ผลของสิ่งสกัดหยาบจากวัชพืชเขตร้อนบางชนิดต่อการงอกและการเจริญเติบโตของเมล็ดไมยราบยักษ์ *Mimosa pigra* L.

นางสาวนราจันทร์ พิมเสน

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ปีการศึกษา 2551



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# EFFECTS OF CRUDE EXTRACTS FROM SOME TROPICAL WEEDS ON SEED GERMINATION AND GROWTH OF *Mimosa pigra* L.

Miss Narachan Phimsen

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Environmental Science
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.....Dean of the Graduate School
(Associate Professor Pornpote Piumsomboon, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Assistant Professor Charnwit Kositanont, Ph.D.)

.....Thesis Advisor
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

.....Member
(Siriporn Zungsonthiporn, Ph.D.)

.....Member
(Assistant Professor Patchra Limpanavech)

นราจันทร์ พิมเสน: ผลของสิ่งสกัดหยาบจากวัชพืชเขตร้อนบางชนิดต่อการงอกและการเจริญเติบโตของเมล็ดไมยราบยักษ์ *Mimosa pigra* L. (EFFECTS OF CRUDE EXTRACT FROM SOME TROPICAL WEEDS ON SEED GERMINATION AND GROWTH OF *Mimosa pigra* L.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร.วรินทร ชวศิริ, 66 หน้า

งานวิจัยนี้เป็นการศึกษาผลทางแอลลิโลพาธิคของสิ่งสกัดหยาบจากวัชพืชเขตร้อนบางชนิดต่อการยับยั้งการงอกของเมล็ดและการเจริญเติบโตของไมยราบยักษ์ สิ่งสกัดหยาบที่สกัดด้วยไดคลอโรมีเทนจากแห้วหมู หญ้างวงช้าง และพญามุตติแสดงประสิทธิภาพในการยับยั้งได้ดีกว่าสิ่งสกัดหยาบที่สกัดด้วยเมทานอล การใช้สิ่งสกัดหยาบความเข้มข้นเทียบเท่าน้ำหนักแห้งของพืชทั้ง 3 ชนิด 1 กรัม พบว่าสิ่งสกัดหยาบจากพืชทั้ง 3 ชนิด สามารถยับยั้งการงอก 86 76 และ73 เปอร์เซ็นต์ ตามลำดับ โดยมีการเจริญเติบโตของความยาวรากลดลงเท่ากับ 81 61 และ 35 เปอร์เซ็นต์ และความยาวต้นลดลง เท่ากับ 62 46 และ 32 เปอร์เซ็นต์ ตามลำดับ ส่วนผลการทดสอบทางแอลลิโลพาธิคของแห้วหมู หญ้างวงช้าง และพญามุตติ กับพืชปลูกบางชนิด ได้แก่ เมล็ดผักบุ้ง คะน้า กวางตุ้ง ข้าวโพด และวัชพืชอื่น ได้แก่ เมล็ดหงอนไก่ป่า ต้อยติ่ง หญ้าปากควาย และหญ้ารังนก ที่ระดับความเข้มข้น 1.0 กรัมเทียบเท่าน้ำหนักแห้ง พบว่าสามารถยับยั้งการงอกของเมล็ดหญ้าปากควาย หญ้ารังนก และหงอนไก่ป่า ได้ 100 เปอร์เซ็นต์ เมล็ดต้อยติ่ง 47 เปอร์เซ็นต์ และยับยั้งการงอกของเมล็ดผักบุ้ง และเมล็ดข้าวโพดเท่ากับ 35 และ 50 เปอร์เซ็นต์ ตามลำดับ แต่ไม่สามารถยับยั้งการงอกของเมล็ดคะน้า และกวางตุ้ง เมื่อเปรียบเทียบกับชุดควบคุม



สาขาวิชา วิทยาศาสตร์สิ่งแวดล้อม ลายมือชื่อนิสิต นราจันทร์ พิมเสน

ปีการศึกษา 2551 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก

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NARACHAN PHIMSEN: EFFECT OF CRUDE EXTRACT FROM SOME TROPICAL WEEDS ON SEED GERMINATION AND GROWTH OF *Mimosa pigra* L. ADVISOR: ASST. PROF. WARINTHORN CHAVASIRI, Ph.D., 66 pp.

The allelopatic effect of 3 tropical weeds, purple nutsedge (*Cyperus rotundus* L.), Indian heliotrope (*Heliotropium indicum* L.) and Pha-yaa mutti (*Granagea maderaspatana* Poir.) were studied. It was found that the dichloromethane crude extract showed higher inhibitory effect than the methanol crude extract. The dichloromethane extract of purple nutsedge at 1 g equivalent (dry weight) exhibited 86, 76 and 73% inhibitory effect on germination of giant mimosa (*Mimosa pigra* L.), respectively. Root growth elongation of the tested species revealed 81, 61 and 35% inhibition, respectively. In addition, shoot elongation inhibition of those plants were 62, 46 and 32%, respectively. The allelopatic effect of Indian heliotrope,purple nutsedge and Pha-yaa mutti crude extracts against crop plants (*Impomoea aqutica, Brassica alboglaba, Brassica chenensis, Zea mays*) and some weeds (*Celosia argentea*, *Ruellia tuberosa*, *Dactyloctenium aegyptium* and *Chloris barbata* were tested at 1 g equivalent (dry weight). *D. aegyptium, C. barbata* and *C. argentea* exhibited 100% germination inhibition activity and *R. tuberosa* displayed 47% germination inhibition. Furthermore, *I. aqutica* and *Z. mays* exhibited 35 and 50% germination inhibition, respectively, but *B. alboglaba* and *B. chenensis* did not show germination inhibition comparing with control.



Field of Study Environmental Science Student's Signature Narachan Phimsen

Academic Year 2008 Advisor's Signature W Chavasiri

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**LIST OF ABBREVIATION S, SYMBOLS AND DEFINITIONS**

| | |
|---|---|
| $CH_2Cl_2$ | Dichloromethane |
| $CH_3OH$ | Methanol |
| CM | Centimeter |
| $^{o}$ C | Degree centigrade |
| d | Day |
| EtoAc | Ethyl acetate |
| EtOH | Ethanol |
| g | Gram |
| gE | Gram dry weight equivalent |
| h | Hour |
| $H_2SO_4$ | Sulfuric acid |
| mm | Millimeter |
| mL | Milliliter |
| NMR | Nuclear Magnetic Resonance |
| RT | Room temperature |
| TLC | Thin Layer Chromatography |
| UV | Ultra Violet |

# CHAPTER I

# INTRODUCTION

## 1.1 Cause of the problem and development

Thailand is an agricultural country and agriculture is a main economic. There are many kinds of serious problems on crop production, such as insect, pathogenic fungi, irrigation, weather, quality of soil and weed. Weeds are defined as the plants growing out of place not intentionally sown, whose undesirable qualities outweigh. Weeds are troublesome in many ways. They reduce crop yield by robbing water, light space and soil nutrient (http://agguide.agronomy.psu.edu) Weed problem is the most tedious and inevitable problem leading to the need for eradication. Therefore, the farmer favorably uses herbicides to control weed because of the convenience, quick and saving labour.

In 2007, Thailand imported approximately 116,322 tons of pesticides (15.2 billion Baht) (http://www.doa.go.th/ard/). Herbicide is applied, several phenomena may take place. It may be adsorbed by foliage, evaporate and drift to another location or enter the soil. Herbicides exist in the environment and are still effective in soil, water and climate.

Although weeds are an undesirable plants, spread rapidly and are highly competition, some weeds produce chemicals affecting for insects, microorganisms and growth of other plants. This phenomenon is well known as allelopathy, the chemicals being called allelochemicals. Therefore, the search for new agrochemicals is fundamentally crucial project for agricultural. Even though the use of synthetic pesticides was found to be more potent and effective than those derived from plant-origin, the synthetic compounds are normally more harmful than the natural ones. Thus, the concept to look for potential chemicals from plant sources which were belived to be environmently friendly should be a challenge topic that still called for seriously extensive study.

## 1.2 Objectives

This research is aimed to utilize the crude extracts of certain tropical weeds in agriculture and the objectives of this research could be summarized as follows:

1. To study allelopathic effects of crude extracts from some tropical weeds on seed germination and the growth of *Mimosa pigra*, other weeds and crop plants.
2. To identify active substance(s) that could display seed germination and growth inhibition of *M. pigra*.

## 1.3 Hypothesis

The crude extracts from some tropical weeds could display the inhibition effect on seed germination and the growth of *M. pigra* seeds.

## 1.4 Anticipated benefits

The inhibitory effect of crude extracts from some tropical weeds on seed germination and the growth of *M. pigra* seeds.

# CHAPTER II

# LITERATURE REVIEWS

## 2.1 Allelopathy

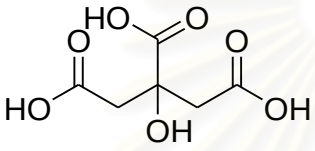
Allelopathy comes from the Greek word *allelo* or "each other". The second root is the Greek *patho* or *pathos*, which means suffering, disease, or intenfelling (Zimdahl, 1999). The term 'allelopathy' has undergone several changes over time. It was first coined by Mollish in 1937. Presently, allelopathy generally refers to the detrimental effects of higher plants of one species (the donor) on the germination or the development or the growth of another (the recipient). Allelopathy can be separated from other mechanisms of plants because the detrimental effect is exerted through release of chemical inhibitors (allelochemicals) by the donor species. Rice (1974) defined "allelopathy" as "any direct or indirect harmful effects of one plant (including microorganisms) on another through the production of chemicals that escape into the environment". The term "allelopathy" should be extended to include the manifold mutual effects of metabolic of both plants and insects. The study of allelopathy has long history, it is utilized for increasing crop productivity and the quality of food for human, decreasing our reliance on synthetic herbicides and improving the environment. As demand increases for sustainable agriculture and concern grows regarding the extensive use of synthetic chemical (*e.g.* contamination of the environment, herbicides resistance, increasing cost), attention is focused on reducing reliance upon synthetic herbicides and finding alternative strategies for weed management. Allelopathy holds great prospect for meeting some of those demands and potential can be used in several ways in agro-ecosystems (Inderjit, 1999).

## 2.2 Allelopathic chemistry

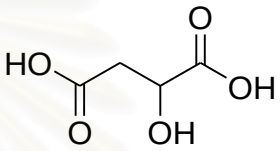
Various types of chemicals implicated in allelopathy have been discussed in details by Rice (1974, 1979, 1984), Thompson (1985) and Putnam and Tang (1986). Most of these chemicals are secondary metabolites and are produced as offshoots of primary metabolic pathways. These secondary products could be

classified into five major categories: phenylpropanes, acetogenins, terpenoids, steroids and alkaloids (Whittaker and Feeny, 1971). It is almost impossible to enumerate each and every chemical identified as an allelochemical. However, the classification into various major chemical groups would be a viable approach. Rice (1984) has classified allelochemicals produced by higher plants and microorganisms into following major categories. Examples of some chemical structures are shown as the followings:

(a) Simple soluble organic acids, straight chain alcohols, aliphatic, aldehyde and ketones

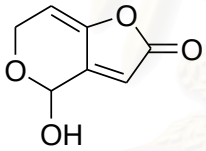



Citric acid

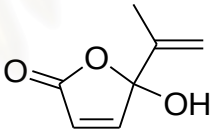



Malic acid

(b) Simple unsaturated lactones




Patulin




Penicilic acid

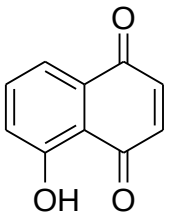
(c) Long-chain fatty-acids

*e.g.* nonanoic acid, decanoic acid, lauric acid, myrisitic acid, palmitic acid, stearic acid, oleic acid and linoleic acid.

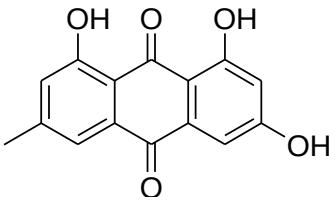
$CH_3 (CH_2)_{12}CO_2H$ $CH_3(CH_2)_{18}CO_2H$

Myristic acid Arachidic acid

(d) Naphthoquinone, anthraquinone and complex quinones

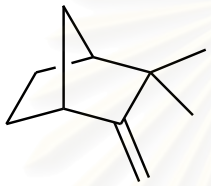



Juglone




Emodine

(e) Terpenoids and steroids

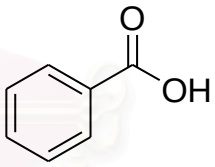


Camphene


Cineole

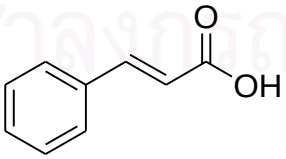
(f) Simple phenols, benzoic acid and derivatives


Phenol

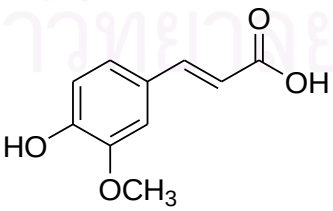



Benzoic acid

(g) Cinnamic acid and derivatives

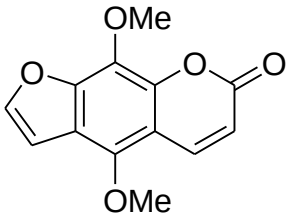



Cinnamic acid

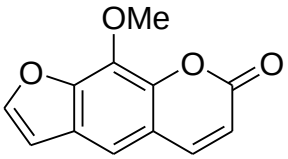



Ferrulic acid

(h) Coumarin

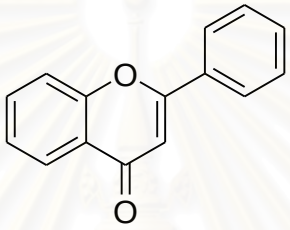



Bergapten



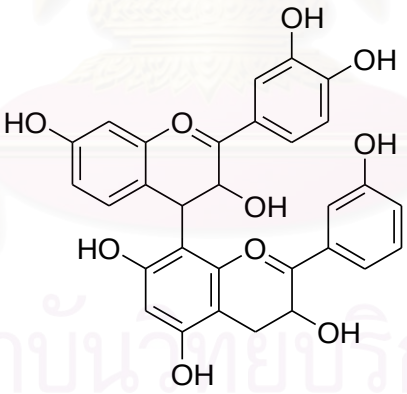

Xanthotoxin

(i) Flavonoids




Flavonoid

(j) Tannins

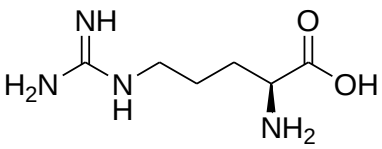



Tannin

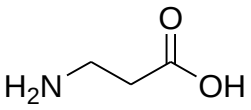


k) Other less common allelochemicals with inhibiting characteristics

- Amino acids and polypeptides

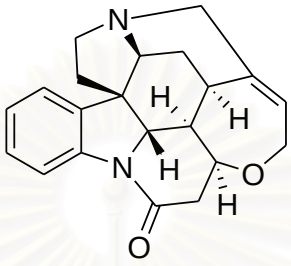



Arginine



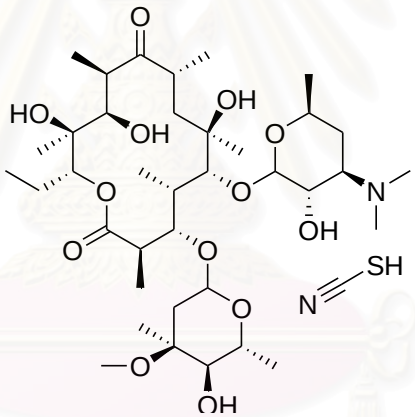
β-alanine

- Alkaloids such as cocaine, strychnine and anthropin



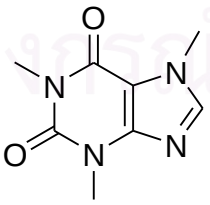
Strychnine

- Sulfides and mustard oil (Sulfides such as SCN thiocyanate)

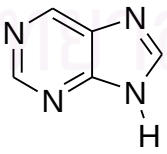


Erythomycin thiocyanate

- Purines and Nucleocides (Caffein, purin**)**

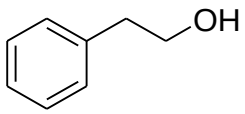


Caffein



Purine

- Ethylene, ABA and phenylethyl alcohol




Phenylethyl alcohol

## 2.3 Production of allelochemicals

Production of allelochemicals varies with environment and associated environmental stresses. They can occur in any plant organ (Rice, 1974), but roots, seeds, and leaves are the most common sources. Source becomes important for exploitation of allelochemicals for weed control. For example, allelochemicals found in flowers or fruits would have less potential value than if they were concentrated in roots or shoots (Putnam, 1985).

