CHAPTER 1
INTRODUCTION

Medicinal plants have been used worldwide for the treatment of various human ailments since antiquity. Their uses are is still quite prevalent in developing countries in the form of traditional medicine. Intensive chemical and pharmacological studies on medicinal plants during the last 5 decades have led to the validation of traditional claims in many cases and facilitated identification of their active principles. The active principles have provided leads in the development of several life-saving drugs that which are in clinical use today.

Traditional medicine is widely used in the rapidly growing health system of under developed and developed continues with considerable economic importance. In Africa, up to 80% of the population uses traditional medicine for their health care needs. In Asia and Latin America, populations continue to use traditional medicine as a result of long historical traditions and cultural believes. In China, traditional medicine accounts for around 40% of all health care delivered [1]. As its name implies, it is part of the tradition of each country and employs practices handed down from generation to generation of healer. Its acceptance by people receiving care is also inherited from generation to generation. The increase in the number of modern medicine practitioners has led to the increased use of modern drugs until they almost entirely replace herbal medicine, particularly in the state-run hospitals and health service centers. However, most people in the rural areas have still used traditional medicine. Hence, the traditional of medicine certainly plays an important role in primary health care with all their riches and potentialities and will help people to reach the goal of "the attainment by all people by the year 2000 of the level of health that will permit them to lead a socially and economically productive life" as declared by the International WHO Conference on Primary Health Care at Alma Ata, 6-12 September 1978 [2, 3]. This announcement urges the member countries to include

medicinal plants in their primary health care system. Traditional medicine, therefore, has a major role in health care delivery systems in terms of numbers of people served by the health care systems throughout the world. Recognizant of this fact, many countries of the world has currently started paying due attention to promote it. Since then the Thai government has integrated the use of medicinal plants and traditional medicine in the primary health care and promotion of research and development of drugs from medicinal plants in its National Economic and Social Development Plant [2, 3].

There have been many reports of compounds being found in plants with anti-inflammatory activity. In addition, many plants with a medicinal reputation for treating inflammatory diseases, and also diseases related to inflammation, have shown anti-inflammatory action in animal or biochemical models used by pharmacologists to screen compounds for anti-inflammatory activity [4]. For example, *Centella asiatica* of the family Umbelliferae, commonly found in India, Asia, Thailand and the Middle East, is used as a traditional medicine for improving memory, for the treatment of mental fatigue, anxiety, eczema [5], anemia, asthma, blood disorders, bronchitis, fever [6], and as an anti-inflammatory and anti-nociceptive agent [7]. Some studies in Thailand reported that the methanol extract of *Clerodendrum petasites* show potent antipyretic and moderate anti-inflammatory activities without ulcerogenic effect [8]. *Cissus quadrangularis*, the medicinal plant indigenous to Asia and Africa is used for many ailments, especially in Thailand and India, for the treatment of hemorrhoids. The methanol extract from *C. quadrangularis* was assessed for anti-inflammatory activity in various acute inflammatory models. It was found that *C. quadrangularis* exerts an anti-edematous activity on ethyl phenyl propiolate-induced ear edema as well as in carrageenin- and arachidonic acid-induced hind paw edema in rats [9]. The mechanism of its anti-inflammatory action is suggested to be due to an inhibitory effect on both the cyclooxygenase and the lipoxygenase pathways of arachidonic acid metabolism. It is likely that *C. quadrangularis* is a dual inhibitor of arachidonic acid metabolism. In the analgesic test, *C. quadrangularis* provoke demonstrates a significant reduction of acetic acid-induced writhing response induced by an intraperitoneal injection of acetic acid and also elicits the significant reduction of licking time on both early and late phases of the formalin test in mice. The results

suggest that *C. quadrangularis* possesses both peripheral and central analgesic activities. Furthermore, *C. quadrangularis* exerts venoconstrictive effect on the isolated human umbilical vein similar to Daflon®, an antihemorrhoidal drug [8]. *Clerodendrum serratum* is another medicinal plant used in Thailand and India for the same purpose as *C. quadrangularis*, i.e., for the treatment of hemorrhoids. Hence the present study was carried out to confirm the traditional use of *C. serratum* for analgesic, anti-inflammatory and venotonic effects that has been implicated for curing pain, inhibiting inflammation and reducing the size of hemorrhoids.

