ผลทางแอลลีโลแพทิกและศักยภาพในการควบคุมวัชพืชของหญ้าท่าพระ

Richardia brasiliensis Gomes

นางสาวพิจารี วิกิจการโกศล

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ALLELOPATHIC EFFECT AND WEED CONTROL POTENTIAL OF *Richardia brasiliensis* Gomes

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พิจารี วิกิจการโกศล : ผลทางแอลลีโลแพทิกและศักยภาพในการควบคุมวัชพืชของหญ้าท่าพระ *Richardia brasiliensis* Gomes (ALLELOPATHIC EFFECT AND WEED CONTROL POTENTIAL OF *Richardia brasiliensis* Gomes) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. วรินทร ชวศิริ, 90 หน้า.

ได้ศึกษาผลทางแอลลีโลแพทิกของสิ่งสกัดจากหญ้าท่าพระ *Richardia brasiliensis* Gomes ที่มีต่อการงอกของเมล็ดและการเติบโตของไมยราบยักษ์ *Mimosa pigra* L. โดยสกัดหญ้าท่าพระทั้งต้นที่แห้งด้วยเฮกเซน ไดคลอโรมีเทน เอทิลแอซีเทตและเมทานอล ตามลำดับ ศึกษาการยับยั้งโดยเปรียบเทียบที่ระดับความเข้มข้นที่แตกต่างกัน 5 ระดับ คือ 0.1, 0.5, 1.0, 2.5 และ 5.0 กรัมสมมูลของพืชแห้ง (gE) สิ่งสกัดเมทานอลแสดงฤทธิ์ยับยั้งการงอกของเมล็ดไมยราบยักษ์ได้ดีที่สุดที่ความเข้มข้นมากกว่าเท่ากับ 1.0 กรัมสมมูลของพืชแห้ง นอกจากนี้สิ่งสกัดเมทานอลที่ความเข้มข้น 1.0 กรัมสมมูลของพืชแห้ง สามารถยับยั้งความยาวรากและความยาวลำต้นได้ 43 และ 48% ตามลำดับ เมื่อนำสิ่งสกัดเมทานอลมาทดสอบการยับยั้งการงอกของเมล็ดและการเจริญเติบโตของวัชพืชและพืชปลูกที่ความเข้มข้น 1.0 กรัมสมมูลของพืชแห้ง พบว่า สิ่งสกัดเมทานอลแสดงฤทธิ์ในการยับยั้งการงอกของเมล็ดและการเจริญเติบโตของวัชพืชได้อย่างมีประสิทธิภาพ ในขณะที่ไม่ส่งผลการยับยั้งต่อพืชปลูก เมื่อแยกสิ่งสกัดเมทานอลด้วยควิกคอลัมน์ ได้ทั้งหมด 4 ส่วน ดังนี้ RBM-1, RBM-2, RBM-3 และ RBM-4 สิ่งสกัดทั้งสี่ส่วนไม่มีผลในการยับยั้งการงอกของเมล็ดและความยาวลำต้น ยกเว้นในส่วน RBM-4 สามารถยับยั้งได้สูงถึง 100% ที่ความเข้มข้น 2.5 และ 5.0 กรัมสมมูลของพืชแห้ง เช่นเดียวกับความยาวราก จากการวิเคราะห์องค์ประกอบของสิ่งสกัดเมทานอลด้วย HPLC พบ 4-hydroxybenzoic acid, *p*-coumaric acid, benzoic acid และ caffeic acid เป็นองค์ประกอบหลัก และทดสอบประสิทธิภาพการยับยั้งการงอกของเมล็ดและการเจริญเติบโตของสารฟีนอลิก พบว่าสารฟีนอลิกแสดงผลการยับยั้งความยาวรากได้ดีที่สุดที่ความเข้มข้น 1 มิลลิโมลาร์ ผลการทดลองเบื้องต้นแสดงให้เห็นว่า หญ้าท่าพระ มีสารสำคัญที่แสดงฤทธิ์ยับยั้งการเจริญเติบโตมีศักยภาพทางแอลลีโลแพทิก จึงมีความเป็นไปได้ที่จะพัฒนาเป็นสารกำจัดวัชพืชที่ได้จากธรรมชาติ ในทางกลับกัน 3,4-dihydroxybenzoic acid และ caffeic acid แสดงฤทธิ์ในการส่งเสริมการงอกความยาวลำต้น และความรากที่ความเข้มข้น 1 มิลลิโมลาร์

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

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5472216923 : MAJOR BIOTECHNOLOGY
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PHIJAREE WIKITKANKOSOL: ALLELOPATHIC EFFECT AND WEED CONTROL POTENTIAL OF *Richardia brasiliensis* Gomes. ADVISOR: ASST. PROF. WARINTHORN CHAVASIRI, Ph.D., 90 pp.

The allelopathic effects of the extracts of *Richardia brasiliensis* Gomes were evaluated on the germination and growth inhibition of *Mimosa pigra* L. Dried whole plant materials were extracted by hexane, CH_2Cl_2, EtOAc and CH_3OH, respectively. The inhibitory activity was compared using five diverse concentrations of 0.1, 0.5, 1.0, 2.5 and 5.0 g dry weight equivalent extract (gE). The CH_3OH extract was completely inhibited the germination of *M. pigra* at ≥ 1.0 gE. Additionally, the root and shoot length were also inhibited by 43 and 48% respectively by the CH_3OH extract at 1.0 gE. The CH_3OH extract was further tested for the inhibition of the germination and growth on weeds and crops at 1 gE. This extract efficiently displayed the inhibition of the germination and growth on weed seeds, while it did not affect on the crop seeds. The CH_3OH extract was separated by quick column to yield four fractions namely RBM-1, RBM-2, RBM-3 and RBM-4. All fractions did not inhibit the seed germination and shoot length, except for RBM-4 showing 100% inhibition at 2.5 and 5.0 gE, same as the root length. Further investigation on its constituents was performed by HPLC analysis and revealed that 4-hydroxybenzoic acid, *p*-coumaric acid, benzoic acid and caffeic acid were main composition. To ascertain the capability of phenolic compounds on germination and growth inhibition, all compounds exhibited the greatest inhibition on the root length of weeds at 1 mM. These results suggested that *R. brasiliensis* contain growth inhibitory substances, possess allelopathic potentials and be possible candidate for developing as natural herbicides. On the other hand, 3,4-dihydroxybenzoic acid and caffeic acid were found to promote the germination, shoot and root length at 1 mM.

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LIST OF ABBREVIATIONS

°C	=	degree Celsius
µL	=	microliter
µm	=	micrometer
AcOH	=	acetic acid
CH_2Cl_2	=	dichloromethane
CH_3OH	=	methanol
d	=	day
EtOAc	=	ethyl acetate
g	=	gram
GC-MS	=	gas chromatography-mass spectrometry
gE	=	gram dry weight equivalent
h	=	hour
H_2O	=	water
HPLC	=	high-performance liquid chromatography
kg	=	kilogram
M	=	molar
min	=	minute
mL	=	milliliter
mm	=	millimeter
mM	=	millimolar
NH_4OAc	=	ammonium acetate
nm	=	nanometer

no.	=	number
R_t	=	retention time
RT	=	room temperature
TLC	=	thin layer chromatography
UV	=	ultraviolet
v/v	=	volume by volume
w/w	=	weight by weight
DAT	=	Day after treatment

CHAPTER I

INTRODUCTION

Weed is a plant considered undesirable in a particular situation and generally difficult to control. One method commonly used to manage weed is using synthetic herbicides. Currently, the amount of herbicides used has been dramatically increased; all of them are imported. According to the Thai agro business association revealed that in 2013, Thailand has imported herbicide 137,000 tons and expect in 2014 to be imported not less than 140,000 tons. The use of these synthetic herbicides has continued to cause the risks to the agro-ecosystem, environment and human health. This leads to search for an alternative for environmentally friendly weed management. Allelopathy is one of those choices through allelochemicals from plants that have the potential to reduce the use of synthetic herbicide, protect environment and prevent the loss of agro-ecosystems, reduce costs and human health for sustainable agriculture.

1.1 Allelopathy

Allelopathy is derived from the Greek allelon, ‘of each other’ and pathos, ‘to suffer’; it means the injurious effect of upon another [1]. The term ‘allelopathy’ was coined by plant physiologist, Hans Molisch in 1973, University of Vienna, Austria and his definition referred to both the harmful and beneficial biochemical interactions among all classes of plants as well as microorganisms [2]. According to the definition given by the International Allelopathy Society (IAS), allelopathy ‘studies any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influence the growth and development of agricultural and biological systems’ [3].

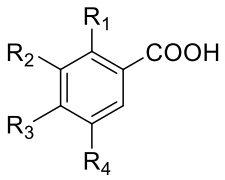
1.2 Allelopathic chemistry

Chemicals that determine allelopathic influences are called allelochemicals. Basically, allelochemicals can be classified as follows: water soluble organic acids,

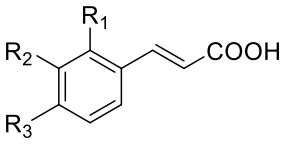
aromatic acids, unsaturated lactones, coumarins, quinones, flavonoids, tannins, alkaloids, terpenoids, toxic gases, long-chain fatty acids, cinnamic acids and derivatives, amino acids, sulfides, purines and cyanogenic glycosides [4].

1.2.1 Phenolic compounds

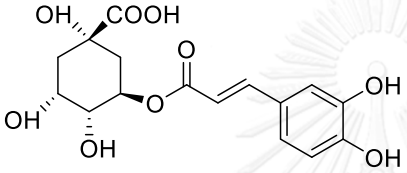
Phenolic compounds are the most important class and have often been reported as allelopathic agents. They are chemicals consisting of a hydroxyl group (-OH) bonded directly to aromatics. Phenolic compounds contain a range of compound types such as simple aromatic phenols, hydroxyl and substituted benzoic acids and aldehydes, hydroxyl and substituted cinnamic acids, coumarins and tannins. Benzoic and cinnamic acids are among the most commonly referred to allelopathic agents (**Figure 1.1**). For example, polyphenols such as ellagic, gallic and pyrogallic acids along with the flavonoid (+)-catechin were isolated from the macrophyte *Myriophyllum spicatum* L. and were found to inhibit the growth of blue-green algae [5]. Protocatechuic acid and cathecol from onion had the ability to prevent diseases infection of *Colletotrichum circinaus* [6]. Experiments were conducted to identify allelochemicals from hull extracts from three rice (*Oryza sativa* L.) cultivars including Janganbyeo, Baekambyeo and Labelle by HPLC analysis. The results showed that Janganbyeo contained salicylic acid, *p*-coumaric acid, *o*-hydroxyphenylacetic acid, syringic acid, ferulic acid, benzoic acid, *p*-hydroxybenzoic acid, *m*-coumaric acid and *o*-coumaric acid. In Baekambyeo include salicylic acid, *o*-hydroxyphenylacetic acid, benzoic acid, *p*-hydroxybenzoic acid and *m*-coumaric acid. Labelle species contained salicylic acid, *o*-hydroxyphenylacetic acid, syringic acid, benzoic acid, *p*-hydroxybenzoic acid, *m*-coumaric acid and *o*-coumaric acid. In bioassay, the inhibition was increased as the concentration of allelochemicals increased from 10^{-5} to 10^{-3} M and found that ferulic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid and *m*-coumaric acid were the most active compounds. However, *p*-hydroxybenzoic acid at concentration 10^{-3} M showed the greatest inhibitory germination and total seedling dry weight [7].



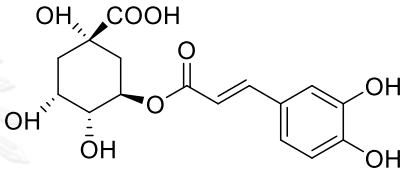
R_1	R_2	R_3	R_4	
H	H	H	H	benzoic acid
H	H	OH	H	*p*-hydroxybenzoic acid
OH	H	H	H	salycilic acid
H	OH	OH	H	protocatechuic acid
H	OH	OH	OH	gallic acid
H	OMe	OH	H	vanillic acid
H	OMe	OH	OMe	syringic acid



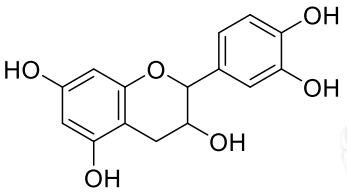
R_1	R_2	R_3	
H	H	H	cinnamic acid
OH	H	H	*o*-coumaric acid
H	H	OH	*p*-coumaric acid
H	OH	OH	caffeic acid
H	OMe	OH	ferulic acid



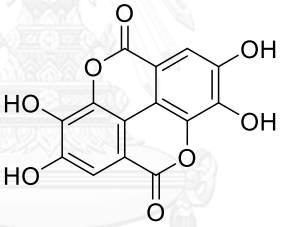
Chlorogenic acid



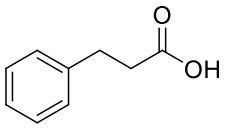
isochlorogenic acid



catechin



ellagic acid



hydrocinnamic acid

Figure 1.1 Common benzoic and cinnamic acid derivatives as allelopathic agents

1.3 Production of allelochemicals

Allelochemicals in plants are mostly secondary metabolites. It means not indispensable constituents in plant and exist only in plant kingdom. The potentials of those allelochemicals turning into new bioactive chemicals useful for more sustainable agriculture and safe food production for humanity have been realized for quite sometime [8]. Allelochemical released into the environment by various ways as presented in **Figure 1.2**

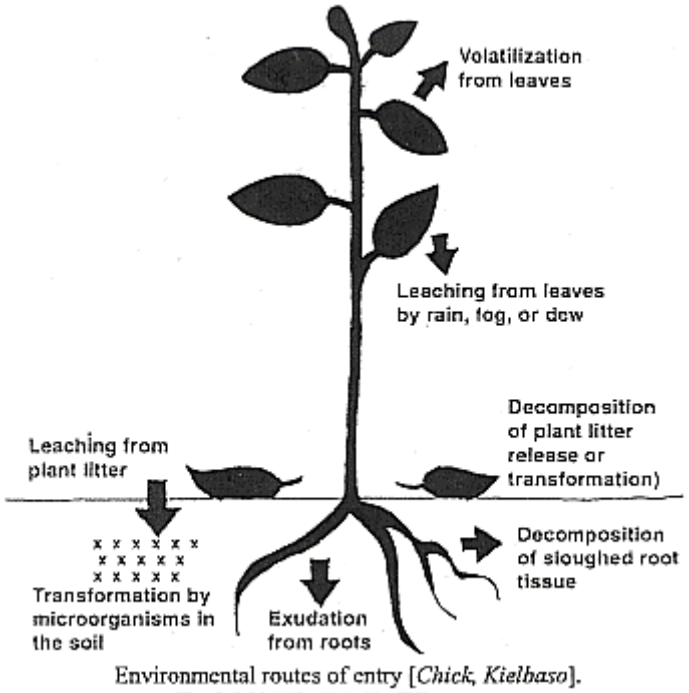


Figure 1.2 Allelochemicals released into the environment [9]

1.3.1 Volatilization from leaves

Allelopathic trees release chemicals in gas from through small openings in their leaves. Other plants absorb the toxic chemical and die [10].

1.3.2 Leaching from leaves by rain, fog or dew and plant litter

Rain causes the leaching of allelopathic substances from leaves which fall to the ground during period of stress; leading to the inhibition of growth and germination of crop plants [11, 12].

1.3.3 Exudation from root

Plants release chemicals into the soil through their roots. The released chemicals are absorbed by the roots of nearby trees. Exuding compounds are selectively toxic to other plants. Exudates are usually various phenolic compounds that tend to inhibit development [10].

1.3.4 Decomposition

Phytotoxic compounds from decomposing plant material, such as rye (*Secale cereal* L.) when used as a mulching material. Apart from shading and keeping the soil moist, rye mulch also inhibits both germination and growth of weed through release of phytotoxins [13].

1.4 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 the effects of allelochemicals on various aspects of plant growth and metabolism, has received fairly wide attention [1].

The followings are some important site 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
- 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

In nature, 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 environmental, 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 entire [1].

1.5 Botanical description of *Richardia brasiliensis* Gomes

Family: Rubiaceae

Synonym: *Richardia adscendens* (DC.) Steud.

Common name: Brazil Pusley

Local name: หญ้าท่าพระ (Ya Tha Pra)

History

Richardia was named for an English physician, Richard Richardson. *Brasiliensis* refers to the country of origin, Brazil.

Seedling

The cotyledons are oblong and smooth, with a distinct maroon area near the base (**Figure 1.3**). The first leaves are creased in the center, covered with stiff hairs and at right angles to the cotyledons.