There is evidence that allelochemical production may be greater when plants suffer from environment stress (Putnam, 1985). Production is influenced by light intensity, quality, and duration, with a greater quantity produced with height UV light and long days (Aldrich, 1984). Weeds, commonly understory plants, might be expected to produce lower quantities of allelochemicals because UV light is filtered by overshadowing crop plants. This, of course, assumes that crops provide shade and that effectively suppresses allelopathic activity. Quantities of allelochemicals produced are also greater under conditions of mineral deficiency, drought stress, and cool temperatures as opposed to more optimal growing conditions (Zimdahal, 1999).

Allelochemicals enter the environment in a number of ways at different times, and mode and time of entry can alter their effects (Fig.2.1). Allelochemicals released into the environment by

- Volatilization from leaves.
- Leaching from leaves by rain, fog or dew and plant litter.
- Exudation from root.
- Decay of plant litter and sloughed tissue from roots.

Although chemicals with allelopathic activity may be present in many species, presence does not mean that allelopathic effects will ensue. Even after a

chemical has been isolated and identified, its placement in the environment after plant release or its time of release may preclude expression of potential activity.

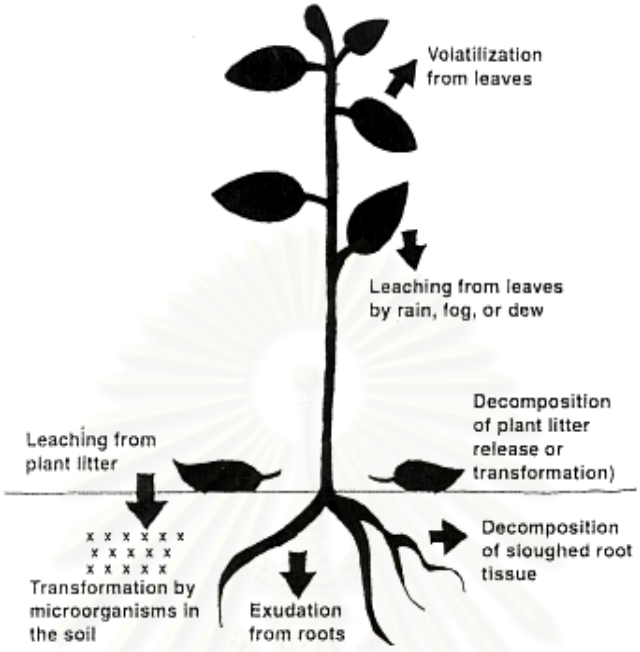



**Figure 2.1** Allelochemicals released into the environment (Zimdhal,1999).

Allelochemicals can be produced by weeds and affected crops, but the reverse is also true, although it has not been as widely studied (Putnam, 1994). It is possible that some crop cultivars produce allelochemicals, and these cultivars could be planted to take advantage of their potential allelochemicals. It has been suggested that crops with allelopathic potential could be planted as rotational crops or companion plants in annual or perennial cropping systems to exert their allelopathic effect on weeds (Zimdhal, 1999).

## 2.4 Mode of action of allelochemicals

The mode of action of allelochemicals can broadly be divided into indirect and direct action. Indirect action may include the effects through alteration of soil property, its nutritional status and an altered population and or activity of harmful/beneficial organisms like microorganisms, insect, 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 (Rizvi, 1992).

The following are some important site and processes know to be attacked or influenced by allelochemicals.

- cytology and ultrastructure
- phytohormones and their balance
- membrane and its permeability
- germination of pollens/spores
- mineral uptake
- stomatal movement, pigment synthesis and photosynthesis
- respiration
- protein synthesis
- leghaemoglobin synthesis and nitrogen fixation
- specific enzyme activity
- conducting tissue
- water relation of plants
- genetic material

Under natural conditions, the action of allelochemicals seems to revolve round a finetuned regulatory process in which, perhaps, many compounds of the act together with one or more than one of the above processes in a simultaneous or sesquential manner. Apart from the above, factors affecting the production of allelochemicals and their release into the environment, their absorption and translocation in the receptor organism, concentration at the site of action and factors determining the effectiveness of allelochemicals after their release from the producing organism, important factors which should be considered if the action of allelochemicals is to be understood in its entirety (Rizvi, 1992).

## 2.5 Allelopathy in crops for weed management

Over time many crops have been reported to possess allelopathic properties (Inderjit and Keating, 1999). However, few reports have been published on variability within crop species as far as allelopathic potential is concerned.

### Rice

Allelopathy in rice was first discovered accidentally in the late 1980s. The field had a natural mono-species weed infestation of duck-salad. Since then

12,000 rice cultivar have been screened for allelopathy and 3.4% of the accessions have been found to be allelopathy against one of the weed species evaluated. Aside from allelopathy against duck-salad, the American group has also screened for rice allelopathy against red-stem (*Ammannia coccinea* Bottb.). Four hundred and twelve rice accessions were identified as having allelopathic potential against duck-salad, 145 against red stem, but only 16 accessions showed effect on both weed species (Dilday *et al*., 1998). This indicates that rice allelopathy may be selective which means that a rice cultiva allelopathic against one weed is not necessarily allelopathic against other weed species. This selective action indicates that several chemical compound are involved in rice allelopathy and/or that weeds have different mechanisms to break down the allelochemicals. Beside the weed species mentioned above, rice with weed suppressive activity against broadleaf signal-grass, rice flat sedge, sprangle top and barnyard-grass have been reported (Olofsdotter *et al*., 1999).

## 2.6 Definition of weed

The common definition of weed mean a plant growing where it is not desired or a plant out of place, or a plants which compete with man for the soil and a plant with a negative value. Weeds are plants that thrive best in an environment disturbed by man. The only characteristic common to all weeds is their excellent adaptation to the disturbed environment in which they are growing (Thomas, Stephen and Floyd, 2002).Weed encompass all types of undesirable plants. For example, tree, broadleaf plants, grass, sedges, rushes, aquatic plants and parasitic flowering plant (Naples and Kesster, 2005).

## 2.7 Methods of weed controlling

### 2.7.1 Mechanical control

Mechanical methods had long use in history and are the primary weed control technique in many crops, such as hand pulling, tillage, cultural weed control. Other techniques for cultural weed control are to perform in crop planting (Lavabre, 1999).

Crop selection

The selection of crop determines the strategies for the subsequent battle with weeds. Crop selection will determine the level of weed control need for

efficient crop production. Chop characteristics that have been shown to be most important in helping crops compete with weed include rapid germination and root development (Smith, 1995).

2.7.2 Biological weed control

Biological weed control is defined as the action of parasites, predators, or pathogens in maintaining another organism'population at a lower average density. (Smith, 1995). The biological control of weeds in Thailand. The pathogens recorded from water hyacinth in Thailand were *Alternaria eichhorniae*, *Myrothecium roridum* and *Rhizoctonia solani*. Only *A. eichhorniae* was found to be specific to water hyacinth, and these pathogens are commonly associated with plant senescence. A combined attack by grasshoppers and pathogens could reduce plant populations, but had no significant effect on overall infestation (Napompeth, 1994).

2.7.3 Allelochemical substances

Allelochemical subatance control involves the natural substance derived from plant could use to protect them-self and released substance into the environment such as plant growth inhibition, insect or microorganism. This phenomenon is called allelopathy. Previous reports showed the evidence for allelopathic phenomenon. For instance, the root seedling extract of rice (*Oryza sativa* L.) inhibited alfalfa seeds and romaine lettuce seeds (Ino, 2001).

2.7.4 Herbicide weed control

Application of herbicides enable to increase the productivity and save labour for field crop cultivation in the tropics. However, in the developing countries in the tropics, problems including size of land holding and economic conditions, use of water for both irrigation and households and use of weed as fodder for animals, are constraints on the use of herbicides. It is necessary to develop safe and effective herbicides which are adapted to the socio-economic and environmental condition in the tropics (AICF, 1996).

## 2.8 Herbicidal measurment of toxicity

LD stands for "Lethal Dose" $LD_{50}$ is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The $LD_{50}$ is one way to measure the short-term poisoning potential (acute toxicity) of a material. Toxicologists can use many kinds of animals but most often testing is

done with rats and mice. It is usually expressed as the amount of chemical administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal. The $LD_{50}$ can be found for any route of entry or administration but dermal (applied to the skin) and oral (given by mouth) administration methods are the most common.(http://www.ccohs.ca/oshanswers/chemicals/ld50.html)

## 2.9 Health effect

Certain herbicides cause a variety of health effects ranging from skin rashes to death. The pathway of attack can arise from improper application resulting in direct contact with field workers, inhalation of aerial sprays, food consumption and from contact with residual soil contamination. Herbicides can also be transported via surface runoff to contaminate distant surface waters and hence another pathway of ingestion through extraction of those surface waters for drinking. Some herbicides decompose rapidly in soils and other types have more persistent characteristics with longer environmental half-lives. Other alleged health effects can include chest pain, headaches, nausea and fatigue. (http://en.wikipedia.org/wiki/Herbicide)

The study of allelopathy has a long time ago. According to Rice (1974), Lee and Monis (1963) was reported the discovery of the report by Banzan Kumazawa in a Japanese document over 300 years old that rain or dew washing of the leaves of red pine (*Pinus densiflora*) was harmful to crop growing under the pine. This was substantiated by these workers in a series of experiments. Historically, this is considered to be the first report on allelopathy. Besides, Bonner (1944) found that the residue of guayule (*Parthenium argentatum*) produced *tran*-cinnamic acid, which is toxic to young guayule plants. In addition, cinnamic acid was slowly decomposed in soil, so that the effect disappeared with time (Bonner, 1944). The following few examples illustrated the importance of allelopathy that affect the growth and development of agricultural crop.One of the most studied aspects of allelopathy is the role of allelopathy in agriculture. Current research is focused on the effects of weeds on crops, crops on weeds (Pheng, 1999) and crops on crops. This research further explores the possibility of using allelochemicals as growth regulators and natural herbicides to promote sustainable agriculture. Leptospermone is a purposed allelochemical in lemon

bottlebrush (*Callistemon citrinus*). It was investigated as a possible commercial herbicide but was found to be too weak. However, a chemical analog of leptospermone was found to be an effective herbicide (Mizutani, 1999). The analog is mesotrione, tradename Callisto. It is sold to control broadleaf weeds in corn but also seems to be an effective control for crabgrass in lawns. One of the most famous cases of purported allelopathy is in desert shrubs. *Salvia leucophylla* was one of the most widely known (Mallik, 1994) Bare zones around the shrubs were hypothesized to cause by volatile terpenes emitted by the shrubs. However, like many allelopathy studies, it was based on artificial lab experiments and unwarranted extrapolations to natural ecosystems. In other studies, allelopathy has been demonstrated to play a crucial role in forests, influencing the composition of the vegetation growth, while also providing an explanation for the patterns of forest regeneration (Christians, 1993). The black walnut *(Juglans nigra)* produces juglone, an allelopathic substance that interferes with the growth of other plants. *Eucalyptus* leaf litter and root exudates are allelopathic for certain soil microbes and plant species (Rick, 1995). The tree of heaven, *(Ailanthus altissima)* produces allelopathic substances in its roots that inhibit the growth of many plants. Furthermore, the pace of evaluating allelochemicals released from higher plants in nature has greatly accelerated, with promising results in field screening. Garlic mustard is an invasive plant in North American temperate forests. Its success may be partly due to its excretion of a not yet identified allelochemical that interferes with mutualisms between native tree roots and their mycorrhizal fungi (Macias, 2000).

## 2.10 Botanical characteristic of selected weeds

*Cyperus rotundus* L. (Family Cyperaceae)

*C. rotundus* is said to be native to India but it is widely distributed in the temperate and topical regions of the world. A perennial herbaceous sedge. The plant has black and hard tuber 2.5 cm. in diameter in soil and forms colonies on the surface by extending rhizomes. Leaves are linear, 15 to 30 cm long, slightly flat to rosette-like, and the base of the leaf-sheaths is reddish brown. Stems are 15 to 80 cm long. Bracts are longer or nearly as long as the inflorescence branches. Spikelets are 2 mm wide 1 to 5 cm long and somewhat flat. Scales are normally

reddish brown. Stigma of florets are trichotomous. Several species of *Cyperus* have tuber connected by rhizome (AICAF, 1996).

**Figure 2.2** Purple nutsedge (*C. rotundus*) (Harada, 1996)

***Heliotropium indicum* L**. (Family Boraginaceae)

*H. indicum*, in native to Asia and is an annual herb widely distributed in the tropics. Stems have many branches and dichotomously acquire a thick plant type. They are 50 to 100 cm long. Whole plant is covered with bristles and has a rough surface. Leaves are oval, creeping, irregularly dentate at the margin, with a short petiole, opposite but sometimes alternate in upper stems. Stipules at the base of the petiole are linear. Inflorescence are terminal. Spikes curl tail-like and become increasingly longer with flowering (15 to 29 cm long). Flower are about 5 mm in diameter, white or pale purple and 5-lobed. Fruits contain 4 nutlets (AICAF, 1996).

**Figure 2.3** Indian heliotrope ( *H. indicum*) (Harada, 1996)

***Granagea maderaspatan* (L.) Poir.** (Family Asteraceae)

*G. maderaspatan* is an annual herb distributed mainly in India and tropical Asia. Whole plant is covered with soft hairs and is fragrant. Stems have many branches at the base, are semi-erect or prostrate, about 50 cm long. Leaves are opposite, 2 to 10 cm long, 1 to 6 cm wide, long ovate, irregularly pinnate tips are acute. Heads with a peduncle 1 to 4 cm long and 1 cm in diameter are solitary on the leaf axial or end of stem. Part of involucres are foliate and small foliated bracts are located at the base of head. Florets are all tubular, yellowish green, marginal flowers are female and sterile, disk flowers are bisexual and fertile. Achene is flat, about 2 mm long and pappus becomes ciliate (AICAF, 1996).

**Figure 2.4** Pha-yaa mutti ( *G. madaspatana*) (Harada, 1996)

***Chenopodium ambrosoides* L.** (Family Chenopodiceae)

It is an annual herb that grows to about 1 m in height. It has multi-branched, reddish stems covered with small, sharply toothed leaves. It bears numerous small yellow flowers in clusters along its stems. Following the flowers, it produces thousands of tiny black seeds in small fruit clusters. It is easily spread and re-grown from the numerous seeds it produces which is why some consider it an invasive weed. The whole plant gives off a strong and distinctive odor. www.rain-tree.com/epazote.htm

**Figure 2.5** Mexican tea (*C. ambrosoides*) www.missouriplants.com

***Rottboellia cochinchinensis* (Lour.) W. Clayton (**Family: Poaceae**)**

Spikelets heteromorphic,awnless, sunken into inflated internode. Internode base truncate,with central peg. Sessile spikelets dorsally compressed, 3.5-6 mm long, 2-3 mm wide, of 1 fertile floret and 1 basal sterile floret. Lower glume indurate, as long as spikelet, 9-11 nerved; upper glume keeled above, boat-shaped, following concavity of internode. Sterile lemma and palea chartaceous, as long as upper glume. Pedicellate spikelets sterile, ovate, 3-5 mm long.
www.issg.org/database/species/ecology.

**Figure 2.6** Itgrass (*R. cochinchinensis*) (Harada, 1996)

***Mimosa pigra* L.** (Family Mimosaceae**)**

*M. pigra* was selected for a main bioassay. It is a perennial woody shrub of family Mimosaceae, known as Giant sensitive Plant, or Thai as Maiyarap Yak. It is a noxious broad leaves weed through out the country. It infests both agriculture or non agriculture area, causes serious problem for irrigation and transportation. Its leaves are bi-pinnate and sensitive to the touch, through movements of the petiole and pinnules. Petioles bear a slender prickle at the junction of each of the 6 to 16 pairs of pinnate leaves and sometimes have stouter prickles between each pair. The stem bear broad-based, sharp thorns up to 7 mm long. Mature plants have many branches growing from the base. The flowers are mauve to pink, massed in globular heads 1 cm in diameter, with each head containing about 100 florets (Waterhouse, 1994)

*M. pigra* was introduced to Mae-teang and Chiang-dao district, Chiangmai province around 1947. The plant grew out of control into the Mae-ping River in which it established itself and thrived very well for many years. Three to four years later, the plant has spread to 3 other nearby provinces, Chiangrai, Lampoon and Lampang. Large infestations could also be seen in Burma, Laos and along the Mae-kong River (Suwannamek, 1983).

**Figure 2.7** Giant mimosa (*Mimosa pigra*) (Harada, 1996)

## 2.11 Relationship studies of crude extract form some tropical weeds on growth of another weeds and crop plants

Some selected plants used for examining the relationship between the extract form some tropical weeds and the growth of other plants are listed as follows in Fig 2.8.

