CHAPTER 5

DISCUSSION AND CONCLUSION

Piper sarmentosum Roxb. (Piperaceae) called "Cha Plu" in local name, is one of the interesting medical plants in Thai traditional medicine. This plant contains many biologically active compounds, for example, sarmentosine, sarmentine (Likhitwitayawuid *et al*., 1987), asaricin, α-asarone (Masuda *et al*., 1991), hydrocinnamic acid and β-sitosterol (Niamsa and Chantrapromma, 1983). Pharmacological activities has been reported to exhibit hypoglycemic activity (Pongmarutai, 1980), antibacterial activity (Matsuda *et al*., 1991), neuromuscular blocking activity (Ridtitid *et al*., 1998), antiplasmodial activity (Rahman *et al*., 1999) and antiprotozoal activity (Sawangjaroen *et al*., 2004). In Malay Peninsula, the boiled leaves and roots were used for the treatment of toothache, relieved fever and pain in influenza and rheumatism patient, respectively (Perry, 1981; Pongboonrod, 1976).

Acute Toxicity Test

In the present study, acute toxicity was carried out in mice to evaluate the toxicities of the methanol extract of *Piper sarmentosum* leaves (MEPS) in mice. The results revealed that the extract at the dose of 5 g/kg given orally as a single dose failed to produce any clinical signs of toxicity such as convulsion, hyperactivity, sedation, loss of righting reflex in the first 8 hours, and the mortality in mice was not found after 7 days of observation. Mokkhasmit and colleagues (1971) has reported that the methanol and water extract with ratio 1:1 of *Piper sarmentosum* leaves at the dose of 10 g/kg p.o. or s.c. did not produce any toxicities in mice. So, methanol extract of *Piper sarmentosum* leaves at dose of 5 g/kg found non-toxicity in mice. Therefore, MEPS at doses of 50, 100 and 200 mg/kg used in the present study are safe.

Analgesic Activity

In analgesic activity test for the methanol extract of *Piper sarmentosum* leaves, we designed different tests to measure different types of pain; thermal stimulus by hot plate and tail flick test; chemical stimulus by writhing and formalin test. These experiments revealed that MEPS had analgesic activity via central and peripheral mechanism.

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Although pain is a natural warning and protection mechanism, patients often seek medical intervention to relieve pain. Pain is generated when mechanical, thermal, chemical or electrical stimuli exceed a certain threshold value. After tissue damage, many chemical mediators are released such as a substance P, histamine, bradykinin and prostaglandins. Bradykinin is an important substance to stimulate nociceptors, and prostaglandins sensitize nociceptor which causes impulses sending to spinal cord. This state is called peripheral sensitization. When spinal cord become hyperexcitable, excitatory amino acid or substance P from A delta fiber and/or C fiber were released and depolarized on its receptor. Signal transduction coming to postcentral gyrus in thalamus which is responsible for the conscious perception of pain. This state is called central sensitization (Gutyon, 1997; William, 2000; Mutscheler and Derendorf, 1995:b).

In analgesic activity test, we designed different tests to measure different types of pain; thermal stimulus by hot plate and tail flick test; chemical stimulus by writhing and formalin test. Moreover, the different features involved in the process, such as intensity, location, quality and duration of noxious stimulus must be considered in the effect of the antinociceptive substance. The measurement of central and peripheral analgesia must be defined by the time to delay on hot plate and tail flick test, the number of abdominal writhes on writhing test and the time to licking the paw on formalin test.

Nociception and drug-induced antinociception are experimentally estimated in animal models by monitoring behavioral motor response resulting from nociceptive stimuli (Le Bar, 2001). Therefore, before test of antinociceptive effect, it is necessary to make sure that neurological or motor deficits are not involved in the response. Only mice and rats that showed nociceptive responses in hot plate test such as jumping or licking the paw less than 15 seconds and withdraw the tail from light beam less than 3 seconds in tail flick test, respectively were used in the experiments. A cut-off time of 45 seconds and 10 seconds was used to avoid tissue damage in hot plate and tail flick tests, respectively. Agents which injected intraperitoneally and subcutaneously such as 0.6% acetic acid, 2.5% formalin, naloxone and morphine were diluted in isotonic solution (0.9% NSS) to prevent local irritation, pain and necrosis (Mutscheler and Derendorf, 1995:a).

