factor for *S. mutans* (He et al., 2019) This biochemical reaction causes demineralization of calcium phosphate in teeth due to the decrease in pH. *S. mutans* are able to metabolise pyruvate (pyruvic acid) further to generate energy and more acid byproducts (Ahn, 2020). When there is an excess of sugars, they tend to use the lactate dehydrogenase (LDH) pathway to produce lactic acid (Chen et al., 2020). Lactic acid production in dental plaque can lead to acid accumulation. When the pH falls below 5.5, demineralization of the tooth surface occurs, which then leads to tooth decay (Larsen & Fiehn, 2017).

As mentioned above, sucrose has a glucose molecule bound to a fructose molecule. The bacteria use an enzyme called invertase to cut the bond between those two monosaccharides (You, 2019), forming polysaccharides, fructans and glucans with the help of fructosyl transferase (FT) and glycosyltransferase (GTF), respectively. Fructans are soluble polysaccharides that can easily be converted back to fructose. As a result, fructans were used as a food reserve for the bacteria and when they required energy, particularly for growth (Cheng et al., 2020).

Glucans, on the other hand, are more difficult to dissolve than fructans (Ishimoto et al., 2018). Therefore, glucans have been used as extracellular polysaccharides that allow the bacteria to attach firmly to the surface of the tooth and form dental plaque, which is a sticky collection of the bacteria, proteins from saliva and dead cells from the lining of the mouth (Bowen et al., 2018; Mieher et al., 2018). Bacteria may adhere considerably more easily to dental plaque in the presence of glucans, which then mature quickly. With the addition of the first effect, in which sucrose is converted to lactic acid, the environment becomes more acidic, allowing the pathogen to grow larger and create dental plaque, which is also known as biofilm.

2.3.1.2 Role of Gene Expression in Biofilm Formation by *S. mutans*

S. mutans adhesion and colonisation on the tooth surface go through both sucrose-independent and sucrose-dependent interactions. Adhesion of a sucrose-independent toward the saliva component in the acquired enamel pellicle will cause the initial adhesion process. However, the adhesion of sucrose-dependent is responsible for the *S. mutans* colonisation on the tooth surface. The existence of *S. mutans* in infants is initiated by transmission from the mother and the rate of bacterial colonisation will be related to the consumption of sucrose. The ability of *S. mutans* to synthesise glucans increases with adhesion and changes in the proportion of *S. mutans* in dental plaque. Thus, the main role of sucrose-dependent adhesion is to create a plaque ecology that can trigger dental caries (He et al., 2019; Lemos et al., 2019).

The presence of sucrose along with glucotransferase B, C and D will form specific glucan binding proteins (GBPs) (Lemos et al., 2019). The *gtfB* uses sucrose as a substrate to produce water-insoluble glucans composed of α1,3-linkages, *gtfC*

produces glucan with mixed α1,3- and α1,6-linkages and the *gtfD* converts sucrose into water-soluble α1,6-linked glucans (Bowen & Koo, 2011). This contributes to the adhesion and accumulation of biofilm. The glucotransferase enzyme in *S. mutans* also initiates the action of the fructosyltransferase enzyme in producing a fructan polymer, which will be used as a nutrient supply and colonisation of oral Streptococcus. Besides the *gtf*, they have been found to be active in acquired enamel pellicle (AEP), providing binding sites to oral microorganisms and even having the ability to bind to other bacteria that may or may not produce *gtf* themselves (Krzyściak et al., 2014). Therefore, the interactions between *gtf* and another microorganism will allow the formation of a strongly attached plate to the tooth surface with stable bonds between bacterial cells (Philip & Walsh, 2019).

In addition, surface-associated protein P1/SpaP, also known as antigen I/II, is a gene associated with the attachment of *S. mutans* onto the tooth surfaces covered with saliva. The protein is a multifunctional adhesin that facilitates bacterial binding to the pellicle component of tooth enamel. Besides the proteins and enzymes in sucrose-dependent adhesion, there are several proteins involved in the metabolism of sucrose, glucans and carbohydrates. This protein will affect the virulence level of *S. mutans*, namely fructosyltransferase (*ftf*), fructanase (*fruA*), extracellular dextranase (*dexA*), and proteins that play a role in the accumulation of intracellular polysaccharides.