Ngonkai dong
(*Celosia argentea*)(Harada, 1996)

Swollen finger grass (*Chloris barbata*)
(Harada, 1996)

Toiting (*Ruellia tuberosa*)
( Harada, 1996)

Bermuda grass
(*Dactyloctenium aegyptiu*) ( Harada, 1996)

Gwarng-toong
(*Brassica chinensi* )

Pakbung
(*Impomoea aquatica*)

Chinese kale (*Brassica alboglabra*)
www.lionseeds.com/.../chinese-kale-yodpha

Corn
(*Zea mays*)

**Figure 2.8** Some selected weeds and crop plants.

Ngonkai dong (*Celosia argentea* L.). Family Amaranthaceae. It is annual herb. Stems are erect, up to 1.5 cm long, with longitudinal green lines and occasionally reddish one. Leaves are alternate. Inflorescence are terminal and appears like a spike with red florets arranged in clusters (AICAF, 1996).

Toi ting (.*Ruellia tuberosa* L.). Family Acanthaceae. It is a perennial herb. Root are branched 6 to 18 tubers, stems are erect and branch, 30 to 60 cm long and reddish green. Leaves are simple, opposite and ovate to obovate. The flowers are apical and axillary, campanulate and pale purple (AICAF, 1996).

Bermuda grass (*Dactyloctenium aegyptium* (L.) P. Beauv.). Family Poaceae. It is perennial grass. Stems are branched, prostrate and spreading on the ground, rooting at the nodes and making a mat. Leaves are linear and covered with long white hairs around the ligule. Culms are 15 to 40 cm long. Spikes are terminal and fingerlike (AICAF, 1996).

Swollen finger grass (*Chloris barbata* Sw.). Family Poaceae. It is annual grass. The plant is glabrous, erect, procumbent at the base of the culms, rooting at the nodes and up to 30 to 60 cm tall. Leaves are 2 to 12 cm long ,( 1 to 2 mm wide, linear and with flat leaf sheaths. Panicles are terminal and fingerlike (AICAF, 1996).

Chinese kale (*Brassica alboglabra* Bailey.) and Kwang-toong (*Brassica chinensis*). Family Cruciferae. Perennial growing to 0.5m at a fast rate. It is not frost tender. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Bees. The plant is self-fertile. (http://plants.usda.gov/java/profile?symbol=BRAL8)

Pakbung (*Ipomoea aquatica* Forsk). Family Convolvulaceae. An annual or perennial vine herb; stems prostrate or floating, thick, herbaceous, 2–3 m long, rooting at the nodes. Leaves are alternate. Flower stalk arises from the leaf axil and bears 1 to several flowers (AICAF, 1996).

Corn (*Zea mays* L.).Family Poaceae. Annual growing to 2 m at a fast rate. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by Wind. (http://en.wikipedia.org/wiki/Teosinte)

All above species that used as plant models could be separated into 2 groups. Group 1 are dicotyledon weeds (Ngonkai dong and Toi ting), monocotyledon weeds (Bermuda grass and Swollen finger grass) and group 2 are dicotyledon plants Pakbung, Chinese kale and Kwang-toong and monocotyledon plants corn.

# CHAPTER III

# EXPERIMENTAL PROCEDURE

## 3.1 Plant materials for crude extract preparation

The whole plants of Indian heliotrpium (*H. indicum*), Pha-yaa mutti (*G. maderaspatana*), Mexican tea (*C. ambrosoides*), Itchgrass (*R. cochinchinensis*) and *M. chanaelea* were collected from Prachinburi while the tubers of purple nutsedge (*C. rotundus* ) were collected from Bangkok.

## 3.2 Model plants for bioassay test

The seeds of giant mimosa collected from Petchburi. The seeds of Ngonkai dong, Swollen finger grass, Toi Ting and Bermuda grass were collected from Kasetsart University, Bangkhen Campus. The seeds of crop plants were bought from Chai tai company Limited (Gwarng-toong seeds: kasorn 013 breed, Chinese kale seeds: 066 breed, corn seeds: top sweet breed and Pakbung seeds: 256 breed).

## 3.3 Instrumental and equipment

Thin layer chromatography (TLC) was performed on aluminium sheets precoated with silica gel (Merck Kieselgel 60 $F_{254}$) and spots on the plate were observed under UV light or visualized by spraying with 10% $H_2SO_4$ in EtOH followed by heating. Silica gel Merck Kieselgel 60 no.7731 and 7734 were used for column and quick column chromatography, respectively.

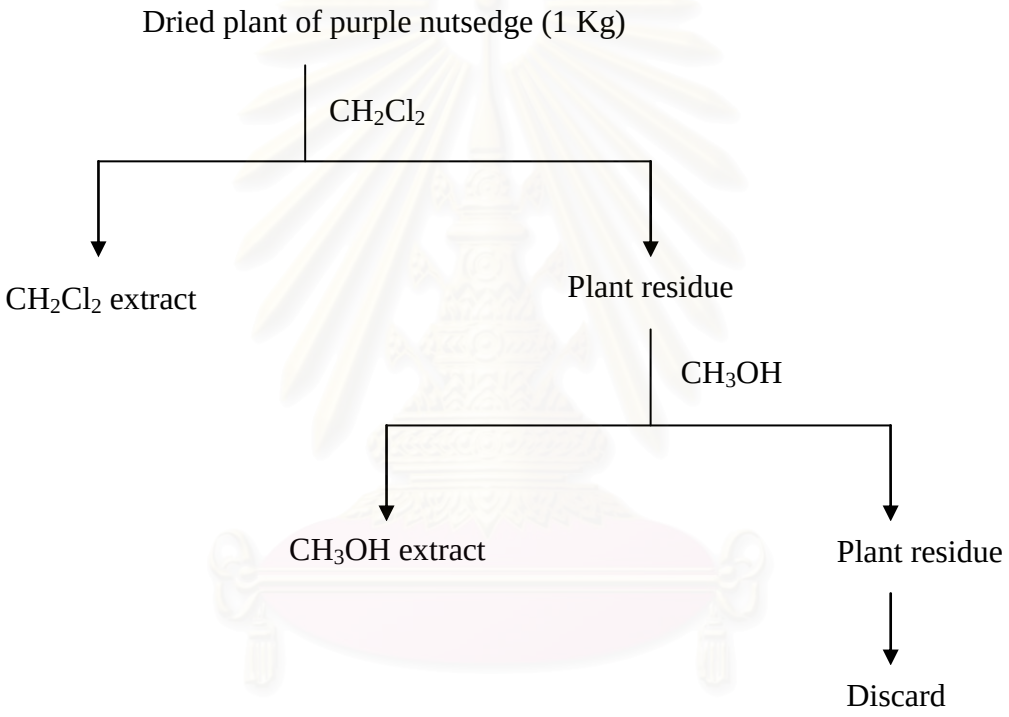
The $^{1}H$- and $^{13}C$-NMR spectra were recorded in $CDCl_3$ on a Bruker ACF 200 and a Jeol NMR 500 spectrometer using tetramethylsilane (TMS) as an internal reference.

## 3.4 Solvents

All commercial solvents employed in this research were purified prior to use by standard methodology except for those which were reagent grades.

### 3.5 Extraction procedure

The whole plant of Indian heliotrpium, Pha-yaa mutti, Mexican tea, Itchgrass and *M. chanaelea* and purple nutsedge (1 kg dry weight) were milled to fine powder and extracted by soaking in $CH_2Cl_2$ for five days at RT. The residue was repeatedly extracted by $CH_2Cl_2$ three times. Evaporation of the solvent afforded the $CH_2Cl_2$ extract as brownish crude for purple nutsedge and as greenish crudes for Indian heliotrpium, Pha-yaa mutti, Mexican tea, Itchgrass and *M. chanaelea*. The marc was then similarly extracted with $CH_3OH$. The extraction procedure for the plants was shown in Scheme 3.1.




**Scheme 3.1** Extraction procedure for the whole plant of purple nutsedge

### 3.6 Experiments for bioassays

Plant growth study was used as a main bioassay to follow the presence of bioactive compounds giant mimosa was used as a model plant in this research.

#### 3.6.1 General procedure for weed germination inhibition test

The crude extract of 0.1, 0.5, 1.0, 2.5, 5.0 g equivalent (gE) in 3 mL of solvents ($CH_2Cl_2$/$CH_3OH$) to dried plant materials was dissolved in a proper, then poured into Petri dishes (diameter 90 mm), each containing a filter paper. The equal amount of the same solvent to dissolve crude extract was added instead of

extracts as control. Leave overnight to remove solvent by air drying, then 5.0 mL of distilled water was added to each plate. Fifty seeds of giant mimosa were put on each filter paper. Petri dishes were closed and placed at RT to observe the growth for 7 days. Ten seedlings were randomly selected to measure the shoot and root elongation (mm) compared with the control experiment. Each experiment was performed in three replications.

The calculation of inhibitory effect of substances could be carried out by equation shown below.

$$\%\text{Germination Inhibition} = \frac{(C-T)\ x100}{C}$$

Where T is germination number of treated seedlings.

C is germination number of controlled seedlings.

*Germination inhibition of 100% means completely inhibitory effect

### 3.6.2 General procedure for weed growth inhibition test

Tested crude extracts were dissolved in a proper solvent at concentration of 0.1, 0.5, 1.0, 2.5, and 5.0 gE. The 3.0 mL of solution was poured into a glass tube (diameter 30 mm and length 120 mm) containing 40 mL of agar. The controlled tube was prepared by water and the same solvent using the same methodology. All tubes were covered with aluminum foil, dried up by heating at 50°C in vacuum oven for 10-12 h, stirred until well-mixed. Six seedlings of giant mimosa with radical root length 1-2 mm (seeds for bioassay were soaked for 12 h and germinated in Petri-dish one night before testing) were transplanted in each tube, 3 tubes for each concentration. The tubes were sealed with transparent vinyl film and kept in growth chamber at 30°C, 24 h daylight. The seedlings were cleared from artificial food, the root and shoot lengths were recorded at 7 d after transplanting. The growth inhibitory effect was calculated with the formula

$$\%\text{Growth Inhibition} = \frac{(C-T)}{C} \times 100$$

where T is root (or shoot) length of treated seedlings.

C is root (or shoot) length of controlled seedlings.

*Growth inhibition of 100% means completely inhibitory effect

## 3.7 Separation

### 3.7.1 Quick column chromatography

The dark brown $CH_2Cl_2$ extract from purple nutsedge (400 g) was subjected to silica gel quick column using gradient solvent starting from hexane and increased polarity by mixing with EtOAc and $CH_3OH$.

### 3.7.2 Silica gel column

According to the biological assay, the fraction that showed the highest %inhibition on giant mimosa seeds was fractionated by silica gel column using gradient solvent starting from hexane and increased polarity by mixing with EtOAc and $CH_3OH$.

# CHAPTER IV

# RESULTS AND DISCUSSION

The objective of this research is to study the allelopathic effect of the crude extracts from some tropical weeds on seed germination and seed growth of giant mimosa (*M. pigra*) and to separate potent bioactive compounds from the crude extract. In addition, the investigation on the inhibitory effect of selected crude extracts on other dicotyledon weeds: Ngonkai dong (*C. argentea*) and Toi Ting (*R. tuberosa*) and monocotyledon weeds: Bermuda grass (*D. aegyptium* )and Swollen finger grass (*C. barbata*) was performed. More exploration using crop plants including Chinese kale (*B. alboglabra*), Pakbung (*I. aquatica*), corn (*Zea mays*) and Kwang-toong (*B. chinensis*) was also carried out.

## 4.1 The preliminary seed germination inhibition test of *M. pigra* with crude extracts from some tropical weeds

Giant mimosa was selected as a weed model for the inhibitory effect study of the crude extracts. This noxious weed is common found throughout the country and has caused serious problems in the environment. Moreover, its seed size is not too small to handle during the experiment and the seed dormancy can be easily broken with high germination percentage.

Six tropical weeds including purple nutsedge, Indian heliotrope, Pha-yaa mutti, Mexican tea, *M. chanaebea* and Itchgrass were chosen. Each plant material was separately extracted with $CH_2Cl_2$ and $CH_3OH$, respectively. Those extracts were further bioassayed on seed germination and growth of giant mimosa and the results are summarized as shown in Figs 4.1 and 4.2.

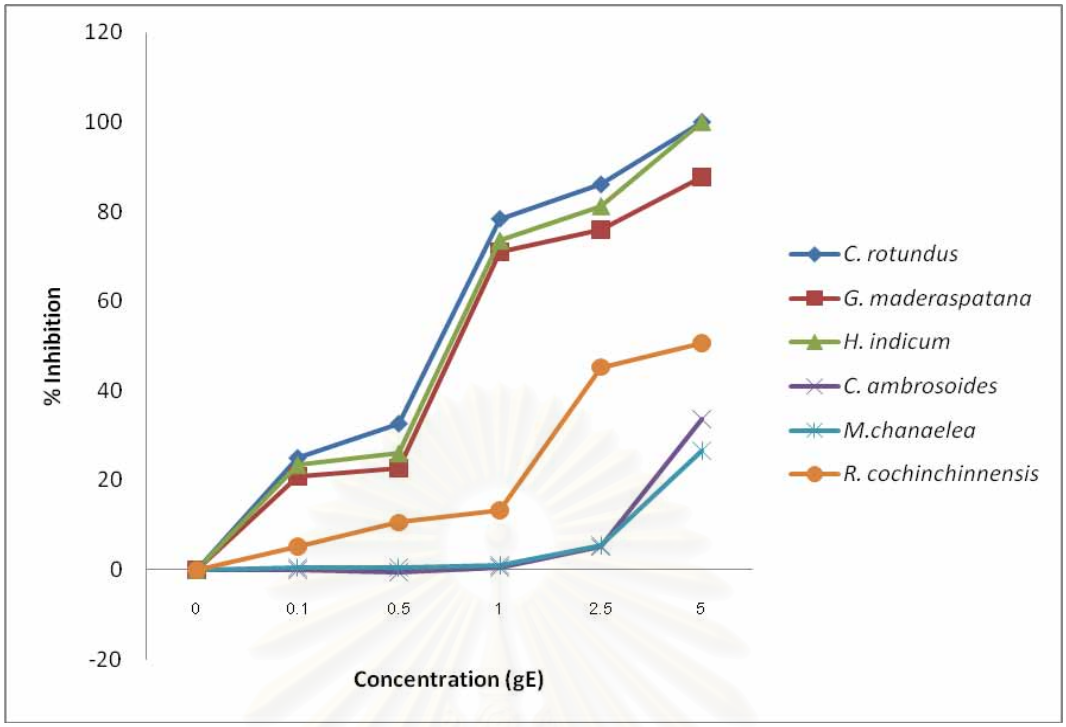



**Figure 4.1** The preliminary seed germination inhibition test with giant mimosa of $CH_2Cl_2$ extracts from selected plants**.**

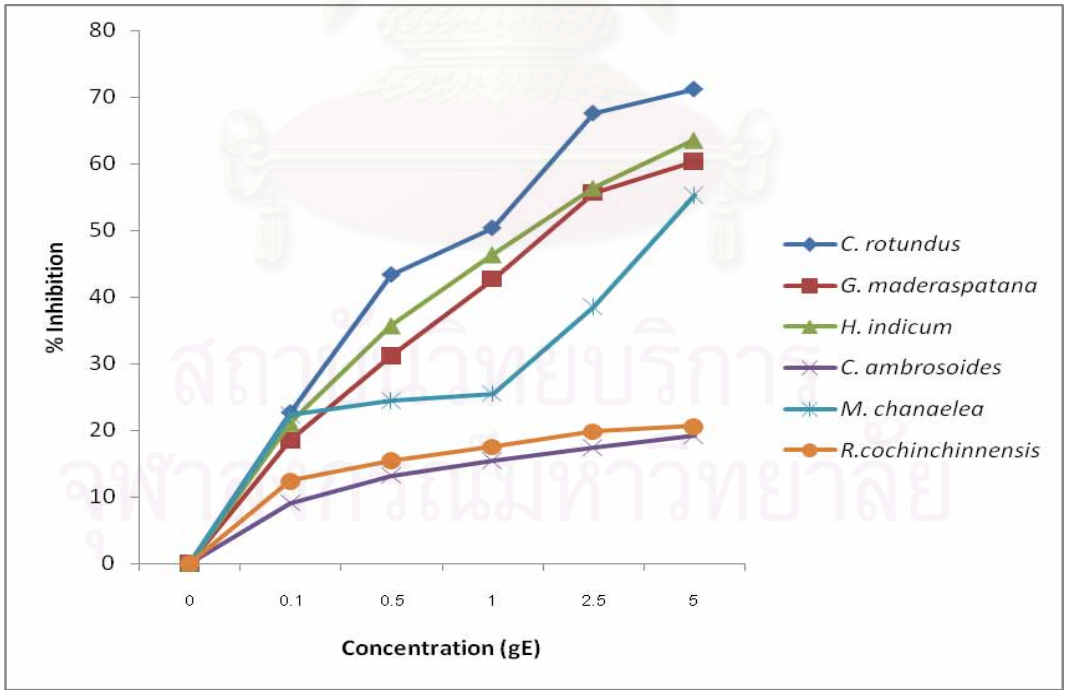



**Figure 4.2** The preliminary seed germination inhibition test with giant mimosa of $CH_3OH$ extracts from selected plants.