In hot plate, tail flick and formalin test, morphine and naloxone were injected subcutaneously and intraperitoneally, respectively to animals. These routes make an absorption of drug more rapidly than oral administration (i.v. > i.p. > i.m. > s.c. > p.o.) (Rang *et al*., 1999:a). The analgesic onset, therefore, was designed to observe 30 min post oral administration for aspirin, 15-60 min after morphine, s.c., and 2-5 min after naloxone, i.m.. These were the reasons why the latency of nociceptive response was initially measured after 30 min for aspirin (p.o.), 15 min for morphine (s.c.) and 10 min for naloxone (i.p.).

In analgesic activity test for the methanol extract of *Piper sarmentosum* leaves, we designed different tests to measure different types of pain; thermal stimulus by hot plate and tail flick test; chemical stimulus by writhing and formalin test to evaluate analgesic activity. Firstly, the potential analgesic effects of methanol extract of *Piper sarmentosum* leaves (MEPS) were investigated by the hot plate test. The results revealed that MEPS at doses of 50 and 100 mg/kg did not significantly alter in reaction time at 30, 45, 60, 75 and 90 minutes after administration when compared with control, whereas MEPS at dose of 200 mg/kg significantly delayed the response of animal to hot plate thermal stimulation at 60, 75 and 90 minutes ($p<0.05$) after administration compared with control. Morphine, a reference analgesic drug, at dose of 5 mg/kg (s.c.) significantly decreased the reaction time to hot plate test at all time intervals measured (30, 45, 60, 75 and 90 minutes) after administration ($p<0.01$). The results indicated that only MEPS at dose of 200 mg/kg decreased the latency of nociceptive response for the thermal stimulation by hot plate test in mice. The antagonistic action of naloxone on effects of morphine or MEPS on the latentcy of nociceptive response in hot plate test was studied to examine the possible analgesic mechanisms of MEPS. The results showed that naloxone at dose of 2 mg/kg, i.p. given before morphine at dose of 5 mg/kg, s.c. completely antagonized the effect of morphine at all time intervals measured ($p<0.01$). Furthermore, naloxone at dose of 2 mg/kg, i.p. given before MEPS at dose of 200 mg/kg significantly delayed the latency of nociceptive response of the extract in hot plate test at 60 ($p<0.05$) and 75 minutes ($p<0.01$) when compared with MEPS at dose of 200 mg/kg alone. The drugs that reduced the nociceptive response induced by cutaneous thermic stimuli in hot plate test may exhibited central analgesic properties or supraspinal analgesia (Matheus *et al.*, 2005).

The hot plate test is widely used to assess analgesic activity of drugs. This test does not directly measure the intensity of the noxious stimulus perceived by the animal, but only

the animal's response to it, and so may be affected by non-analgesic drugs. Sedative and muscle relaxants may impair the ability to response and hence be wrongly considered to have analgesic activity (false positives) (Woolfe and MacDonald, 1944). The test examined response of mice in the hot plate at 55 ºC and cut off at 15 second. Morphine like drugs all impaired motor performance.

The analgesic property was also studied using sensitive model that thermal stimulus on noxious stimuli on C fibers. In present study the thermal test was selected because of several advantage including sensitivity to strong analgesics and limited tissue damage (Prado *et al.*, 1990). This test is suitable for identifying centrally, but not peripherally acting analgesics drugs (Chau, 1989). The validity of this test has been shown even in the presence of substantial impairment of motor performance (Plummer *et al.*, 1996).

The effectiveness of MEPS at the dose of 200 mg/kg in reducing the nociceptive response in hot plate test indicated that MEPS possesses the analgesic activity via central mechanism, and might acts primarily in the spinal medulla, higher central nervous system levels or by an indirect mechanism (Yaksh and Rudy, 1977).

