CHAPTER 1

INTRODUCTION

Background and Rationale

In traditional health care setting, medicinal plant-based systems have a long history of use. Many medicinal plants have been used in the treatment of infectious diseases based on their antimicrobial properties. The use of traditional medicines in many areas of the world are a result of studies directed at the effectiveness of medicinal plants and isolation of the active substances from these plants. Thus, ethnopharmacology, or use of traditional medicines is the new conventional choice in the discovery of new, biologically-active molecules. [1]

Anacardium occidentale, a plant found in southern Thailand, has been reported to have antimicrobial activity against gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and is used to relieve toothache and sore gums. [2] In Thailand, many well-known plants have been used in traditional medicine, for example, *Terminalia bellerica*, *Syzygium cumini, Punica granatum* and *Rhinacanthus nasutus*. *Terminalia bellerica* is used in the treatment of piles, dropsy, leprosy, diarrhea, biliousness, dyspepsia and headache. [3, 4] The ethanolic extract of the bark of *Syzygium cumini* exhibits potent anti-inflammatory action. [5] The pericarp extracts of *Punica granatum* demonstrates antibacterial activity against *Stapphylococcus aureus* strains. [6] The leaves and stems of *Rhinacanthus nasutus* are often used for treating hepatitis, diabetes, hypertention and skin disease. [7, 8] Although there have been many studies conducted on these particular plants, relatively little is known about their effect on oral diseases. The study of antibacterial, anti-inflammatory and toxic activities of these plants is therefore of interest. The scientific knowledge gained from this study will provide further development of using these plants as adjunctive periodontal treatment in the future.

Review of Literatures

1. Periodontium and Periodontitis

The tissue covering and supporting the teeth is known as periodontium and consists of a number of discrete soft (fibrous; gingival and periodontal ligament) and hard (mineralized; cementum and alveolar bone) connective tissues each with a unique architecture, composition and function. [9, 10] The periodontal tissues are among the most biologically active in the body and contain a heterogenous populations of cells with diverse properties and functions. [11] The tissues of fibroblasts involved in soft connective tissue formation, cementoblasts involved in cementogenesis, osteoblasts involved in bone formation, endothelial cells involved in angiogenesis, and epithelial cells involved in the epithelium formation. [12] The most common cells in the periodontal connective tissue, and the most important functionally, are thought to be the residential fibroblasts. [10, 13]

This study focuses on the residential connective tissue cells of the periodontium, the gingival connective tissue fibroblast responsible for the restoration of soft connective tissue.

1.1 Gingival connective tissue fibroblast

The gingival connective tissue is predominantly a fibrous connective tissue that has begining directly from the oral mucosa connective tissues, as well as some fibers arising from the developing dental follicle. A characteristic of gingival connective tissue is rapid remodeling and a high turnover rate. [13] Sixty-five percent of the cells in gingival connective tissue are fibroblasts located in a highly structured extracellular matrix (ECM) containing fibrous and non fibrous proteins, water, growth factors, minerals, lipids, nerves and vascular components. In common conditions, gingival connective tissue fibroblasts produce and maintain the ECM, maintaining homeostasis by also playing a role in modifying parts of the matrix they created. Thus, these cells regulate the constitution and condition of the gingiva and maintain tissue integrity. [9] Most cells that are represented in the gingival connective tissue, besides the fibroblasts, are highly derived from blood vessels and the blood itself. These cells contain endothelial cells, polymorphonuclear leukocytes (PMN), macrophages, lymphocytes, plasma

cells, and mast cells. In healthy gingival connective tissue condition, inflammatory cells are represented in small numbers and increase in number during inflammation, the amount dependent on the type and severity of the inflammatory reaction. [13]

1.2 Pathogenesis of Periodontitis

Periodontal diseases are a group of inflammatory diseases of the gingival tissue and the supporting structures of the periodontium. They are the most common oral inflammatory disease and are described as the bacterially initiated conversion of a healthy gingival region to one characterized by inflammation (gingivitis) and the destruction of the supporting structures of the teeth (periodontitis). [14-16]