1.1 Inflammation

The inflammatory process involves a series of events that can be elicited by numerous stimuli such as infections, ischemia, antigen-antibody interactions, and thermal or other physical injury. At a macroscopic level, the response usually is accompanied by the familiar clinical signs of erythema, edema, tenderness (hyperalgesia), and pain [10]. The inflammatory response consists of two main components, a vascular reaction and a cellular reaction. Many tissues and cells are involved in these reactions, including the fluid and proteins of plasma, circulating cells, blood vessels, cellular and extracellular constituents of connective tissue. The circulating cells include neutrophils, monocytes, eosinophils, lymphocytes, basophils and platelets. The connective tissue cells are the mast cells, which intimately surround blood vessels, the connective tissue fibroblasts, resident macrophages and lymphocytes. The extracellular components consist of the structural fibrous proteins (collagen, elastin), adhesive glycoproteins (fibronectin, laminin, nonfibrillar collagen, tenascin and others) and proteoglycans. The basement membrane is a specialized component of the extracellular matrix consisting of adhesive glycoproteins and proteoglycans [11]. Inflammation is divided into acute and chronic patterns [12].

Acute inflammation is an immediate and early response to the injurious agents. It has relatively short duration, lasting from a few minutes up to a few days [12]. This inflammation is most frequently caused by infectious agents, heat and cold or physical trauma. It may also be a response to immunologic injury. Acute inflammation has three major components. First, alterations in vascular caliber that lead to an increase in blood flow. Second, structural changes in the microvasculature that permit plasma

proteins and leukocytes to leave the circulation. And third, migration of the leukocytes from the microcirculation, their accumulation in the focus of injury and their activation to eliminate the offending agent. It is mediated by the release of autacoids such as histamine, serotonin, bradykinin, prostaglandins (PGs), leukotrienes (LTs), etc. The familiar clinical signs of the acute inflammation such as edema, redness, heat and pain are caused by local vasodilatation and increased capillary permeability. If the initiating stimuli for an inflammatory reaction are not eliminated by the reaction or controlled adequately, a continuing state of inflammation persists [13].

Chronic inflammation implies an inflammatory process that persists for a long time - weeks, months, or years - when there is continuous or multiple episodes of injury. Although it may follow an acute inflammation, as described earlier, a chronic inflammation frequently begins insidiously, as a low-grade, smoldering, often asymptomatic response [12]. The tissues affected by chronic inflammation commonly shows evidence of the following pathologic processes: (1) Immune response; manifestation of the immune response in injured tissue includes the presence of lymphocytes, plasma cells, macrophages and plasma immunoglobulin levels may be elevated. (2) Phagocytosis; immune phagocytosis is mediated by macrophages that have been activated by T-cell lymphokines and involves antigens that have opsonins attached to their surfaces whereas nonimmune phagocytosis is directed against foreign non-antigenic particles. (3) Necrosis; commonly there is some degree of necrosis that may affect only scattered individual cells or may be extensive. (4) Repair; repair of tissue damaged by persistent injury is characterized by new blood vessel formation, fibrotic proliferation and collagen deposition [16]. Chronic inflammation involves the release of a number of mediators such as interleukins (IL) 1, 2 and 3, tumor necrosis factor (TNF) α, interferons (IFN) and platelet-derived growth factor [13]. One of the most important conditions caused by these mediators is rheumatoid arthritis, in which chronic inflammation results in pain and destruction of bone and cartilage that can lead to severe disability and in which systemic changes occur that can result in shortening of life [15].

