CHAPTER I

INTRODUCTION

Since most of Thai population are farmers, the country income is primary demand from agricultural products for example corn, soybean, rice and cotton (Radanachaless, 1997, in Thai). There are many serious problems to effect the decrease production of crops for example, mites, fungi, insect, nematode, gastropods, rodents, quality of soil or especially weed. Weed is an undesirable plant, which can grow in the same planting area as the main. Weed can make huge damage to agriculture, such as diminishing quality and quantity of crops, making main plant decrease. Therefore, weed problem is the main and inevitable problem leading to need eradication.

Nowadays, the farmer favorably uses synthetic herbicides to get rid of weeds. In the midst of pesticides, herbicides are most imported from foreign countries and the tendency of importation is annually increased. To increase the yield of production, large amounts of synthetic herbicides are import in 1993, the total volume of imports was 1,789 million baths. Among those imported herbicides, glyphosate was found to be as the top-rank of imported item, followed by 2,4-D, diuron, atrazine and *etc*, respectively. (Pisitsin *et al*., 1993, in Thai)

Herbicides can be fundamentally classified into two groups: synthetic and natural origin herbicides (Radanachaless, 1997, in Thai). Synthetic herbicides have normally been imitated from natural compounds. However, they have led to the serious problems of health and environment. Even there are some advantages for utilizing herbicides such as broad activities, some non-degradable substances may be left over in soil and persisted for a long time, while natural herbicides, which have been isolated from plants or microorganisms, can be naturally degraded. Therefore, they do not cause damages to physical and biological ecosystems. The search for new herbicides from natural products is therefore a choice to compensate synthetic herbicides.

1.1 Allelopathy

The application of chemicals to eliminate weeds has been widely shown to have negative impact on both environment and living organisms. As a consequence, the idea of using natural products in place of these hazardous chemicals has become more interest for modern scientists at present.

Plants can produce secondary metabolite chemicals and release to the environment. Various plants with allelopatic properties can provide benefit for crop protection. Chemicals from plants may be phytotoxic to some plants but may be a growth regulator of other plants. Allelopatic mechanism can be selected in agronomic crops and bred with good quality cultivates. Allelopatic chemicals from one crop may have selective properties and can be grown with other crops as a bicultural. Chemical substances contained in weeds often play an important role as one of the environmental factors, which affect growth of other plants. The phenomenon is well known as an allelopathy (Holm, 1982), (Rice, 1979).

In agriculture, several allelopathic effects have been illustrated such as the effect among agricultural plants themselves (Young and Chen, 1989), agricultural plants against weeds (Haward *et al.*, 1986), weed effect on agricultural plants (Ito *et al*, 1985) or even among weeds themselves (Simkin and Doll, 1982). The present research focused on the effect among weeds themselves could simultaneously reduce the number of weeds in nature and the utilization of chemicals for herbicides.

1.1.1 Chemical nature of allelochemicals

Various types of chemicals implicated in allelopathy have been discussed in details (Rice, 1974, 1979, 1984). Most of these chemicals are secondary metabolites and are produced as offshoots of primary metabolic pathways. These secondary products could be classified into five major categories: phenyl propanes, acetogenins, terpenoids, steroids and alkaloids (Whittaker and Ferny, 1971). It is almost impossible to enumerate each and every chemical identified as an allelochemical. However, to classify these into various major chemical groups would be a viable approach. (Rice, 1984) has also adopted the same approach and has classified allelochemicals produced by higher plants and microorganisms into the following major categories: simple water soluble organic acids, straight chain alcohols, aliphatic aldehydes and ketones, simple unsaturated lactones, long-chain fatty acids and polyacetylenes, naphthoquinones, anthroquinones and complex quinones, simple phenols, benzoic acid and derivatives, cinnamic acid and derivatives.

1.1.2 Mode of action of allelochemicals (Rizvi and Rizvi, 1992)

The visible of allelochemicals on plant processes is only secondary signs of primary changes. Therefore, studies on the effects of allelochemicals on germination and/or growth are only the manifestation of primary effects occurring at the molecular level. Although a strong tendency is being developed to look into the actual mechanism of action, the experimental work is in its infancy.

The mode of action of allelochemicals can broadly be divided into indirect and direct actions. Indirect action may include effects through alteration of soil property, its nutritional status and an altered population and/or activity of harmful/beneficial organisms like microorganisms, insects, nematodes, *etc*. This is relatively a less studied aspect. On the other hand, the direct mode of action, which includes effects of allelochemicals on various aspects of plant growth and metabolism, has received fairly wide attention.