The results from Figs 4.1 and 4.2 pointed out that among six chosen plant materials, the $CH_2Cl_2$ extract of purple nutsedge 1 gE gave the best inhibitory results on seed germination (78%). The other two extracts which showed high inhibitory effect included those of Pha-yaa mutti and Indian heliotrope (71 and 74%), respectively. The $CH_2Cl_2$ extracts of purple nutsedge., Indian heliotrope and Pha-yaa mutti were selected for further study on the seed germination and growth inhibition with giant mimosa seeds, and with other weeds: Ngonkai dong, Toi Ting, Bermuda grass and Swollen finger grass, and crop plants: corn, Chinese kale, Kwang-toong and Pakbung.

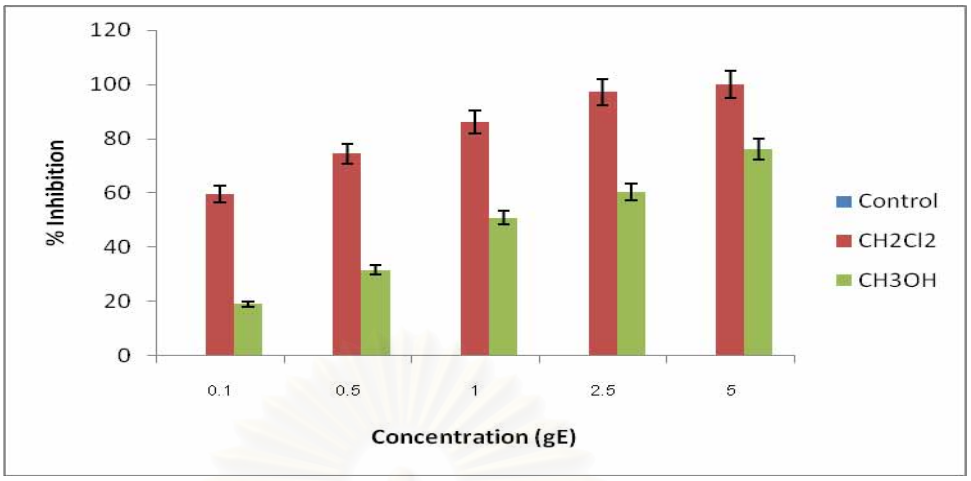
## 4.2 Bioassay experiments

### 4.2.1 Germination inhibition of *M. pigra* by weed crude extract

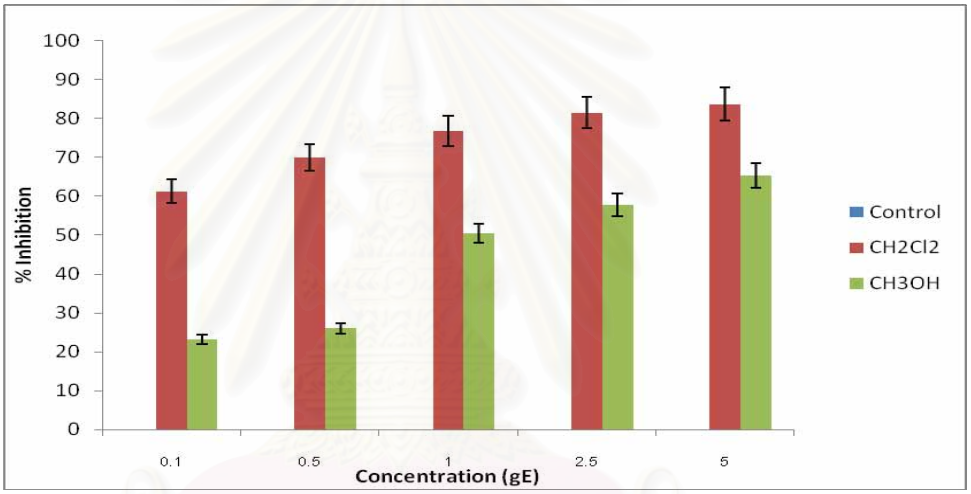
The crude extracts from purple nutsedge, Pha-yaa mutti and Indian heliotrope in $CH_2Cl_2$ and $CH_3OH$ were assayed on giant mimosa again to observe the percent germination inhibition, root and shoot elongation inhibition.

The results of the seeds germination, and root and shoot elongation inhibition of giant mimosa are summarized in Figs 4.3 and 4.4 and 4.5, respectively.

**Purple nutsedge (*C. rotundus*)**




**Indian heliotrope (*H. indicum*)**




**Pha-yaa mutti (*G. maderaspatana*)**

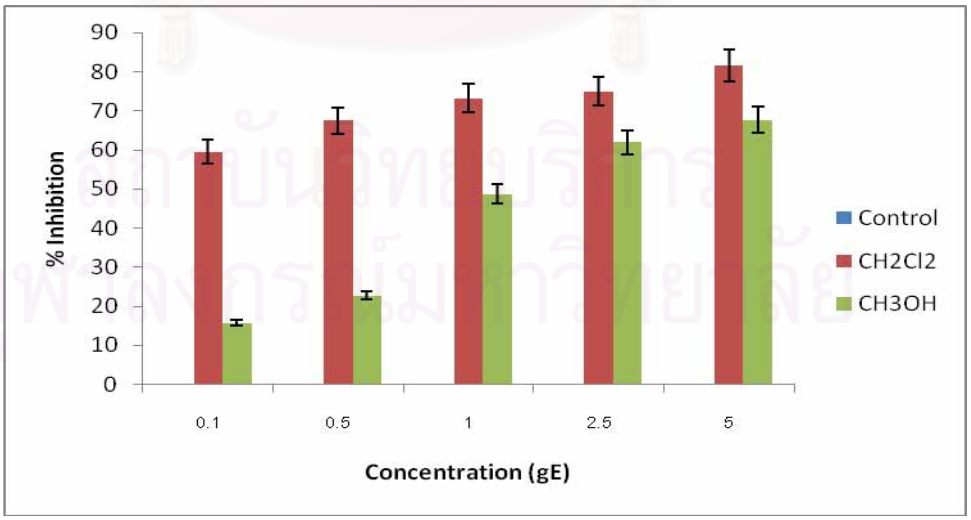



**Figure 4.3** The results of crude extracts from some tropical weeds on seed germination inhibition test of giant mimosa.

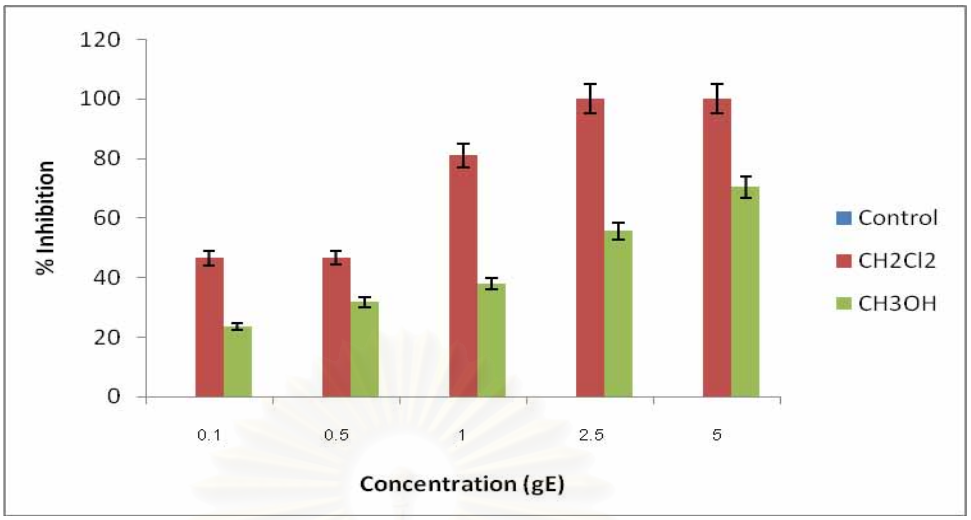
**Germination inhibition**

According to the above results (Fig 4.3), the $CH_2Cl_2$ extracts of the selected tropical weeds generally displayed stronger inhibitory activity against giant mimosa than those obtained from $CH_3OH$ crude extracts except 2.5 and 5.0 g equivalent to dry weight concentration of Indian heliotrope crude extract.

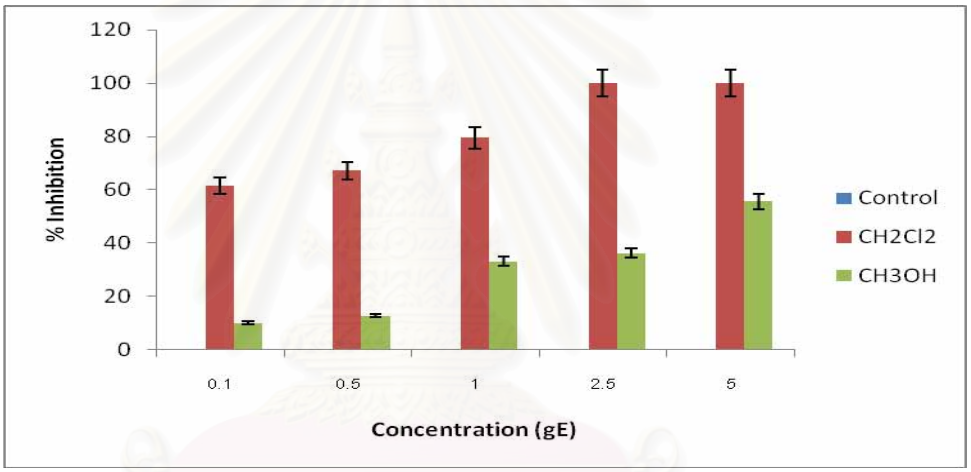
In accordance with the testing result, the $CH_2Cl_2$ extract of purple nutsedge revealed higher % inhibition than those extracts from Indian heliotrope and Pha-yaa mutti.The comparative activity exhibited as follows: 86, 77 and 73%, at 1 gE. The activity influenced by $CH_3OH$ extracts could be arranged as purple nutsedge more than Indian heliotrope and Pha-yaa mutti, respectively at the same concentration.

When the concentration was increased to 2.5 gE of $CH_2Cl_2$ and $CH_3OH$ extracts showed better results than using the crude at lower concentrations.

**Purple nutsedge (*C. rotundus*)**




**Indian heliotrope (*H. indicum*)**




**Pha-yaa mutti (*G. maderaspatana*)**

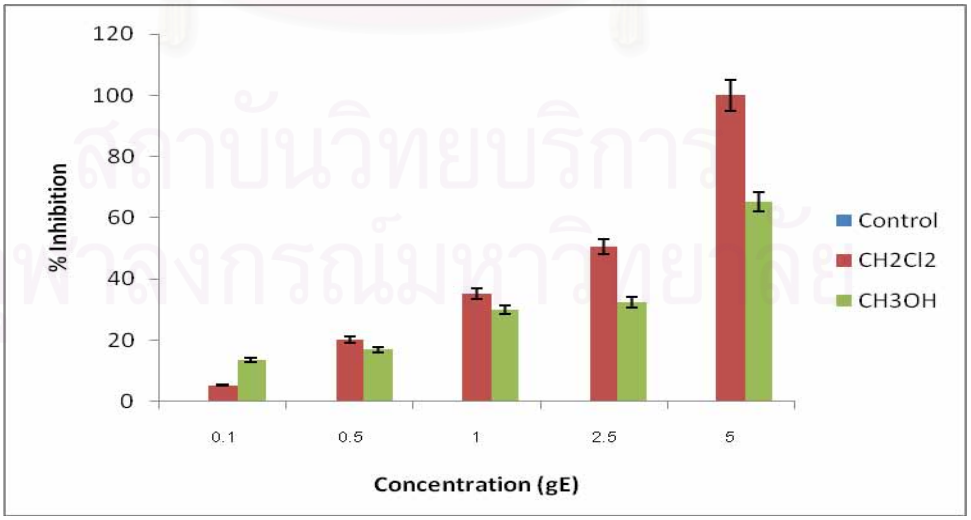



**Figure 4.4** The root elongation inhibition of giant mimosa with crude extracts from some tropical weed.

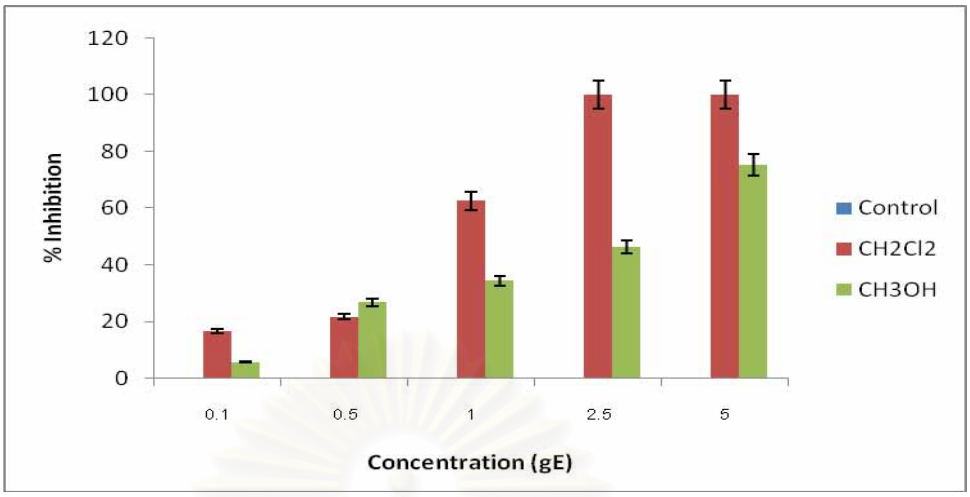
**Root elongation inhibition**

The inhibition of root (Fig 4.4) is thought to be more essential for considering than that of percent shoot growth inhibition. This is because the roots are directly contacted to the tasted chemical while the shoot growth is contributed from root and attributed to accumulated food from seed.

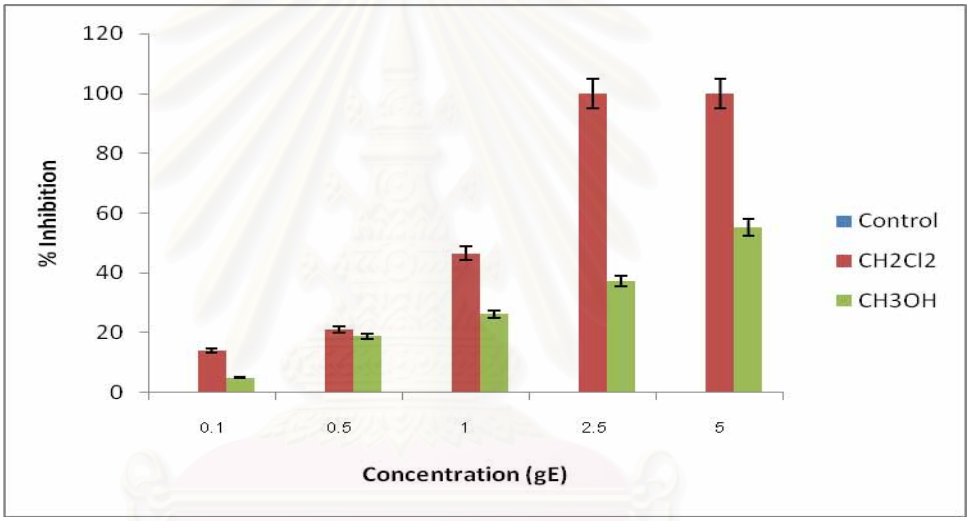
The inhibition of root elongation in $CH_2Cl_2$ extracts was compared with that of $CH_3OH$ extracts at 1 gE. Consequently, the $CH_2Cl_2$ extract displayed strong inhibitory activity against giant mimosa. purple nutsedge showed stronger activity than Indian heliotrope and Pha-yaa mutti, the inhibitory activity could be arranged as 81, 61 and 35%, respectively. For the $CH_3OH$ extracts, it was found that purple nutsedge**,** Indian heliotrope and Pha-yaa mutti showed low activity on root inhibition ranging from 30-38%.

The more concentration up to 5 gE, the more inhibition of root elongation was observed for both $CH_2Cl_2$ and $CH_3OH$ extracts.