Mature plant

Brazil Pusley is an annual or perennial from a thickened rootstock that may be deep (**Figure 1.4**). Its stems are up to 0.4 m long and may be found growing prostrate or ascending. The stems are freely branched, covered with somewhat stiff hairs and rarely root from lower nodes. The leaves are opposite, elliptic to ovate in shape and have a pointed to rounded tip. The leaf base is elongated and the petiole may be almost absent to 1 cm long. The leaves may be up to 6.5 cm long and 2.4 cm wide and are rough textured on both sides. The petioles of opposite leaves are connected by stipules which have become sheath-like. These sheaths have ascending hairs or bristles to about 5 mm long. The flowers are in a terminal head-like cluster, up to 15 mm in diameter, of 20 or more flowers. The flowers typically have 2 pairs of short, broad leaves underneath. The upper-most pair is usually much smaller and at right angles to the lower pair. The outer part of the flower consists typically of 6 narrow lobes, up to 3.5 mm long, which have hairy margins. The lobes are joined at the base, forming a tube up to 1.5 mm long. The petals are also united and are white in coloration. The tube is funnel form in shape and from 3-8 mm long. Each flower usually produces 3 nutlets up to 3 mm long and 2 mm wide. The outside of the nutlet has short thick hairs [14].

Figure 1.3 Seedling, Brazil Pusley, *Richardia brasiliensis* Gomes [14].

Figure 1.4 Mature plant, Brazil Pusley, *Richardia brasiliensis* Gomes [14].

1.6 Chemical constituents studies on *Richardia brasiliensis* Gomes.

In 2008, Danielle reported secondary metabolites isolated from the whole plant of *R. brasiliensis* which was subjected to exhaustive maceration with 95% EtOH for three days. Five compounds including isorhamnetin-3-*O*-rutinoside, oleanolic acid, *m*-methoxy-*p*-hydroxy-benzoic acid, *p*-hydroxybenzoic acid and scopoletin were isolated. The structures were identified using spectroscopic techniques such as IR, one and two-dimensional ^{1}H and ^{13}C NMR besides comparison with literature data (**Figure 1.5**) [15].

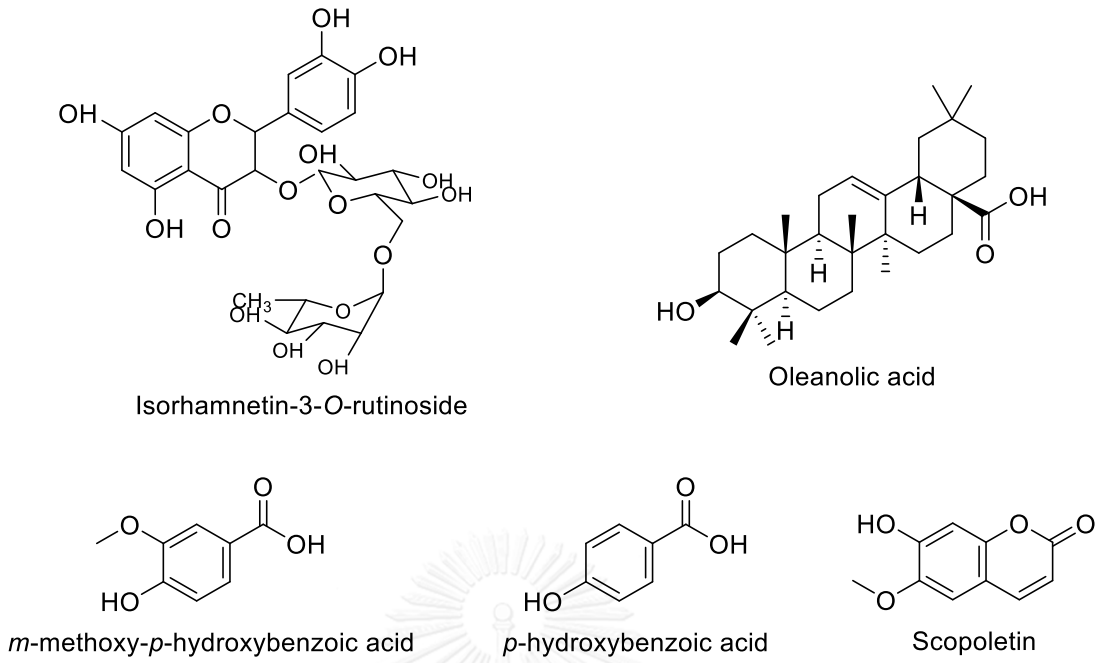


Figure 1.5 Metabolites isolated from *Richardia brasiliensis* Gomes.

1.7 Knowledge about studied weeds and crop plants

In this research *Mimosa pigra* L. was selected for bioassay test. In addition, various weeds including prickly chaff-flower (*Achyranthes aspera* Linn.), phak sian phee (*Cleome viscosa* L.), barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) and swollen finger grass (*Chloris barbata* L.) and crop plants such as corn (*Zea mays* L.), rice (*Oryza sativa* L.), sorghum (*Sorghum bicolor* L.), pakbung (*Ipomoea aquatic* Forssk.), gwarng-toong (*Brassica chinensis* L.) and Chinese kale (*Brassica alboglabra* L.H. Bailey) were chosen for allelopathic study.

1.7.1 *Mimosa pigra* L.

Mimosa pigra L. was invasive weed of the world. It reproduces via buoyant seed pods that can be spread long distances in flood waters. *M. pigra* has the potential to spread through natural grassland floodplain ecosystems and pastures, converting them into unproductive scrubland which are only able to sustain lower levels of biodiversity. In Thailand *M. pigra* has become a real menace in the country. It is difficult

to manage. This weed is blocks irrigation systems that supply rice fields, reducing crop yield and harming farming livelihoods [16].

Family: Fabaceae

Synonym: *Mimosa brasiliensis* Niederl.

Common name: bashful plant (English), catclaw (Puerto Rico), catclaw mimosa (English), chi yop (Thai), columbi-da-lagoa (Portuguese), eomrmidera (Spanish), espino (Spanish), giant sensitive plant (English), giant sensitive tree (English), giant trembling plant (English), juquiri (Portuguese), juquiri grand (Portuguese), kembang gajah (Malay), mai yah raap yak (Thai), maiyarap ton (Thai), malicia-de-boi (Portuguese), mimosa (English), mimose (German), putri malu (Indonesian Bahasa), semalu gajah (Malay), sensitiva (Spanish), trinh nu nhon (North Vietnam), una de gato (Spanish), xao ho (South Vietnam)

Local name: ไมยราบยักษ์ (Mai Yah Laap Yak)

Giant sensitive plant is a much-branched, hairy, perennial shrub typically 1-4 m tall. The alternate leaves are twice compound with 6-12 paired branches (pinnae) each containing 15-25 pairs of leaflets. The stems, branches and leaves contain prickles or thorns which are slightly bent downwards. The flowers are in heads (puffballs) about 1 cm wide, with numerous pink stamens extending outwards. The fruits are flattened, hairy, and the pods are arranged in clusters. Individual 1-seeded sections of the pod break out at maturity leaving the upper and lower margins intact like a frame. The seeds are gray-brown, about 6 mm long and 3 mm wide.

The cotyledons are oblong, about 1 cm long, thick, and blunt at the tip. The stem has a few scattered appressed hairs. The first true leaf is once compound. The next few leaves are twice compound, or divided with two pinnae. Above this, the next leaf or two have 4 pinnae (Figure 1.6) [17].

Figure 1.6 *Mimosa pigra* L. (Giant Sensitive Plant)

(Source: http://bangkrod.blogspot.com/2012/10/blog-post_8.html)

1.7.2 *Achyranthes aspera* L.

Family: Amaranthaceae

Synonym: *Achyranthes acuminata* E.Mey. ex Cooke & Wright

Common name: burweed (English), chaff-flower (English), chaffbur (English), devil's horsewhip (English), prickly chaff-flower (English), grootklits (Afrikaans), langklitskafblom (Afrikaans), na'eem (Arabic) and tu niu xi (Transcribed Chinese).

Local name: หญ้าพันงู (Ya pan ong)

Achyranthes aspera L. is an annual, biennial, lower portion perennial erect under shrub or rather stiff herb growing up to 0.3 to 1.0 m in height. It grows throughout the world in tropical and warmer regions (Figure 1.7) [18].

Figure 1.7 *Achyranthes aspera* L. (Prickly Chaff flower)

(Source: http://herbsdatabase.blogspot.com/2012/07/achyranthes-aspera-linn.html)

1.7.3 *Cleome viscosa* L.

Family: Capparaceae

Synonym: *Cleome icosandra* L.

Common name: Spider Flower and Tickweed

Local name: ผักเสี้ยนผี (Pak Sian Phee)

Cleoma viscosa L. is an annual erect, branched, viscid pubescent herb in 30-90cm height with 3-7 foliate leaves, white, yellow, pink flowers, stems grooved, densely clothed with glandular and simple hairs found in waste grounds and grassy places. The seed are 1.3 mm across, 1 mm thick, reddish brown and cleft narrow (**Figure 1.8**) [19].

Figure 1.8 *Cleome viscosa* L. (Spider Flower)

(Source: http://th.wikipedia.org/wiki/)

1.7.4 *Echinochloa crus-galli* (L.) P.Beauv.

Family: Poeceae

Synonym: *Echinochloa crus-corvi* (L.) P.Beauv.

Common name: barnyard grass

Local name: หญ้าข้าวนก (Ya Kao Nok)

Echinochloa crus-galli L. Beauv. is the most cosmopolitan and economically important member of the genus Echinochloa. Its rapid spread and aggressiveness are attributed to rapid growth, high seed production, low seed dormancy, and wide adaptability under various field conditions. It is a common weed in swamps and aquatic places. It also grows well in drier soil. This weed is an annual, erect, tufted or reclining at base; up to 200 cm tall. Stem are culms rooting at lower nodes, cylindrical, without hairs, and filled with white spongy pith. Leaf are linear with a broad round base and narrow top; blade long 10-40 cm ligule absent. Inflorescences are loose green to purplish, 10-25 cm long comprising compound racemes; spikelets more or less

elliptical and pointed, usually slightly hairy; awns, if present, green to purplish, 2-5 mm long (Figure 1.9) [20].

Figure 1.9 *Echinochloa crus-galli* L. Beauv. (Barnyard grass)

(Source: http://ladda.co.th/jurnal/HerbicideResistance.php)

1.7.5 *Chloris barbata* Sw.

Family: Poeceae

Synonym: *Chloris inflata* Link

Common name: Swollen finger grass (English), giant finger grass (English), purpletop chloris (English), swollen windmill grass (English), bärtiges Gilbgras (German) and paraplygräs (Swedish)

Local name: หญ้ารังนก (Ya Rung Nok)

Chloris barbata Sw. is an annual or short-lived perennial. Culms loosely tufted, ascending or decumbent at base and rooting at lower nodes, 0.2–1 m tall. Leaf sheaths keeled, glabrous; leaf blades flat or folded, 10–40 cm, 4–8 mm wide, glabrous, apex acute; ligule short, ciliate. Racemes digitate, 5–15, erect or ascending, 3–8 cm, often somewhat flexuous and purplish; rachis scabrous. Spikelets with 3 or 4 florets, 3(–4)-

awned; lower glume 1.2–1.5 mm; upper glume 1.7–2.5 mm, shortly mucronate; lemma of fertile floret elliptic in side view, 1.7–2.5 mm, pilose on keel, ciliate on upper margins with 1–1.5 mm hairs; awn 4.5–7 mm; upper florets sterile, lemmas empty, inflated, overlapping to form a knob at side of fertile floret; second lemma turbinate, truncate, 1–1.5 mm, glabrous or sparsely appressed-pilose on back, awn subequaling awn of fertile floret; third (and forth) lemmas orbicular, awn somewhat shorter. This weed causes harmful effect to crop (**Figure 1.10**) [21].

Figure 1.10 *Chloris barbata* Sw. (Swollen finger grass)

(Source: http://www.brrd.in.th/rkb/weed/index.php-file=content.php&id=22.htm)

1.7.6 *Zea mays* var. *ceratina* Kuleshov

Family: Poaeceae

Synonym: *Zea mays* var. *ceratina* Kuleshov

Common name: Corn

Local name: ข้าวโพดข้าวเหนียว (Kao Pod Kao Neaw)

Corn is a robust annual grass, usually single-stemmed, occasionally tillering, with stout culm, sometimes stilt-rooted at the basal nodes, to 1-4 m high, even to 6 m, and 3-4 cm in diameter; The flowers are monoecious and pollinated by wind (**Figure 1.11**) [22].

Figure 1.11 *Zea mays* var. *ceratina* Kuleshov (Corn)

(Source: http://thai.alibaba.com/product-gs/white-waxy-corn-seeds-for-sale-1407882557.html)

1.7.7 *Oryza sativa* L.

Family: Poaceae

Synonym: *Oryza communissima* Lour.

Common name: Rice (Riceberry)

Local name: ข้าวไรซ์เบอรี่ (Kao Rice Berry)

Rice is a typical grass, forming a fibrous root system bearing erect culms and developing long flat leaves. It has a semi-aquatic lifestyle, requiring water particularly during the reproductive growth phase. It forms multiple tillers, consisting of a culm and leaves, with or without a panicle. The panicle emerges on the uppermost node of a culm, from within a flag-leaf sheath and bears the flowers in spikelets. The culm

consists of a number of nodes and hollow internodes that increase in length and decrease in diameter up the length of the culm. Primary tillers emerge from nodes near the base of the main culm and secondary and tertiary tillers emerge sequentially from these. Single leaves develop alternately on the culm, consisting of a sheath, which encloses the culm and a flat leaf blade. The leaf forms a collar or junctura between the sheath and blade and a ligule and two auricles develop on the inside of the junctura and base of the leaf blade respectively. Cultivars can vary widely in the length, width, color and pubescence of the leaves (**Figure 1.12**) [23-25].

Figure 1.12 *Oryza sativa* L. (Riceberry)
(Source: http://www.aecnews.co.th/idea/read/17)

1.7.8 *Sorghum bicolor* L. Moench

Family: Poaceae

Synonym: *Agrostis nigricans* (Ruiz & Pav.) Poir.

Common name: sorghum

Local name: ข้าวฟ่าง (Kao Fang)

Sorghum is an upright, short-day, summer annual that is a member of the Poaceae family. The grass blades are flat, stems are rigid, and there are no creeping

rhizomes. Sorghum has a loose, open panicle of short, few-flowered racemes. As seed matures, the panicle may droop. Glumes vary in color from red or reddish brown to yellowish and are at least three quarters as long as the elliptical grain. The grain is predominately red or reddish brown (**Figure 1.13**) [26-28].

Figure 1.13 Sorghum bicolor L. Moench (Sorghum)

(Source: http://www.doa.go.th/ardc/suphan/sg_grow.htm)

1.7.9 *Brassica chinensis* Jusl var. *parachinensis* (Bailey) Tsen & Lee

Family: Brassicaceae

Synonym: *Brassica chinensis* L.

Common name: Chinese cabbage-PAI TSAI

Local name: กวางตุ้งดอก (Gwarng Toong Dok)

Brassica chinensis is a succulent herb forming rosettes, of open or tight vegetative heads followed by flowering stalks reaching 20-50 cm in height. Leaves are succulent and light green. The leaves are eaten fresh, boiled, fried, or fermented. Some varieties produce seeds that can be pressed for oil. It is perennial, biennial, often grown as an annual (**Figure 1.14**) [29].

Figure 1.14 *Brassica chinensis* Jusl var. *parachinensis* (Bailey) Tsen & Lee (Gwarng Toong)

(Source: https://www.flickr.com/photos/khamin_thai/6152544109/)

1.7.10 *Brassica oleraceae* L. var. *alboglabra* (L.H. Bailey) Musil

Family: Brassicaceae

Synonym: *Brassica alboglabra* L.H.Bailey

Common name: Chinese kale

Local name: คะน้า (Ka na)

Brassica alboglabra, also called Chinese Broccoli, has glossy, blue-green leaves with crisp and thick stems. This vegetable adapts well to cold and hot climates and is grown all year round (**Figure 1.15**) [30].

Figure 1.15 *Brassica alboglabra* L. var. *alboglabra* (L.H. Bailey) Musil (Chinese kale)

(Source: http://www.vegetweb.com/)

1.7.11 *Ipomoea aquatica* Forsk. var. *reptan*

Family: Convolvulaceae

Synonym: *Ipomoea natans* Dinter & Suess.

