CHAPTER I
INTRODUCTION

There are several difficulties frequently found in agricultural processing, for instance, irrigation, weather, quality of soil or especially weed. Weed is the plant, which can grow in the same planting area as the main plant and is undesirable. Weed can make huge damage to agriculture, such as diminishing quality and quantity of crops, blocking dam and irrigation system, causing forest fire, making main plant die, including water pollution and in some cases, irritating human or animals. Thailand is an agricultural country, so weed problem is the main and inevitable problem leading to need eradication. However, in the past, some weeds can be used as medicinal plants indicating that weed are not only useless but also useful in someway, for instance, weeds are also made use as food for human and animals, herbal spice, pulp wood for paper, mulching and environmental conservation.

Nowadays, there are many reports to affirm that some weeds constituted substances, which affected the growth of side-growing plants or other creatures. *Sphenoclea zeylanica* Gaertn., weed growing in rice field, contained betulinic acid which can inhibit the growth of rice and has piscicidal activity and *Imperata cylindrica* can emit substance which controls the growth of Stylosanthes (Sajise and Lales, 1976). Following this evidences, there have been application of weed to kill other weeds; herbicides. Moreover, some weeds can be applied to use as pesticides. Both pesticides and herbicides that make from weed are widely utilized because they have more safety to living things and environment and cause less serious harm than synthetic ones.

The act of plant having the substances to take part in the growth of other plants is so-called allelopathy, and those substances are allelochemic. The study of allelopathy can make useless weed more benefits. As parts of this afford, a serious investigation for naturally occurring substance that posses bioactive compound has been conducted.

Following the above mentioned outcome, naturally occurring organic chemical substances from some parts of four weed species in Compositae family as

Eupatorium odoratum Linn., *E. adenophorum* Spreng., *Ageratum conyzoides* Linn. and *Sphearanthus africanus* Linn. have been selected for agriculturally and medicinally biological activity studies.

1.1 Botanical Description of Family Compositae

The family Compositae is the only family in Asterales order. Compositae (Asteraceae) or sunflower family, is the largest of flower plant families, including more than 1,100 genera and 20,000 species. There are many types of plant in this family, such as noxious weed (*Eupatoruim odoratum, E. adenophorum*), vegetable (lettuce, globe artichoke), flower (sunflower, chrysanthemum) and medicinal plant (*Carthamus tinctorius*).

Plants in Compositae are widely distributed in many region of the world, except freezing pole. They have been found even in arid desert, through tropic area, especially in temperate to cool region which have diversity of genera and species.

Most of the member in this family is shrub or perennial shrub which has rhizome underground. Some of them are annual herb that has rhizome underground or perennial herb or climbing or succulent plant.

The botanist classified Compositae family into two subfamily from difference of characteristics.

1. Subfamily Lactucoideae : inflorescence is discoid, flower homogamous. Most of flowers are white, pink or purple.
2. Subfamily Asteroideae : inflorescence is disciform, flower heterogamous. Most of flowers are yellow (Paisooksantivatana, 1994).

1.2 Allelopathy

Allelopathy refer to any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment (Rizvi and Rizvi, 1992).

1.2.1 Mechanisms of action of allelopathic agents

1. Effects on cell elongation and ultrastructure of root tips

There is much evidence for inhibition of cell division by various allelopathic agents; umbelliferone decreases the cell elongation rate in curcumber roots but increases radial expansion. Exposure of cucumber roots to volatile terpenes from *Salvia leucophylla* Greene. leaves caused accumulation of glubules (which appeared to be lipid) in the cytoplasm of root tip cells, a drastic reduction in the number of a variety of intact organelles including mitochondria, and a disruption of membranes surrounding nuclei, mitochondria, and dictyosomes. This combined disruption of the root meristems resulted in death of cucumber seedling under laboratory conditions.

2. Effects on hormone-induced growth

Much evidence exists that several allelopathic compounds affect hormone-induced-growth. Recent evidence supports the importance of this interaction; T-2 toxin produced by *Fusarium tricinctum* (Cda.) Synd. and Hans. inhibited auxin-promoted elongation in *Glycine max* var. Hawkeye 63. One h preincubation with 5 μm toxin prevented the induction of a faster rate of elongation by auxin. Inhibition of elongation by cytokinin was similar to that of the toxin the mode of action appeared to be different, because the inhibitory effects were additive. Toxin treatment did not diminish cytokinin-induced radial enlargement.