To date, more than 300 bacterial species have been identified in the oral cavity, among these, a relatively small group of gram-negative organisms have been reported. [14-18] These include *Aggregatibactor actinomycetemcomitans* (*Aa*) (formerly *Actinobacillus actinomycetemcomitans*), *Fusobacterium nucleatum* (*Fn*), *Tannerella forsythensis* (*Tf*) (formerly *Bacteroides forsythus*), *Prevotella intermedia* (*Pi*), *Campylobactor* spp., *Capnocytophaga* spp., *Eikenella corrodens* (*Ec*) and *Porphyromonas gingivalis* (*Pg*). These organisms are frequently isolated from infected periodontal pockets and have been recognized as potential periodontal pathogens. [14-18] Virulence factors from these bacteria affect host tissues in 3 ways. Firstly, bacterial antigens interact with the host immune system, particularly PMN, macrophages (MØ) and T-lymphocytes, modulating inflammation. Secondly, the bacterial products such as endotoxins and exotoxins induce tissue destruction that can directly damage host tissues. Thirdly, tissue repair is inhibited by immune evasion strategies. [19, 20]

Gram-negative bacteria possess lipopolysaccharide (LPS) as a cell wall constituent, of which LPS is generally believed to be one of the virulence factors. [21] Early studies have indicated that the LPS levels in gingival crevicular fluid are positively correlated with clinical and histologic signs of gingival inflammation. It is also reported that LPS can penetrate gingival tissues. [21] In addition, host cell activation by LPS produces a spectrum of lymphokines and monokines including the interferons (alpha, beta, gamma), interleukins (IL)-1 and -6, tumor necrosis factor (TNF), platelet activating factor and procoagulant tissue factor. [14, 21]

The presence of the inflammatory mediators IL-1 and prostaglandin E_2 (PGE_2) have been measured in high levels in the gingival crevicular fluid of periodontitis patients and have been associated with attachment and bone loss. [14, 21, 22] IL-1 is a cytokine expressed primarily by monocytes and MØ in response to antigenic challenge, particularly bacterial LPS. PGE_2 is a metabolite of arachidonic acid produced via the cyclooxygenase (COX) pathway. It has been shown to have a number of biological actions, including vasodilation, both anti- and proinflammatory action, modulation of sleep/wake cycles, and facilitation of the replication of human immunodeficiency virus. It elevates Cyclic adenosine phosphate (cAMP) levels, stimulates bone resorption, and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics and a promotor of bone resorption and also causes vasodilation and increased vascular permeability, which enhances clinical signs of inflammation such as redness, pain and edema. High levels of PGE_2 have also been shown to attenuate IgG production and suppress proliferation of gingival fibroblasts, which may affect the humoral immune response and the replacement of damaged connective tissue. [23]

Thus, PGE_2 was selected as the inflammatory cytokine for this study

1.2.1 Prostaglandins

Prostaglandins are arachidonic acid metabolites generated by COX-1 and COX-2. Arachidonic acid is a 20- carbon polyunsaturated fatty acid found in the plasma membrane of most cells. COX-2 is upregulated by IL-1β, TNF-α, and bacterial LPS and appears to be responsible for generating the PGE_2 that is associated with inflammation. The primary cells responsible for PGE_2 production in the periodontium are MØ and fibroblasts. PGE_2 is increased in periodontal sites presenting with inflammation and attachment loss. Induction of matrix metalloproteinases (MMP) and osteoclastic bone resorption is induced by PGE_2. [23- 25]

PGE_2 appears to be partly responsible for bone loss associated with periodontitis. *In vitro* studies demonstrated that bone loss associated with several periodontal pathogens was inhibited in part by inhibitors of prostaglandin synthesis. [23, 24, 25] In addition, use of a nonsteroidal anti-inflammatory agent as an inhibitor of prostaglandin synthesis in human subjects with advanced periodontitis resulted in significantly less bone loss when compared to

placebo. [23-25] PGE_2 release from monocytes from patients with severe or aggressive periodontitis is greater than that from patients with little to no periodontal destruction. It has been postulated that high-risk periodontal patients have a monocyte hypersecretory trait that results in an exaggerated response both locally and systemically to bacterial LPS. [23-25]

1.2.2 Periodontopathic bacteria

Periodontal disease, a polymicrobial mixed infection, is a common oral disease. The oral bacterial flora is always heterogenous and relatively complex, and vary from one patient to another. [23, 26]

Based on the American Academy of Periodontology (AAP) periodontal classification system, 1999, definitions for periodontitis in young patients, including localized and generalized juvenile periodontitis and some forms of rapidly progressive periodontitis, are now classified under "aggressive periodontitis" and adult periodontitis is now classified under "chronic periodontitis". [27]