The followings are some important sites and processes known to be attacked or influenced by allelochemicals: cytology and ultrastructure, phytohormones and their balance, membrane and its permeability, germination of pollens/spores, mineral uptake, pigment synthesis and photosynthesis, respiration, protein synthesis, specific enzyme activity, conducting tissue, water relations of plants, genetic material.

The preliminary screening test for searching new agrochemical is under the cooperative project between Natural Products Research Unit of Department of Chemistry, Faculty of Science, Chulalongkorn University and Weed Science Subdivision, Botany and Weed Science Division, Ministry of Agriculture and Cooperatives. From the preliminary screening test, it was revealed that the ethanolic crude extract of the aerial part of *Hyptis suaveolens* Poit. had a profound inhibitory effect on the growth of *Echinochloa crus-gllli* Beauv.

1.2 Botanical Description of the Family Labiatae.

The Labiatae (Lamiaceae) family contains 200 genera and about 3,000 species (Heywood, 1978), which are distributed chiefly in north temperate regions.

The Labiatae is a family of herbs, rarely shrubs, usually loaded with oil-glands; stem is usually 4-gonous; leaves are opposite or whorled, with stipules. Flowers are irregular, solitary, 2-nate or fascicled and axially, or in centrifugal spicate comes which by their union in pairs form false whorls; fruit of 4 dry, or rarely fleshy 1- seceded lobes (nuttiest) at the base of the calyx; seeds small, erect, albumen sparing (Hooker, 1953).

Hyptis suaveolens Poit. is one of the 400 species of the pantropical *Hyptis*, occurring in tropical America (Willis, 1973). According to the literalness cited (Smitinand, 2001, Radanachaless and Maxwell, 1982 in Thai), there are 3 species of *Hyptis* in Thailand:

1. *Hyptis suaveolens* (L) Poit.

   [kaaraa (การา) (Suratthani), Maeng lak khaa (แมงลักกา) (Chumphon)]

2. *Hyptis brevipes* Poit

   [Chat Pra In (ฉัตรพระอินทร์) (Southern)]

3. *Hyptis capitata* Jacq.

1.3 Literature Review of *Hyptis suaveolens* Poit.

H. suaveolens Poit. is a shrub by herb (Fig.1.1). Its vernacular name is wild spikenard or bush tae (Radanachaless and Maxwell, 1994). In Thailand this plant is called Kaaraa and maeng lak khaa (Smitinand, 2001).

Fig. 1.1 Shoot and flower of *Hyptis suaveolens* Poit.

Family : Labiatae (Lamiaceae)

Genus : *Hyptis*

Species : *Hyptis suaveolens*

Description : (Moody *et al*, 1984). An erect, aromatic annual herb, 0.5-2 m high. *H. suaveolens* is a perennial shrub; *STEM*: more or less hairy, coarse, branched; *LEAVES*: variable, ovate, acute, serrulate, 4-9 cm long; *INFLORESCENCE*: axially, peduncle, 3-4 flowered heads or clusters (some are solitary), racemosely or subpaniculately arranged along the branches; calyx in flower about 4 mm long, soon enlarged and nearly 1 cm long, striate, villous, teeth erect, subulate; flower blue, about 8 mm long; limb 5 mm in diameter; *FRUIT*: a nutlet, compressed, broadly obovoidal, truncate and emarginate at apex, mucronate, 3-4 mm long, 2-3 mm wide; *PROPAGATION*: by seed; *HABITAT*: in dryland field crops, plantation crops, and pastures.

1.4 Utility of *Hyptis suaveolens* Poit. (Chopra, Chopra and Varma, 1969)

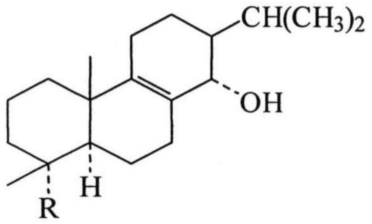
In Thailand, *H. suaveolens* Poit. was used as herbal medicine. Roots were used for appetite stimulation. Stems were used to cure skin diseases, and their leaves were used as anti-pyretic and anti-influenza drug.

