CHAPTER 2

LITERATURE REVIEW

2.1 Oral Health Disease

Oral health is an important component of overall health. Individuals with good oral health can communicate better, enjoy a variety of foods, and have a higher quality of life, self-esteem, and confidence. Nevertheless, a major proportion of the world's population was affected by oral diseases. Dental caries, periodontal disease, oral cancer and other disease are classified as oral health complications (Sahril et al., 2014).

In the majority of countries, periodontal disease is a common type of human infection in the world (Preshaw & Bissett, 2019). Periodontal disease is one of the oral and dental diseases mostly found in humans caused by several factors, one of which is due to the accumulation of bacterial plaque. The chances of getting periodontitis increase along with age especially among diabetic patients (Bascones-Martinez et al., 2011). Bacterial plaque, a sticky, colourless layer that consistently forms on teeth has been identified as the main cause of periodontal disease (Berezow & Darveau, 2011).

From the clinical point of view, an early examination of patient characteristics with standard clinical measures such as probing pocket depth would be quite significant in patients who have already been exposed to oral diseases and treatments to examine and determine the development of future health or disease (Thomson et al., 2012).

2.1.1 Dental Caries

Dental caries is the localised loss of tooth tissue caused by bacterial activity. Cavities occur as demineralization sites on the peripheral of the enamel surface that covers the crowns of teeth. The presence of bacteria aggregates adhered to the tooth surface, which is known as dental plaques (Pitts et al., 2017).

There are reports indicating that *S. mutans* is a key bacterium in the initiation of carious lesions on enamel surfaces. Although both *S. sanguinis* and *S. mitis* are significant teeth colonizers, consequently, it appears to be less significant in tooth decay compared to *S. mutans* (Szafrański et al., 2017). Salivary and bacterial products are the components of the interbacterial matrix, which helps to keep the bacteria population attached. In addition, many types of oral bacteria species colonized teeth. Hence, dental cavities were described as the accumulation of bacteria, especially Streptococci bacteria which are the most prevalent microorganisms on tooth surfaces (Tsai et al., 2018).

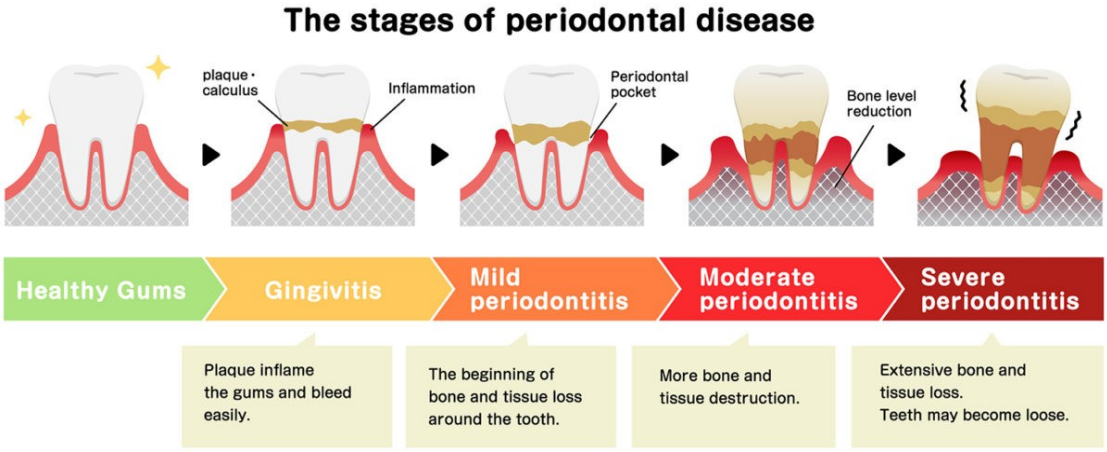
2.1.2 Periodontitis

Periodontitis has a well-established bacterial infectious aetiology (Papapanou & Susin, 2017). These diseases are caused by the periodontium being exposed to dental plaques that build on the teeth and develop bacterial colonies at or below the gingival margins (Slots, 2017). Periodontitis is an attachment loss and inflammatory disease mainly caused by periodontal pathogens in subgingival plaque triggered by the immuno-inflammatory response in a susceptible host (Yucel-Lindberg & Båge, 2013). Inflammation is a complex biological process that occurs by infection, autoimmune, stress, and tissue damage. Due to large number of bacterial strains in periodontal tissues, alveolar bone loss affects periodontal tissue health that can aggravate the periodontitis (Martu et al., 2017).