Victorin, the phytotoxin from the host-specific pathogen, *Helminthosporium victoriae* Meeh. And Murph., inhibited the growth response of susceptible tissue to auxin completely while having no effect on the response of resistant tissue to auxin.

3. Effects on membrane permeability

The most common early sign of damage to cells caused by pathogen produced phytotoxins is an alteration in water and ion permeability of the cytoplasmic membrane; the toxin (victorin) produced by *Heominthosporium victoriae* dramatically increased the permeability of both the plasma membrane and tonoplast of root cells in a susceptible cultivar of *Avena sativa*, but not in a resistant cultivar. The *H. maydis* Nisk. and Miy. race T toxin increased the permeability of plasma membranes of *Zea mays* leaf cells, but the effect was not host-specific. Moreover, this toxin did not affect the tonoplast permeability. Aescin, a triterpeneglycoside, induced leakage of ribonucleotide material, nucleosides, and pentose phosphate or pentose from hyphae of *Ophiobolus graminis* and *Neurospora crassa*. Loss of viability seemed to be correlated with the loss of oligonucleotides from the cells. *Aspergillus niger* was relatively insensitive to the inhibitor and aescin induced leakage of only pentose or pentose phosphate.

Under anaerobic conditions, at low pH and 30°C, commercial baker's yeast lost K^+ ions in the presence of salicylic acid, and glucose utilization was inhibited. Both effects were reversed by washing the cells free of salicylate. A fundamental action of this compound in many organisms may be its ability to reduce the K^+ content of the cells.

Allelopathic extracts of leaf litter of *Tilia cordata* Mill., *Albizzia julibrissin* Durazz., *Aesculus hippocastanum* and *Pinus nigra* decreased the bioelectrical activity of seedlings of *Triticum vulgare* by 96, 93, 86, and 50% respectively. These decreases corresponded with the alleopathic activity of these species.

4. Effects on mineral uptake

Most persons working on allelopathy rapidly become aware of the rapid and the pronounced effects various phytotoxins on the appearance of roots of test plants. These facts, together with the basic function of roots in mineral absorption, have caused much research to be done on effects of allelopathic compounds on mineral absorption. This activity is continuing, and is now being extended to explain how phytotoxins affect mineral uptake.

Susceptible corn roots exposed to the host-specific toxin of *Helminthosporium carbonum* Ullst. race 1 removed nitrate from solution and accumulated it in tissues twice as fast as did of the control roots. Uptake by resistant roots was stimulated also. The stimulation occurred in the presence of tungstate which eliminates nitrate reductase activity, and the toxin did not cause leakage of nitrate from roots under the experimental conditions employed. Thus, the enhanced nitrate accumulation was caused by increased uptake rather than by decreased nitrate metabolism or decreased nitrate leakage.

5. Effects on easily available phosphorus and potassium soils

This is a subject that needs much more study because of its potential significance. An allelochemic from *Agrostemma githago*, significantly increased amounts of easily available phosphorus and potassium in manured eroded Chernozem and hydromorphic black soil in Yugoslavia. Results in unmanured soils were variable, but at least some concentrations of agrostemmin increased amounts of available phosphorus and potassium.

6. Effects on stomatal opening and on photosynthesis

Earlier work indicated that several allelopathic compounds inhibit photosynthesis and recent work has supported earlier conclusions. Boiled aqueous leaf extracts of *Celtis laevigata*, a known allelopathic species, significantly inhibited net CO_2 uptake by three grass species in light when added to the nutrient solution in which the grasses were growing. This effect appeared to be independent of effects on water content.

The phytotoxin produced by *Helminthosporium maydis* caused a rapid inhibition of photosynthesis in whole leaves of *Zea mays* having normal cytoplasm. Electron transport, phosphorylation , and proton uptake activities of isolated chloroplast lamellae were not affected by the toxin in either genetic type. The toxin however, was found to have a direct effect on stomatal functioning. The toxin inhibited light-induced K^+ uptake by guard cells in the Texas strain, and thus the stomates did not open.