1.5 Chemical Constituents Studies on *Hyptis suaveolens* Poit.

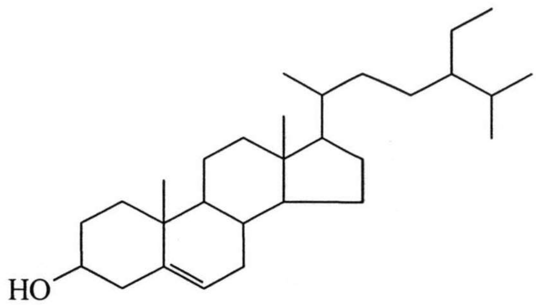
Literature surveys of chemical constituents of plants belonging to *H. suaveolens* Poit. revealed that there have been a variety of organic substances isolated (Table 1.5). The structures of some isolated compounds are shown in Fig 1.2.

Table 1.5 Chemical Contituents of *Hyptis suaveolens* Poit.

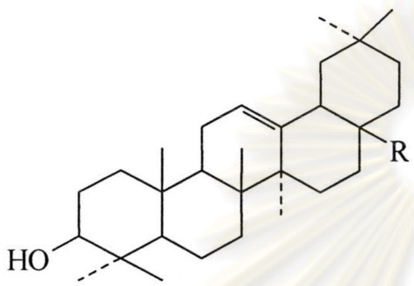
Plant Uses	Extract	Isolated Compounds	References
Leaf	benzene	suaveolic acid, suaveolol	Manchand, White and Fayas, 1974
Root	benzene	*β*-sitosterol, oleanolic acid, *α*-peltoboykinolic acid	Misara *et al.*, 1981
Root	benzene	3*β*-hydroxylup12-en-28-oic acid, *α*-amyrin, *β*-amyrin	Misara, Singh and Upadhyay, 1983
Root	benzene	heptacosanone, sitosterol-*β*-D-glucoside, ursolic acid, betulinic acid, 3*β*-hydroxylup-20(29)-en-27-oic acid, 3*β*-actoxylup-20(29)-en-27-oic acid, methyl 3*β*-hydroxylup-20(29)-en-27-oate	Misara *et al.*, 1983
aerial parts	Petroleum ether	urs-12-en-3*β*-ol-29-oic acid, 3-oxo-urs–12-en-29-oic acid	Mukherjee and Ghosh, 1984
Seed coat	water soak	L-fucose, D-xylose, D-mannose, D-galactose, D-glucuronic acid	Gowda, 1984
aerial parts	Methanol	hyptadienic acid, hyptadienyl acetate, methyl hyptadienate, acetyl methyl hyptadienate, 1,19-α-dihydroxy-urs-2(3),12-dien-28-oic acid	Roa and Roa, 1990



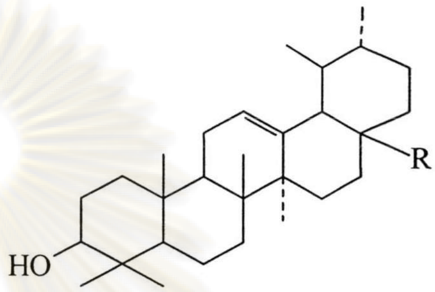
suaveolic acid R = CO_2H
suaveolol R = CH_2OH



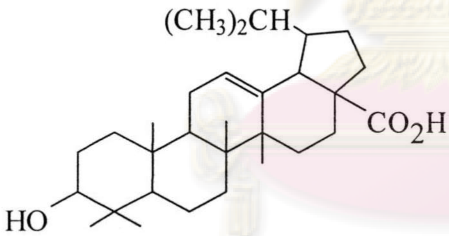
β-sitosterol



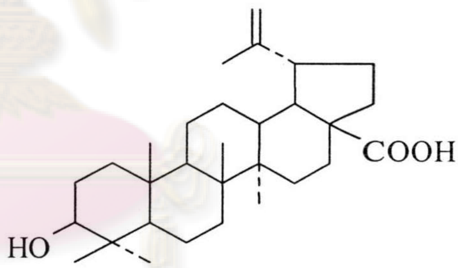
oleanolic acid R = CO_2H
β-amyrin R = CH_3