Common name: Morning-glory-like

Local name: ผักบุ้ง (Pakbung)

Morning-glory-like is a floating herbaceous vine, it has long, branching stems containing a milky sap, with roots extending from leaf nodes. Leaves are alternate, simple, and generally arrowhead-shaped. They are 2-6 inches long and 0.75-2.25 inches wide. Petioles are 1-4 inches long. Flowers are white to lavender and funnel-shaped (morning-glory-like). Fruit is oval to spherical, and is 0.5 inches long and woody when mature. Fruit capsules contain 1 - 4 seeds. Water spinach can grow at a rate of 4 inches per day, producing 84 tons of fresh weight biomass per acre in 9 months. Branching stems can reach 70 feet in length (**Figure 1.16**) [31].

Figure 1.16 *Ipomoea aquatica* Forsk. var. *reptan* (Pakbung)

(Source: http://www.thaiseed.co.th/index.aspx?ProductID=Product-100820102072113)

1.8 The objectives of this research

This research aims to use the extracts of weed in agriculture. The objective of this research can be summarized as follows:

1. To study allelopathic effects of the extracts of *Richardia brasiliensis* Gomes by evaluating seed germination and growth inhibition of weed.

2. To identify active substances that affected on seed germination and the growth inhibition of weed.

CHAPTER II

EXPERIMENTAL

2.1 Plant materials

The whole plants of Ya Tha Pra (*R. brasiliensis*) were collected from a sugar cane field in Khonkaen province, Thailand in October 2012. The plants were dried under the sunlight and then ground into powder.

2.2 Model plants for bioassay test

The seeds of giant mimosa (*M. pigra*) were collected from Nakhonsawan province and stored at 5 ºC until use. Before being used, the seeds were soaked in hot distilled water 70 ºC for 24 h to soften the seed coat. The seeds of selected weeds including; prickly Chaff flower (*A. aspera*), phak sian phee (*C. viscose*), barnyard grass (*E. crus-galli*) and swollen finger grass (*C. barbata*) were collected from Nakhonratchasima and Phachinburi provinces and stored at 5 ºC until use. The seeds of crop plants were bought from Chua Youg Seng seed company Limited including corn (*Z. mays*), rice (*O. sativa*), sorghum (*S. bicolor*), pakbung (*I. aquatic*) Gwarng-toong (*B. chinensis*) and Chinese kale (*B. alboglabra*).

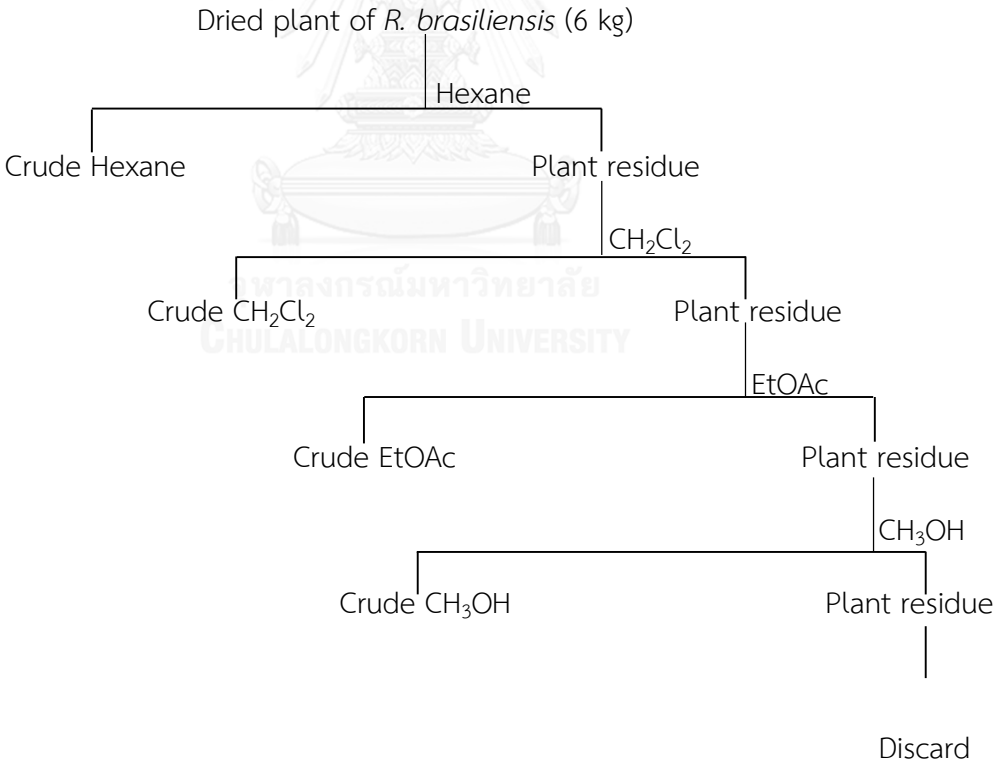
2.3 Instrument and equipment

HPLC was performed on VertiSep UPS C18 4.6×250 mm, 5µm. Silica gel, no.7734 and 7729 were used for column chromatography. TLC was performed on an aluminum sheets percolated with silica gel (Merch's Kiesel gel 60 PF_{254}) and observed under UV light. The GC-MS was performed by Agilent 6890 gas chromatograph in electron impact (EI, 70eV) mode coupled to an HP 5973 mass selective detector and fitted with a fused silica capillary column (HP-5MS) (30 m x 0.25 mm x 0.25 µm film thickness). Helium (1.0 mL/min) was used as a carrier gas. Samples were injected in the split less mode at ratio of 1:10-1:100. The injector was kept at 250 ºC and the transfer line at 280 ºC.

The MS was EM mode at 1,576.5 EM Voltage, in the *m/z* range 50-550. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in the literature and supplemented by the Wiley 7n and Natural Products GC-MS libraries.

2.4 Extraction procedure

The whole plants of *R. brasiliensis* (6 kg dry weight) were ground to fine powder and extracted by soaking in hexane for three days at RT. The residue was repeatedly extracted by CH_2Cl_2, EtOAc and CH_3OH, respectively for three times. The extract was filtered and evaporated with a rotary evaporator at 40 °C. The extraction procedure for the plants was shown in **Scheme 2.1**.



Scheme 2.1 The extraction procedure of *R. brasiliensis*

2.5 Experiments for bioassays

2.5.1 General procedure for seed germination inhibition test

Tested crude extract was dissolved in an appropriate solvent at concentration of 0.1, 0.5, 1.0, 2.5 and 5.0 g equivalent (gE). Three mL of crude extract solution were poured into petri dishes (diameter 90 mm) containing a filter paper, leave overnight to remove solvent. The place 50 seeds of bioassay samples were place on the filter paper for each petri dish and then 5.0 mL of distilled water was added to each plate. Control seeds were sown on the filter paper moistened with water without the extract. The bioassay was repeated three times. Then, petri dishes were closed and incubated at 25 °C, 12/12 light to observe the growth after 7 days. The inhibition percentage was calculated shown below [32].

Germination Inhibition (%) = (C-T) ×100 / C

T is germination number of treated.

C is germination number of controlled.

*Germination inhibition 100% complete inhibitory effect

2.5.2 General procedure for growth inhibition test

The extracts 0.1, 0.5, 1.0, 2.5 and 5.0 g equivalent (gE) were dissolved in an appropriate solvent in 3 mL and poured into test tube (diameter 30 mm and length 120 mm) containing 40 mL of agar, stirred until well-mixed. The controlled tube was prepared without the extract using the same methodology. All test tubes were covered with aluminum foil, dried by oven at 50 °C for 10-12 h, stirred until well-mixed, followed by the addition of 3.0 mL of distilled water to each tube. The bioassay was conducted three times using six selected seeds with radical root length 1-2 mm (seeds for bioassay were soaked in hot distilled water 70 °C for 24 h and germinated in petri dish one night before testing) in each tube. The tubes were sealed with transparent vinyl film and kept in growth chamber at 25°C, 12/12 light. The root and shoot lengths

were recorded at 7 days after transplanting. The %inhibition was calculated as shown below [32].

Growth Inhibition (%) = (C-T) ×100 / C

T is root or shoot length of treated.

C is root or shoot length of controlled.

*Growth inhibition 100% complete inhibitory effect

2.6 Isolation

2.6.1 The separation of CH_3OH extract

The methanol extract 150 g of *R. brasiliensis* was chromatographed on silica gel (No. 7729) for quick column chromatography. The column was initially eluted with EtOAc and increasing polarity by adding CH_3OH from 5%CH_3OH in EtOAc to 80% CH_3OH in EtOAc. Each fraction was examined and combined by TLC. After that, the constituent of CH_3OH extract was analyzed using HPLC.

The analysis of the CH_3OH extract and standard compounds were conducted by HPLC (Waters 600 Controller and Waters 2996) using VertiSep^{TM} UPS C18 HPLC column, 4.6×250 mm, 5µm with inject volume 20 µL. Mobile phase was solvent A: 98%H_2O, and 2%AcOH in 0.018 M NH_4OAc, solvent B: 68%H_2O, 25%CH_3OH, 5%butanol and 2%AcOH in 0.018 M NH_4OAc. Gradient system: (a) 0.0-1.0 min isocratic at 10% B; (b) 1.0-21 min linear gradient from 10 to 25% B; (c) 21.0-36.0 min linear gradient from 25 to 45% B; (d) 36.0-56.0 min linear gradient from 45 to 100% B; (e) 56.0-65.0 min linear gradient from 100 to 10% B; flow rate 1 mL/min. The wavelength of the UV detector was 280 nm. The retention times of mixed reference compounds compared with those of the major peaks of CH_3OH extract were recorded [7].

2.6.2 The separation of CH_2Cl_2 extract

The CH_2Cl_2 extract of the whole plant of *R. brasiliensis* 50 g was dissolved in CH_2Cl_2, mixed with silica gel no. 7729 and dried. Elution was performed in polarity gradient method with a mixture of CH_2Cl_2 and EtOAc by increasing EtOAc from 5% EtOAc in CH_2Cl_2 to 100% EtOAc. The eluted fractions were examined and combined according to TLC behaviors. Each fraction was further analyzed by GC-MS

CHAPTER III

RESULTS AND DISCUSSION

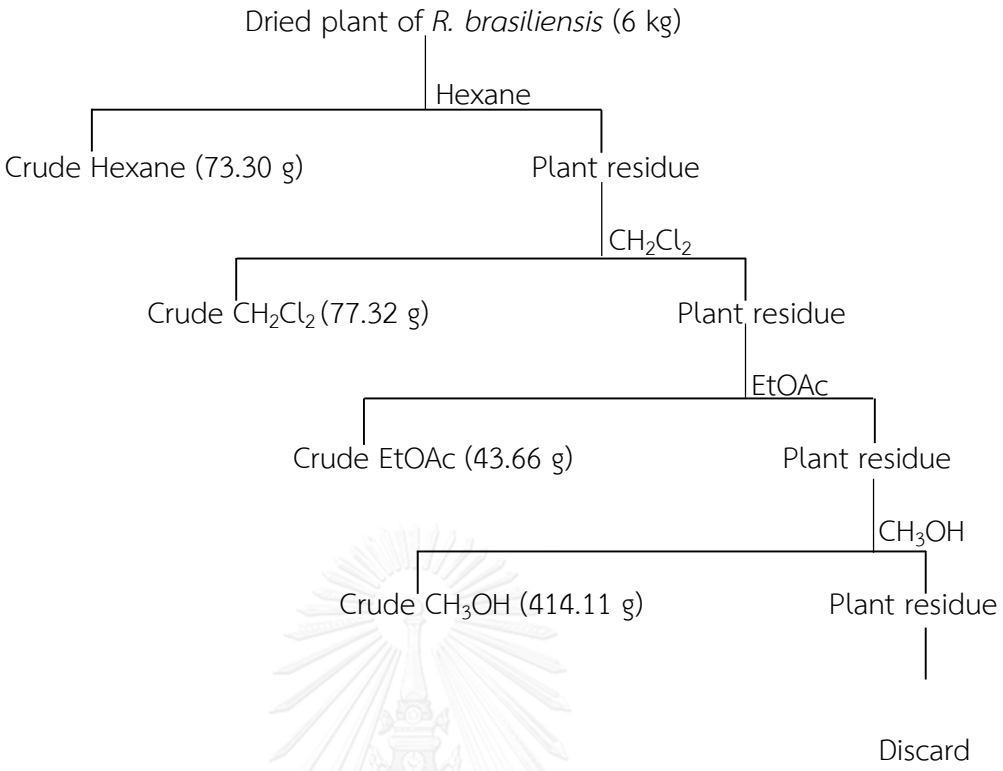
Richardia brasiliensis Gomes. (Rubiaceae) , was one of noxious weeds in Thailand. The main objective of this research is to examine the allelopathic effects of the extracts from *R. brasiliensis* on seed germination and growth inhibition of *M. pigra* and to identify the isolated substances responsible for those inhibitory effects. In addition, the inhibitory effect of the extract on other weeds and crops was conducted. Four tested weeds were prickly chaff-flower (*A. aspera*), phak sian phee (*C. viscosa*), barnyard grass *(E. crus-galli*) and swollen finger grass (*C. barbata*), whereas six crop seeds were corn (*Z. mays*), rice (*O. sativa*), sorghum (*S. bicolor*), pakbung (*I. aquatic*), gwarng-toong (*B. chinensis*) and Chinese kale (*B. alboglabra*).

3.1 The extraction of *Richardia brasiliensis*

The dried whole plants of *R. brasiliensis* (6.0 kg) were milled to fine powder and extracted by soaking in hexane for three days at RT. The residue was repeatedly extracted by CH_2Cl_2, EtOAc and CH_3OH, respectively for three times. The extract was filtered and evaporated with a rotatory evaporator to obtain 73.3, 77.3, 43.7 and 414.1 g (1.22, 1.29, 0.73 and 6.90%yield based on starting material) of hexane, CH_2Cl_2, EtOAc and CH_3OH extracts, respectively. The summary of the extraction is depicted in **Table 3.1** and **Scheme 3.1**.

Table 3.1 Weight and %yield of the crude extracts of *R. brasiliensis*

Solvent	Remarks	Weight (g)	Yield (% w/w)
Hexane	Yellow liquid	73.30	1.22
CH_2Cl_2	Green solid	77.32	1.29
EtOAc	Brown liquid	43.66	0.73
CH_3OH	Dark brown liquid	414.11	6.90



Scheme 3.1 The extraction procedure of *R. brasiliensis*

3.2 Bioassay experiments

3.2.1 Germination inhibition of *M. pigra*

All crude extracts including hexane, CH_2Cl_2, EtOAc and CH_3OH of *R. brasiliensis* were assayed for seed germination inhibition on *M. pigra* at five different concentrations (0.1, 0.5, 1.0, 2.5, and 5.0 gE) compared with the control. The results are summarized as shown in **Figures 3.1** and **3.2**.

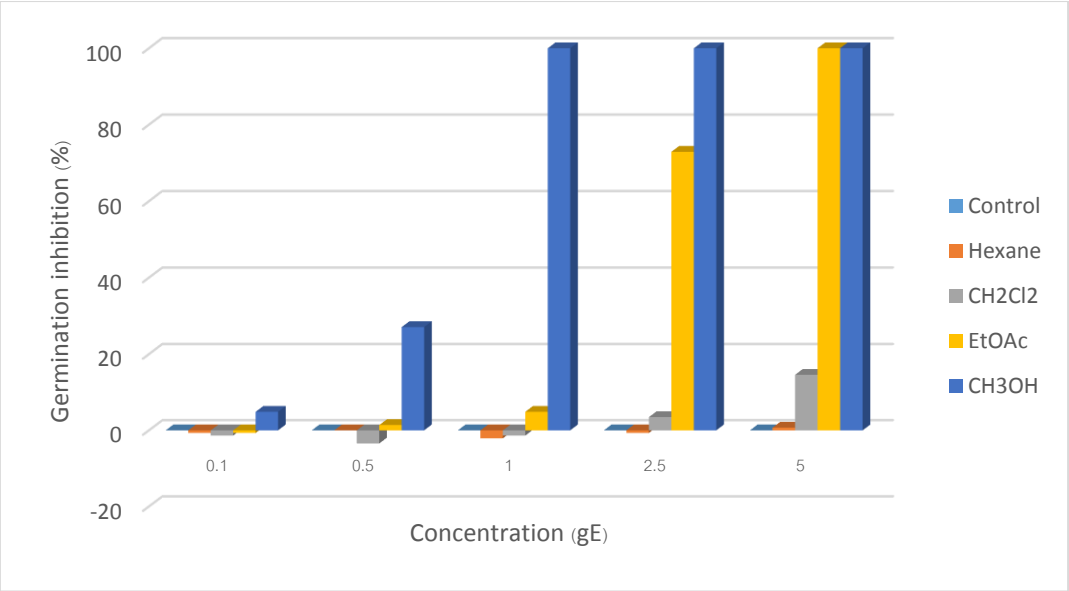


Figure 3.1 The seed germination inhibition of *R. brasiliensis* extracts on *M. pigra*

The CH_3OH extract of *R. brasiliensis* revealed significant inhibition on the seed germination of *M. pigra* higher than the control. The highest germination inhibition of 100% was observed when the concentrations of CH_3OH extract used were 1.0, 2.5 and 5.0 gE. The CH_3OH extract was found to exhibit this activity more than those of hexane, CH_2Cl_2 and EtOAc (**Figure 3.1**), except for in the case of 5.0 gE of EtOAc extract which also displayed the germination inhibition of 100% (see also **Tables A2.** and **A3.** in Appendices) . Therefore, the concentration of 1.0 gE was selected for further investigating on seed germination inhibition with other weeds. The difference in germination inhibitions may cause by different allelochemical quantities and their stability in plant tissues [33]. It should also be noted that the extracts of *R. brasiliensis* have not been reported for allelopathy.