**Purple nutsedge (*C. rotundus*)**




**Indian heliotrope (*H. indicum*)**




**Pha-yaa mutti (*G. maderaspatana*)**

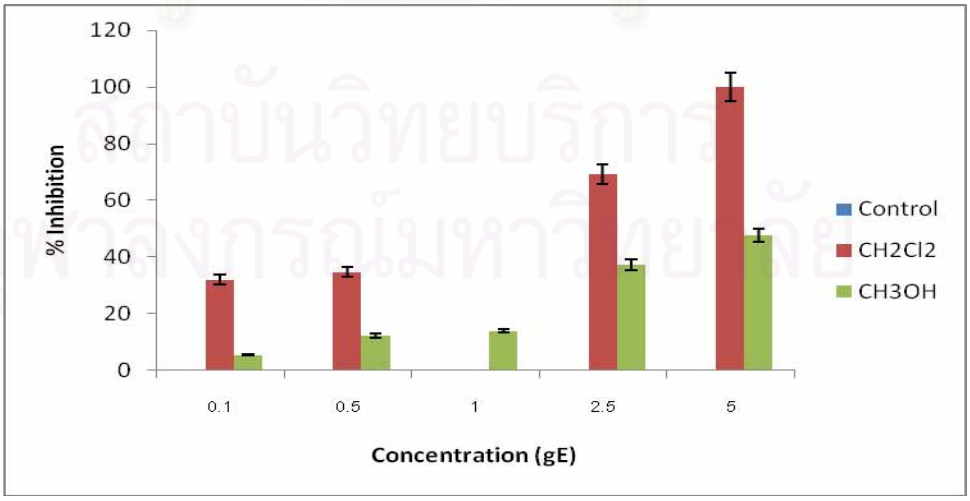



**Figure 4.5** The results of crude extracts from some tropical weeds on shoot elongation inhibition test with giant mimosa.

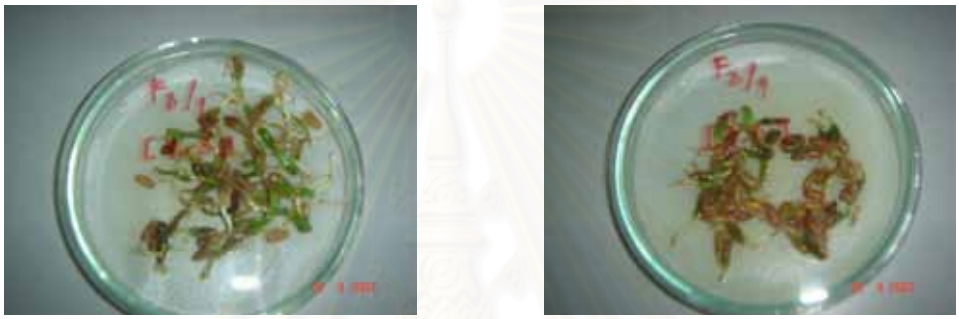
**Shoot elongation inhibition**

The examination on the effect of $CH_2Cl_2$ crude extracts was carried out. By monitoring the percentage of shoot elongation inhibition against of giant mimosaat 1 gE. purple nutsedge displayed the highest activity, followed by Indian heliotrope and Pha-yaa mutti, respectively. For $CH_3OH$ extracts, the comparative order of activity was found to be decreasing as: purple nutsedge, Indian heliotrope and Pha-yaa mutti, respectively.

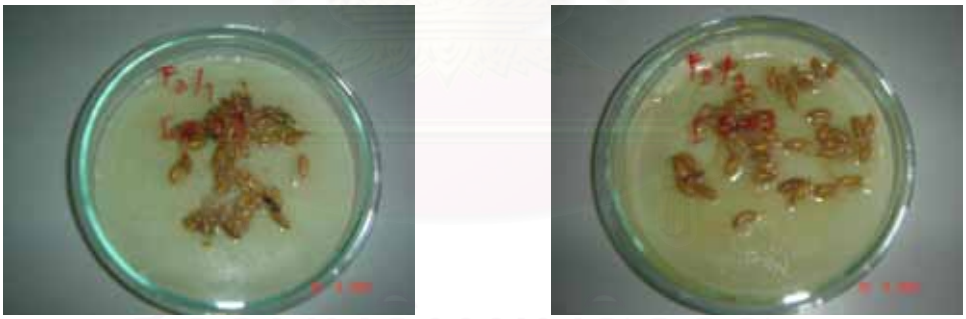
Ultimately, the data from Figs 4.3, 4.4 and 4.5 demonstrated that the $CH_2Cl_2$ and $CH_3OH$ extracts from some tropical weeds had a profound effect on germination, shoot and root elongation inhibition on giant mimosa. Typically, the $CH_2Cl_2$ extract displayed higher activity than the $CH_3OH$ extract.

The effect of crude extracts against giant mimosa for germination inhibition at 0.1 gE compared with control was investigated. When the concentration was increased from 0.5, 1.0, 2.5 to 5.0 gE, the complete inhibition of giant mimosa was noticeable and showed in Fig 4.6.

(A) Control Test. (B) At 0.1 gE



(C) At 0.5 gE. (D) At 1.0 gE.



(E) At 2.5 gE. (F) At 5.0 gE.



**Figure 4.6** The results of various concentrations of the extracts from purple nutsedge on seed germination inhibition against giant mimosa.

### 4.2.2 Growth inhibition of *M. pigra* by weed crude extract

The $CH_2Cl_2$ and $CH_3OH$ extracts from purple nutsedge (*C. rotundus*), Pha-yaa mutti **(***G. maderaspatana***)** and Indian heliotrope (*H. indicum*) were tested against giant mimosa. The growth inhibitory effect, root and shoot elongation inhibition were monitored. The results are presented in Figs 4.7. The raw data were collected in Tables 3 and 4 (see Appendices).

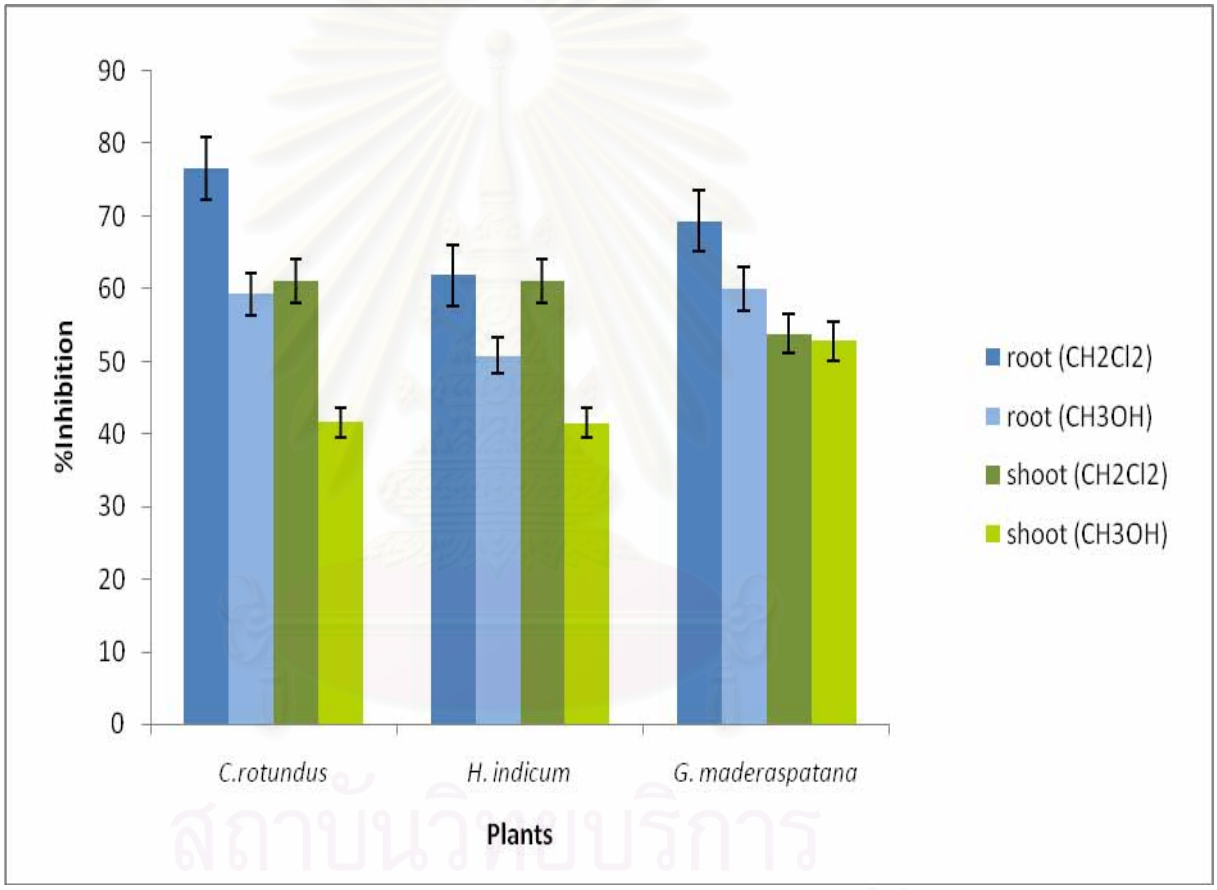



**Figure 4.7** Effect of the $CH_2Cl_2$ and $CH_3OH$ extracts from purple nutsedge Pha-yaa mutti and Indian heliotrope on the growth of giant mimosa, compare with 1 gE.

- **Root inhibition**

The biological activity test of the $CH_2Cl_2$ and $CH_3OH$ crude extracts from Pha-yaa mutti, purple nutsedge and Indian heliotrope against giant mimosa was conducted. Figs 4.7 presents the effect of the crude extracts on root elongation inhibition. The $CH_2Cl_2$ extract, similar to previous observation gave good tendency of root elongation inhibition: for purple nutsedge, Pha-yaa mutti, Indian heliotrope and the percent root elongation inhibition was 77, 69 and 62%, respectively. The $CH_3OH$ extract on the other hand displayed less than 70% of root elongation inhibition activity.

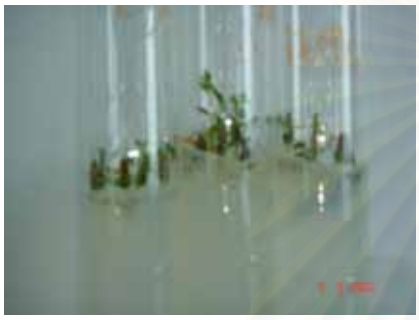
- **Shoot inhibition**

The examination on the effects of the $CH_2Cl_2$ and $CH_3OH$ extracts of purple nutsedge, Pha-yaa mutti and Indian heliotrope with giant mimosa on shoot inhibition was peformed. Considering at 1 gE, the $CH_2Cl_2$ and $CH_3OH$ extracts from purple nutsedge, Indian heliotrope and Pha-yaa mutti gave less than 70% of shoot inhibition activity (Fig 4.8).

From above, the results of growth inhibition with giant mimosa found that purple nutsedge (*C. rotundus*) caused significant reduction in the growth inhibition including root and shoot elongation inhibition (see Appendices on tables4 and 5). The inhibitory effects of $CH_2Cl_2$ extract was more pronounced than $CH_3OH$ extract. This may be due to the presence of higher amounts of germination and growth inhibitory effect.

**(A)** Control test. (solvent)

**(B)** At 0.1 gE



**(C)** At 0.5 gE**.**

**(D)** At 1.0 gE.

**(E)** At 2.5 gE

**(F)** At 5.0 gE

**(G)** Seed growth.

**Figure 4.8** The results of various concentration level of crude extracts from some tropical weeds on seedling growth inhibition of giant mimosa.

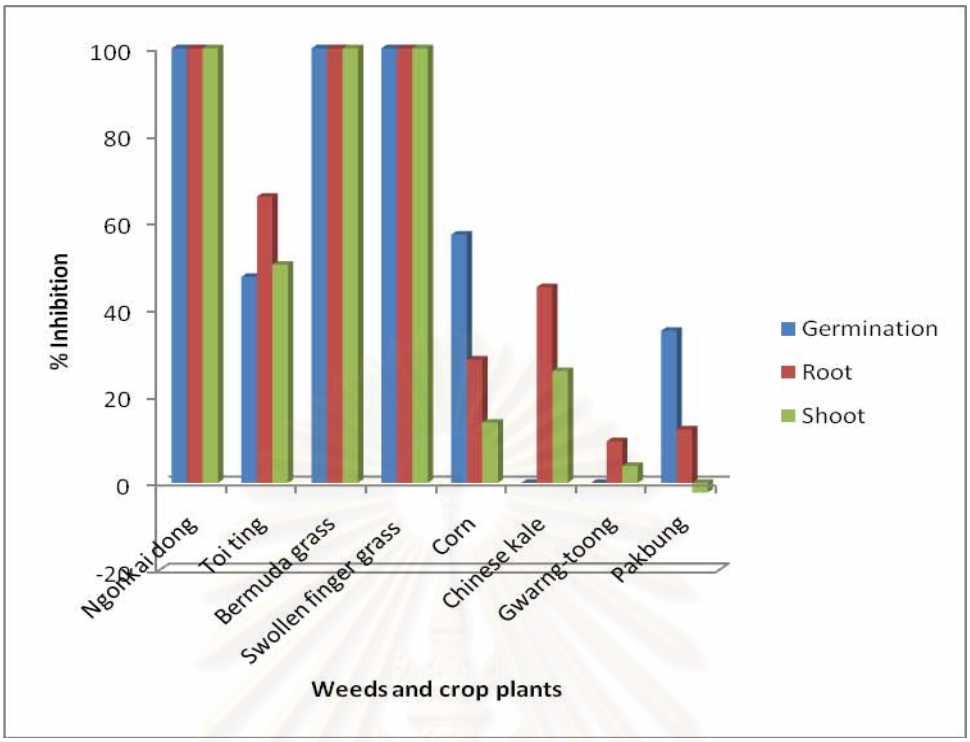
**4.2.3 The effect of crude extract from some tropical weeds on selected weeds and crop plants.**

To clarify that crude extract could inhibit germination or not, certain weeds and crop plants were selected for investigation including Ngonkai dong (*C. argentea*), Toi Ting (*R. tuberosa* ), Bermuda grass (*D. aegyptium*), Swollen finger grass (*C. barbata*) and corn (*Z. mays*), Chinese kale (*B. alboglabra*)**,** Gwarng-toong (*B. chinensis*) and Pakbung ( *I. aquatic*). All of them can be found throughout the country. From the previous study, purple nutsedge, Indian heliotrope and Pha-yaa mutti in $CH_2Cl_2$ and $CH_3OH$ crude extracts were used in this experiment.

Ngonkai dong, Toi Ting, Bermuda grass, Swollen finger grass, Chinese kale, Gwarng-toong, corn and Pakbung were put on filter paper in Petri dishes and incubated at room temperature to observed the germination for 7 days. Ten seedlings were randomly selected to measure the root and shoot elongation (cm) compared with control experiment.

The results were presented in Figs 4.9, 4.10 and 4.11. The tendency of germination inhibition that measured from root and shoot elongation inhibition activity ranged from the highest to the lowest and could divided in 4 groups. The first group was the highest inhibition (70-100%), the second group was inhibited moderately (40-69%), third group was inhibited less than 40% and the final group did not have germination inhibition effect.