Chronic periodontitis is clinically apparent from around the age of 35 years, evolves slowly, PMN leukocyte function is apparently normal and a wide range of microorganisms are involved, such as *Pg*, *Pi*, *Tf* and many others. [14, 23, 27, 28] Aggressive periodontitis usually begins during childhood or in early adulthood, progresses rapidly, shows a poorer response to treatment, is accompanied by a deficit in PMN function and the most prevalent microorganism is *Aa*. [14, 23, 27-30] The microflora associated with bursts of disease activity have been analysed in both the chronic and acute forms of periodontal disease. [23, 30, 31] In studies of patients with aggressive periodontitis (formerly localized juvenile periodontitis), periods of apparent disease activity were reflected in a less diverse microflora in which the absolute counts and proportion of only *Aa* and *Ec* were significantly higher. [23, 30, 31] Similarly, in subjects described as having destructive periodontal disease, the isolation frequency of *Pi, Tf, Aa* and *Campylobactor rectus* (*Cr*) was significantly higher at active sites. *Aa, Pi* or *Pg* was isolated from 99% of progressing pockets but from only 40% of inactive sites. [23, 30, 31] When all three species were absent from a site, the probability of the pocket undergoing future progression was only 1%, but in a prospective study, [23, 30, 31] only 20% of sites suffered attachment loss when one or more of

these microorganisms were present. Thus, three species have more chance of ruling out the possibility of disease when key microorganisms are absent than predicting future episodes of disease when they are present.

The selected periodontopathic bacteria in this study were *Aa, Pi*, and *Pg*.

1.2.2.1 *Aggregatibactor actinomycetemcomitans (Aa)*

Aggregatibactor actinomycetemcomitans (formerly *Actinobacillus actinomycetemcomitans*) was first isolated from cervicofacial actinomycotic lesions in 1912 and initially designated *Bacterium actinomycetemcomitans*. [32, 33] In 1921, this microorganism was refered to as *Bacterium comitans* by Lieske and finally designated *Actinobacillus actinomycetemcomitans* in 1929. [32, 33] The term *Actinobacillus* begins from the likeness of an agar colony to a ray fungus and refers to the internal star-shaped morphology when viewed under a microscope and the short rod like or bacillary nature of the individual cells. The name *actinomycetemcomitans* rises from its association with an actenomycete and frequent isolation from actinomycotic lesion along with *Actinomyces israelii*. [32, 33]

Aa is a gram negative cocco-bacillus approximately 0.4×1.0 μm in size. Microscopically, cultures appear predominantly bacillary with a few coccal forms. *Aa* is capnophilic, requiring an atmosphere containing 5-10% CO_2 for good growth. [32, 33] It is microaerophilic and a facultative anaerobe and can grow under anaerobic conditions. *Aa* is nonsporulating, non motile, non-hemolytic, and oxidase and catalase positive. [32, 33]

Vilulence factors of *Aa* have the ability to be transmitted to susceptible hosts. Numerous studies have focused on the virulence of *Aa* because of its strong association with periodontal diseases and related extraoral infections. [15, 23, 32, 33] *Aa* has been shown to possess a countless number of virulence factors that enhance its survival in the oral cavity and enable it to prevent the host's protective strategies. [15, 32, 33] Many of these virulence factors may be involved in the pathogenesis of periodontitis. [15, 32, 33] They include the ability to attach to ECM proteins and epithelial cells; antibiotic resistance; a bactericin; bone resorption by either endotoxin or surface-associated material; a chemotactic inhibitor; a collagenase; a cytotoxin; Fc-binding proteins; a leukotoxin; immunosuppressive factors; and the ability to invade epithelial cells and tissues. [32, 33]

Many studies have found that *Aa* and *Pi* LPS not only participate in periodontal tissue destruction and alveolar bone resorption, but also inhibit bone formation.[18, 33-35] LPS causes skin necrosis (Schwartzmann reaction), bone resorption and platelet aggregation, and it activates macrophages. Low concentrations of *Aa* LPS stimulate macrophages to produce interleukins and tumor necrosis factor, cytokines involved in tissue inflammation and bone resorption. These data suggest that macrophages that migrate to gingival sites of *Aa* infection will be stimulated to produce these cytokines, which may then be involved in gingival inflammation and alveolar bone resorption. Both LPS and these cytokines, which are known to play an important role in atherosclerosis and coronary heart disease, also appear systemically, where they may function in initiating and or exacerbating conditions associated with cardiac disease.[18, 33-35]

1.2.2.2 *Porphyromonas gingivalis* (*Pg*)