α-amyrin R = CH_3
ursolic acid R = COOH

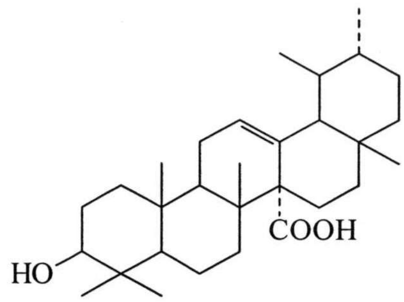


3β-hydroxylup 12-en-28-oic acid

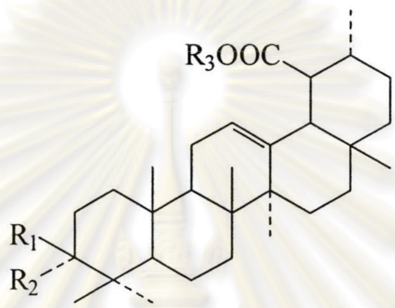


betulinic acid

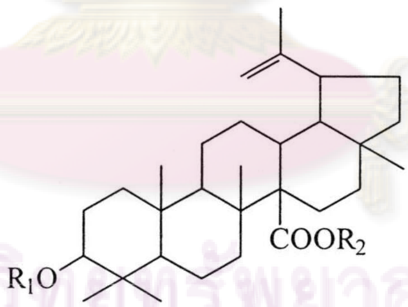
Fig. 1.2 Some isolated compounds from *Hyptis suaveolens* Poit.



α-peltoboykinolic acid

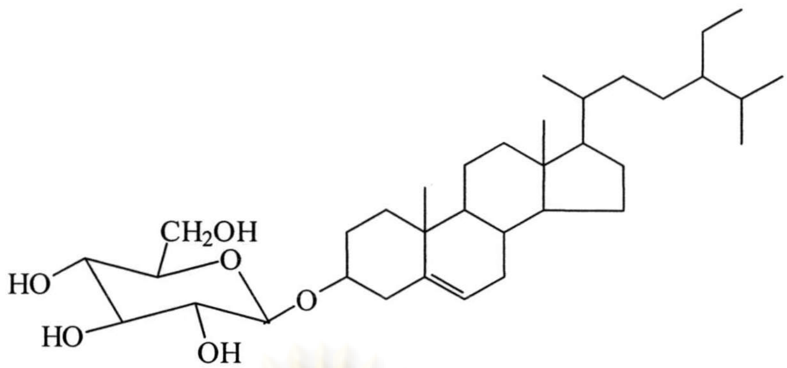


urs-12-en-3β-ol-29-oic acid R_1 = OH, R_2 = H, R_3 = H
3-oxo-urs-12-en-29-oic acid R_1 = R_2 = OH, R_3 = H

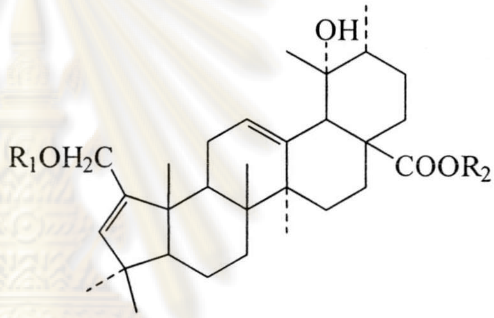


3β-hydroxylup-20(29)-en-27-oic acid R_1 = R_2 = H
3β-acetoxylup-20(29)-en-27-oic acid R_1 = Ac, R_2 = H
methyl-3 β-hydroxylup-20(29)-en-oic acid R_1 = H, R_2 = CH_3

Fig. 1.2 (cont.)



β-sitosterol-β-D-glucoside



hyptadienic acid	R_1 = H ,	R_2 = H
hyptadienyl acetate	R_1 = Ac ,	R_2 = H
methyl hyptadienate	R_1 = H ,	R_2 = CH_3
acetyl methyl hyptadienate	R_1 = Ac ,	R_2 = CH_3

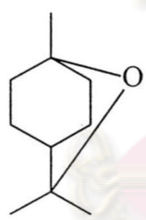
Fig. 1.2 (cont.)

1.6 Essential Oil Composition of *Hyptis suaveolens* Poit.

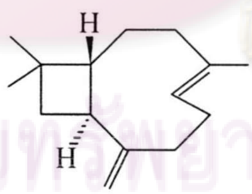
The steam distillate of aerial parts of *H. suaveolens* Poit. has been reported to contain monoterpenes as the major component (Mukhtar *et al.*, 1994), (Hac *et al.*, 1996).

The leaves of *H. suaveolens* in Thailand were found to contain essential oil at 0.1% (v/w) fresh weight. By GC/MS analysis of essential oils, it was found that at least 43 peaks were presented in its GC chromatogram. These peaks were identified as 9 monoterpenes, 3 oxygenated monoterpenes, 17 sesquiterpenes, 5 oxygenated sesquiterpenes and 2 diterpenes. Among these, 1,8-cineole (21.70%) appeared to be the major component, followed by (*E*)-caryophyllene (17.87%) and sabinene (16.92%) (Chouchot, 1998). Based upon isoprene unit classification, monoterpenes, were found to be the major component, accounting for 33% of the essential oil. Sesquiterpenes, oxygenated monoterpenes and oxygenated sesquiterpenes were present in a lesser amount, with 31%, 22% and 2%, respectively (Fig 1.3).