7. Effects on respiration

Macerated leaves and sesquiterpene lactones obtained from *Artemisia tridentata* ssp. *Vaseyama* and other species of sagebrush inhibited growth and stimulated the respiration of *Cucumis sativus*. Arbusculin-A, achillin, desacetoxymatricarin, viscidulin-C, and viscidulin-B were the most active sesquiterpene lactones isolated and showed effects at 10^{-4} M or higher concentrations.

Four of seven flavones and flavone glycosides tested inhibited mitochondria ATPase. Quercetin, in low concentrations, inhibited both solution and particulate mitochondrial ATPase, but had no effect on oxidative phosphorylation in submitochondrial particles. Quercetin also inhibited the ATP-dependent reduction of NAD^+ by succinate in fully reconstituted submitochondrial particles. It was concluded that hydroxyl group at the 3′ and perhaps 3 position are important for the inhibition of ATPase activity by flavones.

The triterpeneglycoside, aescin, in phosphate buffer, reduced the production of $^{14}CO_2$ from uniformly labelled glucose in mycelia of *Ophiobolus graminis* Sacc. and *Neurospora crassa*, whereas aescin in succinate buffer has no effect. The production of $^{14}CO_2$ from glucose or sucrose in *Aspergillus niger* was not affected, however.

8. Inhibition of protein synthesis, and changes in lipid and organic acid metabolism

The important of this mechanism is obvious, but relatively little research has been done on the subject. Recent publications are particularly sparse.

Incubation of Paul's Scarlet rose cells with glucose-UL-^{14}C in the presence of ferulic acid in increased incorporation of ^{14}C into soluble lipid fraction along with a decreased incorporation of ^{14}C into protein, organic acids, and soluble amino acids. Treatment of the cells with cinnamic acid resulted in a significant decrease in incorporation of ^{14}C into protein, but incorporation of ^{14}C into soluble amino acids was significantly increased. There was also a decrease of ^{14}C incorporation into protein amino acids. It was concluded that the reduction in protein synthesis resulting from ferulic or cinnamic acid treatment would lead to a reduction in growth of the cultures, It was inferred also that feruic and cinnamic acids affect protein synthesis in different ways. Cinnamic acid appeared to inhibit the mechanism protein synthesis, whereas ferulic acid caused a diversion of acetate (^{14}C) lipid synthesis rather than into Kreb's cycle and subsequent pathways leading to synthesis of amino acids and proteins.

9. Inhibition or stimulation of specific enzymes

Rhizobitoxine, a phytotoxin produced by some strains of *Rhizobium japonicum*, partially inhibited β-cystathionase of corn and spinach seedlings *in vivo*. Rhizobitoxine-treated corn seedlings allowed to assimilate $^{35}SO_4^{-2}$ for 3 or 6 hr had an increase in radioactive cystathionine up to twenty-two times that in controls. Accumulation of radioactivity in methionine was only slightly inhibited, however. It was concluded that the data did not permit a decision as to whether the pathological effects of rhizobitoxine are due chiefly to inhibition of β-cystathionase.

10. Corking and clogging of xylem elements and stem conductance of water

It was found subsequently that allelopathic agents caused increases in the reduced form ascorbic acid in the xylem vessels, and histochemical evidence indicated that the reduced ascorbic acid played a role in formation of the brown substance. Evidence was obtained also that thiol groups of substances flowing to the area of corking and browning took part in the formation of the brown mass. It was concluded that intensification of redox processes in tissues of plants subjected to allelopathically active substances probably favors formation of the mechnical barriers to toxin penetration of the plant (Rice, 1979).

1.2.2 Chemical Nature of Inhibitor

1. Water-soluble organic acids, straight-chain alcohols, aliphatic aldehydes, and ketones
2. Simple unsaturated lactones
3. Long-chain fatty acids
4. Naphthoquinones, anthraquinones, and complex quinones
5. Terpenoids and steroids
6. Simple phenols, benzoic acid, and derivatives

7. Cinnamic acid and derivatives
8. Coumarins
9. Flavonoids
10. Tannins
11. Amino acids and polypeptides
12. Alkaloids and cyanohydrins
13. Sulfides and mustard oil glycosides
14. Purine and nucleosides
15. Miscellaneous ; ethylene, abscisic acid

1.2.3 Plant parts that contain inhibitors

1. Stems
2. Leaves
3. Roots
4. Flowers or inflorescence
5. Fruits
6. Seeds (Rice, 1974)

1.3 Literature Search of *Eupatorium odoratum* Linn.

Chromolaena odorata (L.) R.M. King & H. Robinson is synonym of *E. odoratum*. Common name are Bitter bush and Siam weed, and common name in Thai is Sapp suea.