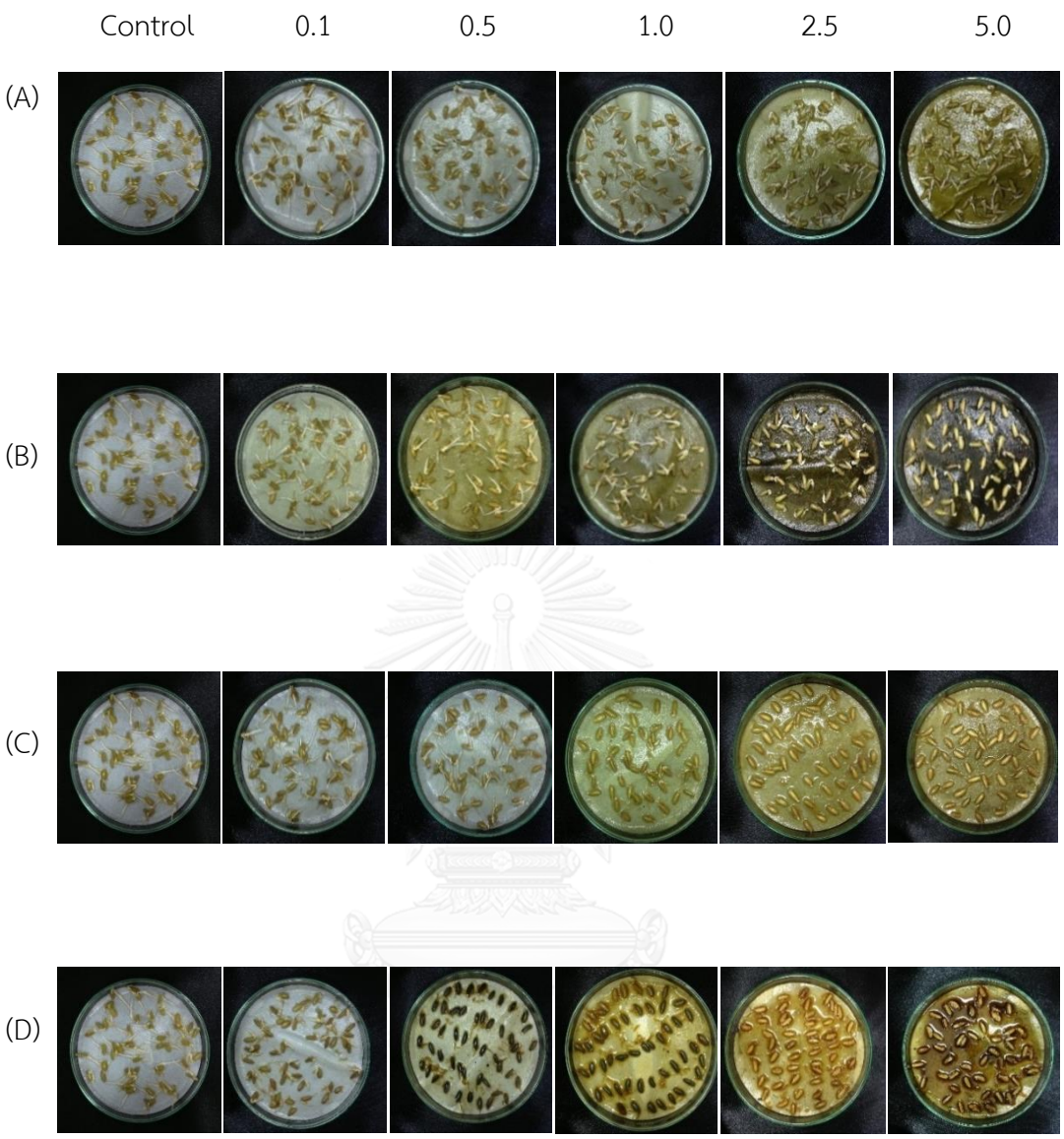


Figure 3.2 Effect of crude extracts of *R. brasiliensis* by various solvents; Hexane (A), CH_2Cl_2 (B), EtOAc (C) and CH_3OH (D) on germination of *M. pigra* (2 DAT)

3.2.2 Growth inhibition of *M. pigra*

Five concentrations of hexane, CH_2Cl_2, EtOAc and CH_3OH extracts from *R. brasiliensis* were assayed for %growth inhibition by observing root and shoot elongation of *M. pigra.* The results of the root and shoot elongation inhibition are presented in **Figures 3.3-3.4**.

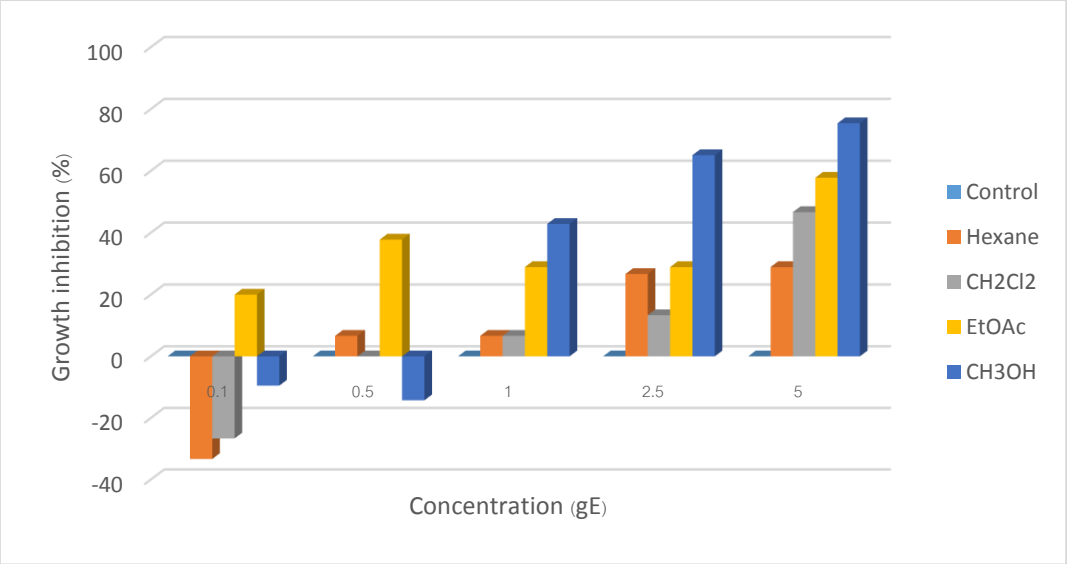


Figure 3.3 The shoot elongation inhibition of the extracts from *R. brasiliensis* on *M. pigra*

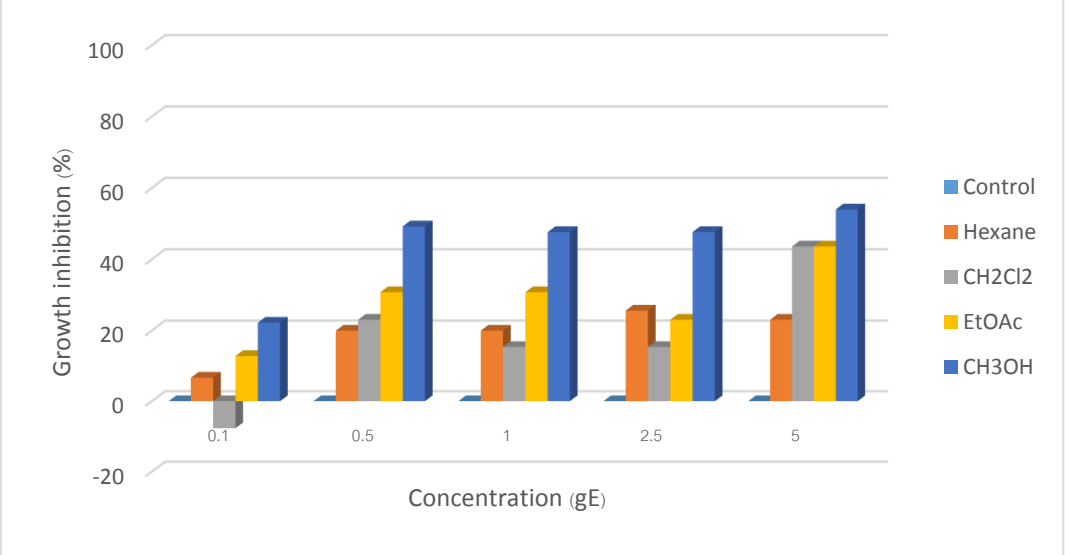


Figure 3.4 The root elongation inhibition of the extracts from *R. brasiliensis* on *M. pigra*

The effects of the extracts from *R. brasiliensis* on the growth inhibition of *M. pigra* exhibited that the CH_3OH extract significantly reduced the shoot length compared with the control and other extracts (**Figure 3.5**). The CH_3OH extract displayed the shoot elongation inhibition 75.39, 65.07 and 42.85% at 5.0, 2.5 and 1.0 gE, respectively. (**Figure 3.3**). The results of the root length inhibition were similar to those for the shoot. The CH_3OH extract at 0.5, 1.0, 2.5 and 5.0 gE concentration could reduce the root length of *M. pigra,* 49.20, 47.61, 47.61 and 53.96% respectively, while the hexane CH_2Cl_2 and EtOAc extracts at the same concentration gave less inhibition (see also **Tables A4.** and **A5.** in Appendices).

The plant growth inhibition was evaluated by percent inhibition of shoot and root length. The CH_3OH extract displayed its potential to inhibit the shoot and root length of *M. pigra* with different extents at different concentrations. Aslani Farzad *et al.* (2014) reported that the different concentrations of CH_3OH extract had various inhibitory impacts on the growth of target plant. It could be a reflection of plant growth inhibitor concentration being released by plant tissues. Higher concentration of the CH_3OH extract would contain greater amount of inhibitory substances, and thus had a higher degree of inhibition [34]. Similarly, in 2010 Md. Abdus Salam reported the allelopathic potential of aqueous CH_3OH extract of neem leaves on seed germination and seedling growth of different plants viz. cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), wild buckwheat (*Eriogonum compositum* Douglas ex Benth.), sand fescue (*Festuca myuros* L.), timothy (*Phleum pratense* L.), barnyardgrass and *Echinochloa colonum* [L.] Link. Inhibitory activity was dependent on the extract concentrations and the higher extract concentration had the stronger inhibitory activity [35].

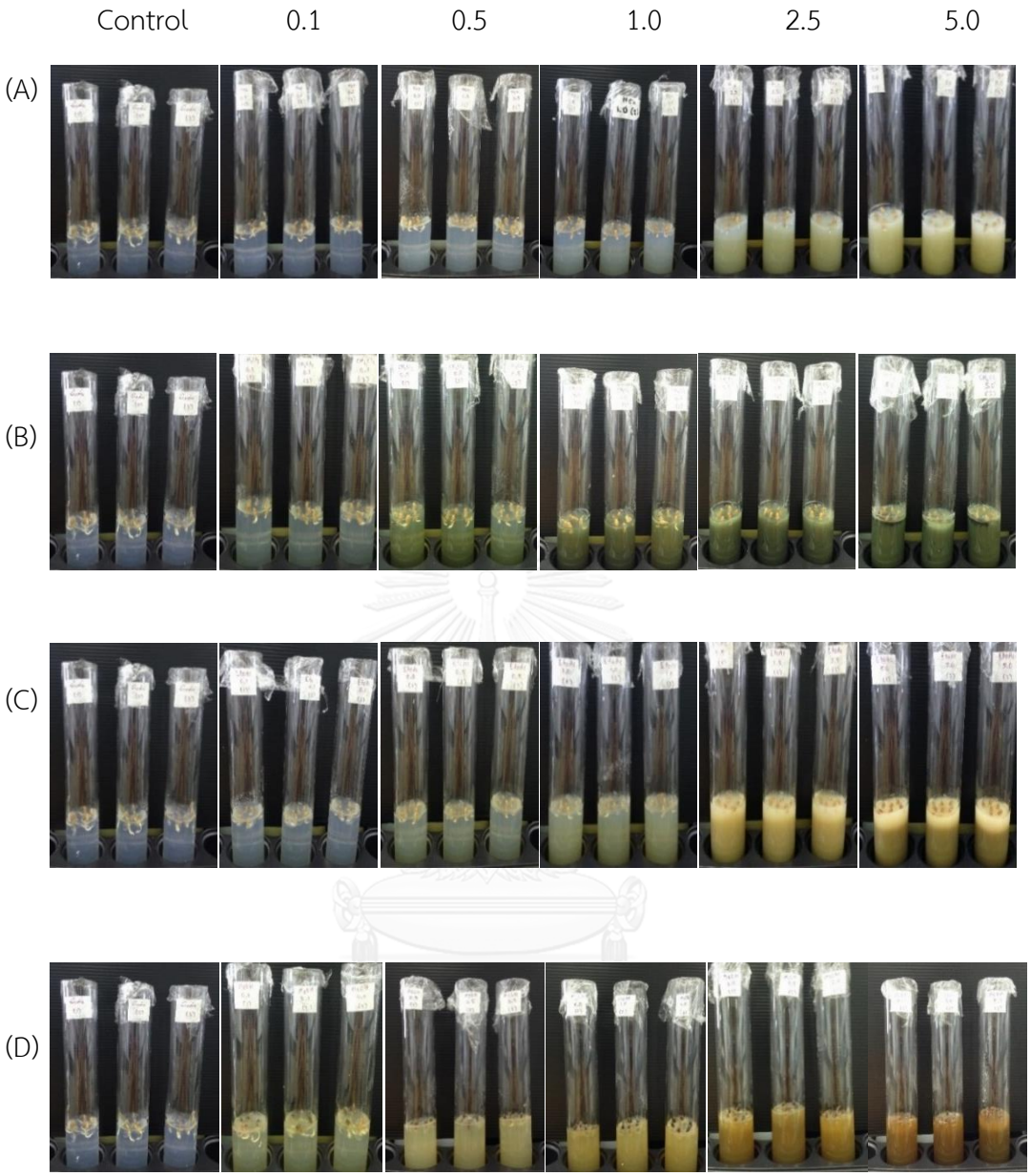


Figure 3.5 Effect of crude extracts of *R. brasiliensis* by various solvents; Hexane (A), CH_2Cl_2 (B), EtOAc (C) and CH_3OH (D) on growth of on *M. pigra* (2 DAT)

3.2.3 The effect of the CH_3OH extract on selected weeds and crops

The potential allelopathic activity of the CH_3OH extract of the whole plant of *R. brasiliensis* at 1 gE concentration was examined on the germination and growth inhibition of prickly chaff-flower, phak sian phee, barnyard grass and swollen finger grass. Furthermore, the examination was also extended on crops including corn, rice, sorghum, pakbung, gwarng-toong and Chinese kale seeds. This investigation was conducted to confirm the effectiveness and possibility of the use of the CH_3OH extract as weed inhibitor from nature. The results of the effect of CH_3OH extract on selected weeds and crops at 1 gE are depicted in **Figures 3.6-3.7**.