Chemical mediators found in inflammatory site include many endogenous ligands, such as arachidonic acid metabolites and others. The two amines, histamine

and serotonin, are especially important because they are present in preformed stores in cells and are therefore among the first mediators to be released during inflammation. Histamine is widely distributed in tissues, the richest source being the mast cells that are normally present in the connective tissue adjacent to blood vessels. It is also found in blood basophils and platelets. Preformed histamine is present in mast cell granules and is released by mast cell degranulation in response to a variety of stimuli: (1) physical injury such as trauma, cold or heat; (2) immune reactions involving binding of antibodies to mast cells; (3) fragment of complement called anaphylatoxins (C3a and C5a); (4) histamine-releasing proteins derived from leukocytes; (5) neuropeptides (e.g., substance P); and (6) cytokines (IL-1, IL-8). In humans, histamine causes dilation of the arterioles and increases the permeability of venules. It is considered to be the principal mediator of the immediate transient phase of increased vascular permeability, causing venular gaps [12]. Histamine can also stimulate free nerve endings (mainly H_1-receptors), cause pain and itching [16]. Thus, inhibition of histamine response delays but dose not prevent the inflammatory response [17].

Serotonin is present in central and peripheral serotonergic neurons. It is released after tissue injury, and it exerts algesic and analgesic effects depending on the site of action and the receptor subtype [18]. Serotonin is also a preformed vasoactive mediator with effects similar to those of histamine. In the microcirculation, serotonin can also cause vasodilatation through serotonin 1 receptors [19], together with constriction of venules, with the result that capillary pressure rises and fluid escapes from the capillaries. In large vessels, both arteries and veins are usually constricted by serotonin. This is a direct action on vascular smooth muscle cells, mediated through serotonin 2A receptors. Serotonin acting in combination with other inflammatory mediators, may ectopically excite and sensitize afferent nerve fibers, thus contributing to peripheral sensitization and hyperalgesia in inflammation and nerve injury [18, 19].

Another mediator, that plays an important role in nociceptive processing is substance P (SP). SP is secreted by nerves and inflammatory cells such as macrophages, eosinophils, lymphocytes, and dendritic cells and acts by binding to the neurokinin-1 receptor (NK-1) [20]. So it is thought to transmit nociceptive

information and contribute to occurrence of pathological pain states such as inflammation and nerve injury [21]; it regulates vessel tone and modulates vascular permeability [22].

Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid (5,8,11,14-eicosatetraenoic acid) that is derived from dietary sources or by conversion from the essential fatty acid linoleic acid [12]. It is released from membrane phospholipids through the activation of cellular phospholipases (e.g., phospholipase A_2) by mechanical, chemical, and physical stimuli or by other mediators (e.g., C5a). AA metabolites, also called eicosanoids, are synthesized by two principal enzyme pathways, the cyclooxygenase (COX) and the lipoxygenase (LOX) pathways [23, 24]. The scheme of the major metabolic transformations of AA is shown in Figure 1.

The COX pathway products are prostaglandin E_2 (PGE_2), PGD_2, PGF_{2_α}, PGI_2 (prostacyclin), and thromboxane A_2 (TXA_2). TXA_2, a potent platelet aggregating agent and vasoconstrictor, is the major PGs product from platelets. Endothelium possesses inhibitor of platelet aggregation. PGD_2 is the major metabolite of the COX pathway in mast cells, along with PGE_2 and PGF_{2_α}. It causes vasodilation and edema formation. The PGs are also involved in the pathogenesis of pain and fever in inflammation [24, 25]. COX exists in two main isoforms, COX-1 and COX-2. COX-1 is expressed in gastric mucosa and mediates a “housekeeping” function [24]. It regulates several homeostatic processes such as renal blood flow, gastric cytoprotection and platelet aggregation, whereas COX-2 is induced in settings of inflammation by cytokines and inflammatory mediators. COX-2 is the enzyme responsible for generation of most of the inflammatory PGs [26, 27]. There are several lines of evidence to support the notion that COX-derived products are important mediators of inflammation, i.e., PGs synthesis is increased at sites of inflammation [12]. COX is an important enzyme that is inhibited by diclofenac and non-steroidal anti-inflammatory drugs (NSAIDs). Recently, an alternative splice variant of COX-1 that is selectively inhibited by acetaminophen has been identified and called COX-3 [28, 29]. There is considerable evidence that the analgesic effect of acetaminophen is due to the activation of descending serotonergic pathway, but its primary mechanism of action may be inhibition of PG synthesis [28].

