# CHAPTER I

# INTRODUCTION

The flavonoids are colouring phenolic substances contributing to the beauty and splendour of flowers and fruits in nature. Their roles are important to plant including antioxidants, antimicrobials, photoreceptors, visual attractors and feeding repellants (Pietta, 2000). The occurrence of this numerous class of oxygen heterocycles is restricted to higher plants, ferns, whereas mosses contain a few flavonoid types (Torssell, 1997).

The flavonoids are structurally characterized as having two hydroxylated aromatic rings, A and B, joined by a three carbon fragment. Structures within these classes are modified by hydroxylation and methoxylation and can be distinguished; chalconoids, flavonoids, isoflavonoids, auronoids and anthocyanidins (Dey and Harborne, 1997). The isoflavonoids possess a 3-phenylchroman skeleton are a major and very distinctive subclass of the flavonoids. They are biogenetically derived by rearrangement of the flavonoid 2-phenylchroman system by means of a 1,2-aryl rearrangement (**Scheme 2, CHAPTER II**). This subclass is almost entirely restricted to the subfamily Papilionoideae of the family Leguminosae. It has been mentioned that this subfamily contains the largest number of plants which are poisonous to fishes and are important from the insecticidal point of view (Perry, 1980). This is a noteworthy reason to decide a work on a leguminous plant, *Pachyrrhizus erosus* (L.) Urban which enriches the insecticidal isoflavonoids.

The genus *Pachyrrhizus* belongs to the tribe Phaseolinae, subfamily, Papilionoideae, the family Leguminosae of the order Rosales. Two species (*P. erosus* and *P. tuberosus*) are cultivated throughout India and particulary in South East Asia and in South America. The plants in the genus *Pachyrrhizus* are wide-climbing herbs. Leaves pinnately 3-foliolate with stipullate lobed leaflets. Racemes long, with tumid nodes and fascicled pedicels; bracts and bracteoles setaceous, caducous. Calyx 2-lipped, the limb as long as the tube, the upper lip emarginated, the lower deeply 3-toothed. Corolla much exserted, the petals subequal; keel obtuse. Stamens diadelphous; anthers uniform. Ovary subsessile, many-ovuled; style long, circinate at the apex, bearded down the inner side below the very oblique stigma. Pod large, linear, turgid, deeply depressed between the seeds (Hooker, 1973).

According to Smitinand (2001), there is only one species of the genus *Pachyrrhizus* found in Thailand as follow.

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| *Pachyrrhizus erosus* (L.) Urban | เครือเขาขน Khruea khao khon, หมาก |
| (*P. angulatus* Rich. ex DC., *P. bulbosus* Kurz, *Cacara erosa* (L.) Kuntze, *Dolichos bulbosus* L., *D. erosus* L.) | บ้ง Maak bong (Phetchabun); ถั่วกินหัว Thua kin hua, ถั่วบ้ง Thua bong, มันแกวละแวก Man kaeo la waek, มันแกวลาว Man kaeo laao, มันละแวก Man la waek, มันลาว Man laao (Northern); มันแกว Man kaeo (Central); หัวแปะก๊วะ Hua pae kua (Peninsular); Yam bean. |

*Pachyrrhizus erosus* (L.) Urban, a native of America, is now pantropic and cultivated in the East for its edible tubers. Root a large tuber, like a turnip, which is eaten both raw and boiled. Stems wide-twining, suffruticose, clothed with short deciduous pubescence. Leaflets large, membranous, glabrous, as broad as long, deeply or shallowly lobed in the upper half, the base deltoid. Racemes lax , 1/2-1 ft., the lower nodes often prolonged into short branches; bracteoles setaceous. Calyx 1/4 in., as long as the pedicel. Corolla reddish, 1 in. or more long. Pod 6-9 in. long, 8-12-seeded, straight, glabrescent (Hooker, 1973).

In China, the fleshy root was used in medicine that was the only place where the root was mentioned as medicinal uses. However, it was noted that, in Rumohius time, the Chinese dried and preserved the tubers as a cooling food for people with fever. In Java, the pulverized seeds mixed with sulphur were applied to a type of skin eruption which spreads quickly by scratching. One-half seed can be taken as a laxative and somewhere used as anthelmintics (Perry, 1980). In addition, the seeds showed a toxicity to several species of insects and have been used as piscicides (Norton, 1943).