Furthermore, in tail flick test, MEPS at dose of 200 mg/kg, orally significantly produced an antinociceptive effect at the time 45 ($p<0.05$), 60 ($p<0.01$), 75 ($p<0.05$) and 90 ($p<0.05$) minutes after administration compared with control. Morphine, a reference analgesic drug, at dose of 5 mg/kg, s.c. significantly delayed the reaction time in all time interval measured in tail flick test in rats. The result also showed that naloxone at dose of 2 mg/kg, i.p. completely antagonized the antinociceptive effect of morphine at dose of 5 mg/kg, s.c. at all time intervals measured ($p<0.01$). In addition, naloxone at dose of 2 mg/kg, i.p. given before MEPS at dose of 200 mg/kg significantly delayed the latency of nociceptive response produced by the extract in tail flick test at 45 ($p<0.05$), 60 ($p<0.05$), 75 ($p<0.01$) and 90 ($p<0.01$) minutes when compared with MEPS at dose of 200 mg/kg. The tail flick test which uses a thermal stimulus is widely used for the evaluation of analgesia, especially the central analgesic response or spinal analgesia (Matheus *et al*, 2005). It was a confirmation test of central effect. It is predominantly a spinal reflex, and is considered to be selective for centrally acting analgesic compounds (Ramabadran *et al*., 1989).

According to Le Bar and colleagues (2001), drug-induced lengthening of the latency for the spinally mediated tail flick reflex is a reliable indicator of opioid-like antinociceptive activity.

In our results, MEPS at dose of 200 mg/kg may act on spinal cord to prolong the time of response of the tail flick reflex. Only the extract at higher dose of 200 mg/kg produced analgesic activity since it may contain bioactive compounds to exhibit antinociceptive response.

In addition, the central analgesic action was confirmed by using naloxone, a specific antagonist of morphinomimetric receptor (Belvisi *et al*., 1998). Naloxone can block the μ, κ and δ opioid receptor on spinal cord and brain and it has a relatively high affinity for opioid binding sites of the μ and κ receptor. When naloxone combined with MEPS at dose of 200 mg/kg, there were no significantly delayed the latency response time at any time intervals because naloxone at dose of 2 mg/kg when given intraperitonelly completely blocked the effect of MEPS on the μ and κ receptor. In the present results, the hot plate and tail flick test were used to demonstrate the central analgesic activity of MEPS. The antinociceptive action of the extract was antagonized by naloxone. Therefore, the antinociception of MEPS was possibly mediated via opioid mechanisms. The other possibility of action of MEPS might be due to direct agonist activities of opioidiometric constituents in the extract, and due to increase release of endogenous opioid peptides (Deraniyagala *et al*., 2003). In humans, the opioid receptors, μ_1 and κ_3 receptors are responsible for supraspinal analgesia, and stimulation of κ_1 receptor is thought to produce spinal analgesia (Mutscheler and Derendorf, 1995). In animal models, the opioid receptor μ, κ and δ receptor are responsible for supraspinal and spinal analgesia (Gutstein and Akil, 2001). Therefore, in our studies the results indicated that the extract may act on μ_1, κ_1, κ_3 and δ opioid receptors in supraspinal and spinal site.

According to Rossi and colleagues (1993), the antinociceptive effect could be due to a direct decrease on the activity evoked by the C fibers in ascending axons, or decrease in the production of prostaglandins responsible for the C fiber stimulation. The mechanism of action for MEPS in hot plate test (supraspinal analgesia) and tail flick test (spinal analgesia) could be involved in the pathway or direct action on opioid receptor on the peripheral terminals of thinly myelinated and unmyelinated cutaneous sensory fibers as well as the morphine-like substance (Conggreshall and Carlton, 1997).

In conclusion, the results evaluated from the hot plate and tail flick tests indicated that MEPS had an analgesic activity-like morphine via central mechanisms, in the level of spinal and supraspinal mechanism to produce analgesia property. So, it was concluded that

MEPS had a central analgesic effect to impair the motor performance by the hot plate and tail flick test.

In acetic acid induced-writhing test, the results indicated that MEPS given orally at doses of 50, 100 and 200 mg/kg significantly decreased the number of writhes in mice induced writhing response by 0.6% acetic acid. Acetic acid ($C_2H_4O_2$) is a strong corrosive agent which obtained in the destructive distillation of wood from acetylene and water via acetaldehyde by oxidation with air (Windholz *et al*., 1976). The intraperitoneal administration of acetic acid irritates serous membrane that will provoke a stereotypical behavior in mice characterized by wave of abdominal contraction, movement of the body as twisting of abdominal muscles, extension of hind limbs and a reaction in motor activity and coordination (Bars *et al.*, 2001). Acetic acid is a chemical stimuli and it is used as an inducer for writhing syndrome, causes algesia by releasing of endogenous substances including serotonin, histamine, bradykinin, substance P and prostaglandins, which then excite the pain nerve endings leading to the abdominal writhing. The acetic induced-writhing test is a visceral pain model (Vyklicky, 1979). Acetic acid induced writhings is a highly sensitive and useful test for analgesic drug development, but the test is not selective pain test, therefore, it is used as a screening test for both peripheral and central acting analgesic activity (Raj, 1996). It gives false positives with sedatives, muscle relaxants (Elisabetsky *et al.*, 1995). Since MEPS at doses of 50, 100 and 200 mg/kg significantly decreased the number of writhing in mice induced writhing response by 0.6% acetic acid, it could be assumed that MEPS decreased visceral pain induced by acetic acid and sites of action for analgesic activity would be mediated both peripheral and central mechanisms.