The LOX pathway utilizes AA by 5-LOX to produce the LOX products which are also involved in inflammatory reactions as pro-inflammatory mediators [30]. These are also involved in the inflammatory processes by enhancing vascular permeability and through chemotactic attraction of leukocytes [31]. The initial products of the LOX pathway are generated by three different LOX, which are present in only a few types of cells; 5-LOX is the predominant enzyme in neutrophils, it gives rise first to the main product, 5-hydroxyeicosatetrenoic acid (HETES) and then to peptidoleukotrienes and leukotrienes (LTs), i.e., LTB_4, LTC_4, LTD_4 and LTE_4. LTB_4 is a very potent chemotactic agent for neutrophils, eosinophils and macrophages, it causes the accumulation of polymorphonuclear leukocyte (PMN), generation of oxygen free radicals and release of lysosomal enzymes [32, 33]. In human skin, LTC_4, LTD_4 and LTE_4 cause transient wheal and flare responses by direct action or through the release of other endogenous mediators such as PGs [23, 34]. Owing to the contribution of LTs to the pathogenesis of many inflammatory processes, they also represent an important target for therapeutic regulation [30].

The kinin system generates vasoactive peptides from plasma proteins, called kininogens, by the action of specific proteases called kallikreins. Activation of the kinin system results in the release of the vasoactive nonapeptide bradykinin. Bradykinin increases vascular permeability and causes contraction of smooth muscle, dilatation of blood vessels, and pain when injected into the skin. These effects are similar to those of histamine [35].

Platelet-activating factor (PAF) is a bioactive phospholipids-derived mediator formed by different cells including eosinophils, macrophages, neutrophils, vascular endothelium and platelets [12]. It activates most inflammatory cells, induces bronchoconstriction, and hyperreactivity. PAF has been shown to induce vasodilatation and increase vascular permeability [36].