E. odoratum is a perrenial shrub. Stem are erected, branched, shortly pubescent or nearly glabrous, striate, up to 3 m tall. Leaves opposite , triangular-oblong with serrate-dentate margin, hairy. Inflorescence of heads on peduncles, corolla light violet. Seed (fruit) long with a well developee pappus of whitish scales. On preferably well-drained soils, roadsides, waste lands and brushwood. Found in lower altitudes, compare with *E. adenophorum*. Propagated by seeds. Native of Central and South America. Distributed throughout Thailand, from near sea level to above 2000 m. Whole plant odoriferous. Show a strong allelopathy. Cause cattle poisoning (Association for International Cooperation of Agriculture & Forestry, Japan (AICAF). 1997).

Utility of this plant ; leaf use for dysentery, applied on fresh cuts and wounds to stop bleeding (Jain and Robert, 1991).

1.4 Literature Search of *Ageratum conyzoides* Linn.

A. conyzoides is native to tropical America and became naturalized in the tropical and subtropical regions of the world. Annual herb. Stem are 30 to 120 cm long, hairy. Leaves are hairy, ovate or cordate, serrate, 2 to 10 x 0.5 to 5 cm in size. Flowers are terminal, white or pale purple. Flower heads consist of 60 to 70 tubulous flowers arranged in clusters. The plant grows in somewhat wet agriculture land, wastelands, along roadsides and in plantations and becomes and important weed in upland fields. The species also preferably grows in areas at high elevations. Propagation by seeds. In the early growth stage, application of phenoxy herbicides, 2,4-D and MCPA is effective. The plant is toxic to livestock. Leaves are used as medicine for the treatment of cold, malaria, external wounds and skin diseases (AICAF, 1997).

Utility of this plant ; Root use for antilithic juice, abdominal problems (decoction), and promotes digestion (raw root). Leaf use for fever (made into paste with ginger and mustard seed and applied on skull to bring down body temperature).

For snake bite a paste is made with leaves of *Galinsoga parviflora, Drymaria cordata, Bidens biternata* and *ginger* and is heated with mustard oil and applied on wounds. Prevent bleeding, for headache (a snuff of powdered leaf of white-flowered form is mixed with black pepper), pulp for pneumania. Fruit use for prevents bleeding. Whole plant use for fresh juice applied to wounds, boils and skin diseases, for catarrh, diarrhea, rheumatism, pounded and applied on tumours and swellings, paste prepared from leaves and slender stem used as scabocide, decoction with honey for dy sentery. Part unspecificed use as Antidote to snakebite and stings, on cuts and uterine problems (Jain and Robert, 1991).

1.5 Literature Search of *Sphaeranthus africanus* Linn.

S. africanus is native to Africa and is distributed widely in the tropical regions of South and Southeast Asia. It is an annual herb, 20 to 110 cm tall. Stem are erect of oblique, with many branches, winged and slightly fragrant. Leaves are obovate-oblong, spiral and sessile. Inflorescence has a globose head, is 8 to 12 mm in diameter and purple. It grows in wet areas in lowland rice fields, levees and around ponds. Sometimes it becomes a harmful weed in rainfed and upland rice fields. Propagation by seeds. Usually control by hand weeding. Occasionally used as feed for livestock (AICAF, 1997).

1.6 Literature Search on the Chemical Constituents of *Eupatorium adenophorum* Spreng.

There are four species member in Genus Eupatorium in Thailand that is *Eupatorium adenophorum* Spreng., *E. capillifofium* Small, *E. odoratum* Linn. and *E. stoechadosmum* Hance (Smitinand, 1980).

Ageratina adenophora King & Robinson and *E. glandosum* Kunth are synonyms of *E. adenophorum* Spreng. Common name in Australia are crofton weed, cat weed, hemp agrimony; in New Zealand, it is called Mexican devil; in Hawaii, it is called pamakani and in the USA. are called sticky agrimony or sticky eupatorium (Parsons and Cuthberson, 1992).