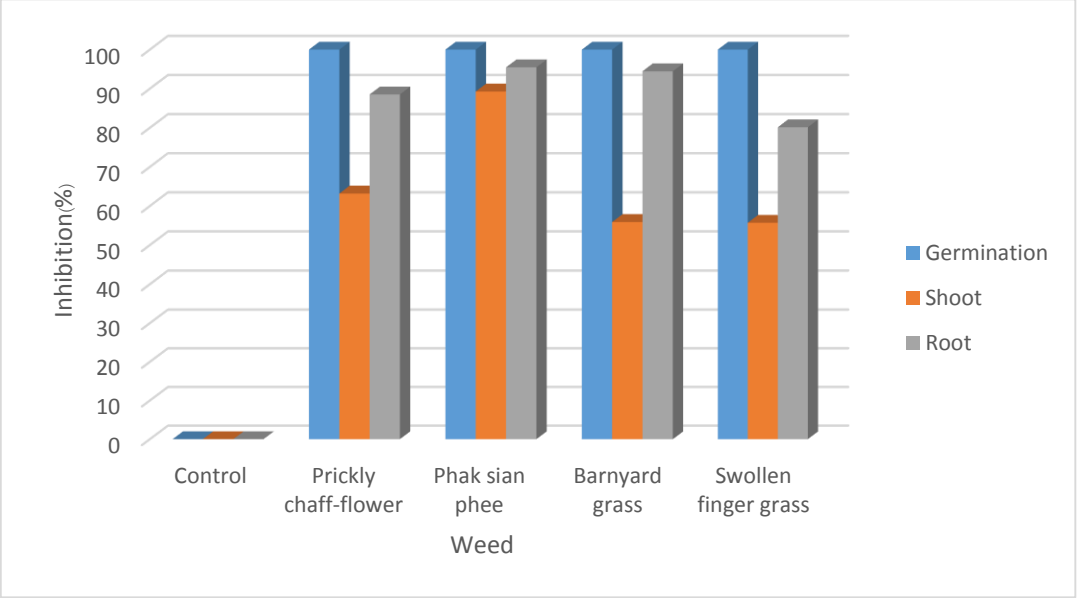


Figure 3.6 The effect of the CH_3OH extract on selected weeds at 1 gE

The results displayed that the CH_3OH extract inhibited 100% germination of prickly chaff-flower, phak sian phee, barnyard grass and swollen finger grass. The shoot and root lengths of the tested plants were reduced as 63.20 and 88.54%, respectively. Thus, this extract affected on the germination and growth inhibition of these weeds at 1gE concentration.

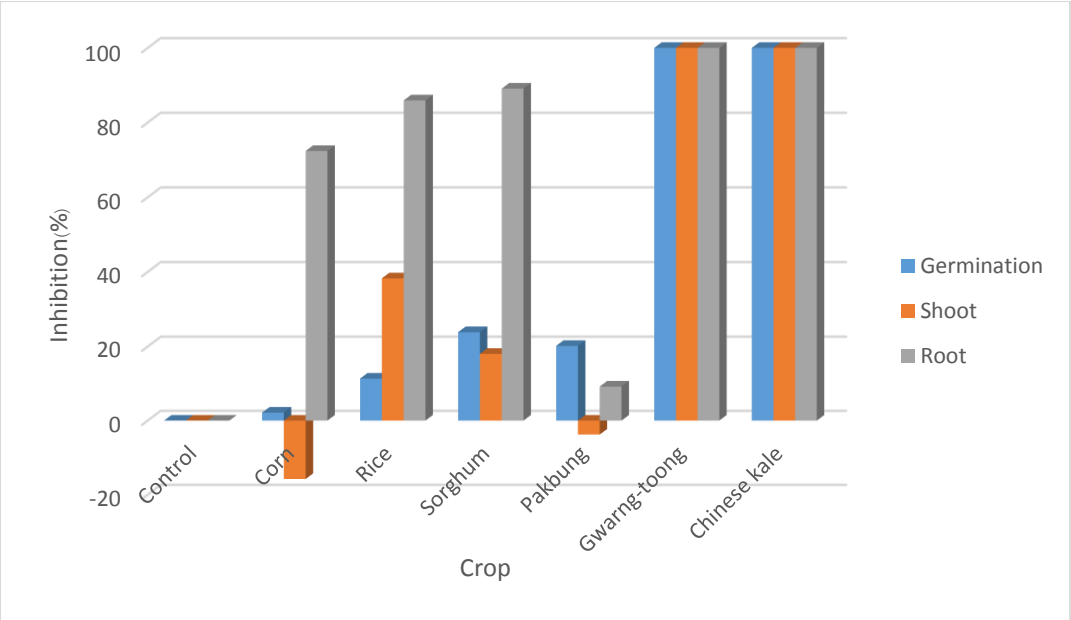


Figure 3.7 The effect of CH_3OH extract on selected crop at 1 gE

The results revealed that different crops showed different response to the CH_3OH extract. To illustrate this, this extract could inhibit the seed germination and growth inhibition of gwarng-toong and Chinese kale. On the other hand, the germination inhibition percentage of 2.15, 20.17, the percent of inhibition for shoot length of 15.76, -3.75 and the root length of 72.39, 9.20 were observed for corn and pakbung, respectively. Although the CH_3OH extract inhibited the root length of corn, but it was not considered as a serious impact because corn is classified as a C4-photosynthetic plant roots which are fibrous root system and strong upright stems [36]. Therefore, corn and pakbung showed excellent tolerance to the CH_3OH extract as compared to the gwarng-toong and Chinese kale.

The results above demonstrated the allelopathic potential of CH_3OH extract from *R. brasiliensis*. The CH_3OH extract did not affect on the crops, thus it is a great feature if it can be applied in field crops for management of weeds.

Figure 3.8 The germination and growth inhibitions of CH_3OH extract on selected weeds at 1 gE Prickly chaff-flower (A), Phak sian phee (B), Barnyard grass (C) and Swollen finger grass (D) (7 DAT)

Figure 3.9 The germination and growth inhibitions of CH_3OH extract on selected crops at 1 gE Corn (A), Rice (B), Sorghum (C), Pakbung (D), Gwarng-toong (E) and Chinese kale (F) (7 DAT)

3.3 Fractionation and allelopathic activity test of the CH_3OH extract

3.3.1 Fractionation by quick column

The CH_3OH extract 150 g was chromatographed on silica gel (No. 7729) quick column. The column was initially eluted with EtOAc and increasing polarity with CH_3OH from 5% CH_3OH-EtOAc to 80% CH_3OH-EtOAc. Each fraction was examined by TLC and combined according to its TLC pattern. The results of fractionation are shown in **Table 3.2.**

Table 3.2 The separation of the CH_3OH extract from *R. brasiliensis*

Eluent (V/V)	Fraction code	Remarks	Weight (g)
100% EtOAc-5% CH_3OH/EtOAc	**RBM-1**	Green solid	2.15
5-10% CH_3OH/EtOAc	**RBM-2**	Brown solid	2.19
20% CH_3OH/EtOAc	**RBM-3**	Brown liquid	7.42
20-80% CH_3OH/EtOAc	**RBM-4**	Dark-brown liquid	83.83

3.3.2 Allelopathic activity assays

The separation of the CH_3OH extract from *R. brasiliensis* by quick column gave four fractions including **RBM-1**, **RBM-2**, **RBM-3** and **RBM-4**. Those four fractions were subjected to germination and growth inhibition test on *M. pigra* at 0.1, 0.5, 1.0, 2.5 and 5.0 gE. The results are presented in **Figures 3.10-3.12**.

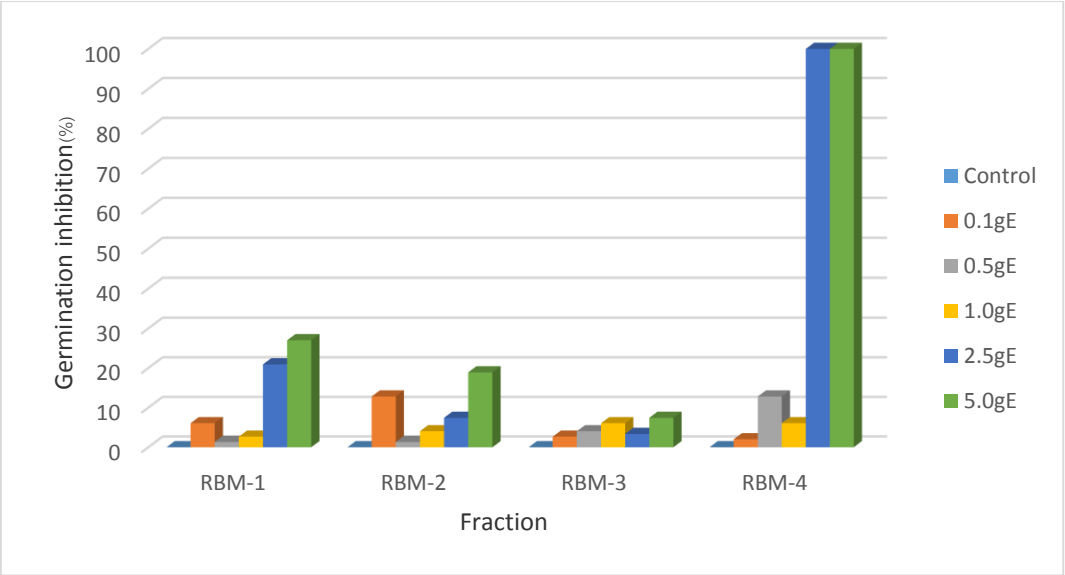


Figure 3.10 The germination inhibition of fractions derived from the separation of the CH_3OH extract on *M. pigra*

For germination inhibition of CH_3OH fractionations on *M. pigra*, **RBM-1**, **RBM-2**, **RBM-3** and **RBM-4** (0.1, 0.5 and 1.0) did not inhibit the seed germination of *M. pigra* compared with the control, except for **RBM-4** at 2.5 and 5.0 gE being exhibited significant inhibition on the seed germination of *M. pigra* by 100% (**Figures 3.10** and **3.13**). From the above experiments, it was thought that the separation of the extract would lead to the sub-fractions which may display more effective germination inhibition compared with the CH_3OH extract (**Figure 3.1**). The suppression of the germination of seed weeds by allelochemicals may stem from two processes. First, at least from germination until emergence, since the small seeds have large surface area, they can be easily exposed to allelochemicals. Second, when residue is used as mulch, the allelopathic toxins are released onto the soil surface. Thus resulting buildup of allelochemicals are beneficial to the crop next season. [37].

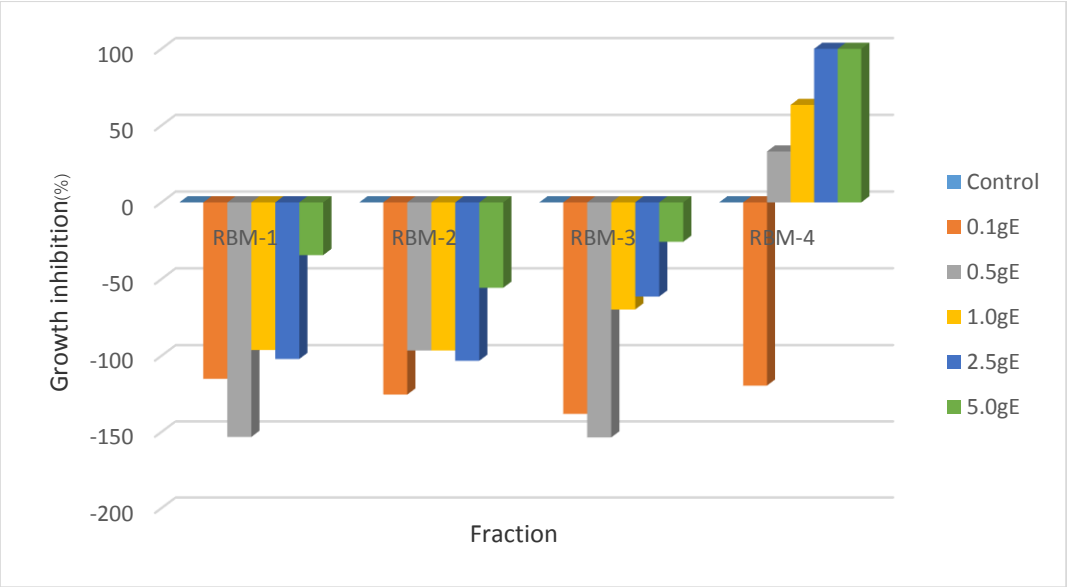


Figure 3.11 The shoot inhibition of the fractions derived from the separation of the CH_3OH extract on *M. pigra*

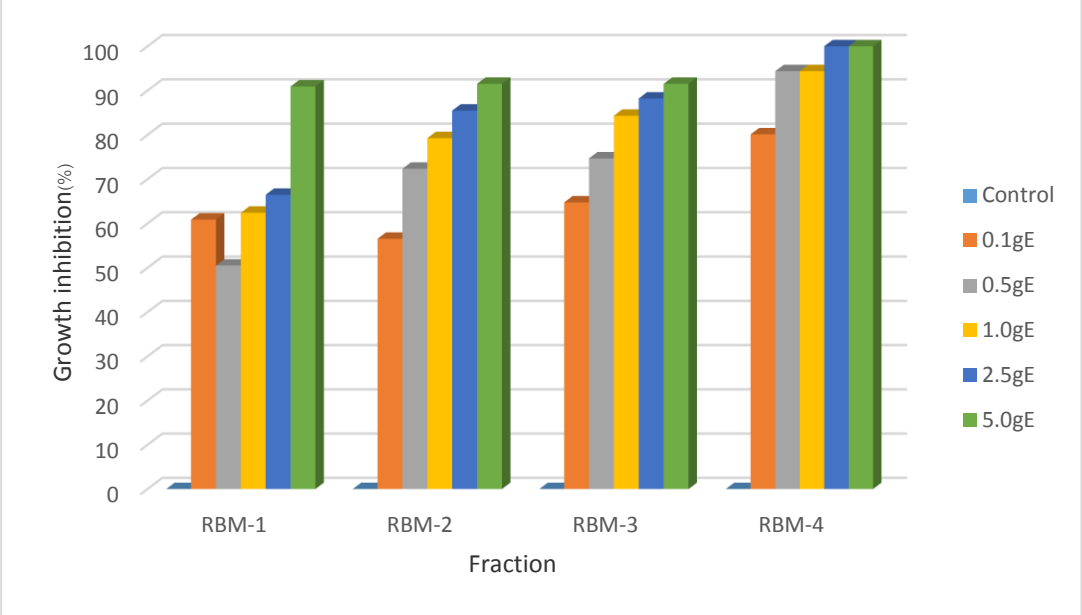


Figure 3.12 The root inhibition of the fractions derived from the separation of the CH_3OH extract on *M. pigra*

The effects of the CH_3OH fractionations (**RBM-1**, **RBM-2**, **RBM-3** and **RBM-4**) on the shoot and root length of *M. pigra* at concentrations of 0.1, 0.5, 1.0, 2.5 and 5.0 gE were examined. The results displayed that with five concentrations of all fractionations, at 0.1 gE the promotion of shoot length of *M. pigra* could be observed **(Figures 3.11** and **3.14)**. On the other hand, **RBM-4** at 0.5, 1.0, 2.5 and 5.0 gE was significantly suppressed the shoot length with %inhibition of 33.13, 63.49, 100 and 100, respectively. The growth inhibition of the root of all four CH_3OH fractionations at 0.1, 0.5, 1.0, 2.5 and 5.0 gE was reduced in response to the different concentrations of CH_3OH fractionations compared with the control **(Figures 3.12** and **3.14)**. Generally, the roots of plants are the first tissue to contact allelochemicals, so the inhibition of root length is observed. Wherewith, the allelochemicals affected the performance of ion uptake and water uptake reduced [38, 39]. The inhibition of ion uptake is directly related to membrane perturbation. The inhibited ion uptake in many studies would directly lead to disruption of plant water balance, or the resulting mineral deficiency would indirectly change the water relation. Some suggested that these observations be due to interference with normal membrane function and disruption of active transport [40, 41].