Previous phytochemical studies on *P. erosus* have been reported (Norton and Hansberry, 1945; Bickel and Schmid, 1953; Eisenbeiss and Schmid, 1959; Crombie and Whiting, 1963; Krishnamurti and Seshadri, 1966; Krishnamurti , Sambhy and Seshadri, 1970; Kalra, Krishnamurti and Nath, 1977; Kardono *et al.*, 1990). The major components of the seeds of *P. erosus* have been known to be isoflavonoids including 3-arylcoumarins, coumaronochromene, coumestan, isoflavanones, isoflavone, pterocarpan, and rotenoids, but the full $^{1}$H- and $^{13}$C-NMR assignments for some compounds have not been completed. Some previous reports on the HSV inhibitory activity of flavonoids (Kaul *et al.*, 1985; Meyer *et al.*, 1997) prompt the author to investigate the possibility of acquisition of other anti-HSV agents from *P. erosus*.

Besides the isoflavonoids, other flavonoids as chalcones, flavones and flavanones, which have biogenetic relationships (biosynthetic pathways shown in **Schemes 1-3**, **CHAPTER II**) are also enriched in the leguminous plants, involving the plants in the genus *Millettia*, of which occurring many species grown in Thailand. The genus *Millettia* belongs to the tribe Tephrosieae, subfamily Papilionoideae. There are 40-50 species of this genus spread throughout the tropics of the old world (Hooker, 1973).

The plants in the genus *Millettia* are trees or large shrubs, usually climbers. Leaves odd-pinnate. Flowers showy, in axillary racemes, often fascicled, simple or paniculate and terminal. Calyx campanulate; teeth generally short or nearly obsolete. Corolla much exerted; petals with long claws; standard broad; keel not beaked. Stamens monodelphous or diadelphous, filaments filiform; anthers uniform. Ovary sessile, linear, few-ovuled; style filiform, incurved, glabrous, stigma capitate. Pod linear or oblong, 1- or few-seeded, flat or turgid, late in dehiscing or hardly dehiscent (Chopra, Budhwar and Ghosh, 1965).

According to Smitinand (2001), the species of the genus *Millettia* found in Thailand are as follows.

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| *Millettia atropurpurea* Wall. | = *Collerya atropurpurea* (Wall.) Schott |
| *M. brandisiana* Kurz | กระพี้จั่น Kra phi chan, จั่น Chan, |

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| | พี้จั่น Phi chan (General); ปี๊จั่น Pi chan (Northern). |
| *M. caerulea* Baker. | ป๊วเปาะเด๊าะ Pua-po-do (Karen-Mae Hong Son); ผักเยี่ยววัว Phak yiao wua (Nakhonsawan, Northern); หางไหลแดง Hang lai daeng (Kanchanaburi). |
| *M. decipiens* Prain | ปารี Pa ri (Malay-Narathiwat). |
| *M. extensa* Benth. | ก๋าวเครือ Kao khruea, กวาวเครือ Kwao khruea (Chiang Mai); ตานครบ Tan krop (Lampang) |
| *M. glaucescens* Kurz | ยะดา Ya-daa (Malay-Narathiwat); หยีน้ำYi nam (Peninsular). |
| *M. kangensis* Craib | กระเจาะ Kra cho, ขะเจาะ Kha cho, ขะเจาะน้ำ Kha cho nam (Chiang Mai). |
| *M. kityana* Craib | เครือข้าวเย็น Khruea khao yen, ลางเย็น Lang yen, ฮางจืด Hang chuet, ฮางเย็น Hang yen (Northern). |
| *M. latifolia* Dunn | ขะเจาะ Kha cho (General). |
| *M. leucantha* Kurz var. *leucantha* | กะเซาะ Kaso (Central); กระเจาะ Kra cho, ขะเจาะ Kha cho |