$CH_2Cl_2$




$CH_3OH$

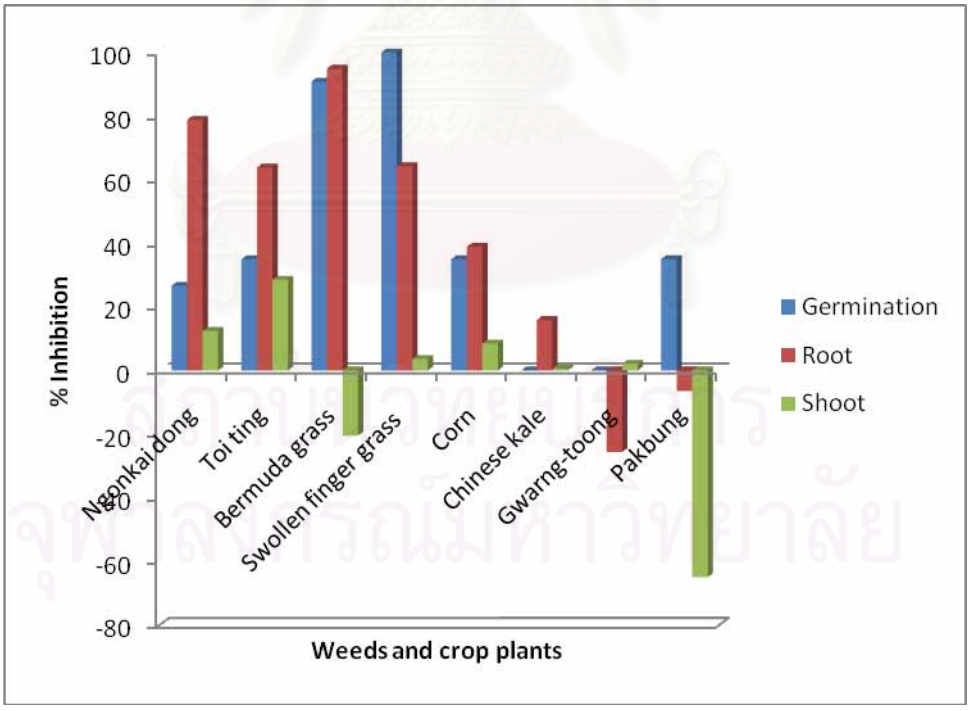


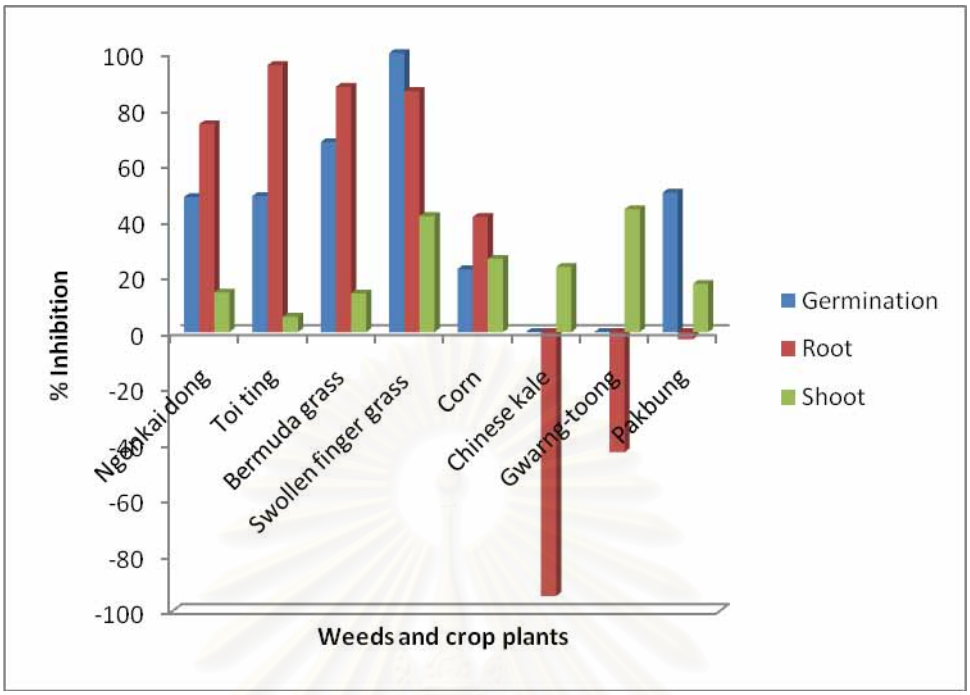

**Figure 4.9** The effect of purple nutsedge extracts on selected weeds and crop plants at 1 gE.

From Fig 4.9, the results of root and shoot elongation inhibition were found that the $CH_2Cl_2$ extracts of purple nutsedge showed effect on Ngonkai dong, Bermuda grass and Swollen finger grass were inhibited completely. Second group, corn and Toi ting was inhibited moderately. The final group Chinese kale, Gwarng-toong and Pakbung were not inhibition.

In $CH_3OH$ crude extracts, Bermuda grass and Swollen finger grass showed more than 80% germination inhibition. In addition, Pakbung, corn and Toi ting and Ngonkai dong displayed less than 50% germination inhibition activity and Chinese kale, Gwarng-toong were not inhibited. In the part of root and shoot elongation inhibition, Bermuda grass gave good tendency of inhibition but Gwarng-toong and Pakbung were not inhibited. For the shoot elongation inhibition, the activity was observed less than 30% shoot inhibition.

From above, the inhibitory effect of purple nutsedge extracts on germination inhibition, root and shoot elongation inhibition, it was found that the $CH_2Cl_2$ crude extracts showed activity better than $CH_3OH$ crude extracts at 1 gE.

$CH_2Cl_2$




$CH_3OH$

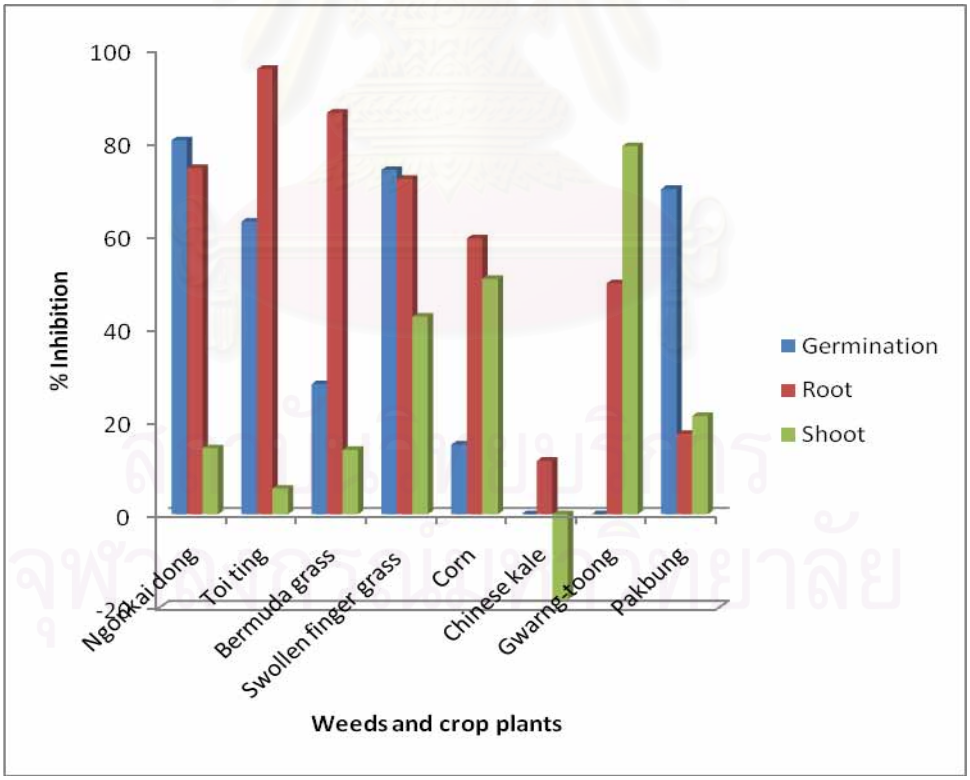
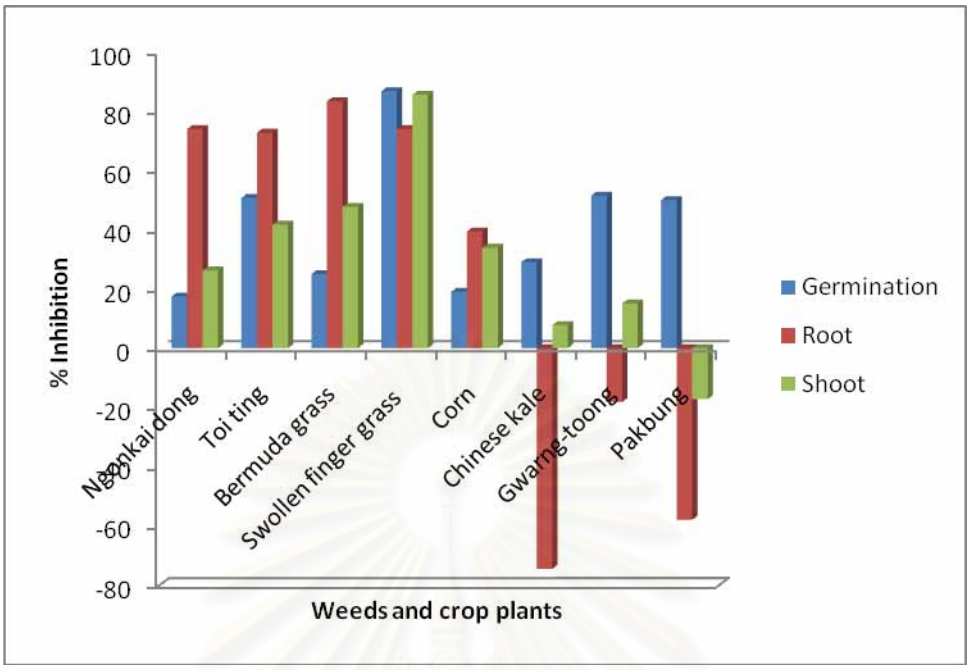



**Figure 4.9** The effect of Indian heliotrope extracts on selected weeds and crop plants at 1 gE

From Fig 4.10, it revealed that the $CH_2Cl_2$ extracts of Indian heliotrope showed high germination inhibition on Swollen finger grass where as in the case of Bermuda grass, Toi ting, Ngonkai dong, corn and Pakbung, it displayed less than 70% germination inhibition activity. For Chinese kale and Gwarng-toong showed low inhibition activity. Percent of root elongation inhibition against some weeds and crop plants found that Ngonkai dong, Bermuda grass and Swollen finger grass provided the inhibition activity from 70 to 90%, the others were inhibited less than 40%, while shoot elongation activity, Swollen finger grass was the highest inhibition activity.

In the part of $CH_3OH$ crude extract, Swollen finger grass, Bermuda grass and Toi ting displayed less than 80% of germination inhibition activity, Chinese kale and Gwarng-toong were not inhibited. In addition, root elongation of Toi ting, Swollen finger grass, Bermuda grass and Ngonkai dong exhibited more than 70%. The other plants revealed low inhibition activity. Furthermore, shoot elongation inhibition of Gwarng-toong was the highest and Chinese kale was the lowest shoot inhibition activity.

$CH_2Cl_2$




$CH_3OH$

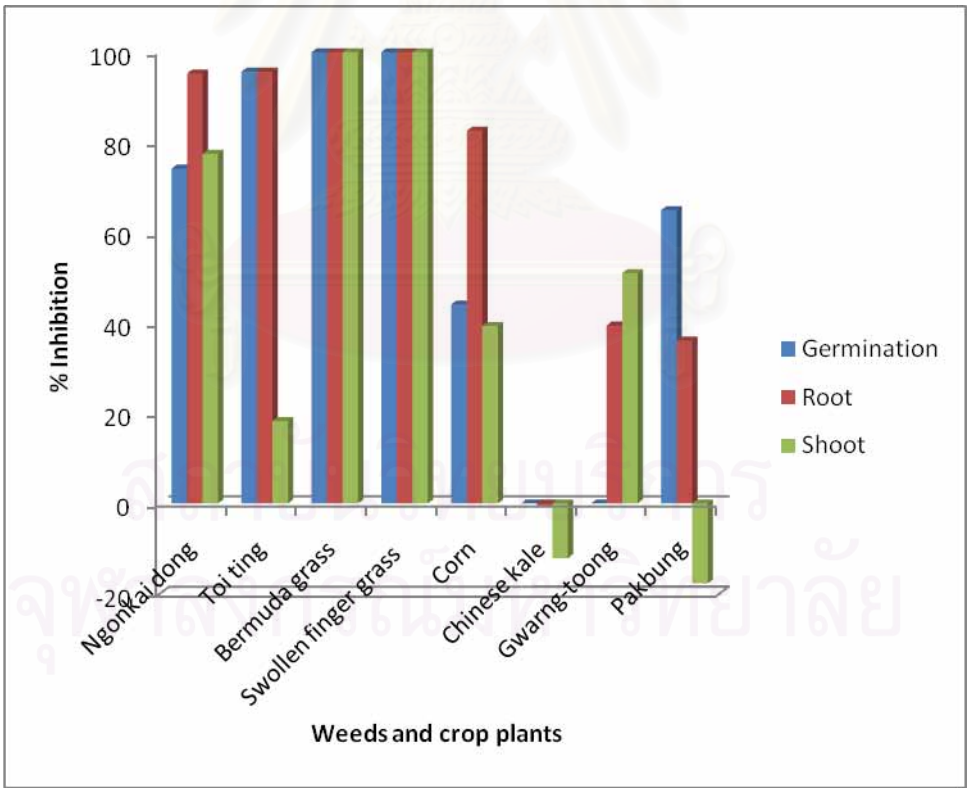



**Figure 4.10** The effect of Pha-yaa mutti extracts on selected weeds and crop plants at 1 gE.

The biological test of the $CH_2Cl_2$ extract from Pha-yaa mutti, it was found that Swollen finger grass gave good tendency of germination inhibition activity while Gwarng-toong, Toi ting, Pakbung, Chinese kale, Bermuda grass, corn and Ngonkai dong revealed less than 55% of germination inhibition. In the case of root elongation inhibition, Bermuda grass, Ngonkai dong and Toi ting displayed more than 70% inhibition activity. The other plants revealed low inhibition activity. Finally, shoot elongation inhibition, it was noteworthy that Swollen finger grass exhibited more than 80% shoot inhibition activity.

In $CH_3OH$ crude extract, particularly, Swollen finger grass, Bermuda grass Ngonkai dong and Toi ting revealed very interesting activity that gave 95 to100 % germination, root and shoot elongation inhibition activity. The other plants showed low or did not have inhibition.

However, the $CH_2Cl_2$ and $CH_3OH$ crude extracts of purple nutsedge, Indian heliotrope and Pha-yaa mutti exhibited the high germination inhibition activity on Swollen finger grass, Bermuda grass Ngonkai dong and Toi ting while the inhibitory effect on crop plants showed low activity. In the further should be studied and developed crude extract from tropical weeds for agriculture.

From Figs 4.9, 4.10 and 4.11, the biological test of purple nutsedge, Indian heliotrope and Pha-yaa mutti on selected weeds and crop plants, clearly revealed that monocotyledon and dycotyledon of plants had not effected on germination inhibition.

## 4.3 Fractionation

### 4.3.1 Fraction of $CH_2Cl_2$ crude extract from purple nutsedge (*C. rotundus*)

The dichloromethane extract from purple nutsedge was concentrated to gave 400 g of brown sticky liquid. TLC of the concentrated extract showed that there were at least five components in this crude (solvent system: 40% EtOAc-hexane). The concentrate 400 g was chromatographed on silica gel (size 7729) for quick column. The column was initially eluted with hexane and increasing polarity by mixing with EtOAc. The results of fractionation are shown in Table 4.1.

**Table 4.1** Fraction of $CH_2Cl_2$ crude extract from purple nutsedge**.**

| Eluents(V/V) | Fraction no. | Remark | Weight (g) |
|---|---|---|---|
| 100%hexane | CRS1 | Yellow oil, wax | 30.19 |
| 5-10%EtOAc-hexane | CRS2 | Brown mix oil | 45.63 |
| 20%EtOAc-hexane | CRS3 | Brown liquid | 69.61 |
| 40% EtOAc-hexane | CRS4 | Dark-brown liquid | 63.39 |
| 60-100% EtOAc-hexane | CRS5 | Dark-brown gum | 44.22 |
| 5-10%MeOH-EtOAc | CRS6 | Dark-brown gum | 14.65 |

### 4.3.2 Bioassay test of fraction

**Germination Inhibition against with giant mimosa (*M. pigra*)**

Six fractions namely CRS1, CRS2, CRS3, CRS4, CRS5 and CRS6 were collected from the chromatographic separation. Each fraction was monitored for bioactive portion using various concentrations of 0.1, 0.5, 1.0, 2.5 and 5.0 gE compared with control, each concentration 3 replications, 50 seeds of giant mimosa /Petri dish, incubated for 7 d at RT. The seed germination, root and shoot elongation inhibition were examined and the percentage of inhibition against giant mimosa seeds was calculated. The results are displayed in Figs 4.11, 4.12 and 4.13, respectively.

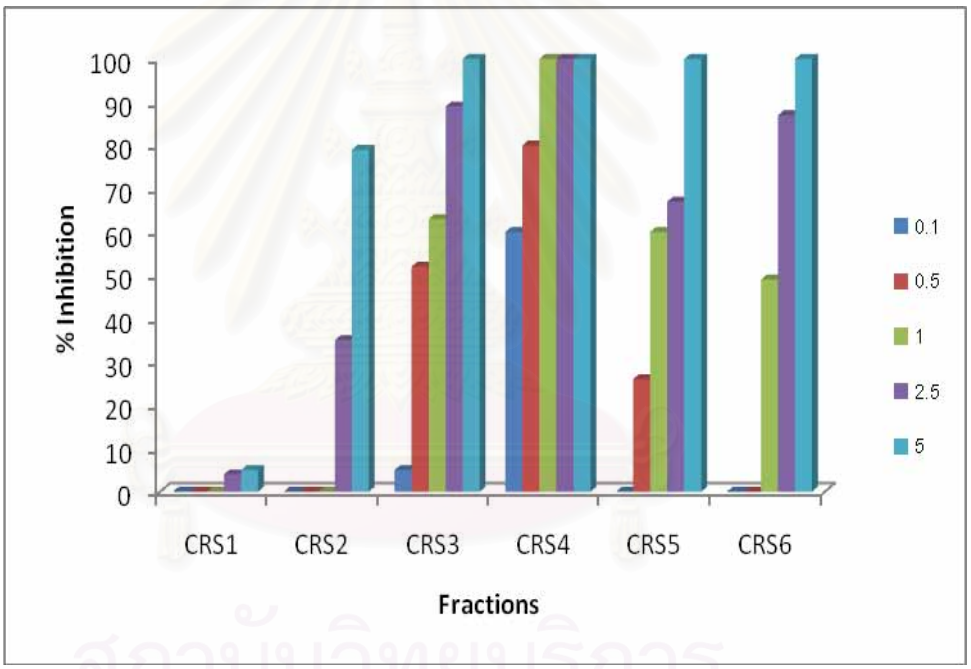



**Figure 4.12** %Inhibition of germination of giant mimosa seeds affected on the fractions derived from the $CH_2Cl_2$ extract.