Gingivitis is caused directly by dental biofilm. Gingivitis is characterised by red, swollen gums and bleeding during tooth brushing and if left untreated, it can progress to periodontitis. Biofilm growth and development are divided into four stages, including initial adhesion, lag phase, rapid growth, and steady state. As the biofilm matures, it shifts from Gram-positive, aerobic flora to one dominated by Gram-negative, anaerobic bacteria (Berezow & Darveau, 2011).

Chronic periodontitis is characterised as the destruction of the periodontal ligament and loss of adjacent bone, which may be caused by inflammation of the gingival and the adjacent attachment apparatus as shown in Figure 2.1 (Tsai et al., 2018). Chronic periodontitis develops more slowly than aggressive periodontitis. In chronic inflammatory diseases such as periodontitis, fibroblasts play an important role in maintaining over-activated host immune response. Fibroblasts have also been reported to detect pathogenic antigens and produce inflammatory mediators such as CXCL8 and TGF-β (Bengtsson et al., 2015).

The subgingival microbial biofilm discards bacterial cell surface components such as lipopolysaccharide into the gingival sulcus and may invade gingival cells and tissue. Plaque biofilm can spread and grow in the subgingival region over time, and the toxins generated by the bacteria in plaque biofilm damage the gums. The toxins cause a continuous inflammatory response in which the body turns on itself, destroying the tissues and bones that support the teeth. Gums loosen from teeth during this process, generating periodontal pockets that get infected (Papapanou & Susin, 2017). Moreover, certain pathogenic bacterial species stimulate the host's immunological and inflammatory responses, resulting in bone and soft tissue damage (Yucel-Lindberg & Båge, 2013).



Source: Retrieved from https://drtomwright.com/periodontal-disease/

Figure 2.1: The stages of periodontal disease from healthy gums until chronic periodontitis

Diabetes, cardiovascular disease, lung disease and obesity are the disease that may be linked to severe periodontitis (Tsai et al., 2018). Some nutrients are known to alter gene expression, resulting in the suppression of inflammatory driving cytokines such as IL-1, IL-6 and TNF-α (Hajishengallis, 2014). Despite the fact over 400 distinct bacterial species have been identified in the oral cavity, only a small number have been identified as periodontal pathogens (Pitts et al., 2017).

According to Zhang (2016), *P. gingivalis* and *A. actinomycetemcomitans* have been linked to the pathogenesis of localised juvenile periodontitis and severe adult periodontitis, respectively. *P. gingivalis* is a Gram-negative anaerobe bacterium that is the primary causal agent (Zhang et al., 2016). Bacterial diversity among periodontal health and disease status using 16S rRNA sequencing revealed a variation in the composition of the oral microbiota (Chen et al., 2015; Liu et al., 2012). Periodontal infections are considered to have an impact on systematic health due to the large number of oral bacteria involved.

2.2 Oral Pathogens

For decades, the microbial diversity in the oral cavity has been recognised and identified over 700 distinct bacterial species (Parahitiyawa et al, 2010), whereas the periodontal pocket area is inhabited by over 400. In addition, the bacterial diversity of oral biofilm varies across individuals and is affected by several factors, such as oral hygiene, genetics, and age.

Bacteria surfaces are more sensitive to detachment, which enables movement and the formation of new biofilm colonies on adjacent oral structures and tissues. Pathogenic microorganisms in the biofilm or dental plaque that develops adjacent to the teeth by attaching to host-derived glycoproteins, phosphoprotein and lipids coating tooth surface (Marsh et al., 2016) cause the common inflammatory forms of the disease, which can result in tooth loss (Tsai et al., 2018).

The main oral bacteria involved with periodontal disease progression, including bone and tissue damage is the anaerobic bacteria of *P. gingivalis* (Bengtsson et al., 2015; Darveau et al., 2012). Gram-negative bacteria, including *P. gingivalis*, *T. denticola* and *T. forsythia* are commonly isolated from periodontal plaques and were considered to be periodontal pathogens (Hajishengallis, 2014).