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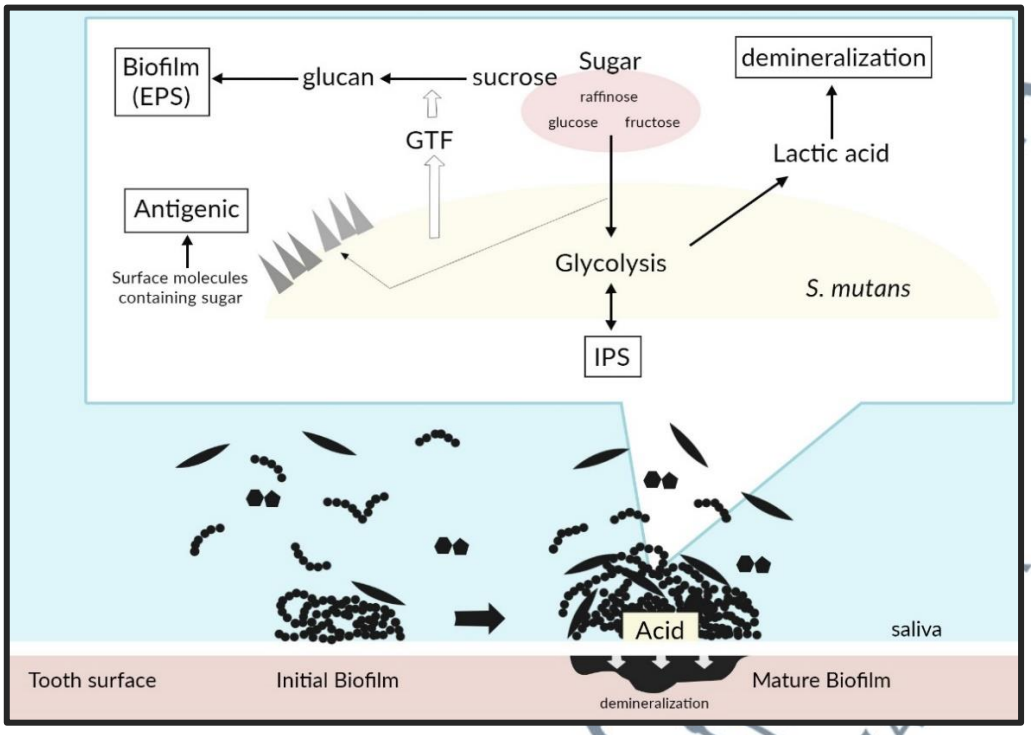

Figure 2.2: *S. mutans* Virulence Factor is Related to Sugars. *Gtf*: Glucosyltransferase; IPS: Intercellular Polysaccharide; EPS: Extracellular Polysaccharide.

Sucrose can enter *S. mutans* through three different pathways. The presence of operons containing sucrose phosphorylase (*gtfA*) and intracellular dextranase indicates the presence of multiple sugar metabolism systems (*dexB*). This system can transport isomaltosaccharides, which can activate *dexA*'s extracellular dextranase activity. In other words, *Ftf* can create fructan from sucrose, allowing *fruA* to enter the cell (Kawada-Matsuo et al., 2016).

As a result of carbohydrate fermentation, *S. mutans* has a complex glycolytic pathway that can produce lactate, formate, acetate, and ethanol. When glucose is abundant, the exact distribution of the fermented product depends on the growing conditions, with lactate being the main product. A lack of lactate dehydrogenase strain reduces *S. mutans'* cariogenic potential (LDH). As a result, the lack of LDH in the

biofilm can kill *S. mutans*. This is the foundation for the advancement of genetic engineering for the prevention of dental caries.

2.4 Toothache Treatments

Biofilm formation on the teeth is a significant issue in maintaining the health of the teeth and gums. This biofilm, as previously stated, is made up of a group of bacteria that adhere to the surface of the tooth and allow the bacteria to multiply quickly. Biofilm can also serve as the foundation for the formation of dental plaque, which can lead to gum disease and tooth decay. As a result, various preliminary preventive measures have been implemented to address this issue. There are several treatment methods for biofilm formation on the teeth, including the most basic use of fluoride toothpaste and mouth washes.

2.4.1 Fluoride Toothpaste

It has been shown that fluoride toothpaste helps stop biofilm from building up on teeth. When bacteria make acid, it can dissolve the minerals in the tooth structure. This process is called demineralization, and it can lead to tooth decay. At this point, fluoride, a naturally occurring mineral, may help lower the amount of acid in the mouth (Nascimento et al., 2019). This means that fluoride toothpaste can be beneficial in inhibiting the demineralization of tooth structure by reducing the acid production of bacteria in the mouth.