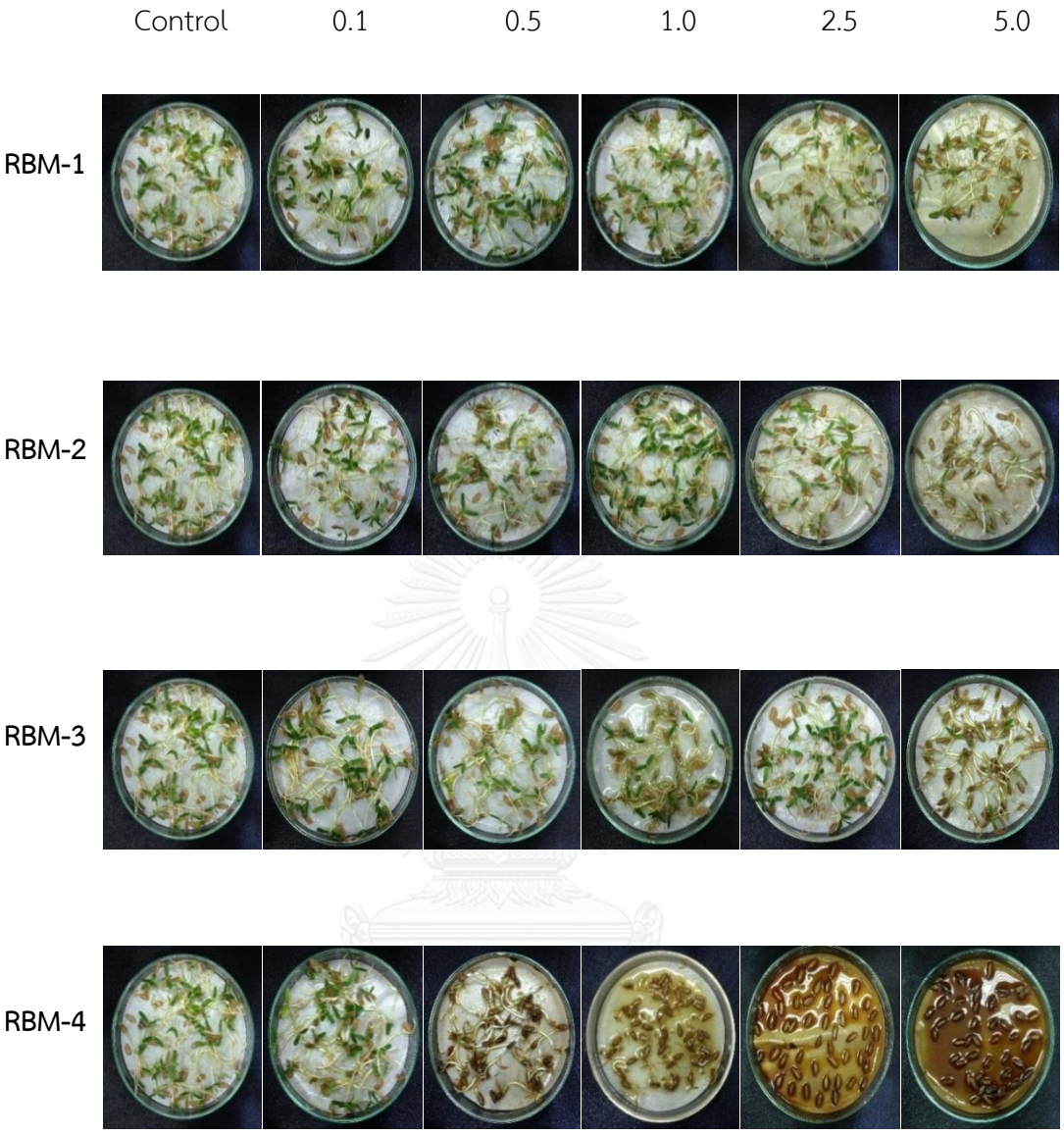


Figure 3.13 The germination inhibition of the CH_3OH fractionations at control, 0.1, 0.5,1.0, 2.5 and 5.0 gE on *M. pigra* (7 DAT)

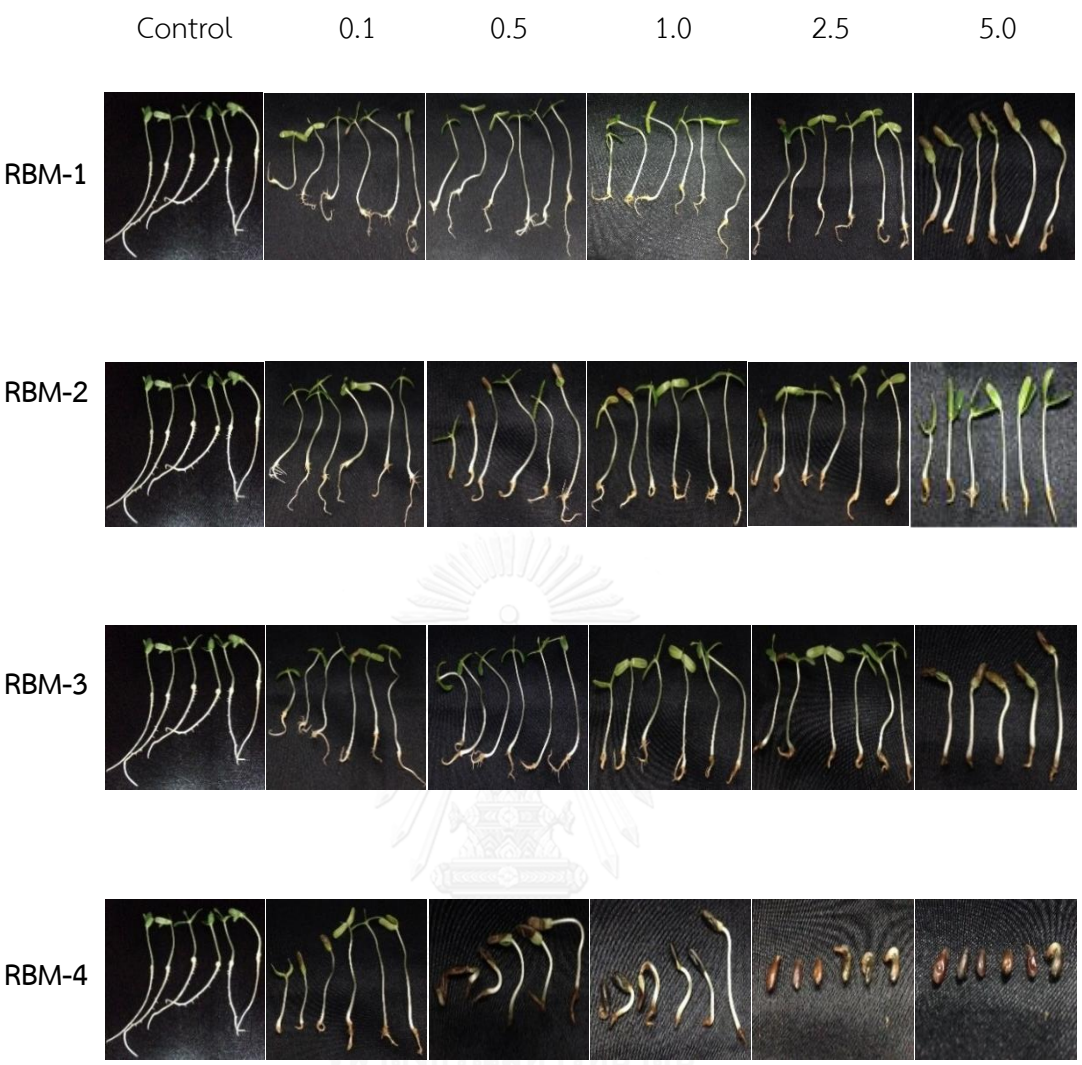


Figure 3.14 The shoot and root inhibition of the CH_3OH fractionation at control, 0.1, 0.5, 1.0, 2.5 and 5.0 gE on *M. pigra* (7 DAT)

3.3.3 Chemical constituent study of the CH_3OH extract using HPLC

This study was conducted with HPLC to investigate the presence of allelopathic compounds from the fractionations of the CH_3OH of *R. brasiliensis* which displayed the greatest inhibitory effect of germination and growth inhibition on *M. pigra*. The results are presented in **Figure 3.15** and **Table 3.3**.

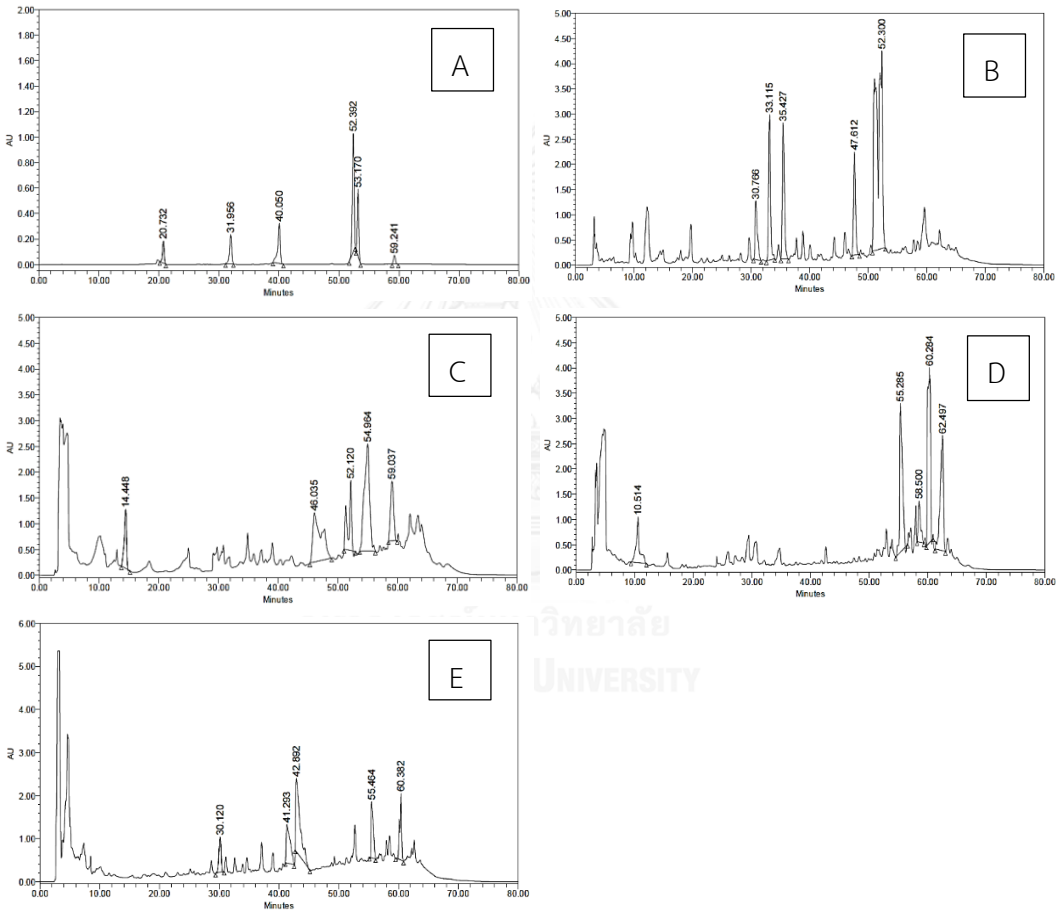


Figure 3.15 Comparison of HPLC chromatograms of (A) a mixture of standard chemicals (3,4-dihydroxybenzoic acid (1), 4-hydroxybenzoic acid (2), caffeic acid (3); *p*-coumaric acid (4), ferulic acid (5), benzoic acid (6)). (B) **RBM-1** (C) **RBM-2** (D) **RBM-3** and (E) **RBM-4**

The HPLC analysis obviously demonstrated the presence of allelopathic substances in the CH_3OH fraction with different extent in each fraction **(Table 3.3)**. The CH_3OH fraction was further separated into four fractions **RBM-1 (Figure 3.15B)** contained 4-hydroxybenzoic acid and *p*-coumaric acid. The detected component in **RBM-2** (**Figure 3.15C**) was *p*-coumaric acid which was the same as that in **RBM-1**. **RBM-3 (Figure 3.15D)** revealed the presence of benzoic acid, while **RBM-4 (Figure 3.15E)** contained 4-hydroxybenzoic acid, caffeic acid and benzoic acid. All compounds were identified by comparing their retention times on HPLC chromatogram with authentic standards **(Figure 3.16)**.

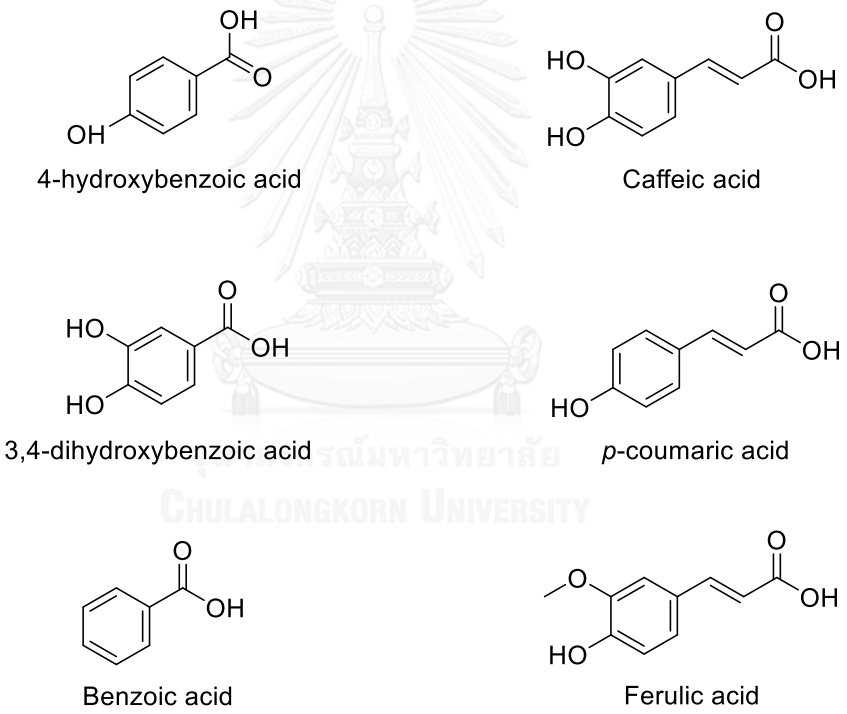


Figure 3.16 The structures of selected phenolic compounds

Table 3.3 The HPLC analysis of the fractions from the CH_3OH extract

Fraction	Retention time (min)	%Area	Chemicals
RBM-1	30.76	6.19	4-hydroxybenzoic acid
	33.11	13.09	Unknown
	35.42	13.26	Unknown
	47.61	9.63	Unknown
	52.30	57.84	*p*-coumaric acid
RBM-2	14.44	9.02	Unknown
	46.03	27.25	Unknown
	52.12	14.60	*p*-coumaric acid
	54.96	36.76	Unknown
	59.03	12.37	Unknown
RBM-3	10.51	9.69	Unknown
	55.28	26.93	Unknown
	58.50	5.94	Unknown
	60.28	33.77	Benzoic acid
	62.49	23.67	Unknown
RBM-4	30.12	9.75	4-hydroxybenzoic acid
	41.29	19.26	Caffeic acid
	42.89	39.82	Unknown
	55.46	15.17	Unknown
	60.38	15.99	Benzoic acid

3.4 Bioassay test of selected compounds

1 mM of selected six commercial compounds namely 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, benzoic acid, caffeic acid, *p*-coumaric acid and ferulic acid was tested for their performance on allelopathic activity in germination, and root and shoot inhibition on corn, rice, sorghum, pakbung, Chinese kale and gwarng-toong seeds. In addition, the examination was also carried out on weed seeds including giant mimosa, prickly chaff-flower, phak sian phee, barnyard grass and swollen finger grass. The results are presented in **Figures 3.17-3.18**.

