CHAPTER 1

INTRODUCTION

Chromolaena odorata (L.) R. M. King & H. Rob., formerly *Eupatorium odoratum* L., is a medicinal plant known in Thai as Sap Suea (1) and commonly called Siam weed or Christmas bush (2). This genus belongs to the Eupatorieae, one of the thirteen tribes of the Asteraceae. The genus *Eupatorium* has been recently taxonomically revised to contain 44 species (3) and, found in Thailand, 3 species (1) as follows :

1. *Eupatorium capillifolium* (Lam.) Small โกฏจุฬา Kot chula (Bangkok); Dog fennel
2. *Eupatorium odoratum* L. = *Chromolaena odorata* (L.) R.M.King & H.Rob. ช้าผัก-คราด Cha phak khrat, ยี่สุ่นเถื่อน Yisun thuean (Surat Thani); บ้านร้าง Ban rang, ผักคราด Phak khrat (Ratchaburi); เบญจมาศ Benchamat (Trat); ฝรั่งรุกที่ Farang rukthi, ฝรั่งเหาะ Farang ho (Suphanburi); มนทน Mon-thon (Chaobon-Phetchabun); มุ้งกระต่าย Mung kratai (Udon Thani); รำเคย Ram khoei (Ranong); **สาบเสือ** Sap suea (Sing Buri); หญ้าค่าพั้ง Ya-kha-phang (Shan-Mae Hong Son); หญ้าดงร้าง Ya dong rang, หญ้าพระศิริไอยสวรรค์ Ya phra siri aiya sawan (Saraburi); หญ้าดอกขาว Ya dok khao (General); หญ้าฝรั่งเศส Ya farangset (Chanthaburi, Trat); หมาหลง Ma long (Chon Buri); หญ้าเมืองวาย Ya mueang wai, หญ้าเมืองฮ้าง Ya mueang hang (Northern); หญ้า-ลืมเมือง Ya luem mueang (Nong Khai); หญ้าเลาฮ้าง Ya lao hang (Khon Kaen); หญ้า-เหม็น Ya men (Northeastern); Siam weed
3. *Eupatorium stoechadosmum* Hance เกี๋ยงพาไย Kiang pha yai (Northern); ซะเป Sa-pe, มอกพา Mok-pha, หญ้าลั่งพั้ง Ya-lang-phang (Shan-Mae Hong Son); พอกี๋ Pho-ki, พ่อสู่เจาะ Pho-su-cho (Karen-Mae Hong Son); สะพัง Sa phang (Loei); สันพร้าหอม San phra hom (Central)

According to the taxonomic classification, 5 species of the genus *Eupatorium* are altered to be the members of the genus *Chromolaena* (4). In Thailand, only one species of *Eupatorium* was classified to be in the genus *Chromolaena* and that was *Eupatorium odoratum* L. which was altered to be *Chromolaena odorata* (L.) R. M. King & H. Rob. (2, 5).

Chromolaena odorata is an erect or scrambling much-branched shrub, growing up to about 3 m high in open land and reaching up to 20 m as a scrambling climber on trees (Figure 1). The leaves are alternate or opposite in pairs; usually simple, ovate-lanceolate, with abruptly narrowed base and an acute or acuminate apex; 5-15 cm long, toothed and three nerved; rarely whorled and without stipules; odorous, gland-dotted beneath. It produces numerous small individual flowers called florets (Figure 2), usually white to light mauve in color; achenes are 5 angled, 4-5 mm long with many spreading bristles. The florets are of one or two kinds in each capitulum; hermaphrodite, unisexual or neutral, rarely dioecious. The seed has no endosperm but the embryo is straight with planoconvex cotyledons (6-7).

Figure 1. The plant of *Chromolaena odorata* (L.) R. M. King & H. Rob.

Figure 2. The inflorescence of *Chromolaena odorata* (L.) R. M. King & H. Rob.

C. odorata is a native to rainforest areas of the Americas from southern Florida in the United States to Mexico, the West Indies and the drainage begin of the Amazon in Brazil and Bolivia. In tropical America it is now widespread and abundant along the roads and rivers, in fallow and waste land, forest edges, and in neglected pastures and plantation crops. The weed was first introduced into Calcutta, India in the 1840s from where it spread very widely through the wet tropics of Asia including Bangladesh, Nepal, Sri Lanka, Burma, Thailand, Malaysia, Singapore, Indonesia and elsewhere in Southeast Asia. It is a very serious weed of forestry and agriculture in many wet tropical areas of the world (Figure 3). It is in the top three worst weeds of plantation crops in West Africa, the Philippines, Sri Lanka, India, Indonesia and south-west China. Several attempts to eradicate this weed have been undertaken in many countries using both herbicides and biocontrol. In various parts of the world, *C. odorata* is called Siam weed, Christmas bush, Triffid weed, Devil weed, Bitter bush and pesebrito (7-8).