It has been suggested that acetic acid stimulates the vanilloid receptor (VR1) and bradykinin B_2 receptor in the pathway comprising sensory afferent C-fibers (Ikeda *et al*., 2001). The prostaglandin biosynthesis by arachidonic acid via cyclooxygenase plays a role in the nociceptive mechanism (Franzotti *et al.*, 2002). The quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of mice obtained after the intraperitoneal injection of acetic acid demonstrated high levels of prostaglandins PGE_2 and $PGF_2\alpha$ during 30 minutes after stimulus (Deraedt *et al.*, 1980). Of the prostanoids, PGI_2 has been mainly responsible for the causation of pain following acetic acid administration (Murata *et al*., 1997). Thus, the abdominal constriction is related to the sensitization of nociceptors to prostaglandins.

In writhing test, results suggested that MEPS at doses of 50, 100 and 200 mg/kg was likely to inhibit the number of writhes induced by acetic acid as a standard drug aspirin at the dose of 200 mg/kg. Aspirin and other NSAIDs can inhibit cyclooxygenase in peripheral tissues, thus, interfering with the mechanism of transduction in primary afferent nociceptors via inhibiting the synthesis of prostaglandins (Fields, 1987). The mechanism of analgesic action of MEPS could probably be due to interfere with the synthesis, release of endogenous substances, blockade of the effect or desensitization of nerve fibers (Collier *et al*., 1968), that excite pain nerve endings similarly to aspirin and other NSAIDs.

The formalin test is another pain model which assesses the way of an animal responds to moderate, continuous pain generated by injured tissues (Tjolsen *et al.*, 1992). Centrally acting drugs such as morphine inhibited both of the early and late phases equally while peripherally acting drugs as aspirin only inhibited the second phase (Dubisson and Denis, 1977).

The 2.5% formalin was injected subcutaneously to hindpaw of mice. The time spent licking in the injected paw was recorded in 2 phases ; 0-5 minutes as early phase and 15-30 minutes as late phase. Synonyms for formaldehyde include formalin, methyl aldehyde and methylene oxide. The chemical formula is CH_2O. Commercially available formalin is a 37 – 50% aqueous solution of formaldehyde (Ellenhorn *et al*., 1997).

The formalin test was selected because of several advantages including the ability to mimic human clinical pain condition, production of tonic stimulus and sensitivity to NSAIDs. This test possesses 2 distinctive phase, possibly reflecting different types of pain. The early phase reflects direct effect of formalin on nociceptors (non-inflammation pain, neurogenic pain) and the late phase reflects inflammatory pain (Hunskaar and Hole, 1997). The licking time of the formalin test showed biphasic responses. The early phase (0-5 minute) was mediated by the central effect via a direct stimulation of the nociceptor and releasing substance P or bradykinin. The late phase (10-30 minute) was mediated by the peripheral effect via the release of some chemical transmitters such as histamine, serotonin, prostaglandins and bradykinin (Shibata *et al.*, 1989).

In the present study, the results showed that MEPS had central and peripheral analgesic properties via antinociceptive action on sensory receptor stimulation and anti-inflammatory action, respectively since MEPS significantly reduced the licking time both in early and late phase in mice induced nociceptive response by formalin. The formalin test normally

postulates the site and mechanism of action of the drug (Chau, 1989). Since the MEPS extract could reduce the licking time in late phase in the formalin test, the possible site and mechanism of action was probably due to inhibition of inflammatory mediators, notably prostaglandins synthesis as well as receptor blockade. The formalin test is the test which is indicative of chronic inflammation, whereas other tests such as acetic acid induced writhing or hot plate tests are indicative of acute pain (Cowan, 1990). Moreover, MEPS at dose of 200 mg/kg showed only slight analgesic activity on the early phase and exhibited a pronounced effect on the late phase. The slight inhibition in the early phase may be due to direct effects on nociceptor via blockade on nociceptors and/or inhibited to releasing substance P and bradykinin. The analgesic effect on the late phase of *Piper sarmentosum* methanol extract from leaves indicates its inhibitory activity on pain arise from inflammation, which reflects the effect on the synthesis and/or release of prostaglandins, histamine, serotonin and bradykinin (Trongsakul *et al.*, 2002).