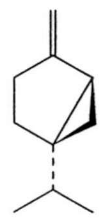
Structurally, the major components, 1,8-cineole and sabinene, belong to the monoterpenes group of menthane and thujane, respectively, while (*E*)-caryophyllene belongs to the sesquiterpenoid group of caryophilane.



1,8 cineole
(menthane)



E-caryophyllene
(caryophilane)



sabinene
(thujane)

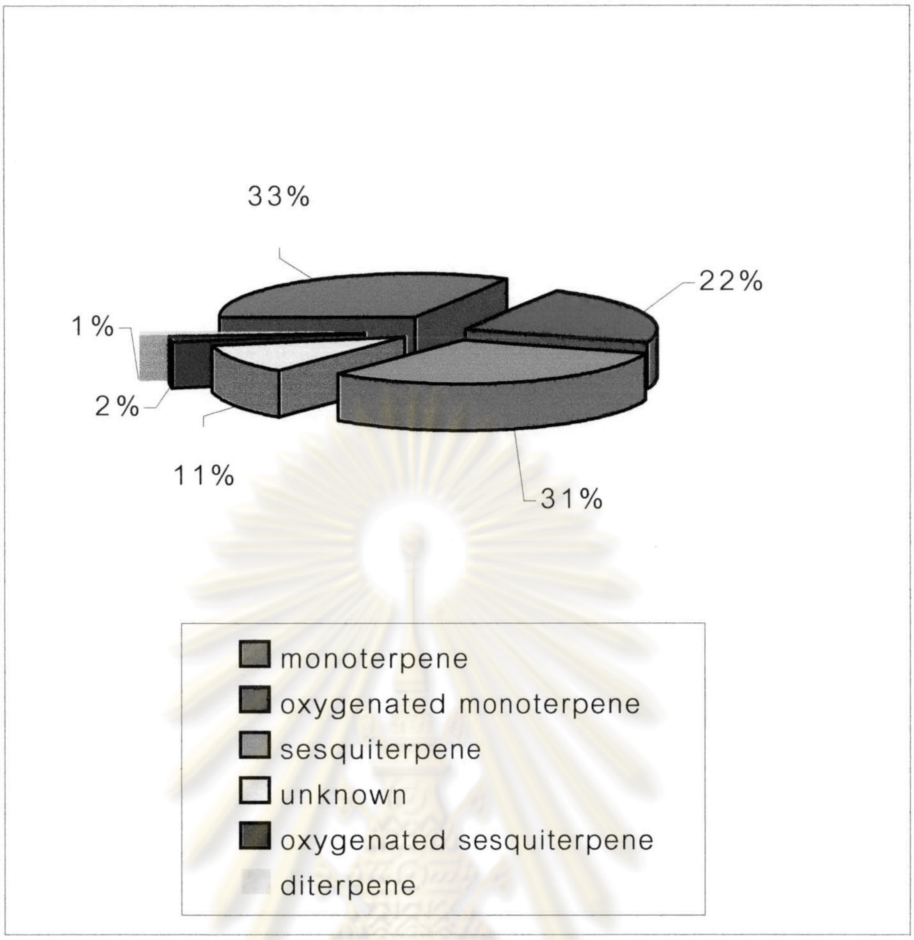


Fig. 1.3 Percentages of various terpenoid groups found in essential oil of *Hyptis suaveolens* Poit. leaves in Thailand.

1.7 Biological Activity Studies of Extracts of *Hyptis suaveolens* Poit.

The biological activites of the extracts of *H. suaveolens* Poit. are reported as listed in Table 1.7.

Table 1.7. Biological activities of the extracts of *Hyptis suaveolens* Poit.