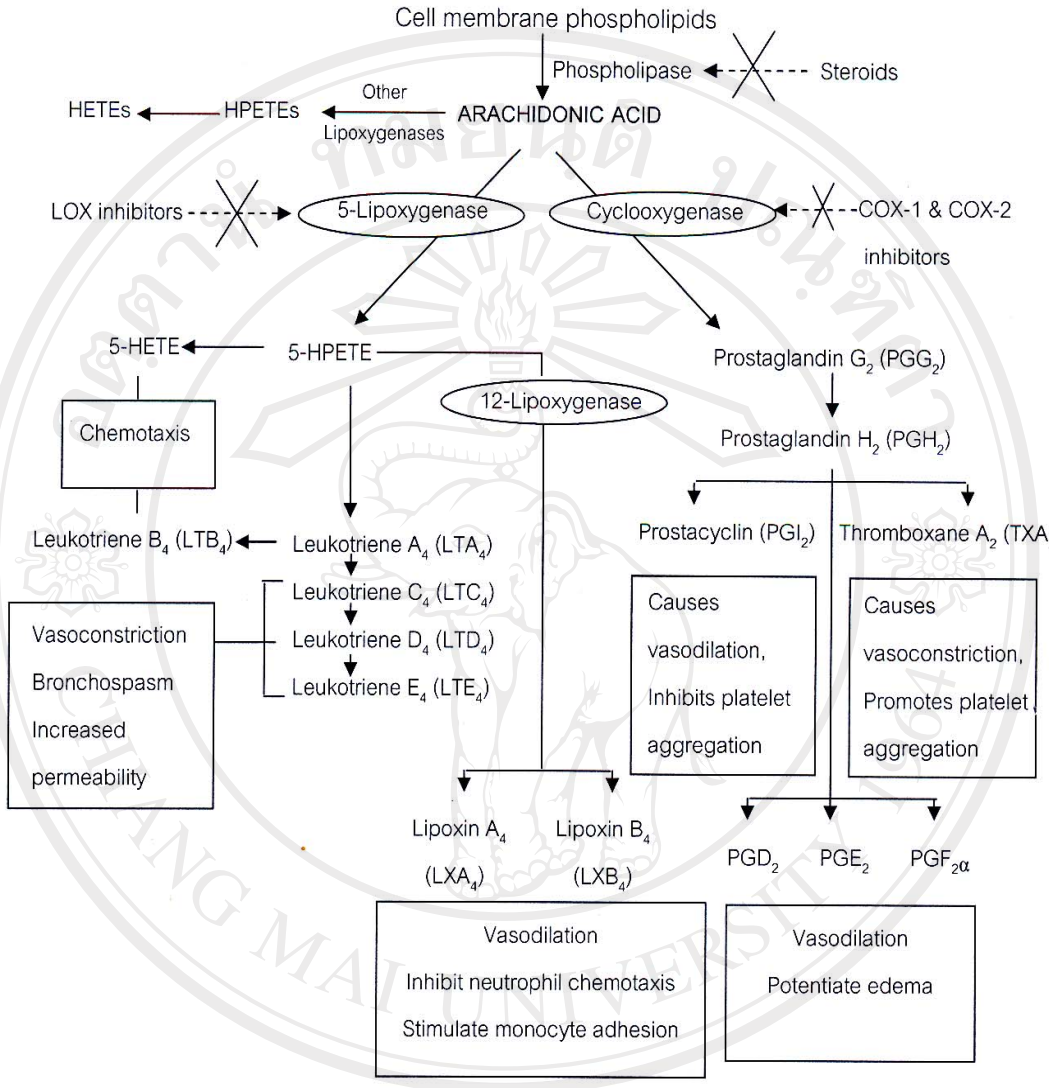

Figure 1. Generation of AA metabolites and their roles in inflammation and the molecular targets of action of some anti-inflammatory drugs [12].

Cytokines are polypeptide products of many cell types (but principally activated lymphocytes and macrophages) that modulate the function of other cell types. Long known to be involved in cellular immune response, these products have additional effects that play important roles in both acute and chronic inflammation [12]. TNF and IL-1 are the most relevant of cytokines, which exercise an influence on the inflammatory process [12, 37]. They produce many of the same proinflammatory

responses which include mobilization and activation of PMNs; induction of COX and LOX enzymes; increasing in adhesion molecule expression; activation of B-cells, T-cells, and natural killer cells; and stimulation of production of other cytokines. Clearly, many of the events associated with acute inflammatory reaction can be mediated by TNF and IL-1. Other actions of these agents likely contribute to the fibrosis and tissue degeneration of chronic proliferation phase of inflammation; stimulation of fibroblast proliferation, induction of collagenase and activation of osteoblasts and osteoclasts [38]. As well as their important local effects, the cytokines produced by macrophages and neutrophils have long range effects that contribute the host defense. One of these is the elevation of body temperature, which is caused by IL-1, IL-6, TNF-α, and other cytokines. These are termed "endogenous pyrogens" [39]. Other cytokines, including IL-8 and IFN-γ, exert additional effects such as increased chemotaxis for leukocytes and increased phagocytosis. All these effects result in the accumulation of fluid (edema) and leukocytic cells in the injured areas [38]. The role of mediations in different reactions of inflammation is shown in Table 1.

Nitric oxide (NO) is short half-life, soluble, free radical gas produced by a variety of cells. It plays multiple roles in inflammation including relaxation of vascular smooth muscle, promotes edema and vascular permeability. It stimulates the synthesis of inflammatory PGs by activation of COX-2. Thus, inhibition of the NO pathway may have a beneficial effect on inflammatory diseases [40].