Anti-inflammatory Activity

Inflammation is the reaction of tissue and its microcirculation to a pathogenic insult and generated the inflammatory mediators and movement of fluid and leukocytes from the blood into extravascular tissue. The causes of inflammation are microbial infections, chemicals or physical agents. It had five cardinal signs as redness, heat, swelling, pain and loss of functions. Inflammatory response following injury area of tissues suggests that endogenous chemical mediators as histamine, lysosomal compound, prostaglandins, leukotrienes, serotonin, bradykinin and prostaglandins are released, and cause vasodilation, chemotaxis and increase in vascular permeability (Fantone and Ward, 1999; Denis, 2003).

The carrageenan-induced paw inflammation has been accepted as a useful phlogistic tool for investigation of systemic anti-inflammatory agent. This test is sensitive to most clinically effective anti-inflammatory drugs, and it is commonly used as an experimental animal model for acute inflammation. Edema formation due to carrageenan injected in the rat paw is the biphasic events ; the initial and second phase (Vineger *et al.*, 1969). In addition, the second phase is sensitive to most clinically effective anti-inflammatory drugs (DiRosa *et al.*, 1971). Carrageenan is a polysaccharide of the red seaweed (Rhodophyceca). The structure consists of alternating copoly of beta-(1→3)-D-galactose and (1→4)-3, 6, anhydro-D-galactose (Windholz *et al*., 1989).

The carrageenan- induce paw edema has been reported 2 phases; the initial phase occurs within the first to second hour after carrageenan injection. It derives from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandin, histamine and bradykinin in the inflammatory area. The second phase occurs 3-5 hours after carrageenan injection. In this phase, the macrophages in carrageenan-insulted dermal tissue release much interleukin-1 (IL-1) to induce accumulation of polymorphic nuclear cells (PMNs) in the inflamed area. The activated PMNs then release the lysomal enzymes and active oxygen, especially superoxide to destroy connective tissues and induce paw swelling (Vinerger *et al.*, 1987). Other mediators such as prostaglandins, bradykinin, protease and lysosomal enzyme could be found in this phase (Crunkhon and Meacock, 1971). In addition, one of the most important features of inflammation is edema which is caused by the action of some inflammatory autacoids like kinins, prostaglandins especially the E series, leukotrienes, etc. resulting in vasodilation, enhancement of capillary permeability, plasma exudation and these mediators also cause pain and fever (Silbernagl and Lang, 2000). So, inhibition of these mediators from injured sites or from bringing out their pharmacological effects will normally ameliorate the inflammation and other symptoms (Asongalem *et al.*, 2004).

In the present results, MEPS at doses of 50, 100 and 200 mg/kg significantly exhibited and sustained in the inhibition of the rat paw edema in the second phase, the possible mechanism of the observed anti-inflammatory activity might be its ability to inhibit the release of histamine, serotonin, kinin substance, lysozymes synthesis or biosynthesis of prostaglandins which is consistent with the observation of anti-inflammation activity of a standard drug aspirin.

Antipyretic Activity

Fever may be a result of infections or one of the sequelae of tissue damages, inflammation, graft injection or other disease states. Antipyretics are drugs which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever, this set point elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Prostaglandins, mainly PGE_2 alters metabolism of thermoregular cells in the hypothalamus resulting in an increase of the set point for thermoregulation to a higher temperature (Wemger1, 1995; Vander, 2001; Guyton and Hall, 2000:a).