Plant uses	Crude extract	Activities	References
Root	Benzene	Antifungal	Misra *et al.,* 1981
Aerial parts	Oil	Antimicrobial	Iwu *et al.*, 1990
Leaf	Ethanol	Plant growth regulator	Premasthira and Zungsontiporn, 1997
Leaf	Ethanol, water	Anti-phytopathogenic fungi	Rachtanaketakul, 1991 (in Thai)
Leaf	Ethanol, water	Molluscicide	Okunji and Iwu, 1988
Leaf	Oil	Anti-phytopathogenic fungi	Pandey and Dubey, 1994
Leaf	Oil	Antifungal	Singh and Handique, 1997
Not specify	Oil	Antibacterial	Jain, Jain and Jain, 1974
Not specify	Oil	Antifungal, Antibacterial	Singh and Upadhyay, 1993
Not specify	Acetone, ethanol	Xanthine oxidase inhibition	Gonzaler *et al.*, 1995
Not specify	Water	Insecticide	Uraisakul and Puthakarn, 1994 (in Thai)
Not specify	Oil	Anti-phytopathogenic fungi	Weerated, 1997 (in Thai)
Not specify	Not specify	Insecticide	Palsson, Thomas and Jaenson, 1999

1.8 Knowledge about *Echinochloa crus-galli* Beauv. Weed

Echinochloa crus-galli Beauv. is a grass (Fig. 1.4). It is a common and very important weed of most agricultural areas of the world. *E. crus-galli* Beauv. is said to be a native of Europe and India (Waterhouse, 1994).

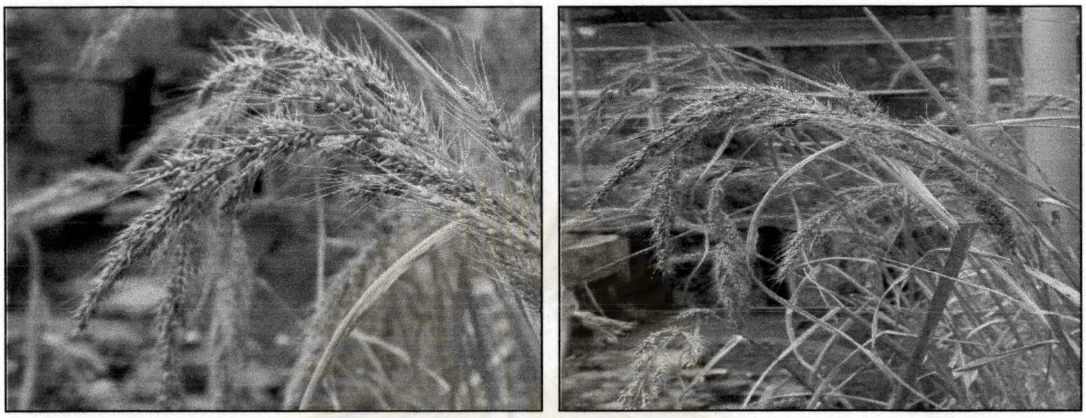


Fig. 1.4 *Echinochloa crus-galli* (L.) Beauv.

Genus : *Echinochloa*

Family : Poaceae

Vernacular Names : barngard grass, Dutch kut grass and yah kow nohk.
(Teerawafsakul, 1984)

E. crus-galli Beauv., is an annual, noxious, narrow leaf weed in the Family Poaceae. The common name is barnyard grass, or baronet grass (Smith and Shaw, 1968). This weed is known as Yha Khow Nok in Thai. Barnyard grass is a damaging weed in rice paddies and cultivated areas of other crops (Holm and Herberger, 1970). Leaves are linear and acuminate with 6-30 centimeters long. Flowers are in fluorescence, panicle 10-20 centimeters long, composed of 9-12 branches (spikes). It is a vigorous, warm season annual grass, reaching 1 to 5 feet in high with bases of many stems reddish to dark purple (Whitson, 1996). This weed is not a noxious weed in Thailand but also of the world. Holm reported it as a top-ten world's worst weed, distributing both in the tropical and temperate zones of the world, both in paddy and upland fields. This weed causes a harmful effect to crop especially to rice (Chisaka, 1970).

1.8.1 Method of Controlling *Echinochloa crus-galli* (L.) Beauv.

Methods to control growth of barnyard grass could be classified into many types such as (Wongsaroj, 1997):

1. Chemical controlling method: chemical control includes all control techniques that involve the use of chemical agents (herbicides) to kill *E. crus-galli* Beauv.

2. Biological controlling method: biological control is defined as "the action of parasites, predators and pathogens in maintaining another organism's density at a lower level than would occur in their aberrance" and "the action of fungus and insects".

1.9 Goal of Research

The goal of this research could be summarized as follows:

1. Preliminary screening for herbicidal activity from *H. suaveolens* Point
2. To elucidate structures of the isolated substances from the most interesting crude extract.
3. To search for compounds with plant growth inhibition activity against *E. crus-galli* Beauv. and others.