Besides that, studies have shown that fluoride toothpaste helps make and keep teeth healthy and strong by strengthening the tooth enamel (Clark-Perry & Levin, 2020).

This is known as remineralization, where it is a process of replacing minerals lost from the enamel. When fluoride is present in the mouth, it can be taken up by the tooth structure and incorporated into the enamel, which helps to strengthen it. This condition is known as fluorapatite formation, which is more resistant to acid attacks.

Fluoride is generally safe and effective when used in the right amounts. However, there are some things that could go wrong if you use it excessively, for example, fluorosis (Whelton et al., 2019; Fiorillo et al., 2020). Fluorosis is a cosmetic condition that can occur if too much fluoride is consumed during the development of the teeth. Mild fluorosis appears as white specks or streaks on the tooth surface, while severe fluorosis can cause brown discoloration and pitting of the tooth surface. Besides that, Biria et al. (2022) reported that routine daily toothbrushing with fluoride-containing toothpastes can also cause allergic reactions in some people who may be allergic to fluoride or other ingredients in fluoride-containing products, which can cause a skin rash or hives.

2.4.2 Mouth Washes

Mouthwash is also an oral care product used to help maintain the health of teeth and gums. Basically, mouthwash contains antibacterial ingredients that help kill bacteria in the mouth (Su et al., 2019). As we all know, bacteria in the mouth are the initiating cause of dental problems. Therefore, developing a method to kill those bacteria can help prevent plaque formation, bad breath, and other dental and gum problems. Besides that, it can also reduce the risk of tooth decay and gum disease because the bacteria and debris that accumulate on the surface of the teeth cannot form

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a sticky layer on the surface of the gums that has been called plaque (Alshehri, 2018; Halboub et al., 2020).

While mouthwash can be an effective part of a good oral hygiene routine, there are also some potential disadvantages to using it. Some types of mouthwash contain alcohol, which can be harsh and irritating to the tissues in the mouth (Aspinall et al., 2021). This can make your mouth feel dry or burn, and people with sensitive gums or mouth sores may not be able to use it (Kaur & Sharma, 2020; Nardi et al., 2020). Besides that, many mouthwashes contain artificial colours and flavours that may cause allergic reactions or other sensitivities in some people (Ramesh & Muthuraman, 2018). As mentioned earlier, most mouthwash contains antibacterial agents, so it is obviously able to kill both harmful and beneficial bacteria in the mouth (Meyer & Enax 2019), potentially disrupting the natural balance of oral flora.

Apart from having antibiotics, there are also mouthwashes that contain antiseptics. Antiseptics are substances that stop microorganisms from growing and spreading on living tissues. It prevents the formation of biofilms by killing or inhibiting the growth of the microorganisms by disrupting the bacteria's and other microorganisms' cell membranes, making them less able to adhere to surfaces and form biofilms (Zayed et al., 2023). Antiseptic mouthwash contains an active ingredient such as cetylpyridinium chloride (CPC) or chlorhexidine (CHX) that helps to kill bacteria and reduce plaque and gingivitis (Verspecht et al., 2019; Tsuda et al., 2020). This type of mouthwash is often recommended for people with gum disease or those who are at risk for developing gum disease.

For example, Rajendiran et al. (2021) stated that CPC has been shown to be effective at stopping biofilms from forming and killing microorganisms in biofilms. The positively charged molecule of CPC sticks to the negatively charged cell surface of a

microorganism (Mao et al., 2020). This breaks the cell membrane and lets intracellular parts leak out, which kills the cell. CPC has broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, fungi, and viruses. Dong et al. (2020) found in their study that CPC was effective in reducing the viability of *Pseudomonas aeruginosa* biofilms after 2 hours of treatment.

Like other antimicrobial agents, there is a risk of developing resistance to the CPC of CHX with repeated and prolonged use. The way CPC works is similar to how other quaternary ammonium compounds work, which may cause different types of disinfectants or antiseptics to stop working (Mao et al., 2020). Verspecht et al. (2019) have reported that some of the oral bacterial isolates can become resistant to CHX after being exposed to it. For instance, *Porphyromonas gingivalis* isolates increase their minimal inhibitory chlorhexidine concentration up to fourfold after exposure to 20 passages.

2.5 Toothache Plant

The toothache plant is an ethnospecies, defined as any species in the genus *Acmella* of the aster family (Asteraceae), with *Acmella paniculata* being one of these Compositae families found in both tropical and subtropical regions. It has been reported to be beneficial in traditional medicine and nutritional systems among various tribal societies worldwide. But in the past, the scientific names *Acmella olearcea* and *Spilanthes acmella* were often used in phytochemical and ethnobotanical studies (Panyadee & Inta, 2022).