Bacteria in periodontal pockets typically penetrate the gingival epithelial barrier (Zhang et al., 2016). Furthermore, there is evidence that there are variations in the distribution of periodontal infections across different groups (Heller et al., 2012). Even though the aetiology of chronic periodontitis is multifactorial, studies indicated that specific Gram-negative bacteria in subgingival plaque biofilm plays a significant role in development of periodontal lesions (Preshaw et al., 2012).

2.2.1 *Streptococcus mutans*

S. mutans is a Gram-positive, facultatively anaerobic cocci with oval-shaped cells. In recent years, collective data has been accumulated indicating that *S. mutans* appears to be a part of the plaque flora, mostly located on carious lesions (Lemos et al., 2019). *S. mutans* is considered to be the major oral bacteria in the initiation of dental caries (Matsumoto-Nakano, 2018) and the acidic condition in the oral favour the selection of microorganisms with aciduric physiology resulting in enamel decay (Dani et al., 2016).

In 1924, *S. mutans* was discovered from caries lesions (Szafrański et al., 2017). Although the aetiology of caries is multifactorial, *S. mutans* are thought to be the main microorganisms involved (Sabbagh et al., 2020). The interaction of bacteria with each other is intended to form a biofilm on the surface of the host. The control of gene expression in response to changes in the signalling system in cell population density is known as quorum sensing (QS) (Leung et al., 2015). Caries development factors include levels of *S. mutans* as well as poor metabolic control of diabetes (Ramli et al., 2016).

There are multiple pathways for *S. mutans* to evolve for catabolizing sucrose for acid production. Moreover, several glycosyltransferase (gtf) enzymes convert sucrose into glucan binding protein (gbp), a glue-like extracellular polymer that promotes biofilm formation via cellular adhesion to the tooth surfaces and other oral bacteria. Several gtfs synthesize sucrose into glucan, a glue-like extracellular polymer that promotes biofilm formation by cellular adhesion to tooth surface and other oral pathogens (Bowen & Koo, 2011).

S. mutans is not the only microbe involved in the formation of dental caries. *S. mutans* could change the local environment by developing an extracellular polysaccharide-rich and low-pH, thus providing a suitable niche for other acidogenic

and aciduric bacteria to thrive (Lemos et al., 2019). Moreover, gtfs can bind to other oral microorganisms and transform them into glucan producers (Bowen & Koo, 2011). There is a study that discovered 73 distinct core genes solely in *S. mutans* which are involved in glucose metabolism and acid tolerance (Cornejo et al., 2013).

S. mutans is an active microbiota that triggers QS in oral biofilms. Besides that, these signalling systems of QS also act as cell communication that is widely used to coordinate and carry out colony-wide functions including virulence factors, formation of biofilms, bioluminescence, sporulation, and conjugation. In QS, *S. mutans* will be generated and emitted chemical signal molecules, which increase in concentration as cell density increases. In addition, QS mechanisms have developed in *S. mutans* to regulate the synthesis of bacteriocins or known as mutacins which are involved in defending against other oral bacteria (Leung et al., 2015).

S. mutans has multiple virulence factors that can produce large amounts of organic acids from carbohydrates, able to tolerate environmental stress, has low pH, the production of many secreted proteins, and water-insoluble glucan exopolysaccharides which enhance adhesion of bacteria and accumulate on the tooth surface with other bacteria (Huang et al., 2018). Furthermore, bacteriocins (peptide antibiotics) will be produced by *S. mutans*. The mutacins promote bacteria in competing for limited nutrients in their environment (Matsumoto-Nakano, 2018) and inhibit the growth of adjacent bacterial species (Kamiya et al., 2011).

2.2.2 *Porphyromonas gingivalis*

P. gingivalis has long been suspected to be a major member of the periodontal microbiota, playing a role in the development of periodontal disease as well as bone and tissue damage (Bengtsson et al., 2015). Figure 2.2 shows the colonies of *P. gingivalis*

on media. The Gram-negative oral anaerobe of *P. gingivalis* implicated in the development of periodontal disease, an inflammatory disease that can destroy the tooth-supported tissues that lead to tooth loss (Mysak et al., 2014). *P. gingivalis* has been linked to the development of systemic disease via systemic inflammation with elevated circulating cytokines and mediators (Hajishengallis, 2014).