Brewer's yeast is the most important microorganism for producing fermented beverages. It is a eukaryote and belongs to the Fungi. All beer-brewing stains of yeast are placed in the genus Saccharomyces and species cerevisiae. Viewed under the light microscope, a single cell is spherical or ellipsoidal in shape and is 5-13 μm across (Lewis and Young, 1995). Yeast-induced fever is called pathogenic fever. Its etiology include production of prostaglandins, which set the thermoregulatory center at a higher set point. It could stimulate phagocytes to release endogenous pyrogen which circulates in blood to act on the thermoregulatory center in the hypothalamus. The endogenous pyrogen produce and activate IL-1 and prostaglandins, mainly PGE_2 which alter metabolism of thermoregulatory cells via cAMP secondary messenger-mediated mechanism. The result is an increase in set point for thermoregulation to a higher temperature. So, inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as acetylsalicyclic acid (Howard, 1993; Rawlins and Postgrad, 1973). Akio and colleagues (1988) suggested that there are several mediators or multi-processes underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis.

Since, antipyretic activity is commonly mentioned as a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis (Vane, 1987), the yeast-induced hyperthermia in rat model was employed to investigate the antipyretic activity of MEPS. In the present study it was found that MEPS at doses of 50, 100, 200 mg/kg does not show significantly decreased the rectal temperature in yeast induced fever. This result does not support the view that MEPS has some influence on prostaglandin biosynthesis in the CNS which is the final common pathway responsible for fever induction. Therefore, the results indicated that MEPS did not suppress the fever induced by yeast in rats, and it would be assumed that MEPS at doses of 50, 100 and 200 mg/kg had no antipyretic activity in rat induced fever by brewer's yeast.

Conclusions

As previous mentioned in the experiment, aspirin and morphine were used as the reference drugs to investigate the possible antinociceptive, anti-inflammatory and antipyretic activities of MEPS incorporated with various essential tests for analgesic (hot plate, tail flick, writhing and formalin tests), anti-inflammatory (carrageenan-induced paw edema) and antipyretic (brewer's yeast-induced fever) activities. The finding results from this study are summarized as followings;

1. MEPS showed the antinociceptive responses in hot plate, tail flick, acetic acid-induced writhing and formalin test and the analgesic mechanisms were likely to aspirin via peripheral mechanisms and/or morphine via central mechanisms depending on the test used.

2. MEPS exhibited the anti-inflammatory activity by inhibition of the rat paw edema, the possible mechanism of the observed anti-inflammatory activity might be its ability to inhibit the release of histamine, serotonin, kinin substance, lysozymes synthesis or biosynthesis of prostaglandins which is consistent with the observation of anti-inflammation activity of a standard drug aspirin.

3. MEPS did not have antipyretic activity in rats induced fever by brewer's yeast.

The antinociceptive and anti-inflammatory activities of MEPS found in the present study may be due to effects of a single or many constituents or the compounds in the plant. The future investigations to isolate and purify only the active constituents presented in *Piper sarmentosum* leaves should be done to further studies for the antinociceptive and anti-inflammatory activities. However, it is possible that its analgesic, anti-inflammatory action may be involved in some different mechanisms.

The chemical constituents found in *Piper sarmentosum* Roxb. leaves were hydrocinnamic acid, β-sitosterol (Niamsa and Chantrapromma, 1983), volatile oil such as asarone, α-asarone, asaricin (Masuda *et al*., 1991), longifolene, β-caryophyllene, allo-aromadendrene and 9-epi-(E)-caryophyllene (Aunpak *et al*., 1997), and the other compounds such as vitamin C, E, carotenes, xanthophylls, tannins and phenolic groups (Chanwitheesuk *et al*., 2004). The phytochemicals would be bioactive compounds to show analgesic and anti-iflammatory activities.

Giusti and colleagues (1985) reported that hydrocinnamic acid may decrease pain by blocking the carboxypeptidase A which is responsible for breakdown endorphins and enkephalins. The other effect of hydrocinnamic acid such as anti-diabetic effect via increase glucose transporter (GLUT-4) activity was also reported (Kim *et al*., 2006).

Schulz and colleagues (1998) and Von Holtz and colleagues (1998) reported that β-sitosterol was useful in the treatment of benign prostatic hyperplasia (BPH). It affected on prostaglandins synthesis to reduce pain in prostate gland. In addition, Villasenor and colleagues (2002) showed that β-sitosterol possessed antinociceptive activity on hot plate and acetic acid-induced writhing test. The other effect of β-sitosterol such as cytoprotective activity in U937 cells of atherosclerosis disease was reported (Wang *et al*., 2006).