The widespread use of this toothache plant in modern medicine has made it necessary for the confirmation of this particular species at the molecular level to avoid

confusion regarding the prevailing taxonomic position worldwide. According to Paulraj et al. (2013), there is frequent confusion among these species and name misuse is common among traditional and complementary medicine practitioners, herbal users, and especially anonymous sources of information on the internet. Similar morphological and spectral characteristics of these species and even genera, have continued to mislead. Patel et al. (2019) stated in their writing that there are differences based on morphology, chromosomes and molecules in each of these species. For example, Genus *Acmella* has a luminous head and no pappus, while *Spilanthes* has a discoid head. At the chromosome level, *Spilanthes* has 16 chromosomes and Acmella has 12–13 (Reshmi & Radhakrishnan, 2016; Monroe et al., 2016). Therefore, differences in chromosomes can also play a role in the evolution and differentiation of species and can affect the physical and physiological characteristics, growth patterns and reproduction of plants. However, more clear and in-depth scientific studies are still lacking and limited. According to Panyadee & Inta (2022), more comparative studies of *Acmella* spp. are still needed.

In spite of this, they are considered to be synonyms because of the similarities that exist between them, such as their function as a "toothache plant" and existing studies that prove there is a similar therapeutic effect that these two plants are capable of producing when used to treat toothache (Baset al., 2019; Rani et al., 2019; Salehuddin et al., 2020; Abdul Rahim et al., 2021; Vishwanathan et al., 2021; Patel et al., 2022).

2.5.1 *Acmella paniculata*

Acmella belongs to the family Asteraceae and as a genus, it comprises over 60 species that are widely distributed in tropical and subtropical regions of the world, such

as Africa, America, Brazil, Malaysia, Borneo, India, Sri Lanka and Asia (Singh & Upadhyay, 2018; Abdul Rahim et al., 2021). *A. paniculata* is an annual or short-lived herb that is 40–60 centimetres tall. It is grown in damp areas and has a low rate of germination or poor vegetative propagation. In other words, this plant crops up in the wild and can be cultivated as well. The flowers and the leaves have a pungent taste and when consumed, cause tingling and numbness (Uthpala & Navaratne, 2021).

Figure 2.3: *Acmella paniculata* Plant.

As mentioned earlier, *A. paniculata* is also known as the "toothache plant" because of its wide use by tribals to cure toothache and gum infections. The other common names include Eyeball Plant, Spot Plant, Brazil Cress, Alphabet Plant and Australian Cress (Paulraj et al., 2013). While it is known in Hindi as "Akarkara," it is widely used in traditional and herbal medicine to help regulate the immune system (Usmani et al., 2016; Arif et al., 2017). However, the local Malaysians called it the "subang nenek" plant (Ong et al., 2011; Anee et al., 2018)

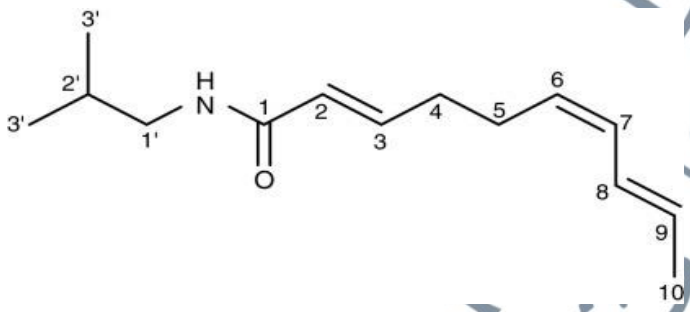
A. paniculata is a food product that is believed to have many therapeutic effects, primarily in pain-relieving properties. Many studies have found that *A. paniculata* can

help treat rheumatic, diuretic, anti-inflammatory, fever, flu, cough, and even dental problems (Singhal et al., 2016; Uthpala & Navaratne, 2021). Therefore, it has been recognised as an important medicinal plant and is increasingly well received by people worldwide. Traditionally, the native people used this plant to make salads due to its spicy aroma and some cooked it as part of their meals. Because bioactives are absorbed topically, eating them in salads has been claimed to help with toothaches and other oral diseases that cause pain. As a result, it became much more popular to be called "toothache plant" by the practitioners.