Pg has long been considered an important member of the periodontopathic microbiota involved in periodontal disease progression and bone and tissue destruction.[35, 36] This organism is essentially absent during periodontal health, and during disease progression to periodontitis can reach a very significant percentage of the pathogenic microbiota.[18, 35, 36]

All species of the genus *Porphyromonas* have been found to be associated with both human and animal hosts. These species have been isolated from the oral cavities of humans, dogs, cats, and nonhuman primates.[35,36]

Members of the genus are approximately 0.65 × 2.25 μm in diameter and are obligately anaerobic coccobacilli exhibiting smooth, raised colonies, asaccharolytic, non-spore forming, and contain non motile rods. When grown on a blood agar surface, the colonies are initially white to cream colored.[35] With time (4-8 days) these colonies darken from their edge towards the center to a deep red to black color, which correlates with the concentration of protoheme.[35] The species produce a large number of enzymes, proteins and end products of their metabolism that are active against a broad spectrum of host proteins and provide mechanisms for evasion of host defenses. Most of the enzymatic activity is due to the production of cysteine proteinases or trypsin-like proteinase (called gingipain).[35] Metabolically, the ability of *Pg* to secrete these cysteine proteinases in a host provides them with distinct advantages for its survival

and growth, including the ability to use large host proteins for their growth and metabolism. Moreover, the gingipains are chemotactic for PMN, and there is an increased concentration of these host cells at sites of potential tissue and bone destruction. [35, 36]

1.2.2.3 *Prevotella intermedia* (*Pi*)

Pi was previously characterized as a black-pigmented bacteroides. [20, 32, 38] *Pi* has been isolated in association with both aggressive periodontitis and advancing periodontitis. [20, 32, 38] It is found prominently in gingivitis and rapidly returns after therapy. It has many of the virulence factors such as the gelatinases MMP-2 and MMP-9, which are known to be stimulators of bone resorption. [20, 32, 37, 38]

Pi has recently been subdivided into two species, *Pi* and *Prevotella nigrescens*. Recent studies indicate that in plaque samples from patients with periodontitis, *Pi* was detected more frequently than *Prevotella nigrescens*, whereas samples from healthy gingival sites were dominated by *Prevotella nigrescens*. [20, 32, 37, 38] No studies have evaluated the relative pathogenic potential of the two species, but it is hoped that separate monitoring of them will clarify the role of *Pi* in periodontitis. [38]

1.3 Antibiotics in Periodontitis Treatment

An increased interest in antibiotic therapy as an adjunct to standard treatment regimens began in the late 1970s with the realization that certain bacteria were frequently associated with the disease process. [39-42] The addition of antimicrobial control directed toward putative periodontal pathogens was thought to offer an opportunity to enhance the treatment of periodontal diseases. Mechanical and surgical treatments, combined with diligent oral hygiene measures, were usually successful in controlling periodontal destruction. [43, 44] In some instances, the combination of antibiotic therapy in conjunction with conventional periodontal treatment yielded a favorable clinical response not obtained with conventional therapy alone. As a result, confusion developed as the practitioner concerned over antibiotic selection, risk/benefit ratio, dosing regimen, length of treatment, evaluation parameters, clinical outcome assessments, and short-and long-term benefits from adjunctive antibiotic therapy. [39- 41]

There is some strong evidence that supports the use of an adjunctive antibiotic in the treatment of certain forms of periodontal diseases. [39-41] Aggressive forms of periodontal disease frequently progress more rapidly than the more common, non-aggressive forms (chronic periodontitis); and as such, require more aggressive treatment. [45] Patients presenting with aggressive periodontitis or with periodontitis that has not responsed favorably to previous therapy; e.g., refractory disease, most likely will benefit from the additional use of an antibiotic along with conventional periodontal therapy. [45] The concept of administering an antibiotic to the immediate area of infection is attractive. It enables delivery of high drug concentrations directly to the infected site and eliminates, or decreases, the potential for creating antibiotic resistance. [46-49] So local delivery antibiotic is of interest.

Local delivery of antibiotics into the periodontal pocket

Within the past decade, several locally applied controlled delivery products have been approved by the Food and Drug Administration (FDA) for the treatment of periodontitis. [49, 50] The concept that local delivery of an antibiotic into the periodontal pocket achieves a greater, more potent concentration of drug than available with systemic delivery is very attractive. [46-50] The amount of drug delivered often exceeds the equivalent of 1 mg/ml. This level is considered bacteriocidal for most bacteria that exhibit resistance to systemically delivered concentrations. [46-50] Likely important, local delivery of an antibiotic would have a minor impact on the microflora residing in other regions of the body.