The treatment of patients with inflammatory diseases involves two primary goals; first, the relief of pain which is often the presenting symptom and the major continuing complaint of the patients; and second, slowing of tissue-damaging processes [15]. NSAIDs are among the most commonly used for inflammation therapy [41, 42]. The mechanism of action of NSAIDs involves inhibition of COX. NSAIDs have three major actions which are anti-inflammatory action, analgesia and antipyrexia [43]. The anti-inflammatory action is exerted via the decreasing in vasodilator PGs (PGE_2 and PGI_2), thus inhibiting their effects and resulting in less vasodilation and edema. This antiprostaglandin effect is considered to be the primary mechanism by which these drugs produce both therapeutic and adverse effects. Diclofenac and traditional NSAIDs are nonselective COX inhibitors because they inhibit both COX-1 and COX-2. COX-1 is expressed in the gastric mucosa, and the

mucosal PGs generated by COX-1 are protective against acid-induced damage [38]. Thus, inhibition of COX by diclofenac or NSAIDs also causes gastric ulceration [44]. To preserve the anti-inflammatory effects of COX inhibition but still prevent the harmful effect on gastric mucosa, highly selective COX-2 inhibitors are now available [45]. They were designed to relieve pain, fever, and inflammation as effectively as older NSAIDs, but with fewer adverse effects, especially stomach damage [38]. Analgesic effect of NSAIDs is due to a decreasing of PGs generation resulting in less sensitization of nociceptive nerve endings to inflammatory mediators.

Anti-inflammatory corticosteroids such as dexamethasone and prednisolone, have powerful anti-inflammatory effect. They block all the known pathway of eicosanoid synthesis, perhaps by stimulating the synthesis of several inhibitory proteins collectively called anexins or lipocortins. They inhibit phospholipase A_2 activity, probably by interfering with phospholipid binding and thus preventing the release of AA [14]. Although the use of corticosteroids as anti-inflammatory agents does not address the underlying cause of the disease, the suppression of inflammation is of enormous clinical utility and has made these drugs among the most frequently prescribed agents. Topical corticosteroid is widely used and very useful for the treatment of acute inflamed and painful hemorrhoids [46].

Hemorrhoids, the mass of dilated vein in the anorectal area that are caused by increased pressure in the vein adjacent to the anus. Analgesic and anti-inflammatory agents with venotonic effect, such as the combination of diosmine and hesperidine, are of benefit for the treatment of hemorrhoids [46].

Table 1. Role of mediators in different reactions of inflammation [12].

Action	Mediators
Vasodilation	PGs NO Histamine
Increased vascular permeability	Vasoactive amines Bradykinin LTC_4, LTD_4, LTE_4 PAF Substance P
Chemotaxis, leukocyte recruitment and activation	C5a LTB_4 Chemokines IL-1, TNF
Fever	IL-1, TNF PGs
Pain	PGs Bradykinin
Tissue damage	Neutrophil and macrophage lysosomal enzymes Oxygen metabolites NO

1.2 Experimental models

1.2.1 Inflammatory models

1.2.1.1 Ethyl phenylpropiolate [EPP]-induced rat ear edema

Edema is a cardinal sign of acute inflammation. Thus, edema is a useful parameter to look at when testing for agents which may be active in treating acute inflammation [47]. Ear edema induced in rats by EPP has been suggested to serve as a more useful model for the rapid *in vivo* screening of agents with anti-inflammatory activity, since only small amount of a test substance is needed. By using edema inducer [EPP], the mechanism involved can be suggested. Histamine, serotonin, bradykinin and PGs are released in EPP induced ear edema [48].

1.2.1.2 Carrageenin-induced hind paw edema in rats

The rat hind paw edema still remains the most commonly test used for anti-inflammatory activity. The edema is produced in hind paw of rats by injection of phlogistic agents such as formalin, dextran, egg albumin, carrageenin, arachidonic acid. The most commonly used phlogistic agent was carrageenin [49, 50]. The edema is produced by a sequential release of pharmacological mediators; histamine, serotonin, bradykinin, and PGs [50]. It is important when using this model to assess the effect of the potential anti-inflammatory agent at the appropriate time during the swelling of the hind paw. Ideally the foot should be measured at more than one time point but certainly at 3–4 h. This allows for the participation of all the chemical mediators. The test is excellent for detecting inhibitors of COX [47].