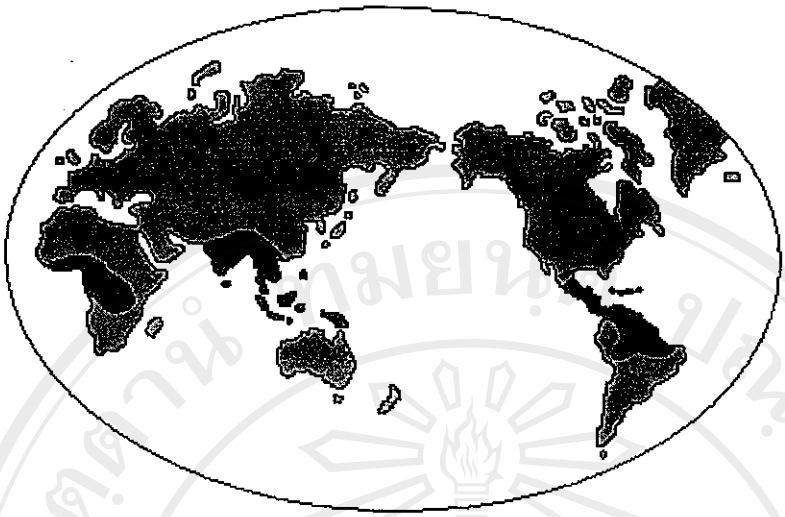

Figure 3. The distribution of Siam weed (indicated by the darker area)

C. odorata has also been used as a traditional medicine in many countries. In Thailand, Nepal and Nigeria, juice of the crushed leaves is topically applied to cuts to arrest bleeding and to promote wound healing (3, 9). In Nigerian ethnomedicine, a decoction of leaf is valued in traditional medicine as a cough remedy and as an ingredient with lemon grass and guava leaf for the treatment of malaria. The fresh leaf in limited quantities has been used to enrich the fodder for domestic animals. Other medicinal uses include antidiarrheal, astringent, antihypertensive, antispasmodic, anti-inflammatory and diuretic (6). In Vietnam and other tropical countries, a decoction of fresh leaves is used for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection and dento-alveolitis (10). This plant is also used as a fish poison in Himalayas (3).

Several biological activities of *C. odorata* have been reported including :

1. The chloroform and acetone extracts of the plant exhibited significant *in vitro* antimicrobial activity against the bacteria *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and the fungus *Aspergillus niger*, while the alcoholic and aqueous extracts were found to be inactive. The chloroform extract, which contained mainly isosakuranetin and kaempferide, displayed the maximum inhibitory activity on all the microorganisms tested (11).

2. The essential oil from leaves demonstrtated antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (12).

3. The essential oil from leaves showed toxicity against the maize grain weevil (*Sitophilus zeamais*) with the LD_{50} of 6.78% (13).

4. The leaf extract showed antimicrobial activity against *Pseudomonas aeruginosa* and *Streptococcus faecalis* (14).

5. The 50% alcohol and tincture extracts of the leaves showed activity against five strains (100%) of *Neisseria gonorrhoeae* which were isolated from symptomatic patients. This plant is one of the popular herbals used in Guatemala for the treatment of gonorrhoea (15).

6. The aqueous extract of the leaf showed hepatotropic activity *in vitro* (6).

7. The aqueous alcoholic extract has been found to possess *in vitro* antispasmodic activity in the guinea pig ileum and blocked the histamine-induced spasms (6).

8. The chloroform-soluble extractive has been shown to be cytotoxic to cell line CA-9KB, with an IC_{50} of 20 μg/ml (however, the isolated flavonoids did not show any significant activity) (6).

9. The aqueous extract of the leaves (Eupolin) showed an inhibitory effect on hydrated collagen lattice contraction by normal human dermal fibroblasts (16). This work illustrates that traditional remedies that are used by folk practitioners to improve healing can be examined in a scientific manner using *in vitro* wound-healing models.

10. Eupolin ointment, a topical agent used in the treatment of soft-tissue wounds and burns in Vietnam, is made from an aqueous extract of the leaves of *C. odorata*. Clinical studies using this extract have shown antimicrobial and anticoagulation

effects as well as the promotion of tissue remodeling in the wound healing process. Fibroblasts and endothelial cells (keratinocytes), two cell types that play a crucial role in wound healing, were used to investigate some of the effects of Eupolin extract *in vitro*. The result of the study demonstrated that Eupolin extract enhanced proliferation and migration of human keratinocytes. This evidence might explain, in part, the beneficial clinical effects that have been observed (17-18).

11. Eupolin increased expression of several components of the adhesion complex and fibronectin by human keratinocytes. The adhesion complex proteins are essential to stabilised epithelium and this effect could contribute to the clinical efficacy of Eupolin in healing (19).

12. The antioxidant effects of the ethanol extract were found within the range of 50 to 800 μg/ml. The extract showed significant protection on cultured fibroblasts and keratinocytes against hydrogen peroxide and superoxide radical damage. Further fractionation by CC was performed and the results revealed that the fractions containing phenolic acids and complex mixtures of lipophilic flavonoid aglycones were found to be major and powerful antioxidants to protect cultured skin cells against oxidative damage (10, 20).

13. The purified compound, 4', 5, 6, 7-tetramethoxyflavone, which was isolated from the leaves, was studied *in vitro* for the effect on blood clotting factor activities. It was found that the compound enhanced blood coagulation, the observed APTT being shorter than that observed in the control. The result suggested that the compound accelerated clotting time through the intrinsic pathway of the coagulation which may involve the reaction of factor XII, factor XI, factor IX or factor VIII (21).

14. This plant has been reported to poison cattle and its use to fertilize rice fields resulted in the killing of fish (22).

15. Some flavonoid derivatives isolated from the flowers of this plant exhibited moderate antimycobacterial activity against *Mycobacterium tuberculosis* and cytotoxic activity against human small cell lung cancer cells and human breast cancer cells (23).

According to the literature, many biological activity results of *C. odorata* extract have been revealed, however, only two papers that have demonstrated active components isolated from this plant (21, 23). In the current study, the biological screening tests of this plant were investigated and the results showed that the crude extracts exhibited significant activity in several tests. Thus, this plant was selected for recollection and investigation. The objectives of this project were to determine the biological and pharmacological activities of this plant and to isolate the chemical components from the plant extract by means of bioassay-directed fractionation. Then, the structures of the isolated compounds were to be elucidated using spectroscopic methods.