2.5.2 Bioactive Metabolites of *A. paniculata*

Phytochemicals are bioactive metabolites found naturally in plants that have protective or disease-preventive properties. Uthpala and Navaratne (2020) report the chemical contents of this plant are alkaloids, terpenoids, flavonoids, glycosides, carbohydrates, fatty acids and amino acids. They are the major compounds that are considered to be responsible for the pharmacological activity of the plant. Alkaloids are the most common elements found in this genus of *Acmella* and have proven to have diverse biological activities (Joshi et al., 2020). However, alkamides, on the other hand, are often found in plants from the Apiaceae and Asteraceae families. Amides are derived from fatty acids and are typically less potent than alkaloids and the fatty acid portion of the alkamides gives them special properties and biological activity. Many reports stated that they have anti-inflammatory, immunostimulatory, and analgesic effects. The most common alkamide presence in *Spilanthes acmella* (*A. paniculata*) is spilanthol (Barbosa et al., 2016; Patel et al., 2019), and it is believed that the pungent effect that causes the taste of the plant to be "spicy" comes from this compound (Figure 2.4). Xu et al. (2019)

mentioned that fatty acid amides produce numbing and tingling sensations by inhibiting 2-pore-domain potassium leak channels on trigeminal sensory neurons, causing depolarization and triggering an action potential that transmits a signal perceived as numb or tingling to the brain.

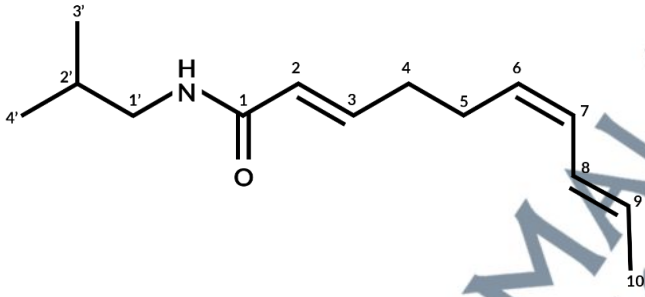


Source: Barbosa et al. (2016)

Figure 2.4: N-Isobutyl-2,6,8-decatrienamide (Spilanthol) the Same Component Found in *Spilanthes acmella* and *Acmella oleracea*.

According to research, spilanthol is chemically identical to affinin (Figure 2.5) (Barbosa et al., 2016; Castro-Ruiz et al., 2017). Many of the articles describe affinin as the major alkamide present in *Heliopsis longipes* instead of spilanthol (Castro et al., 2014; Castro-Ruiz et al., 2017). *H. longipes*, also known as chilcuague, is a perennial herbaceous plant in the *Asteraceae* family that is a common spice and a medicinal plant widely used in Mexico and South America for its anaesthetic, analgesic, antiulcer, anti-inflammatory, and antiparasitic properties (Willig et al., 2019; Parola-Contreras et al., 2021). It is also used as a culinary seasoning and insecticide (Correa-Delgado et al., 2021). This demonstrates that, in addition to having the same chemical structure, these two alkamides have the same properties in medicine related to their function as bioactive compounds. On the other hand, affinin is the term that is used in the species *Heliopsis longipes*, whereas spilanthol is a term that is found in the *Spilanthes* plant;

however, in the field of phytochemistry, the two terms are typically related to one another (Maggini et al. 2021).



Source: Castro-Ruiz et al. (2017)

Figure 2.5: Chemical Structure of affinin, (2E,6Z,8E)-N-isobutyl-2,6,8-decatrienamide with CAS Number 25394-57-4. The Major alkamide in *H. Longipes* Roots.

Sharma & Arumugam (2021), in their study of the ethanolic extract from the plant *S. acmella*, used LC-MS (liquid chromatography-mass spectrometry) to separate and identify the different compounds present in the extract, which revealed that the extract contained seven different N-alkylamides, which are a type of natural compound often found in plants. Specifically, the extract contained five N-isobutylamides (IBA), one 2-methylbutylamide (MBA), and one 2-phenylethylamide (PEA), and one of them is spilanthol.

2.5.3 Biological Activities of *A. paniculata*

The presence of bioactive compounds within a plant is the primary factor responsible for the plant's pharmacological activity. These compounds may influence crucial physiological processes that take place in the human body. It is essential to conduct research into the nature of a plant's phytochemical constituents in order to gain

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an understanding of both the chemistry of the plant and the potential pharmacological action it may have.