1.2.1.3 AA-induced hind paw edema in rats

AA-induced hind paw edema in rats provides a valuable tool for evaluating the *in vivo* anti-inflammatory activity of LOX inhibitors and other agents with a mechanism of action different from COX inhibition. 5-LOX products of AA are involved as inflammatory mediators. LTB_4, LTC_4, LTD_4, and LTE_4 cause edema together with increase microvascular permeability [51]. The subplantar injection of AA into the hind paw of rats produces edema within 5 min and reaches peak response by 1 h after injection. One of the unique aspects of AA-induced rat paw edema is its

sensitivity to dual inhibitors of AA metabolism, LOX inhibitors and corticosteroids but is insensitive to selective COX inhibitors [52].

1.2.2 Analgesic models

The formalin test is very useful for not only assessing the analgesic activity but also for elucidating the mechanism of pain [53, 54]. This test consists of two distinct phases which possibly reflecting different types of pain mechanisms. The first phase starts immediately after injection of formalin and lasts about 5 min. This is due to direct chemical peripheral stimulation of nociceptors [55] that seems to be caused predominantly by C fiber activation [38]. In this phase, first response is evoked by the direct formalin stimulation of the nerve endings followed by SP release and SP may play a role through cooperation with bradykinin in this phase [54]. The second phase starts approximately 15-20 min after formalin injection and lasts for 20-40 min [56]. The second phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord [57, 58]. The response of the early phase can be inhibited by centrally acting analgesics such as morphine and codeine. In contrast, the late phase which seems to be due to an inflammatory response is partly mediated by PGs and can be inhibited by NSAIDs (e.g., aspirin and diclofenac), corticosteroids (e.g., dexamethasone and prednisolone) as well as the centrally acting analgesics [59].

1.2.3 Vascular model

The term “hemorrhoids” describes a plexus of veins located between the lamina muscularis mucosa and sphincter muscle structures. With an extension of veins, this plexus becomes enlarged and plays an important role in fine continence of the anal canal. For testing of the effects of compounds with venotonic effects, the isolated human umbilical vein is widely used as a representator for human veins. In the human umbilical vein, bradykinin promotes a potent and effective venoconstrictor response [60]. Thus, isolated human umbilical vein was used in this study to confirm the traditional use of *C. serratum* in the treatment of hemorrhoids which caused by venous insufficiency and loss of contractility of vein.

1.3 Historical background of *Clerodendrum serratum* Linn.

Clerodendrum serratum Linn. is commonly known in Thai as "Ak-khi-tha-wan" or "Lua-sam-kian (Chiang Mai)" or "Tri-cha-wa" or "Sa-mao-yai" (Nakhon Ratchasima) [61, 62, 63]. It is a herb in the family Verbenaceae [61]. Botanical characteristics of this plant are as follows: a shrub 1–4 m high. Branchlets densely yellow pubescent especially on nodes when young, becoming dark brown to gray-yellow and glabrous. Leaves opposite or in threes; petiole to 5 cm or leaf subsessile; leaf blade oblong, obovate-oblong, elliptic, or ovate, 6–30 × 2.5–11 cm, papery, pubescent, margin subentire to serrulate or sparsely coarse serrate, apex acuminate to acute; veins 10 or 11 pairs, abaxially prominent. Inflorescences terminal thyrses, densely yellow-brown pubescent, cymes sometimes monochasial; bracts sessile, ovate to broadly ovate, 1.5–4.5 × 0.5–1.8 cm, pubescent; bractlets lanceolate to ovate. Calyx ca. 5 mm, truncate to minutely 5-dentate, pubescent. Corolla white, bluish, or purplish, tube ca. 7 mm; lobes oblong to obovate, 6–12 mm. Stamens ca. 2–4 cm, long exserted, base pubescent. Ovary glabrous. Style long exserted. Drupes green when young, becoming black, subglobose [64]. The picture of *C. serratum* is shown in Figure 2.

Phytochemical studies have shown that the stem of *C. serratum* contains β–sitosterol, 24(S)–ethyl cholesta-5,22,25-trien-3β-ol, 5-hydroxy-7,4'-dimethoxy flavone, luteolin, apigenin, scutellarien, and ursolic acid [65]. The root bark of *C. serratum* contains three major triterpenoids, oleanolic acid, queretaroic acid, and serratagenic acid. The leaves of *C. serrutum* contain stigmasterol, a quinone pigment, α-spinasterol, luteolin, luteolin-7-0-glucuronide, apigenin, baicalin, scutellarien 7-0-glucuronide, and a few plant acids [66].