E. adenophorum Spreng. is native plant of central America and Mexico but widely distributed in the tropical and temperate regions of the world. This plant occur as weed in grasslands, upland fields, and burnt fields and occurs in forest border in tropical highlands. It is distributed in the north of Thailand at 1,000-1,500 m altitude. The common name of *E.adenophorum* Spreng. in Thai is "Sapp Ma" (Smitinand, 1980). Whole plant is odoriferous and shows strong allelopathy properties and is toxic to cattle.

E. adenophorum Spreng. is a perennial herb or shrub. Stems are erected, branched, pubescent, dark purple in color with short and soft hairs, up to 2 m tall. The whole plant is hairy and crushed leaves emit an offensive smell. Leaves are opposite, ovate, ovate-oblong with serrate margin, 3-7.5 cm wide, 6-12 cm long. Inflorescence are heads in dense terminal corymbs, peduncled, corolla white, sometime pinkish; Seed (fruit) long with well-developed pappus of white scales. Found in abundance in ravine-slopes after shifting cultivation, at rather higher altitudes compared with *E. odoratum.* Propagated by seeds. Blooming period is February-July (Harada, Paisooksantivatana and Zungsontiporn, 1987).

There was a major cadinene that exhibited appreciable antifeedant action against *Philasomia ricini* Hutt. (Bordoloi, Shukla and Sharma, 1985). The mince freeze-dried of the leave was toxic to mouse liver (Sani *et al*, 1992). The leave shed control and decrease dense of plants grown around (Tripathi, Singh and Rai, 1982).