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| | (Northern); กระพี้เขาควาย Kra phi khao khwai (Prachuap Khiri Khan); ขะแมบ Kha maep, คำแมบ Kham maep (Chiang Mai). |
| *M. leucantha* Kurz var. *buteoides* (Gagnep.) P.K.Loc (*M. buteoides* Gagnep.var. *siamensis* Craib, *M. pendula* Benth.) | กระเจ๊าะ Kra cho, ขะเจ๊าะ Kha cho (Lampang); กระท้อน Kra thon (Phetchabun, Phitsanulok); ไม้กระทงน้ำผัก Mai kra tong nam phak (Loei) สะท้อน Sa thon (Saraburi); สาธร Sa thon (Ubon Ratchathani). |
| *M. macrostachya* Collett & Hemsl. var. *macrostachya* | ขะเจาะน้ำ Kha cho nam (Chiang Mai). |
| *M. macrostachya* Collett & Hemsl. var. *tecta* Craib | ขะเจาะหลวง Kha cho luang, ขะเจาะใหญ่ Kha cho yai (Narathiwat). |
| *M. pachycarpa* Benth. | เกถะ Ke-tha (Karen-Chiang Mai); เครือไหล Khruea lai (Chiang Mai). |
| *M. peguensis* Ali (*M. ovalifolia* Kurz) | ตอหิ To-hi (Karen-Kanchanaburi). |
| *M. pulcha* Benth. Kurz | จันพอ Chan pho (Northern). |
| *M. racemosa* (Roxb.) Benth. | = *Endosamara racemosa* (Roxb.) R. Geesink |

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| *M. sericea* (Vent.) Benth. | จะไนโค๊ะ Cha-nai-kho, ปาตู Paa-tu (Malay-Narathiwat); นอเราะ No-ro (Malay-Yala, Pattani); ยิมแม เก๊าะ Yim-mae-ko (Malay-Yala); อ้อยสามสวน Oi sam suan (Nong Khai). |
| *M. thorelii* Gagnep. | = *Derris thorelii* Craib |
| *M. utilis* Dunn | สะท้อนน้ำผัก Sathon nam phak (Loei). |
| *M. xylocarpa* Miq. (*M. hemsleyana* Prain , *M. pubinervis* Kurz) | กะเจ๊าะ Ka cho, ขะเจาะ Kha cho (General); คะแมด Kha maet (Chiang Mai); จักจั่น Chakkachan (Loei); พี้พง phi phong (Phrae); ยะดา Ya-da (Malay-Yala); ไย่ยี Yai-yi (Karen-Mae Hong Son); สาธร Sa thon, หยีน้ำ Yi nam (Pattani,Yala). |

*Millettia leucantha* Kurz var. *leucantha* is indigenous plant known in Thai as Kra cho, Kha cho, Kra phi khao khwai, Kaso, Kha maep and Kham maep. This plant is an erect tree, with thinly silky branchlets, and leaves 1/2 ft. long. Leaflets thin, flexible, 2-3 in. long, dull green, thinly silky above, when mature densely clothed with adpressed grey silky pubescence below, the lowest as long as broad; petiolules under 1/4 in. Flowers in short dense racemes in the axils of the leaves; pedicels densely fascicled, 1/8-1/6 in. Calyx 1/6-1/5 in., shortly grey-silky; teeth deltoid, shorter than

the tube. Corolla shorter than the calyx. Pod oblong, 3-5 in. long, 1 ¼ in. broad, tubercled, 1-3 seeded (Hooker, 1973).

Many plants in genus *Millettia* have been used as traditional medicine for several purposes in tropical region countries. In particularly, most of the seeds and some of the roots of *Millettia* plants are known for their insecticidal, molluscicidal and piscicidal properties (Mabberly, 1987). In India, dried leaves of *M. auriculata* have been used for male anti-fertility activity (Choudhary *et al.*, 1990). In Cameroon, *M. conraui* and *M. sanagana* were used in the treatment of intestinal parasites and cholic in children (Fuendjiep *et al.*, 1998a; Mbafor *et al.*, 1995). Additionally, the bark of *M. erythrocalyx* grown in the central part of Thailand have been used for treating stomach pain (Sritularak *et al.*, 2002). In Ethiopia, dried roots of *M. ferruginea* were used for gonorrhea (Desta, 1993).