A. paniculata is widely used in India as a natural remedy to treat toothache. That is why *A. paniculata* is famously known as the "toothache plant," because the reason is that the flowers contain a natural anaesthetic that will numb the gums and the teeth and cause at least a comfort situation in that localised region. The roots and flowers have the most medicinal value. Researchers believe that the compound called spilanthol, which is a fatty acid amide, is responsible for the local and aesthetic properties of the plant. It can reduce the pain by blocking the receptors that produce the sensation of aching in the gums and even induce the secretion of saliva (Xu et al., 2019; Stein et al., 2021).

Krishna et al. (2014) investigated the basis of ethnopharmacological and antimicrobial activity of aqueous extracts from various parts of *A. paniculata*. The screening was done by using the agar diffusion method against some pathogenic bacteria such as *Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Vibrio cholerae* and *Vibrio parahaemolyticus*. The result concludes that the crude extract of *A. paniculata* has potent antimicrobial activity against most of the pathogenic bacteria tested. They also proved that all parts of *A. paniculata* have potential as sources of antimicrobial activity, as the screening results show the *A. paniculata* extract contains alkaloids, tannins, saponins and flavonoids.

Rani et al. (2019) investigated the impact of varying concentrations of *Spilanthes acmella* flower head extract on the ability to inhibit the growth of certain fungi. These species included *Fusarium oxysporium, Fusarium moniliformis, Aspergillus niger,* and *A. paraciticus*. All of the concentrations of the test solution were effective in preventing the fungi from reproducing. The highest concentration resulted

in the largest inhibition zone, which grew proportionally with increasing dose. Among the organisms that were put to the test, *F. oxysporium* and *F. moniliformis* were found to have the highest inhibition zones, followed by *A. niger* and *A. paraiticus*.

As for anaesthetic activity of *Spilanthes acmella* was carried out using two different animal models: (i) intracutaneous wheals in guinea pigs to determine the degree of anaesthesia and (ii) plexus anaesthesia in frogs to determine the onset of anaesthesia. Both models used 2% xylocaine as the standard drug. The result shows that the aqueous extracts of *S. acmella* produced 70%–90% anaesthesia by the intracutaneous wheal and the mean onset of local anaesthetic action was very strong with $P<0.001$ (Chakraborty et al., 2010). This has also been supported by Barbas et al. (2017), where their study found that the extractives of *S. acmella* and *N. grandiflora* induced deep anaesthesia in juvenile *C. macropomum*, with antioxidant components reducing or maintaining lipid peroxidation in assessed organs. A concentration of 10 mg L−1 of *S. acmella* extract was suitable for fast anaesthesia and safe recovery, while lower concentrations enhanced protection against oxidative damage and have potential for use as chemoprotectants during transport.

Antipyretic effects of *S. acmella* was determined by using a general procedure in detecting the induction of pyrexia which been called as yeast induced methods (Liu et al., 2017). In the study involving 100, 200 and 400 mg of aqueous *S. acmella* extracts with aspirin as the standard drug shows that the extracts produced dose-dependent reduction in mean temperature at various periods of observation (Vishwanathan et al., 2021).

Anti-inflammatory effects in some cases show that in vitro and in vivo studies of *S. acmella* has been shown to inhibit nitric oxide (NO) release, prevent inflammatory cytokines (IL-1, IL-6, and TNF-α), weaken COX-2 and iNOS expression, suppress NF-

κB, and inhibit MAPK signalling pathways (Rahim et al., 2021). Researchers believe that spilanthol is responsible for causing the anti-inflammatory effects (Bakondi et al., 2019). To date, Sharma et al. (2021) have done a study to evaluate the anti-inflammatory activities of the *S. acmella* ethanol extract using the carrageenan-induced rat paw edema method. This method is a suitable test and is frequently used to evaluate anti-inflammatory drugs. The results showed the extract at doses of 200 and 400 mg/kg significantly suppressed the edema in the rats by 52% and 60%, respectively.

There is also a diuretic effect on *S. acmella*, where the results of the study found that there is a group of various compounds capable of increasing urine production. In traditional medicine, *S. acmella* flowers are believed to have a strong diuretic activity. This has been proven by conducting a study to evaluate the potential of plant diuretics on rats using cool water extract, where the highest dose (1500 mg/kg) of flowers tested possessed strong diuretic activity when given orally in a single dose (Sruthi et al., 2017). Ali et al. (2015) also concluded the *S. acmella* plant has shown a diuretic effect on the rats, where there was a significant increase in both ion natrium and ion potassium levels in the urine when treated with three different doses (100 mg/kg, 300 mg/kg, and 500 mg/kg).

UNIVERSITI SAINS ISLAM MALAYSIA
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