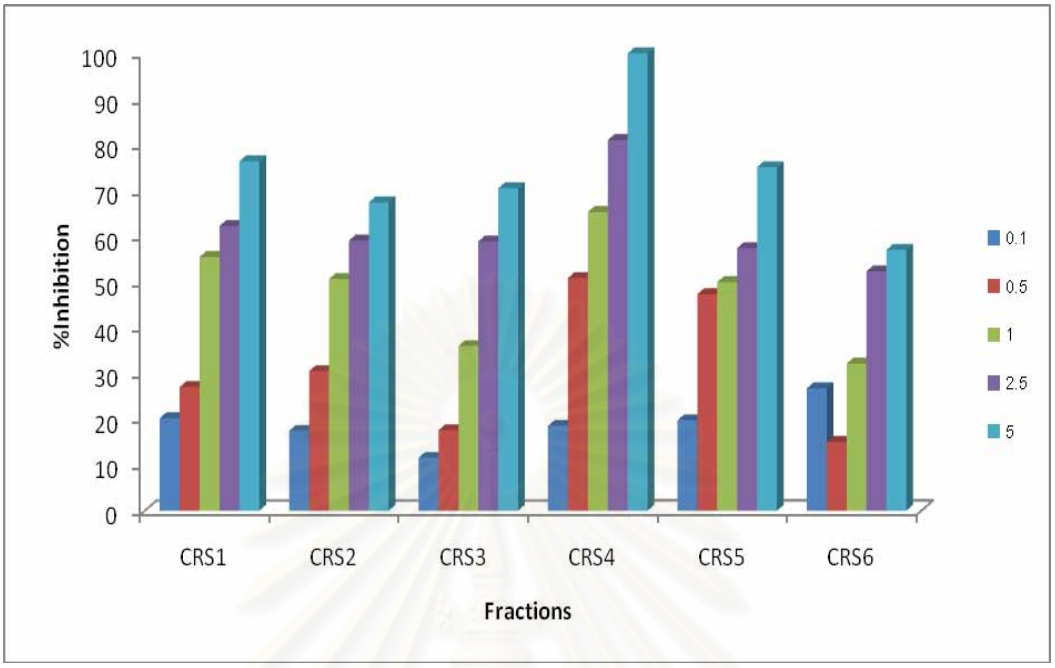



**Figure 4.13** %Inhibition of root elongation inhibition of giant mimosa seeds affected by the fractions derived from the $CH_2Cl_2$ extract .

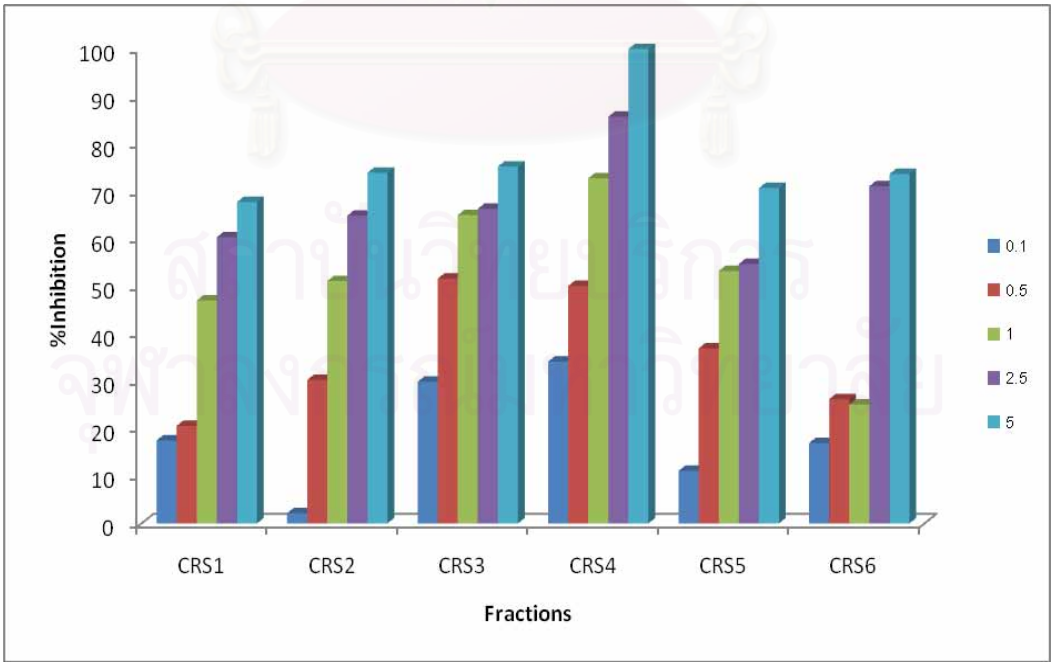



**Figure 4.14** %Inhibition of shoot elongation inhibition of giant mimosa seeds affected by the fractions derived from the $CH_2Cl_2$ extract.

The above figures revealed the correlation between tested $CH_2Cl_2$ crude extract from purple nutsedge at 1 gE with germination, root and shoot elongation of giant mimosa. The CRS4 fraction exhibited the germination inhibition activity more potency than other fractions. The inhibition of root is more essential for considering because the roots are directly contacted to the test. Therefore, fraction CRS4 possessing high activity of root and shoot inhibition. It gave 65% root inhibition followed by fractions CRS1, CRS2, CRS5, CRS3 and CRS6, respectively. The comparative activity is exhibited as follows: 55, 50, 50, 36 and 32%, respectively and percentage of shoot elongation inhibition found CRS4 was inhibited more than each fraction.

# CHAPTER V

# CONCLUSION

During this course of research, biological activity of crude extracts from some tropical weeds were thoroughly examined on seed germination and growth inhibition of giant mimosa.

The preliminary test of allelopathic from purple nutsedge, Indian heliotrope, Pha-yaa mutti, Mexican tea, *M. chanaelea* and Itchgrass in $CH_2Cl_2$ and $CH_3OH$ extracts were considered at 1 gE. It was found that the $CH_2Cl_2$ crude extracts from purple nutsedge, Indian heliotrope and Pha-yaa mutti, displayed high inhibitory activity on the growth of giant mimosa (78, 74 and 71%, respectively). The other weeds revealed less than 30% of growth inhibition activity. Thus purple nutsedge, Indian heliotrope and Pha-yaa mutti extracts were selected for bioassay test on germination and growth inhibition with giant mimosa.

For germination and growth inhibition test at concentration of 0.1, 0.5, 1.0, 2.5 and 5.0 gE, the efficiency of the $CH_2Cl_2$ extract exhibited the growth activity more than $CH_3OH$ extract. Considering at concentration of 1 gE, purple nutsedge was noteworthy and revealed more than 80% growth inhibition activity. Indian heliotrope and Pha-yaa mutti displayed more than 70% growth inhibition activity. Percentage of root elongation inhibition against giant mimosa, purple nutsedge showed the most activity than Indian heliotrope and Pha-yaa mutti. The order of activity could be arranged as follows: 81, 61 and 35%, respectively. Finally, shoot elongation inhibition, the $CH_2Cl_2$ extract from purple nutsedge exhibited good tendency of shoot inhibition in contrast with Indian heliotrope and Pha-yaa mutti.

The inhibitory effects of tropical crude extract at low concentration (0.1 or 0.5 gE) were grossly encountered to be plant promoters: hormone-like herbicide and crude extract with high concentration (2.5 or 5.0 gE) always desisted herb growth at high level (high percent growth inhibition). The concentration at such, the difference of inhibition activity of each tested substance would probably vanish.

Furthermore, this research was forward on the effect of crude extracts from some tropical weeds on Ngonkai dong, Toiting, Swollen finger grass and Bermuda grass including crop plants such as Pakbung, Chinese kale, Gwarng-toong and corn.

Weed germination inhibition against crop plants and some weeds comparing at concentration of 1 gE, was found that the $CH_2Cl_2$ extract of purple nutsedge revealed very interesting activity and gave 100% growth inhibition activity on Ngonkai dong, Swollen finger grass and Bermuda grass. The extracts of Indian heliotrope and Pha-yaa mutti exhibited high activity on Swollen finger grass while Ngonkai dong, Toiting, Bermuda grass, Pakbung and corn displayed less than 70% germination inhibition activity. Chinese kale and Gwarng-toong did not inhibit from tropical crude extract. The $CH_3OH$ extracts, some weeds exhibited more than 80% germination inhibition where for crops plants revealed low inhibition. The percentage of root elongation inhibition in $CH_2Cl_2$ and $CH_3OH$ c extracts from some tropical weeds found that some weeds displayed high inhibition activity, for crop plants exhibited low or no inhibition activity.

In addition, the crude extracts from purple nutsedge, Indian heliotrope and Pha-yaa mutti gave good tendency of weed growth inhibition activity. So that in the future it can develop for farmer and decrease contamination of chemical synthesis in the nature.

In the part of separation, it was found that the $CH_2Cl_2$ extract of purple nutsedge displayed higher activity than Indian heliotrope and Pha-yaa mutti on germination and growth inhibition activity. Thus purple nutsedge was selected for quick column chromatography. The column was initially eluted with hexane and increased polarity by mixing with EtOAc, fractions were combined together about 6 fractions. After that, each fraction was bioassayed on seed growth inhibition with giant mimosa, at concentration of 1 gE. The results of germination and root elongation inhibition, comparative order of activity observed was displayed as follows: CRS4›CRS1 ›CRS2 ›CRS5 ›CRS3 ›CRS6, respectively. The effect of shoot elongation inhibition could be arranged as follows: CRS4 ›CRS3 ›CRS5 ›CRS2 ›CRS1 ›CRS6, respectively.

**Proposal for the future work**

From the research, the results of weed germination and growth inhibition against giant mimosa, weeds and crop plants of crude extracts from some tropical weeds disclosed the relationship of these studied activities. The possibly future work related to this research would be the study of crude extracts from tropical weeds and other weeds that caused problems in Thai agriculture.

# REFERENCES


AICAF. 1996. **Weed in the tropics**. Sanbi Printing.

Barnes, J.P., Putnam A.R.1986. Evidence for allelopathy by residues and aqueous extracts of rye (Secale cereal). *Weed Sci*. 34:384-390.

Bonner, J and Galston, A.W. 1944.Toxic substance from the culture media of guayule which may inhibit growth. *Bot.* 106-185.

Chistian, N.1993. **A nature product for the control of annual weed**. Golf Course Management.

Dilday, R.H., Yan, W.G, Moldenhuer K.A. and Gravois K.A.1998. **Allelopathic activity in rice for controlling major aquatic weed**s. In Allelopathy in Rice M. Olofsdotter, ed. Los Banos, Philippines: International Rice Research Insitite.

Inderjit. 2004. **Weed biology and management**. Boston: Kluwer Acedamic Publishers.

Inderjit, Keating K. I. 1999. Allelopathy: principle, procedure, processes and promises for biological control. *Adv Agron*. 67: 141-231.

Lavabre, E.M. 1991. **Weed control**. London : Macalian.

Macias, F.A., Castellano, D., Molinillo, J.M.G. 2000.Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. *Agric. Food Chem*. 48: 2512-2521

Mizutani,J.1999. Select allelochemicals. *Critical Reviews in Plant Sciences*. 18: 221-223.

Molish, H and Der Einfluss einer Pflanze anf die andere. 1937. **Allelophatic**. Fishcher, Jena.

Naples, M.L. and Kesster, P.J. 2005. **Weed of rain fed lowland rice fields of Laos and Cambodia.**

Napompeth, B. 1994. **Biological Control of Paddy and Aquatics Weeds in Thailand**. National Biological Control Research Center (NBCRC) Kasetsart University.

Olofsdotter, M.,Navarez D., Rebulanan M. and Steribig J.C. 1999. Weed suppressing rice cultivas dose allelopathy play a role. *Weed Res*. 39: 444-454.

Putnam, A.R and Duke, W.B. 1974. Biological suppression of weed: evidence for allelopathy in accessions of cucumber. *Science*. 185: 370-371.

Putnam, A.R and Tang, C.S. 1986. **The Science of Allelophathy**. John Wiley and Sons, New York, NY.

Rice, E.L. 1974. **Allelophaty**, Second editition, Academic Press, New YorK, NY.

Rice, E.L. 1984. **Allelophaty**, Academic Press, 2nd Ed, Orlando, FL.

Rick, W. 1995. **Allelopathy in Eucalypts**: A Review. Australia; CRC Press LLC.

Rizvi, S. J. H. and Rizvi, V. 1992. **Allelopathy : Basic and applied aspects**. London: Chapman & Hall published.

Smith, A.E. 1995. **Handbook of weed management systems**. New York: M. Dekker.

Suwannamek,U.1983. **A Series of Research Work on Control of *Mimosa pigra* Linn**. Agronomy Department Kasetsart University.

Thomas J.M., Stephen C.W. and Floyd M.A. 2002. **Weed science: principles and practices**. New York: Willy.

Thompson, A.C. 1985. **The Chemistry of Allelophaty.** ACS symposium Series 268, American Chemical Society, Washington, DC.

Waterhouse, D.F. 1994. **Biological Control of weed**. Southeast Asian Prospects Australian Center for International Agriculture Research, Canberra, Australia.

Zimdahl, L.R.. 1999. **Fundamental of weed science**. San Diego: Academic Press.

Available from: http: // www. agguide.agronomy.psu.edu/ [2008, January 2].

Available from: http: // www. doa.go.th/ard /[2009, April 5].

Available from: http: // www. ccoh.ca/oshanswers/chemicals/ ld 50.html. [2009, April 5].

Available from: http: // www. en.wikipedia.org/wiki/Herbicid [2009, April 12].

Available from: http: // www. rain-tree.com/epaazolte.html/ [2009, April 12].

Available from: http: // www. missouriplants.com/[2009, April 12].

Available from: http: // www. issg.org/ database/ species/ ecology
[2009, April 12].

Available from: http: // www.plants.usda.gov/ java/profile/[2009, April 12].

Available from: http: // www. en.wikipedia.org/wiki/Teosinte[2009, April 12].

# APPENDICES

**BIOASSAY EXPERIMENT**

- **Screening of crude extract activity from some tropical weeds on *Mimosa pigra* L.**

**Table 1** The result of preliminary test crude extract from some tropical weed on seeds germination of *M. pigra* seeds.

| Plant | %Germination Inhibition(average) | | | | | |
|---|---|---|---|---|---|---|
| | Concentration( g / dry weight equivalent) | | | | | |
| | Control | 0.1 | 0.5 | 1.0 | 2.5 | 5.0 |
| **$CH_2Cl_2$** | | | | | | |
| *C. rotundus* | 0 | 25.04 | 32.70 | 78.38 | 86.06 | 100 |
| *G. maderaspatana* | 0 | 20.85 | 22.77 | 70.89 | 75.91 | 87.66 |
| *H. indicum* | 0 | 23.44 | 26.04 | 73.52 | 81.23 | 100 |
| *C. ambrosoides* | 0 | 0 | -0.51 | 0.51 | 5.10 | 33.67 |
| *P. amaranthus* | 0 | 0.54 | 0.54 | 1.09 | 5.43 | 26.63 |
| *R.. cochinchinnensis* | 0 | 5.32 | 10.64 | 13.30 | 45.21 | 50.62 |
| **MeOH** | | | | | | |
| *C. rotundus* | 0 | 22.70 | 43.40 | 50.41 | 67.63 | 71.23 |
| *G. madaspatana* | 0 | 18.52 | 31.21 | 42.73 | 55.73 | 60.43 |
| *H. indicum* | 0 | 21.06 | 35.72 | 46.38 | 56.38 | 63.57 |
| *C. ambrosoides* | 0 | 9.10 | 13.21 | 15.42 | 17.42 | 19.23 |
| *P. amarathus* | 0 | 22.28 | 24.46 | 25.48 | 38.46 | 55.24 |
| *R.. cochinchinnensis* | 0 | 12.37 | 15.46 | 17.53 | 19.88 | 20.62 |

- **Weed Germination Inhibition against *Mimosa pigra* L.**

**Table 2** Effect of dichloromethane ($CH_2Cl_2$) crude extract form *C. rotundus* *G. madaspatana* and *H. indicum* on the germination seed of *M. pigra* .

| Plants | %Inhibition | | | | | |
|---|---|---|---|---|---|---|
| | Concentration (g/dry weight equivalent) | | | | | |
| | Control | 0.1 | 0.5 | 1.0 | 2.5 | 5.0 |
| **Germination Inhibition** | | | | | | |
| *C.rotundus* | 0 | 59.50[a] | 74.50[a] | 86.06[a] | 97.25[a] | 100[a] |
| *H. indicum* | 0 | 61.25[a] | 70.00[ab] | 76.75[b] | 81.50[b] | 83.75[b] |
| *G.maderaspatana* | 0 | 59.50[a] | 67.50[b] | 73.25[b] | 75.00[c] | 81.50[b] |
| **Root Inhibition** | | | | | | |
| *C.rotundus* | 0 | 46.45(1.95)[a] | 46.63(1.83)[a] | 81.04(1.09)[b] | 100(0.00)[a] | 100(0.00)[a] |
| *H. indicum* | 0 | 69.14(2.67)[b] | 79.52(1.88)[a] | 61.47(1.42)[c] | 100(0.00)[a] | 100(0.00)[a] |
| *G.maderaspatana* | 0 | 5.31(3.47)[c] | 20.30(2.97)[b] | 35.07(0.67)[a] | 50.62(0.62)[b] | 100(0.00)[a] |
| **Shoot Inhibition** | | | | | | |
| *C.rotundus* | 0 | 16.54(4.43)[a] | 21.75(2.45)[a] | 62.56(1.17)[a] | 100(0.00)[a] | 100(0.00)[a] |
| *H. indicum* | 0 | 14.12(4.78)[b] | 21.06(4.09)[b] | 46.60(3.71)[b] | 100(0.00)[a] | 100(0.00)[b] |
| *G.maderaspatana* | 0 | 34.79(5.04)[c] | 58.18(4.67)[c] | 32.00(4.58)[c] | 69.35(0.00)[b] | 100(0.00)[c] |

( ) average of root and shoot elongation (cm) calculated from 3 replications.( each replication about 10 seedling)

[a]Different letter in a column indicate values significantly different at 0.05 level determined by one-way ANOVA followed Duncan's test.