In the United States of America (USA), five locally delivered antibiotic regimens have received FDA approval for use in the treatment of periodontitis. These include tetracycline fiber, chlorhexidine chip, and doxycycline polymer. [46, 50] Metronidazole gel and minocycline ointment are not marketed in the USA at this time. [50] In most cases, all products have been tested primarily in the treatment of "chronic periodontitis" either as an adjunct to scaling and root planing or as an alternative to scaling and root planing. Positive clinical results were reported in each study. [46, 50] Presently, data are insufficient to evaluate the potential efficacy of these regimens in the treatment of refractory periodontal disease. Instinctively, these regimens would seem to be ideal for the treatment of recurrent periodontitis or for the treatment of individual sites that have not responded as well as desired.

Another FDA approved drug for the treatment of periodontitis is doxycycline hyclate (SDD). Prescribed at a dosage of 20 mg, bid, doxycycline has no detectable antimicrobial efficacy on the oral flora or on the flora in other regions of the body. [51, 52] A 20 mg dose exerts its effect not as an antibiotic, but as a collagenase inhibitor as well as an anti-inflammatory agent. This product has been extensively tested as an adjunct to scaling and root planing in multi-centered, double-blind, placebo controlled studies. [51, 52] Significant improvements in clinical attachment level and probing depth were present at 3, 6, and 9 months of treatment in the SDD/ scaling and root planing subjects relative to scaling and root planing alone, with more severely diseased sites showing the greatest improvement. [51, 52]

The use of local antibiotics such as tetracycline, doxycycline, minocycline and metronidazole, which aims to maintain a high concentration of the drug within a well-confined area at the diseased site, has thus been introduced. [46, 50] Many reports have shown that the side effects and adverse drug reactions of locally administered therapy are lower than therapy administered systemically such as; hypersensitivity or allergic reactions, induction of bacterial resistance, super infection by other classes or species of microorganisms, toxic side-effects, and drug interactions with concomitant medications. [40, 41, 46] Due to crevicular fluid flow and anatomic difficulties, such as furcations, the chemical compound cannot reach some areas and in spite of high concentrations, the clinical efficacy is limited. [41, 42] For these reasons locally delivered regimens have been developed in various compounds and concentrations. Studies have shown that the use of tetracycline fibers and metronidazole gel combined with thorough scaling and root planing resulted in statistically significant reductions in pocket depth when compared to scaling and root planing alone. Moreover, metronidazole gel also reduced bleeding when compared to scaling and root planing alone. [41, 43, 44, 46, 53] Mombelli, et al found that the local application of a tetracycline fiber resulted in a reduction of *Aa* and *Pg* but failed to eradicate these periodontal pathogens. [53] Other studies have shown that minocycline ointment and minocycline HCl microsphere, the additive effect of minocycline, improve pocket depth when compared to scaling and root planing alone. [42, 46, 47]

2. Thai medicinal plants

The Thai people have been using traditional medicinal herbs as an alternative treatment for many infectious diseases due to their proven antimicrobial properties and also because of the prohibitive cost of commercial drug equivalents. Based on these two factors, there is an opportunity to further research the active components of a variety of locally available plants from the southern region of Thailand as an adjunctive treatment for patients with periodontal diseases. Promising plants include *Ao* and *Sc* (leaves and bark), *Pn* (pericarp), *Rn* (leaves) and *Tb* (dried-fruit).

2.1 *Anacardium occidentale* (*Ao*)

Figure 1-1 *Anacardium occidentale* (original picture)

Ao is better known as the Chashew nut tree. This plant has been reported to have antimicrobial activity against the gram-negative bacteria *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*).[2, 55] Akinpelu reported that infusion of *Ao* bark and leaves can be successfully used to relieve toothache and sore gums while young leaves can be used for the treatment of dysentery, diarrhoea and piles.[55] Stem bark can be used for the treatment of pellagra.[55] *Ao* is a plant indigenous to Africa and employed traditionally in Nigeria for a variety of ailments in addition to the edible nature of its fruits and seeds. The plant is used

for various inflammatory conditions, particularly arthritis. It is also used for treating fevers, aches and pains, inflammation on the extremities and asthma. This plant is popular in traditional medicine. This plant has been demonstrated to possess anti-inflammatory properties, thereby validating its folkloric use. An extract of the plant demonstrated 90% inhibition of prostaglandin synthesis from bovine seminal vesicles and had an anti-inflammatory property.[56, 57]