Concerning *M. thonningii*, a plant growing in the savanna of West tropical Africa, the root was used to treat female troubles and used as an anthelmintics, whereas dried entire plant was used for malaria in Sudan (Khalid *et al.*, 1986). Moreover, this plant was also used as a laxative, a blood purifier, a dewarmer, an analgesic and for the treatment of diarrhoea throughout the sub-region of Africa (Irvine, 1961; Abbiw, 1990)

The stem of *M. auriculata* was used as an emmenagogue for anti-fertility purpose in China (Kong *et al.*, 1976) and the dried root was used to inhibit blood coagulation (Kosuge *et al.*, 1984). The root of *M. usaramensis* has been used as a remedy for snake bite in East Africa (Kokwaro, 1976). The bark pulp of *M. zechiana* found in Guinea and Cameroon was used with sea water and Guinea grains diluted with warm water as a gargle for rhinopharyngeal and bronchial troubles and purple leaves were rubbed on painful parts (Kerharo and Bouquet, 1950). In Thailand, the dried leaves and stem of *M. caerulea* were used to reduce infection in cuts and burns (Anderson, 1986), whilst dried leaves of *M. kitjana* were used for diabetes (Oerlinghausen, Ngamwathana and Kanchanapee, 1971).

Several phytochemical studies on other species of this genus have been reported but none on *M. leucantha*. Those reports showed many subtype of the flavonoids found in the plants in this particular genus. There are many reports concerning biological activities of the flavonoids including antimicrobial (Saxena *et al.*, 1987; Inamori *et al.*, 1991; Hufford *et al.*, 1993; Hunter and Hull, 1993; Batista *et al.*, 1994; Rao and Krupadanam, 1994; Perrett *et al.*, 1995; Vijaya, Ananthan and

Nalini, 1995; Borris, 1996; Rao, Prashant and Krupadanam, 1996; Afolayan and Meyer, 1997; Deng *et al.*, 2000), antiviral (Kaul *et al.*, 1985; Meyer *et al.*, 1997), anti-inflammatory (Baumann *et al.*, 1980; Landolfi *et al.*, 1984; Wagner, Knaus and Jordan, 1987; Noreen *et al.*, 1998; Liang *et al.*, 1999;;Wang *et al.*, 1999; Kim *et al.*, 2002; Yamaki *et al.*, 2002) and cytotoxic (Soulinna *et al.*, 1975; Edwards *et al.*, 1979; Kandaswami *et al.*, 1991; Scambia *et al.*, 1991; Ramanthan, Tan and Das, 1992; Beutler *et al.*, 1993; Fu *et al.*, 1993; Yit and Das, 1994) activities. Additionally, pharmacological screening activities of the extract of *M. leucantha* showed these significant activities as well. It is of interest to investigate this plant for the above activities.

The main objectives in this investigation are as follows.

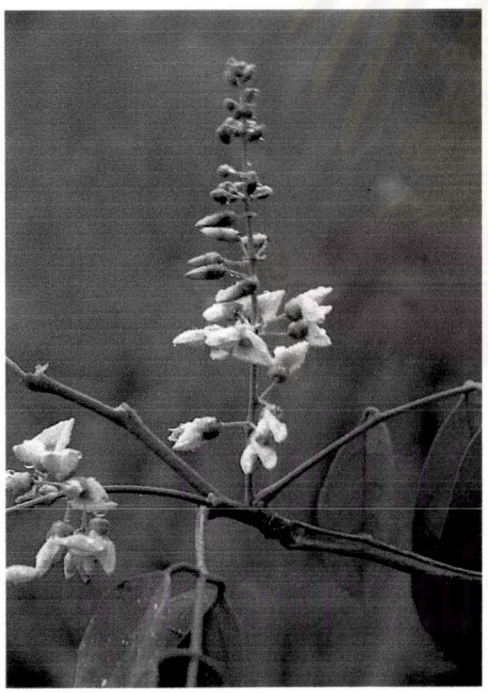
1. to isolate and purify compounds from the seeds of *Pachyrrhizus erosus* (L.) Urban and the stembark of *Millettia leucantha* Kurz var. *leucantha*
2. to determine the chemical structure of each isolated compound
3. to evaluate antimicrobial, anti-HSV, anti-inflammatory and cytotoxic activities of each isolated compound

A

B

**Figure 1** *Pachyrrhizus erosus* (L.) Urban (Nanthawan and Oranuch, 1999); A) Whole plants with inflorescence and pods, B) Seeds

A



B

C

**Figure 2** *Millettia leucantha* Kurz var. *leucantha* (Tongchai and Niwatra, 2001); A) Whole plant with stem bark, B) Inflorescence, C) Leaves and Pods