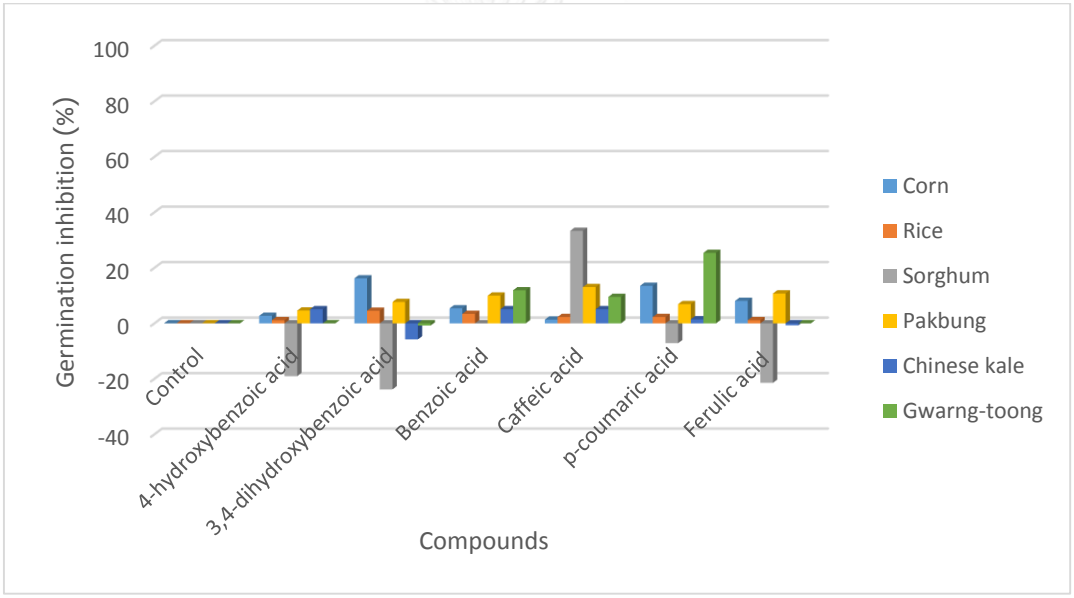


Figure 3.17 The effects of selected compounds (1 mM) on the germination inhibition of six crop seeds

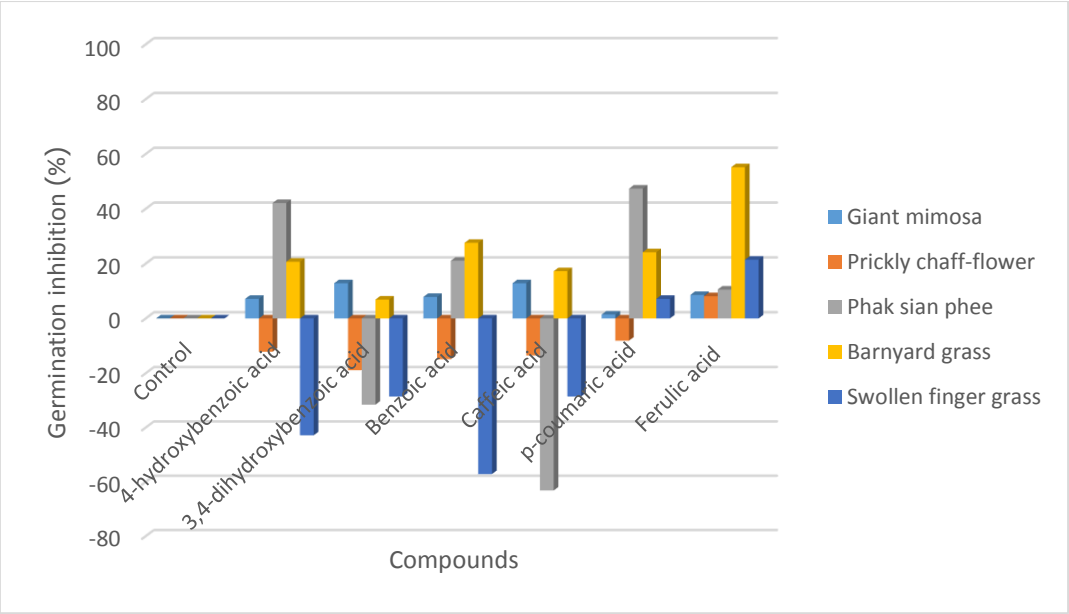


Figure 3.18 The effects of selected compounds (1 mM) on the germination inhibition of six weed seeds

The seed germination inhibition of selected crops for six phenolic compounds at 1 mM are presented in **Figures 3.17** and **3.18**. All chosen compounds could not inhibit the germination of crop seeds (see also **Tables A10.** and **A11.** in Appendices). The similar trend of the inhibitory effect on weed germination was found for the crop. Ferulic acid, however, exhibited the germination inhibition on barnyard grass more than other plants (55.17%). Interestingly, the inverse effect was observed. To illustrate this, the promotion of seed germination of sorghum seeds could be found in the case of 4-hydroxybenzoic acid (-19.04%), 3,4-dihydroxybenzoic acid (-23.80%), *p*-coumaric acid (-7.14%) and ferulic acid (-21.42%). For prickly chaff-flower, the similar results were observed for 4-hydroxybenzoic acid (-12.16%), 3,4-dihydroxybenzoic acid (-18.91), benzoic acid (-14.86), caffeic acid (-13.51%) and *p*-coumaric acid (-8.10%). However, the description of germination shown in **Figures 3.19** and **3.20**. More details will be discussed in Topic 3.4.1. To summarize, there was no inhibitory effect on germination at 1 mM of six selected phenolic compounds on crops and weeds, but enhancing the promotion of seed germination.

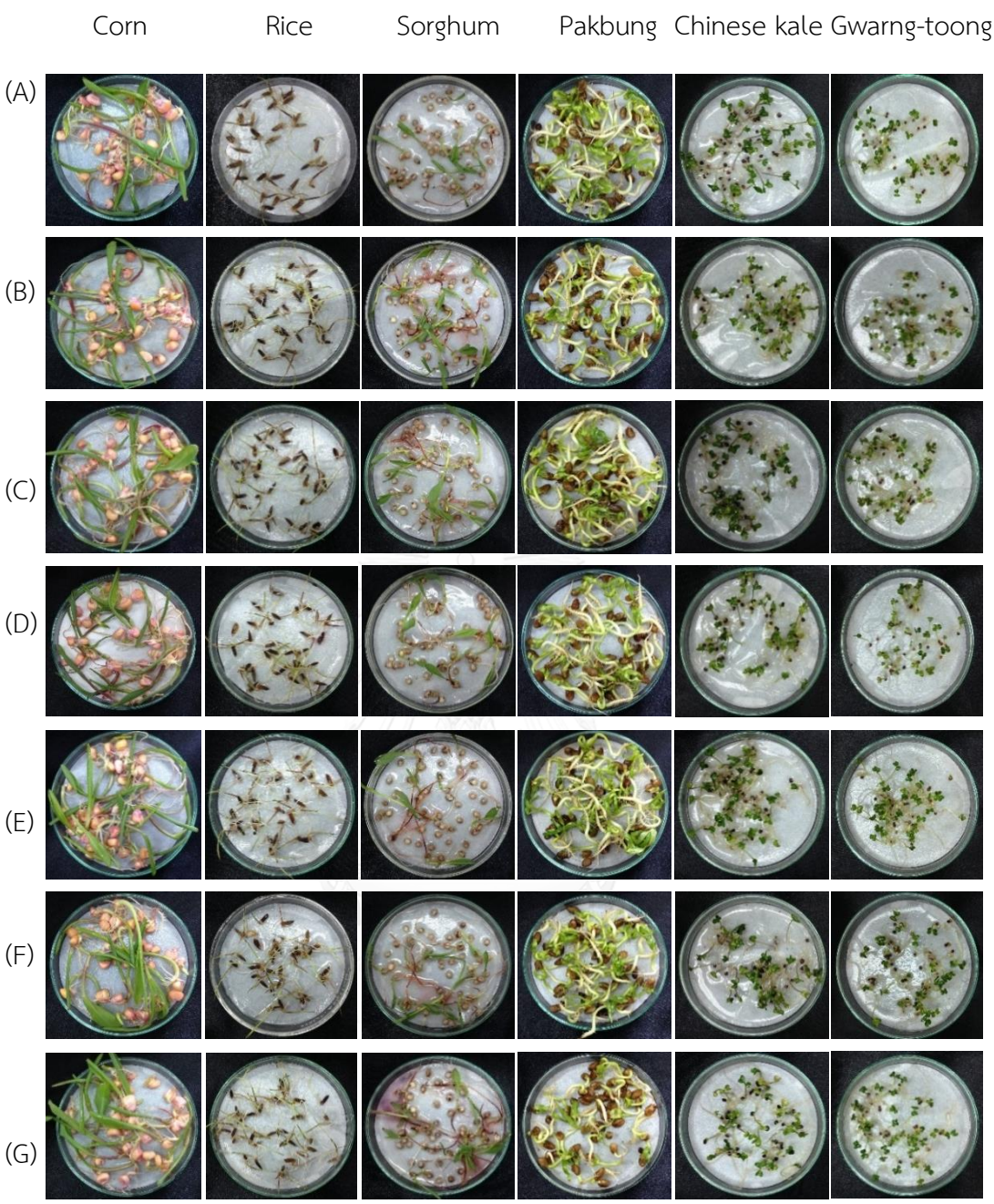


Figure 3.19 The germination inhibition of corn, rice, sorghum, pakbung, Chinese kale and gwarng-toong on phenolic compounds at 1mM (A) control, (B) 4-hydroxybenzoic acid, (C) 3,4-dihydroxybenzoic acid, (D) benzoic acid, (E) caffeic acid, (F) *p*-coumaric acid and (G) ferulic acid (7 DAT)

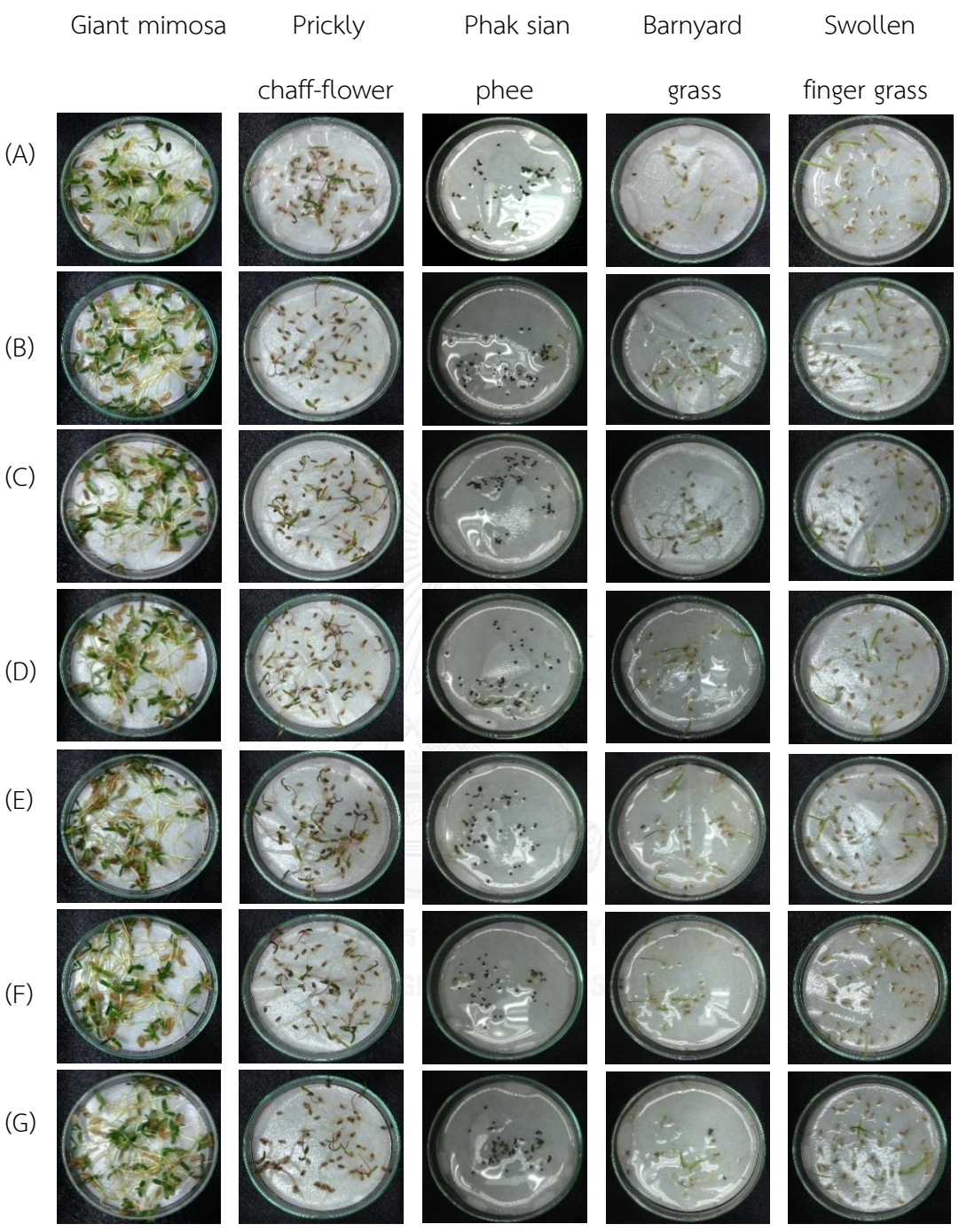


Figure 3.20 The germination inhibition of giant mimosa, prickly chaff-flower, phak sian phee, barnyard grass and swollen finger grass on phenolic compounds at 1 mM (A) control (B) 4-hydroxybenzoic acid (C) 3,4-dihydroxybenzoic acid (D) benzoic acid (E) caffeic acid (F) *p*-coumaric acid and (G) ferulic acid (7 DAT)

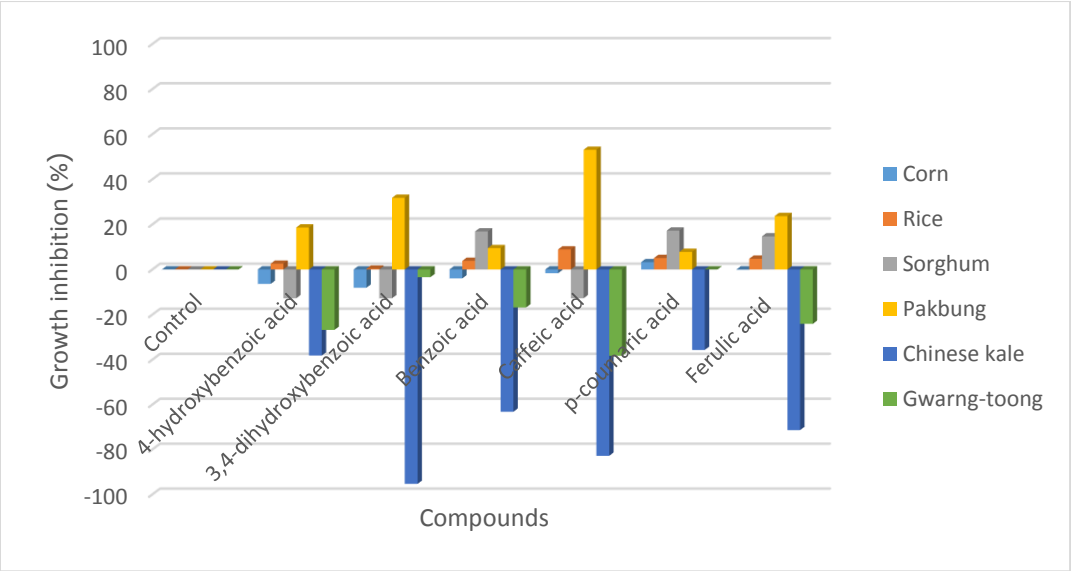


Figure 3.21 Inhibitory effect of compounds on shoot growth of selected crop plants at 1 mM

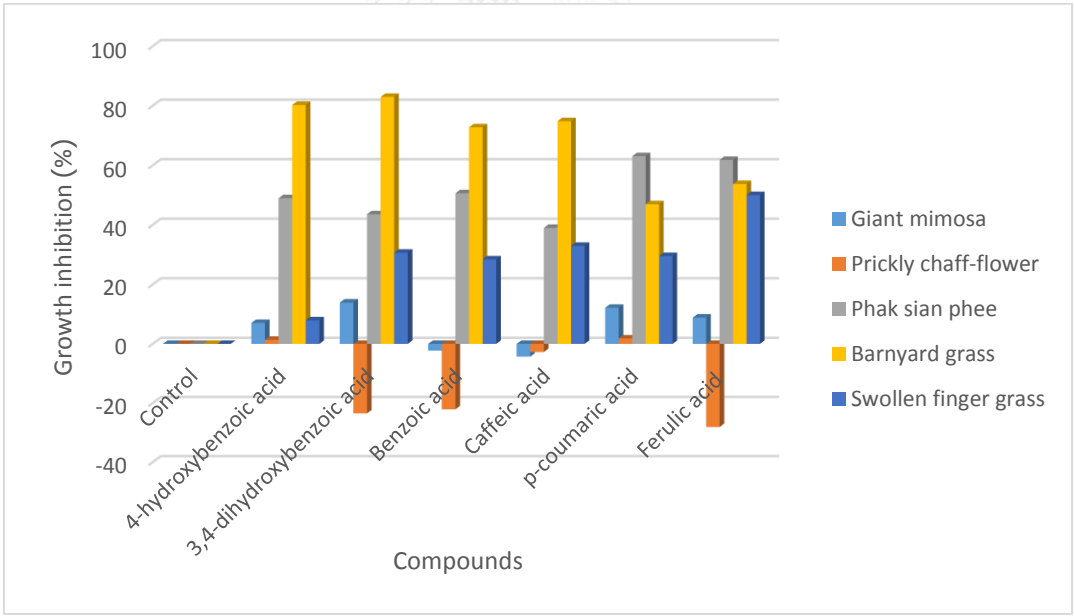


Figure 3.22 Inhibitory effect of compounds on shoot growth of selected weeds at 1 mM

The effects of selected six compounds on the shoot length of selected crops and weeds are shown in **Tables A12.** and **A13.** in Appendices. It is manifest that these compounds had no effect on the shoot length of most crops (**Figure 3.21**). The shoot length of weeds was affected by phenolic compounds. For phak sian phee, *p*-coumaric acid showed the best result on the reduction of the shoot length 63.07%. For barnyard grass, 3,4-dihydroxybenzoic and ferulic acids displayed the reduction the shoot length of 82.99 and 50.00%, respectively. The same observation for swollen finger grass could be seen (**Figure 3.22**). However, all six compounds did not show the inhibitory effect on the shoot length of the crops, but affected on the shoot length of weeds.