2.2 *Terminalia bellerica* (*Tb*)

Figure 1-2 *Terminalia bellerica* (original picture)

Tb is a useful tree which occurs widely in the valleys of India. Its common name in Hindi is Bahera and in Thai, it is called Samoor-pipek.[3, 4] Parts of the tree have been used in traditional medicine for treatment of piles, dropsy, leprosy, diarrhea, biliousness, dyspepsia and headache.[3, 4] It has been reported to contain β-sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, mannitol, glucose, galactose, fructose and rhamnose.[3]

2.3 *Syzygium cumini* (*Sc*)

Figure 1-3 *Syzygium cumini* (original picture)

Sc is a medicinal plant, whose parts have been pharmacologically proven to possess hypoglycaemic, antibacterial and anti-HIV activities. [5] In English it is better known as the black or purple plum tree. It is called "Whau", in Thai. [5] The bark of the plant is employed in folk medicine for treatment of inflammations. [5] The ethanolic extract of the bark of *Sc* was investigated for its anti-inflammatory activity in animal models and has potent anti-inflammatory action against different phases of inflammation without any side effect on the gastric mucosa. [5]

2.4 *Punica granatum* (*Pn*)

Figure 1-4 *Punica granatum* (original picture)

Pn is a native shrub of occidental Asia and Mediterranean Europe, popularly refered to in English as pomegranate. Brazilians call it "Roma" and its common name in Thai is "Tab-tim". In Brazil, the fruit is used to treat aptha, diarrhea and intestinal parasites. [58] For centuries the bark, leaves, flowers, and fruit of this plant have been used to ameliorate diseases ranging from conjunctivitis to hematuria. This plant was used by Egyptians as a treatment for tapeworm and other parasitic infestations. [6, 58, 59] In recent decades, *Pn* has been studied for many potential uses including: immunomodulation, atherosclerosis, bacterial infection, fungal infection, parasitic infection, periodontal disease, and food poisoning. [59] One study reported that the extracts of the pericarp and fresh fruit of *Pn* demonstrated antibacterial activity against *Stapphylococcus aureus* (*S. aureus*) strains. [6]

2.5 *Rhinacanthus nasutus* (*Rn*)

Figure 1-5 *Rhinacanthus nasutus* (original picture)

Rn is a plant widely distributed throughout the tropics including Southeast Asia, South China and India. [60] Its common name in Thai is "Thong-phan-chang". This traditional medicine plant has been used for the treatment of skin diseases such as ringworm, amputating

necrosis of the penis, cancer, rash, falling hair, and abscess pain. In addition, the leaves and stems have been used for health promotion. [60]

Relatively little is known about the effect of Thai medicinal plants on periodontopathic bacteria and their anti-inflammatory activity and toxicity. This study is designed, firstly, to investigate the effect of the five previously mentioned promising local Thai medicinal plants on the antibacterial activity of the well known periodontal organisms. Secondly, the plant extracts will be examined for anti-inflammatory activity. The investigation of these extracts and the toxicity on HGF will provide more information and direction on developing these plants in locally administered periodontal treatment in the future.

Objectives and expected benefits

Objectives

The objectives of this study are:

1. to investigate the antibacterial activities of five Thai medicinal plant extracts, namely
   - *Anacardium occidentale (Ao)* leaves and bark
   - *Terminalia bellerica (Tb)* dried-fruit
   - *Syzygium cumini (Sc)* leaves and bark
   - *Punica granatum (Pn)* pericarp
   - *Rhinacanthus nasutus (Rn)* leaves

   on three periodontopathic bacteria, namely
   - *Aggregatibactor actinomycetemcomitans (Aa),*
   - *Prevotella intermedia (Pi)*
   - *Porphyromonas gingivalis (Pg)*
2. to investigate the toxicity of these selected plant extracts on gingival connective tissue fibroblasts
3. to study the anti-inflammatory activity of the selected plant extracts.

Expected Benefits

The results of this preliminary study will provide:

1. The knowledge of seven Thai medicinal plant extracts that have antibacterial activity on periodontopathic bacteria.
2. The baseline data of the efficacy [Minimal Inhibitory Concentration (MIC)] of the studied plant extracts compared to standard antibiotics.
3. The knowledge of the toxicity of these plant extracts on gingival connective tissue fibroblasts.
4. The knowledge of the anti-inflammatory activity of the studied plant extracts.
5. The beginning step of developing the use of Thai medicinal plants as an adjunctive locally administered treatment for periodontal diseases.