**Table 3** Effect of methanol (MeOH) crude extract form *C. rotundus*, *G.maderaspatana* and *H. indicum* on the seeds germination of *M. pigra*.

| Plants | %Inhibition | | | | | |
|---|---|---|---|---|---|---|
| | Concentration (g/dry weight equivalent) | | | | | |
| | Control | 0.1 | 0.5 | 1.0 | 2.5 | 5.0 |
| **Germination Inhibition** | | | | | | |
| *C.rotundus* | 0 | 19.04[a] | 31.70[a] | 50.78[a] | 60.43[a] | 76.06[a] |
| *H. indicum* | 0 | 23.24[a] | 26.04[a] | 50.52[a] | 57.73[b] | 65.35[b] |
| *G.maderaspatana* | 0 | 15.85[a] | 22.77[a] | 48.78[a] | 61.91[a] | 77.66[a] |
| **Root Inhibition** | | | | | | |
| *C.rotundus* | 0 | 23.47(1.79)[a] | 31.76 (1.74)[a] | 37.84(1.47)[a] | 55.58(0.54)[a] | 70.25(0.00)[a] |
| *H. indicum* | 0 | 12.83(2.44)[b] | 10.05(2.36)[b] | 36.23(1.24)[a] | 33.23(0.60)[b] | 55.58(0.00)[a] |
| *G.maderaspatana* | 0 | 13.49(2.98)[b] | 17.00(2.68)[b] | 30.00(2.41)[b] | 32.44(2.20)[b] | 65.25(0.62)[b] |
| **Shoot Inhibition** | | | | | | |
| *C.rotundus* | 0 | 3.81(4.31)[a] | 26.78(1.99)[a] | 34.50(1.14)[a] | 46.27(0.29)[a] | 75.50(0.00)[a] |
| *H. indicum* | 0 | 5.06(4.54)[a] | 18.84(4.23)[b] | 26.24(3.93)[b] | 37.22(3.15)[c] | 55.16(0.99)[b] |
| *G.maderaspatana* | 0 | 5.37(4.99)[a] | 12.21(4.98)[c] | 13.92(4.81)[c] | 37.26(1.24)[b] | 47.59(1.10)[b] |

( ) average of root and shoot elongation (cm) calculated from 3replications (each replication about 10 seedling)[a]Different letter in a column indicate values significantly different at 0.05 level determined by one-way ANOVA followed Duncan's test.

**Table 4** The bioassay results of $CH_2Cl_2$ crude extracts form *C. rotundus G. maderaspatana* and *H. indicum* with *M. pigra*.

| Plant | %Inhibition | | | | | |
|---|---|---|---|---|---|---|
| | Concentration (g/dry weight equivalent) | | | | | |
| | Control | 0.1 | 0.5 | 1.0 | 2.5 | 5.0 |
| **Root Inhibition** | | | | | | |
| *C. rotundus* | 0 | 16.54(1.34)[a] | 23.80(1.45)[b] | 76.60(1.27)[b] | 100(0.00)[a] | 100(0.00)[a] |
| *H.indicum* | 0 | 12.65(1.28)[a] | 12.72(1.27)[c] | 61.85(2.12)[c] | 100(0.00)[a] | 100(0.00)[a] |
| *G.maderaspatana* | 0 | 34.79(1.23)[a] | 62.58(1.26)[c] | 69.35(1.74)[a] | 89.35(1.40)[b] | 100(0.00)[a] |
| **Shoot Inhibition** | | | | | | |
| *C. rotundus* | 0 | 46.44(1.02)[a][b] | 46.63(0.96)[a] | 61.04(0.75)[a] | 100(0.00)[a] | 100(0.00)[a] |
| *H.indicum* | 0 | 35.35(0.72)[a] | 49.19(0.44)[a] | 61.04(0.46)[a] | 100(0.00)[a] | 100(0.00)[a] |
| *G.maderaspatana* | 0 | 12.05(1.73)[b] | 15.07(1.00)[a] | 53.80(1.11)[a] | 75.76(0.82)[b] | 100(0.00)a |

( ) average of root and shoot elongation (cm) calculated from 3 replications ( each replication about 6 seedling)

[a]Different letter in a column indicate values significantly different at 0.05 level determined by one-way ANOVA followed Duncan's test.

**Table 5** The bioassay results of MeOH crude extracts form *C. rotundus G. maderaspatana* and *H. indicum* with *M. pigra*.

| Plant | %Inhibition | | | | | |
|---|---|---|---|---|---|---|
| | Concentration (g/dry weight equivalent) | | | | | |
| | Control | 0.1 | 0.5 | 1.0 | 2.5 | 5.0 |
| **Root Inhibition** | | | | | | |
| *C. rotundus* | 0 | 22.35(0.40)[a] | 33.33(0.64)[a] | 59.27(0.86)[a] | 72.16(0.50)[b] | 85.75(0.18)[a] |
| *H.indicum* | 0 | 20.87(0.44)[a] | 24.88(0.51)[a] | 50.79(0.53)[a] | 64.75(0.47)[ab] | 78.04(0.27)[b] |
| *G.maderaspatana* | 0 | 12.58(0.69)[a] | 29.72(0.47)[a] | 67.00(0.21)[a] | 93.94(0.33)[a] | 100(0.00)[a] |
| **Shoot Inhibition** | | | | | | |
| *C. rotundus* | 0 | 1.89(0.93)[a] | 10.50(0.75)[a] | 41.59(1.52)[b] | 56.80(1.44)[b] | 78.74(0.67)[ab] |
| *H.indicum* | 0 | 16.79(0.87)[a] | 32.64(0.70)[a] | 41.53(1.33)[ab] | 55.96(1.61)[b] | 67.78(1.39)[b] |
| *G.maderaspatana* | 0 | 12.58(1.46)[a] | 17.51(1.06)[a] | 52.77(0.82)[a] | 70.59(0.30)[a] | 100(0.00)[a] |

( ) average of root and shoot elongation (cm) calculated from 3 replications ( each replication about 6 seedling)

[a]Different letter in a column indicate values significantly different at 0.05 level determined by one-way ANOVA followed Duncan's test.

- **Germination Inhibition on selected weeds and crop plants.**

**Table 6** The percentage of germination inhibition on selected weed and crop plants of *C. rotundus* crude extract.

| Plants | % Germination Inhibition at 1 g dry weight equivalent | | |
|---|---|---|---|
| | Crude extract from *C. rotundus* | | |
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn | 0 | 100 | 26.60 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 47.41 | 34.96 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 100 | 90.80 |
| 4. *C. barbata* (L.) Sw. | 0 | 100 | 100 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 57.15 | 35 |
| 6. *B. alboglabra* Barley. | 0 | 0 | 0 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | 0 | 0 |
| 8. *I. aquatica* Forsk.. | 0 | 35 | 35 |

**Table 7** The percentage on germination inhibition on selected weeds and crop plants of *H. indicum* crude extract.

| Plants | % Germination Inhibition at 1 g dry weight equivalent | | |
|---|---|---|---|
| | Crude extract from *H. indicum* | | |
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn. | 0 | 48.39 | 80.50 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 48.80 | 63 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 68.13 | 28 |
| 4. *C. barbata* (L.) Sw. | 0 | 100 | 74.09 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 22.62 | 15.00 |
| 6. *B. alboglabra* Barley. | 0 | 0 | 0 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | 0 | 0 |
| 8. *I. aquatica* Forsk.. | 0 | 50 | 70 |

**Table 8** The percentage on germination inhibition on selected weeds and crop plants of *G. maderaspatana* crude extract.

| Plants | % Germination Inhibition at 1 g dry weight equivalent | | |
|---|---|---|---|
| | Crude extract from *G. maderaspatana* | | |
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn. | 0 | 17.39 | 74.19 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 50.74 | 95.68 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 25.00 | 100 |
| 4. *C. barbata* (L.) Sw. | 0 | 86.77 | 100 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 19.05 | 44.09 |
| 6. *B. alboglabra* Barley. | 0 | 29.00 | 0 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | 51.50 | 0 |
| 8. *I. aquatica* Forsk.. | 0 | 50.00 | 65.00 |

**Table 9** The percentage on root elongation inhibition on selected weeds and crop plants of *C. rotundus* crude extract.

| Plants | % Root Inhibition at 1 g dry weight equivalent | | |
|---|---|---|---|
| | Crude extract from *C. rotundus* | | |
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn. | 0 | 100 | 78.78 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 65.88 | 63.79 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 100 | 94.90 |
| 4. *C. barbata* (L.) Sw. | 0 | 100 | 64.20 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 28.36 | 38.88 |
| 6. *B. alboglabra* Barley. | 0 | 45.05 | 15.83 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | 9.58 | -25.66 |
| 8. *I. aquatica* Forsk.. | 0 | 12.29 | -6.47 |

**Table 10** The percentage on root elongation inhibition on selected weeds and crop plants of *H. indicum* crude extract.

| Plants | % Root Inhibition at 1 g dry weight equivalent | | |
|---|---|---|---|
| | Crude extract from *H.indicum* | | |
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn. | 0 | 74.50 | 74.50 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 95.73 | 95.83 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 88 | 86.41 |
| 4. *C. barbata* (L.) Sw. | 0 | 86.42 | 72.16 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 41.27 | 59.37 |
| 6. *B. alboglabra* Barley. | 0 | -94.61 | 11.50 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | -43.08 | 49.71 |
| 8. *I. aquatica* Forsk.. | 0 | -2.61 | 17.29 |

**Table 11** The percentage on root elongation inhibition on selected weeds and crop plants of *G. maderaspatana* crude extract.

| Plants | % Root Inhibition at 1 g dry weight equivalent | | |
|---|---|---|---|
| | Crude extract from *G.madaspatana* | | |
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn. | 0 | 74.02 | 95.29 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 72.77 | 95.68 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 88.37 | 100 |
| 4. *C. barbata* (L.) Sw. | 0 | 73.98 | 100 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 39.39 | 82.58 |
| 6. *B. alboglabra* Barley. | 0 | -74.54 | -0.55 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | -18.19 | 39.43 |
| 8. *I. aquatica* Forsk.. | 0 | -58 | 36.08 |

**Table 12** The percentage on shoot elongation inhibition on selected weeds and crop plants of *C. rotundus* crude extract.

| Plants | % Shoot Inhibition at 1 g dry weight equivalent | | |
|---|---|---|---|
| | Crude extract from *C. rotundus* | | |
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn. | 0 | 100 | 12.42 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 50.22 | 28.5 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 100 | -20.51 |
| 4. *C. barbata* (L.) Sw. | 0 | 100 | 3.59 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 13.86 | 8.45 |
| 6. *B. alboglabra* Barley. | 0 | 25.76 | 0.52 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | 3.89 | 2.06 |
| 8. *I. aquatica* Forsk.. | 0 | -2.23 | -65 |

**Table 13** The percentage on shoot elongation inhibition on selected weeds and crop plants of *H. indicum* crude extract.

| Plants | % Shoot Inhibition at 1 g dry weight equivalent | | |
|---|---|---|---|
| | Crude extract from *H. indicum* | | |
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn. | 0 | 14.21 | 14.21 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 5.51 | 5.52 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 13.85 | 13.85 |
| 4. *C. barbata* (L.) Sw. | 0 | 41.61 | 42.54 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 26.24 | 50.65 |
| 6. *B. alboglabra* Barley. | 0 | 23.38 | -18.38 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | 44.11 | 79.23 |
| 8. *I. aquatica* Forsk.. | 0 | 17.29 | 21.14 |

**Table 14**The percentage on shoot elongation inhibition on selected e weeds and crop plants of *G. maderaspatana* crude extract.

| Plants | % Shoot Inhibition at 1 g dry weight equivalent Crude extract from *G. madaspatana.* | | |
|---|---|---|---|
| | Control | $CH_2Cl_2$ | MeOH |
| **Weeds** | | | |
| 1. *C. argentea* Linn. | 0 | 26.22 | 77.48 |
| 2. *R. squarrosa* (Fenzl.) Cufod | 0 | 41.73 | 18.25 |
| 3. *D. aegypt*ium (L.) P.B. | 0 | 47.73 | 100 |
| 4. *C. barbata* (L.) Sw. | 0 | 85.58 | 100 |
| **Crop plants** | | | |
| 5. *Z. mays* Linn. | 0 | 33.89 | 39.32 |
| 6. *B. alboglabra* Barley. | 0 | 7.80 | -12.16 |
| 7. *B. chinensis* var.chinensis Mansf | 0 | 15.09 | 51.03 |
| 8. *I. aquatica* Forsk.. | 0 | -17.23 | -17.73 |

**Table 15** Effect of fractionated from dichloromethane ($CH_2Cl_2$) crude extract on *M. pigr*a**.**

| Fraction | Part | %Inhibition | | | | | |
|---|---|---|---|---|---|---|---|
| | | Concentration (g dry weight equivalent) | | | | | |
| | | Control | 0.1 | 0.5 | 1.0 | 2.5 | 5.0 |
| CRS1 | Germ. | 0 | 0 | 0 | 0 | 4 | 5 |
| | Root | 0 | 20.24 | 27.03 | 55.49 | 62.26 | 76.40 |
| | Shoot | 0 | 17.50 | 20.58 | 46.99 | 60.33 | 67.73 |
| CRS2 | Gem. | 0 | 0 | 0 | 0 | 35 | 79 |
| | Root | 0 | 17.47 | 30.52 | 50.66 | 59.05 | 67.42 |
| | Shoot | 0 | 2.16 | 30.24 | 51.09 | 64.88 | 73.93 |
| CRS3 | Germ. | 0 | 5 | 52 | 63 | 89 | 100 |
| | Root | 0 | 11.57 | 17.59 | 35.99 | 58.88 | 70.52 |
| | Shoot | 0 | 29.85 | 51.60 | 65.00 | 66.33 | 75.23 |
| CRS4 | Germ. | 0 | 0 | 60 | 80 | 100 | 100 |
| | Root | 0 | 18.55 | 50.08 | 65.32 | 81.03 | 100 |
| | Shoot | 0 | 34.10 | 50.06 | 72.76 | 85.74 | 100 |
| CRS5 | Germ. | 0 | 0 | 26 | 60 | 67 | 100 |
| | Root | 0 | 19.78 | 47.35 | 50 | 57.42 | 75.11 |
| | Shoot | 0 | 11.08 | 36.91 | 53.21 | 54.69 | 70.69 |
| CRS6 | Germ. | 0 | 0 | 0 | 49 | 87 | 89 |
| | Root | 0 | 26.77 | 15.04 | 32.17 | 52.36 | 57.06 |
| | Shoot | 0 | 16.95 | 26.19 | 25.05 | 71.09 | 73.65 |

# VITAE

Miss Narachan Phimsen was born on September 22, 1981 in Nakornsrithammarat province, Thailand. She graduates a Bachelor Degree of Science in Pest Management, from the Department of Agriculture, Kasetsart University, Bangkok, Thailand in 2004. She graduates in Master degree majoring in environmental science in 2009, Chulalongkorn University, Bangkok, Thailand .