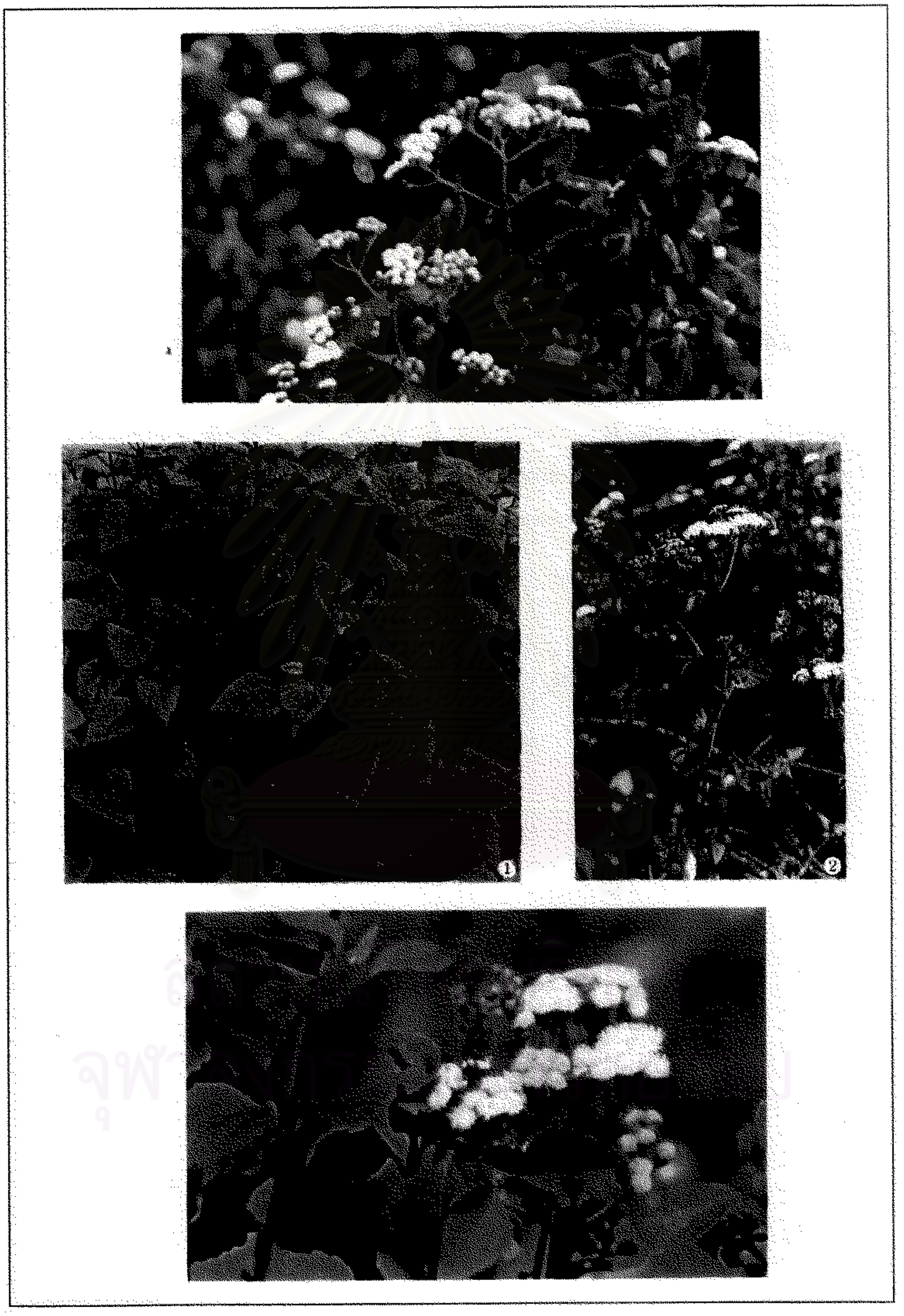


Fig. 1.1 *Eupatorium adenophorum* Spreng.

1.7 Utility of *Eupatorium adenophorum* Spreng.

Boiled leaves taken for stomachache. Ground fresh leaves then applied on fresh cuts, injuries or piles. It used as antiseptic and blood coagulant (Jain and Robert, 1991).

1.8 Chemical constituents Studies on *Eupatorium adenophorum* Spreng.

Literature surveys of chemical constituents of the plant belonging to Eupatorium genus that have been a variety of organic substances isolated (Table 1.1). The structures of some isolated compounds are shown in Fig. 1.2.

Table 1.1 Chemical constituents of *E. adenophorum* Spreng.

Plant parts	Crude extract	Isolated compounds	References
aerial part	chloroform	Three-known cadinenes (1>4>3), β-sitosterol	Baruah *et al* (1994)
	Petroleum ether ethanol	isohexacosane *β*-amyrin *n*-hexacosanoic acid lupeol taraxasterol stigmasterol salvigenin epifridelinol	Ansari *et al* (1985)
	---	6-cadinene derivatives (I → ageraphorone)	Bohlmann and Gupta (1981)
seedling	---	chromene- demethoxyencecalin encercalin demethoxylececalin	Proksch , Palmer and Hartmann (1986)
leaves of seedling	---	chromene sesquiterpenes chlorogenic acid	Proksch *et al* (1990)
leaves and inflorescence	chloroform	*n*-dotriacontane β-sitosterol stigmasterol taraxasteryl palmitate taraxasteryl acetate	Xu *et al* (1988)
dried whole plant	Petroleum ether	β-sitosterol Eupatorenone (sesquiterpenoid) triterpene (unidentified)	Ananvoranich , Likhitwitayawuid and Ruangrungsi (1989)

Table 1.1 (cont.)

Plant parts	Crude extract	Isolated compounds	References
		stigmasterol lupeol epifriedelind Cadinene:Eupatorinonr phytosterol	
---	ethanol	friedelin taraxasterol steroid stigmastadienone stigmasterol	Gonzalez *et al* (1987)
---	---	9-oxo-10,11 dehydroageraphorone	Oelrichs *et al* (1996)
---	---	Major cadinene	Bordoloi *et al*, (1985)
---	---	2 new cadinenes	Shukla *et al* (1983)