C. serratum is one of the most frequently used medical plants in Thailand. It can be found throughout the country. The crushed fresh roots of *C. serratum* mixed with lime juice are used topically for the treatment of hemorrhoids [31]. The crushed fresh leaves are taken orally for the treatment of hemorrhoids [67]. The fresh leaves of this plant are boiled in water and the extracted solution is used orally for the treatment of stomach cramp and vomiting [68, 69]. The aqueous extract of fresh stems of *C. serratum* is used orally as a diuretic. The mixture of fresh stems and leaves of the

plant crushed to wet mass is topically used for the treatment of ringworm and leprosy. Its fruit is traditionally used for the treatment of cough and conjunctivitis [69]. This plant, commonly known as the " Bharangi" in Ayurveda, and "Sirutekku" in Siddha system of medicine, is claimed to be useful in treating pain, inflammation, rheumatism, respiratory disorders, fever and malarial fever [70]. A dictionary of Indian Raw Material and Industrial Products also reports its medicinal uses in rheumatism, dyspepsia, and the decoction of its root mixed with ginger and coriander is used for relieving nausea [71].

Gupta *et al* (1968) had studied the anti-asthma effect of the saponin isolated from *C. serratum*. The results showed that the protective effect of *C. serratum* saponin on asthma is found to be associated with the augmentation of anti-allergic activity in the lung tissues as the lung extracts from the treated animals could inhibit histamine and Slow reacting substance of anaphylaxis (SRS–A) responses on guinea pig ileum to a greater extent and for longer periods as compared to the extract from untreated control animals [72]. The saponin from *C. serratum* was studied by Gupta *el at* (1973a) and found to accord protection of sensitised guinea pigs against histamine as well as antigen (egg albumin) micro-aerosols [73]. Gupta *el at* (1973b) studied the effect of chronic treatment of the saponin from *C. serratum* on the disruption of mesenteric mast cells. The results showed that the saponin protects sensitised mast cells from degranulation on antigen shock, thus confirming the immuno–suppressive and membrane stabilizing effect in the similar way as does sodium chromoglycate [74]. Narayanan *et al* (1999) studied the antinociceptive, anti-inflammatory and antipyretic effects of the extract of the root of *C. serratum* in experimental animals. The results support the traditional claimed of *C. serratum* as a remedy for pain, inflammation and fever [75].

Although various phytochemical and some biological information have been distributed, the vascular effects of *C. serratum* have not yet been reported. As flavonoids present in the drugs used for the treatment of hemorrhoids, i.e., Daflon® and Sidual® have been reported to have anti-inflammatory and analgesic activities as well as venotonic effect [76, 77, 78], it was therefore reasonable to study *C. serratum* for these effects in detail, with the hope for verifying and developing traditional medicine which could have potential for the treatment of hemorrhoids.

1.4 Hypothesis

C. serratum is widely used for the treatment of hemorrhoids. Drugs used for this purpose such as Daflon® and Sidual® contain compounds which can cure pain, inflammation and influence bleeding tendency. *C. serratum* is also used in traditional medicine for the treatment of hemorrhoids, the hypothesis of this study was that the extract of *C. serratum* possessed analgesic, anti-inflammatory and venoconstrictive effects.

1.5 Purposes of the study

The purposes of the present study were to study anti-inflammatory and analgesic activities of the methanol extract of the aerial part of *C. serratum* in various animal models in comparison with reference drugs such as diclofenac, prednisolone, and Daflon®. The mechanisms of action of the methanol extract of *C. serratum* on the inflammatory process and pain pathway were also examined in comparison with reference drugs. Moreover, the venoconstrictive effect of the methanol extract from *C. serratum* was investigated using the isolated human umbilical vein.

Figure 2. *Clerodendrum serratum* Linn.