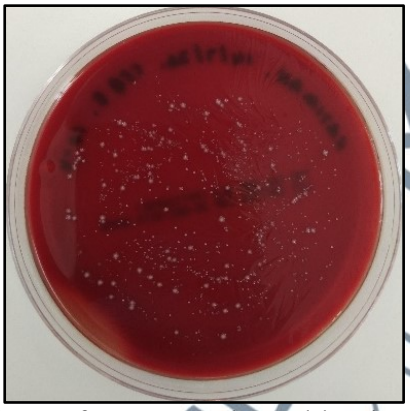


Figure 2.2: The morphology of *P. gingivalis* on blood agar was black-pigmented.

The ability of *P. gingivalis* to invade host cells, including gingival epithelial cells (Bengtsson et al., 2015), heart and aortic endothelial cells, indicates a feasible mechanism for its establishment and subsequent pathogenesis to evade the host immune system (Bengtsson et al., 2015). Amino acids as an energy source will be used by *P. gingivalis* to live in deep periodontal pockets as there is less sugar for energy sources (Hajishengallis, 2014).

Cysteine proteinases are classified as arginine-specific (Rgp) and lysine-specific (Kgp) which are linked to the development and establishment of *P. gingivalis* (Zhang et al., 2016). Through the production of microvesicles carrying proteinase, *P. gingivalis* still may cause harm and alter inflammatory response in host cells (Bengtsson et al., 2015).

Fimbriae, capsules, lipopolysaccharide, cysteine proteinases and gingipains are some of the virulence factors found in *P. gingivalis* (Zhang et al., 2016). Gingipains are essential for the formation of peptides or amino acids, which act as an energy and carbon source for the organism (Zhang et al., 2016). Gingipains can be found in the culture supernatant, membrane surface and cytoplasm. Rgp cleave peptide bonds selectively at arginine residues, whereas Kgp cleave peptide bonds specifically at lysine residues (Zhang et al., 2016).

Capsules have a role in *P. gingivalis* virulence by inhibiting the host of immunological response to bacteria, reducing phagocytosis, and allowing bacteria to grow. Moreover, *P. gingivalis* has been shown to reduce cytokines IL-1, IL-6, and IL-8 in fibroblast which suggests that they can trigger the host responses (Septiwidyati & Bachtiar, 2020). Lipopolysaccharide has the potential to induce inflammation in periodontal tissue because it is one of the primary virulence factors for *P. gingivalis*.

2.3 *Salvadora persica* as a Therapeutic Agent

Medicinal plants are now well-known and frequently used as an alternative treatment. It is presently acknowledged as significant due to the presence of a variety of pharmacological and biologically active chemicals inside. There are various functions of plants that are rich with the source of phenolic compounds. Most plant-derived biologically active compounds with anti-cancer, anti-viral, and anti-bacterial activities. *Salvadora persica* (*S. persica*) is a native plant from Iran and the Middle East that has historically been used for oral hygiene (Noumi et al., 2017).

The most prevalent *S. persica* is obtained from the Arak tree, which grows mostly in Saudi Arabia and other regions of the Middle East (Sabbagh et al., 2020). Arak tree seldom grows larger than a foot in size and can reach a height of three meters.

The leaves are thick and small, rounded to ovate, and have a strong mustard scent. The fruits which look like fleshy berries are small and difficult to see. They may be eaten both fresh and dried. *S. persica* tree can survive in challenging environments and withstands soils ranging from extremely dry to highly saturated (Aumeeruddy et al., 2018).

Miswak or *S. persica* is a *Salvadoraceae* family member known locally as the toothbrush tree. It is a tiny, small tree with soft, pale, yellow wood that has been used as a traditional oral hygiene tool throughout Africa, South America, the Middle East, and Asia (Khan et al., 2020). Oral health is a sunnah, which means Prophet Muhammad used the miswak stick (Figure 2.3) as a toothbrush for every prayer (Rispler-Chaim, 1992), upon awakening, after meals and before reciting prayers. Arab people regarded the oral cavity as an important aspect of the human body and treated it with care when it came to personal dental hygiene (Aboul-Enein, 2014).