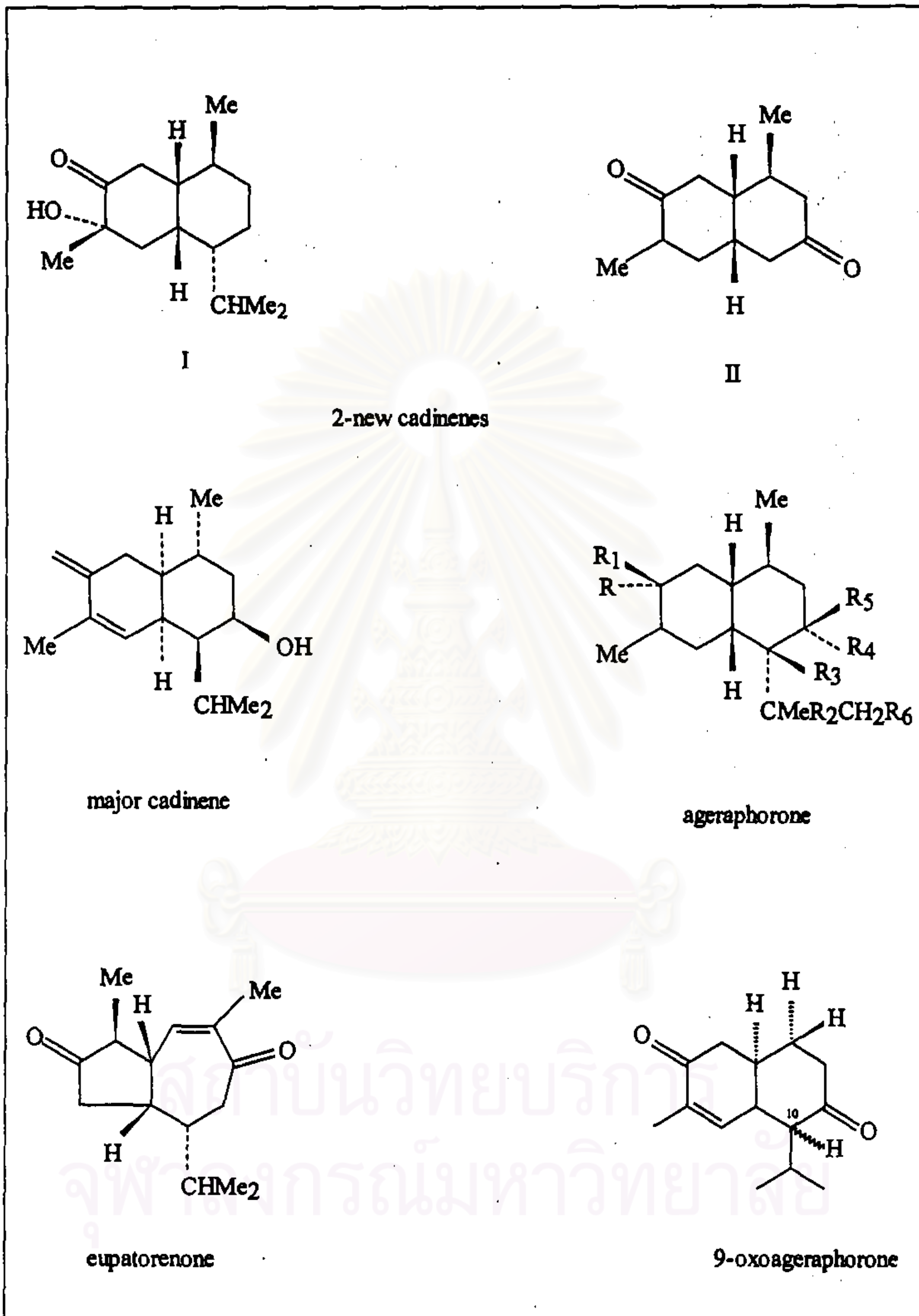
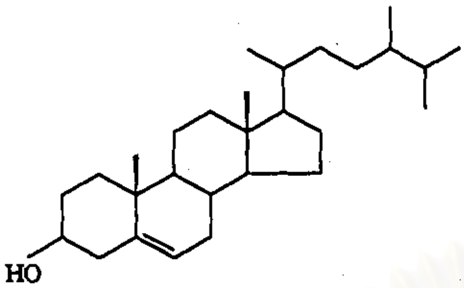
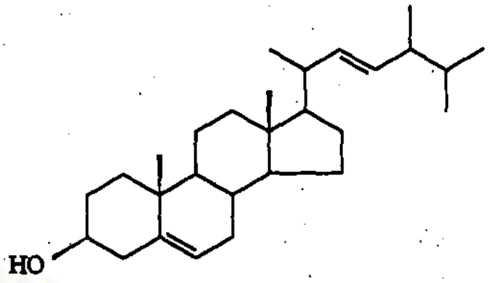


Fig 1.2 Some compounds isolated from *Eupatorium adenophorum* Spreng.