Momin and colleagues (2003) investigated the pharmacological activity of *Daucus carota* L. seed extract. The results revealed that α-asarone, one of compound in seed, inhibited the cyclooxygenase I and II isoforms. So, α-asarone may be an active compound to decrease prostaglandins synthesis. Moreover, it possessed an antioxidant property against noise-stress induced changes in the rat brain (Manikandan and Devi, 2005), hypocholesterolemic and cholelitholytic effects in hypercholesterolemic rats (Rodriguez-Paez *et al*., 2003).

Agarwal and Rangari (2003) reported that β-caryophyllene, phytochemical of essential oil of *Strobilanthus ixiocephala* Benth., demonstrated the anti-inflammatory activity in carrageenan-induced paw edema and cotton pellet granuloma in arthritis model in rats. Lampronti and colleagues (2006) showed that β-caryophyllene possessed the antiproliferative activity on human erythroleukemic K562 cells. Moreover, Sybulal and colleagues (2006) showed that β-caryophyllene had an antimicrobial activity against the fungi, *Candida glabrata*, *Candida albican*, *Aspergillus niger*, and the bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa*.

Rosa and colleagues (2005) reported that vitamin C (ascorbic acid) had an analgesic effect in formalin-induced licking and glutamate-induced nociception. Furthermore, it caused marked inhibition of nociceptive response induced by intrathecal injection of glutamate, NMDA, AMPA, kainite and substance P. In addition, Carson and colleagues (2006) reported the antioxidant property of vitamin A, C and E. These vitamins had been reported to abrogate cardiomyocyte secretion of inflammatory cytokines, and improve myocardial contractile function.

Sedative and muscle relaxant agents such as benzodiazepine and tubocurarine, respectively were prescribed in the treatment of various diseases for examples anxiety, insomnia and back pain. These agents were reported to cause a false positive on analgesic activities by increasing the latency of nociceptive response in hot plate and tail flick tests, and decreasing the number of writhings in acetic acid-induced writhing test (Woolfe and MacDonald, 1944; Elisabetsky *et al.*, 1995). The active compounds in plant material which possessed sedative or muscle relaxant activities would affect on the analgesic activities. The study of Ridtitid and colleagues (1998) showed that the methanol extract of *Piper sarmentosum* leaves possessed the neuromuscular blocking activity (muscle relaxant effect). Therefore, the active compounds in MEPS might interfere analgesic activity in the present study.

In conclusion, *Piper sarmentosum*, a Thai herbal plant, is used widely for preparing of herbal medicine. Various parts of this plant, for examples fruit, leaves and root

contain many biologically active compounds and possess pharmacological activities. In this study, MEPS at doses of 50, 100 and 200 mg/kg exhibited the analgesic activity by decrease the response of the animal in hot plate and tail flick test (pain induced by thermal stimuli), reduced the number of writhing in acetic acid-induced writhing test and the licking time both in early phase and late phase in formalin test (pain induced by chemical stimuli). The mechanisms of the MEPS that possess analgesic action are mediated central nervous pathway via supraspinal and spinal site, and peripheral nervous pathway. In addition, it also had the anti-inflammatory activity in acute phase by reducing the volume of rat paw edema in carrageenan-induced paw edema. But, MEPS did not have antipyretic activity in reduction of the rectal temperature on brewer's yeast induced pyrexia. Therefore, the extract used in traditional medicine is assumed to have an analgesic and anti-inflammatory activities confirming the traditional use of this plant. The summarized mechanisms of MEPS were shown in Table 13 as below.

Table 13. Summary of possible mechanisms of the methanol extract of *Piper sarmentosum* leaves (MEPS) to produce analgesic, anti-inflammatory and antipyretic activities.

MEPS (mg/kg), p.o.	Central analgesic activity		Analgesic activity			Anti-inflammatory activity	Antipyretic activity
	Hot plate test (supraspinal mechanism)	Tail flick test (spinal mechanism)	Writhing test (visceral pain)	Formalin test (chemical pain)		Carrageenan-induced paw edema	Brewer's yeast-induced pyrexia
				Early phase	Late phase		
50	-	-	*	-	-	*	-
100	-	-	***	-	*	***	-
200	* (60min)	** (60 min)	***	*	***	*** (3 h)	-

* = p <0.05

** = p <0.01

*** = p <0.001 (ANOVA followed by Bonferroni's test)