The World Health Organization (WHO) also promotes and recommends individual's daily physical removal or disruption of dental plaque with oral hygiene tools (toothbrush or traditional brushing sticks), as well as the removal of subgingival calculus by a specialized dentist is the treatment accessible for treating periodontal disease (WHO, 1984).

In response to mechanical debridement in periodontal therapy, different medication delivery mechanisms, mouth rinses and long-term drug release systems are commonly performed as therapeutic agent (Eid Abdelmagyd et al., 2019). There are several strategies for periodontal therapy, including mechanical and chemotherapeutic therapy. Periodontal scaling is the most effective way to inhibit this harmful inflammatory process. Dental scaling procedures to remove soft and hard dental residues are used by dentists (Sahni et al., 2012). However, bacteria can live inside

tissues or in deep pockets that are inaccessible to instruments, removing all pathogens during scaling and root planning techniques is not always effective. This resulted in the use of antibacterial and chemotherapeutic agents as adjuvant and complementary therapy (Mekhemar et al., 2021).

2.3.1 Composition in *Salvadora persica* Extracts

Recent studies of *S. persica* showed a secondary metabolite profile. The sticks of *S. persica* were found to be more effective when compared with synthetic toothbrushes for oral hygiene and they can treat gum inflammation (El-Latif Hesham & Alrumman, 2016). This is because, *S. persica* has anti-microbial, anti-inflammatory (Balto et al., 2017), anti-plaque, aphrodisiac, alexiteric, analgesic, antipyretic, astringent, diuretic, and bitter stomachic actions (Khan et al., 2020). When using *S. persica* as a chewing stick, it possesses cleansing efficacy, the ability to remove plaque, and the potential to reduce gingival bleeding (Al-Judaibi, 2020).

S. persica therapeutic agents for oral health can be explained by their mechanical action when used for brushing, as well as their pharmacologic active components as shown in Table 2.1. These include chemically active substances such as tannins, which inhibit the glucosyltransferase enzyme to reduce plaque and periodontal disease and resins used to protect against dental caries (Al Bratty et al., 2020). It is possible to state that *S. persica* extracts are mainly safe substances with a significant history of human therapeutic treatment and no serious safety concerns (Mekhemar et al., 2021).

Table 2.1 Important bioactive constituents found in *S. persica* extracts.

Bioactive constituent	Biological activity	References
Benzylamides	Antibacterial activity	(Khalil, 2006)
Trimethylamine and salvadorine	Antibacterial and gingiva-stimulating effects	(Ahmad & Rajagopal, 2014)
Benzaldehyde, benzyl nitrile and benzyl isothiocyanate	Antimicrobial effects	(Haque & Alsareii, 2015)

2.3.2 Antibacterial Actions of *Salvadora persica* Extracts Against Oral Pathogens

Fresh leaves, twigs and roots of its small tree can be used in traditional herbal medicine for asthma, cough, rheumatoid arthritis and oral hygiene (Aboul-Enein, 2014). The existence of a volatile active antimicrobial agent resulted in a significant antibacterial activity against the tested microorganisms. *S. persica* extracts enhance oral health and prevent the formation of dental caries. Herbal remedies are medicinal plant ingredients that have been shown traditionally and scientifically to have therapeutic advantages (Eid Abdelmagyd et al., 2019). *S. persica* (Figure 2.3) is a widely used plant in traditional medicine in Africa, the Middle East and Asia for the treatment of a wide range of diseases, especially for periodontal health and treatment (Nordin et al., 2020).

It has been discovered that *S. persica* is efficient against microorganisms involved in the formation of dental plaque, such as *S. aureus*, *S. mutans*, *S. faecalis*, *S. pyogenes*, *L. acidophilus*, *P. aeruginosa,* and *C. albicans* (Noumi et al., 2017). Further study has shown that both ethanol and hexane extracts had the highest antibacterial action against *E. faecalis,* and *C. albicans* (Balto et al., 2017).

Several studies have identified *S. persica* extracts have high sensitivity against Gram-negative bacteria, especially *P. gingivalis*. This might be due to Gram-positive bacteria that have thick peptidoglycan cell wall, which provides higher resistance to penetration by compounds in *S. persica* (Rafiei et al., 2017).

Source: Retrieved from https://www.healthbenefitstimes.com/miswak/

Figure 2.3: Chewing stick of *S. persica*.