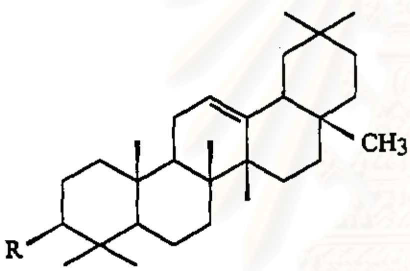


B-sitosterol

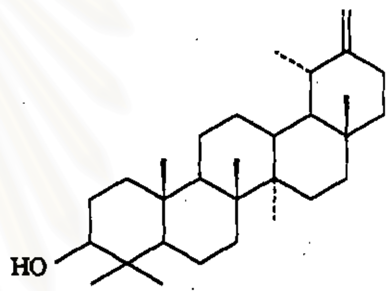


stigmasterol

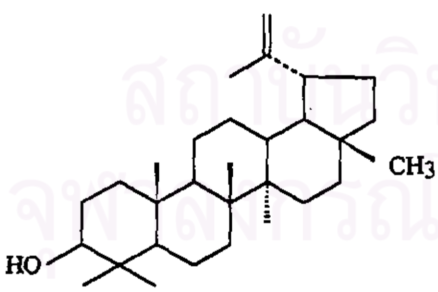
Steroids found in *E.adenophorum*



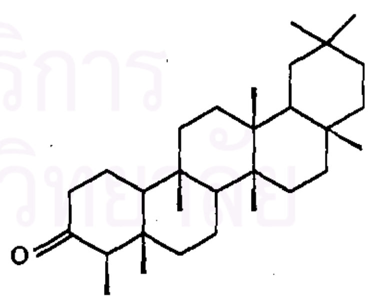
B-amyrin R=OH(H)



taraxasterol

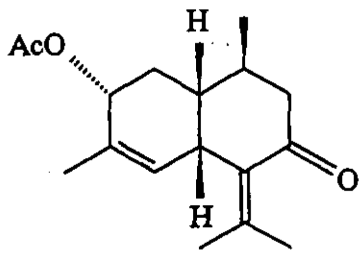


lupeol



friedelin

Fig. 1.2 (cont.)



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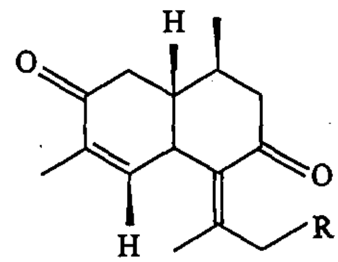
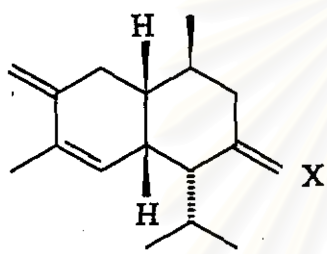
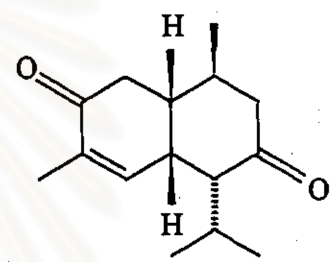


2 R=H
3 R= OH



4 X= O
5 X= *B*-OH, H



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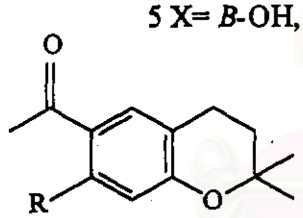
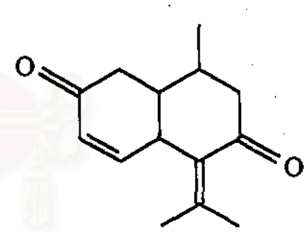
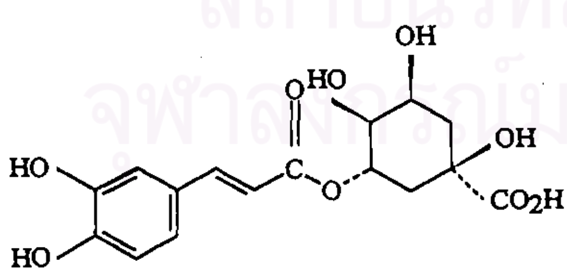


chromene derivatives
1 demethoxyencecalin R=H
2 demethylencecalin R=OH
3 encecalin R=OCH_3



sesquiterpene derivative



chlorogenic acid

Fig. 1.2 (cont.)

1.9 Goal of Research

The goal of this research could be summarized as follows:

1. Preliminary screening for bioactive compounds of 4 species from Compositae weeds.
2. To extract and fractionation the organic compounds from the weeds.
3. To elucidate the structure of the isolated substances from the most interesting crude extract.
4. To search for plant growth inhibition compounds.