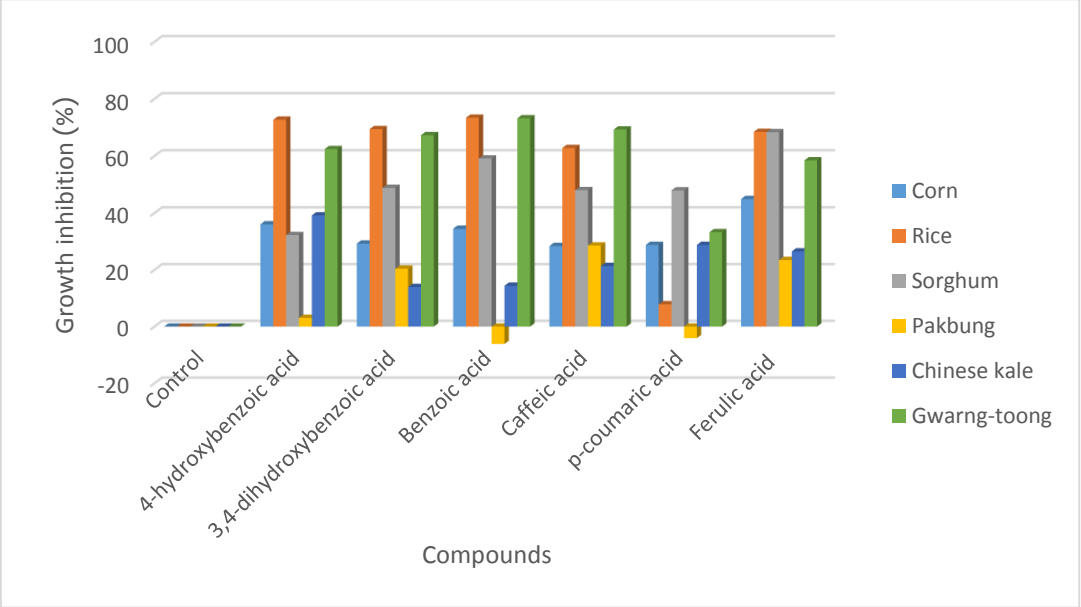


Figure 3.23 Inhibitory effect of compounds on root growth of selected crop plants at 1 mM

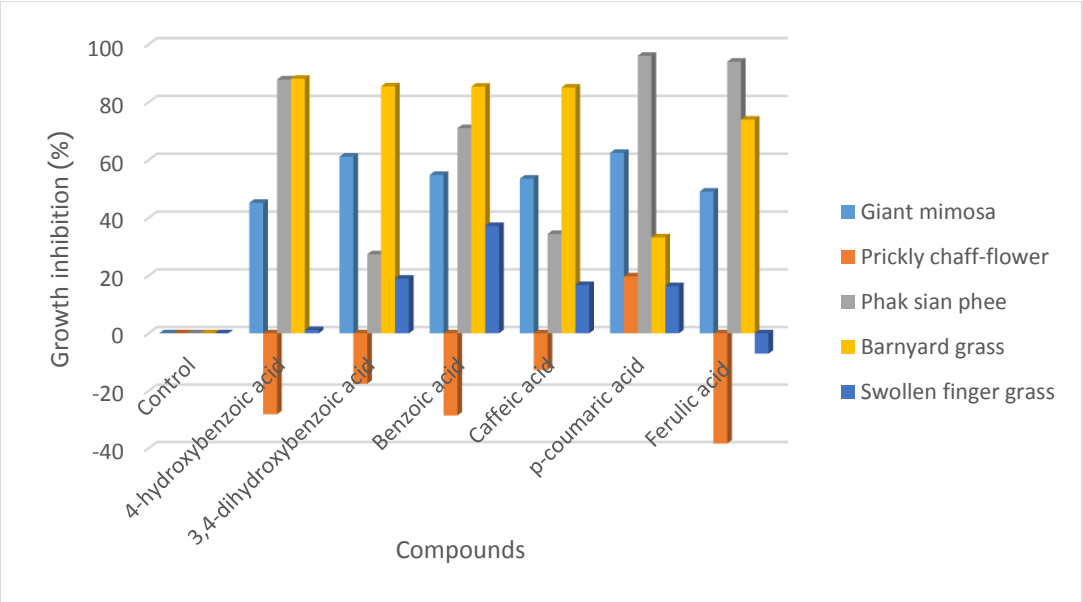


Figure 3.24 Inhibitory effect of selected compounds on the root growth of selected weeds at 1 mM

All six phenolic compounds at 1mM inhibited the root length of crops more than 50% including rice, gwarng-toong. In sorghum, benzoic and ferulic acids inhibited the root length more than 50% (**Figures 3.23** and **3.25**). The greatest inhibitory effect on the root length of weeds was more than 60% with 3,4-dihydroxybenzoic acid and *p*-coumaric acid on giant mimosa. The inhibition on phak sian phee (87.84%) was observed for 4-hydroxybenzoic acid, 71.01% for benzoic acid, 96.04% for *p*-coumaric acid and 93.99% for ferulic acid. In addition, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, benzoic acid, caffeic acid and ferulic acid were found not to inhibit the root length of barnyard grass more than 70%. Whereas all six phenolic compounds, except *p*-coumaric acid promoted the root length of prickly chaff-flower (**Figures 3.24** and **3.26**). The present results revealed that the root length of weeds was very sensitive to phenolic compounds. In addition, Chou *et al.* reported that *p*-coumaric acid, *o*-hydroxyphenylacetic acid, syringic acid, ferulic acid, benzoic acid, 4-hydroxybenzoic acid, *m*-coumaric acid, *o*-coumaric acid and salicylic acid inhibited the growth of barnyard grass [7].

It could be concluded that phenolic compounds could inhibit the root length more than the shoot length in weeds. The present study therefore provided the evidence of allelopathic potential of these compounds.

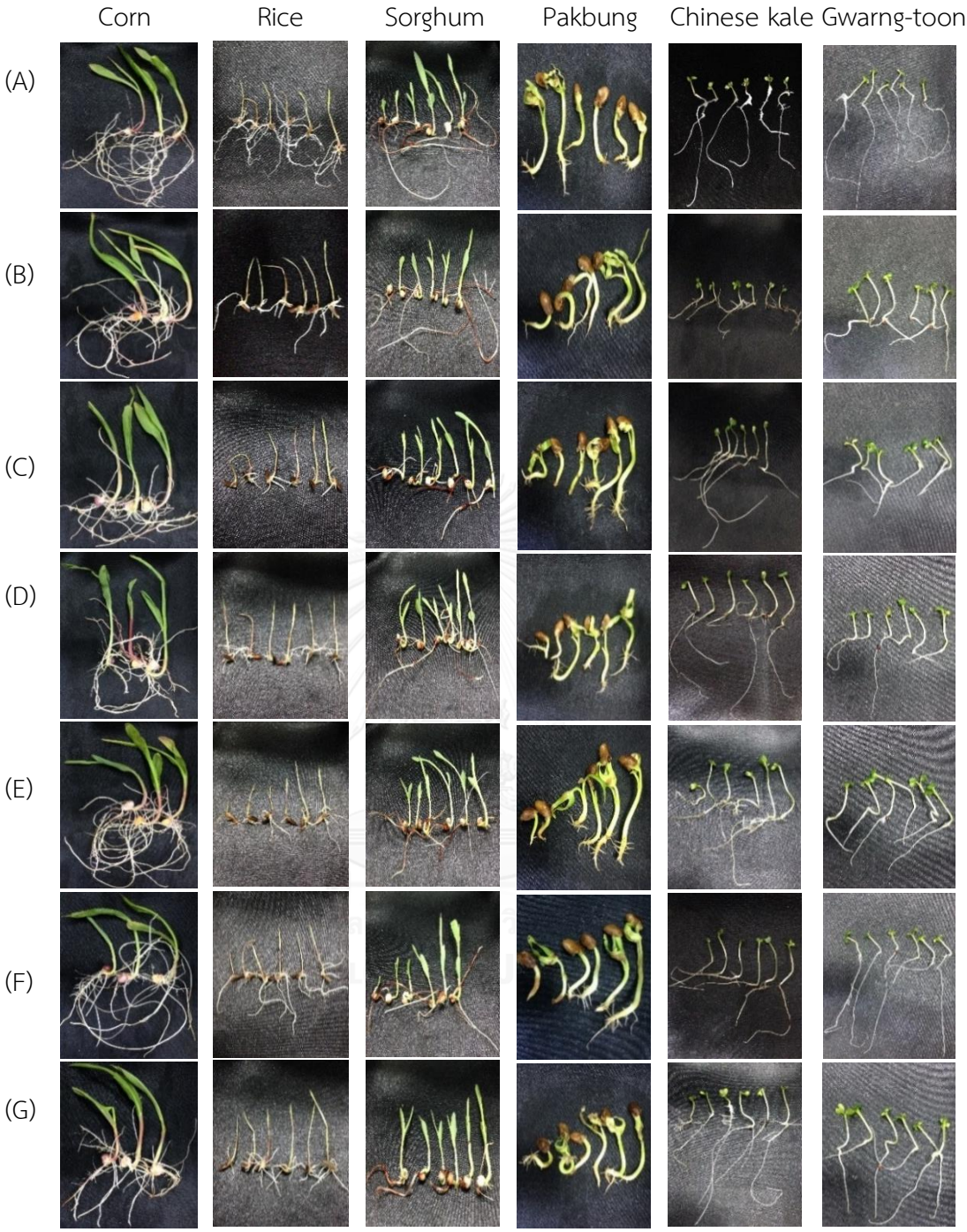


Figure 3.25 The shoot and root length of corn, rice, sorghum, pakbung, Chinese kale and gwarng-toong on phenolic compounds at 1mM (A) control (B) 4-hydroxybenzoic acid (C) 3,4-dihydroxybenzoic acid (D) benzoic acid (E) caffeic acid (F) *p*-coumaric acid (G) ferulic acid (7 DAT)

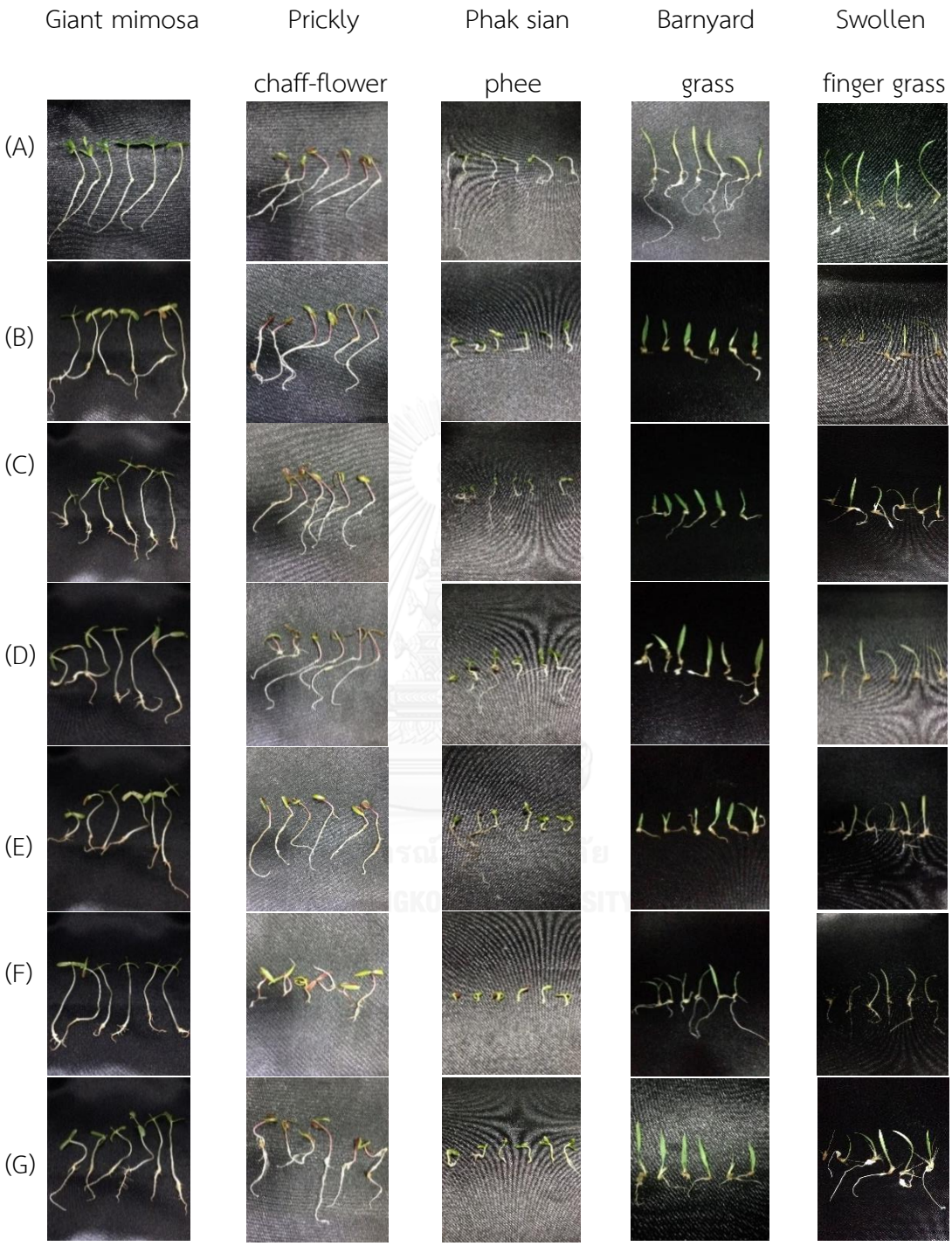


Figure 3.26 The shoot and root length of giant mimosa, prickly chaff-flower, phak sian phee, Barnyard grass and swollen finger grass on phenolic compounds at 1 mM (A) control (B) 4-hydroxybenzoic acid (C) 3,4-dihydroxybenzoic acid (D) benzoic acid (E) caffeic acid (F) *p*-coumaric acid (G) ferulic acid (7 DAT)

3.4.1 The promotion of selected compounds

According to the results from previous section, some selected compounds were able to inhibit the germination and growth of weed. On the other hand, some were found with surprise to promote the germination and growth of the plants (**Figure 3.27**).

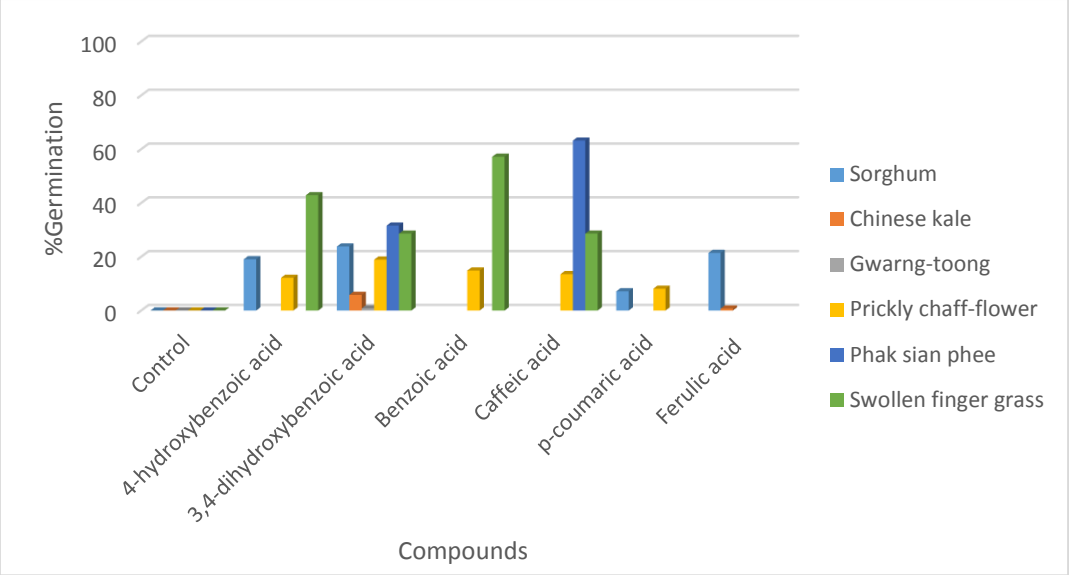


Figure 3.27 The effects of selected compounds (1 mM) on the germination of selected weeds and crops

The results above showed that all six compounds are able to promote the germination with different extent. Among selected six compounds, caffeic acid was the highest effective promotor for the germination of phak sian phee with 63.15% (**Figure 3.27**).

In addition, six compounds also have the ability to show the effect on promoting elongation of shoot and root as presented in **Figures 3.28-3.29**.

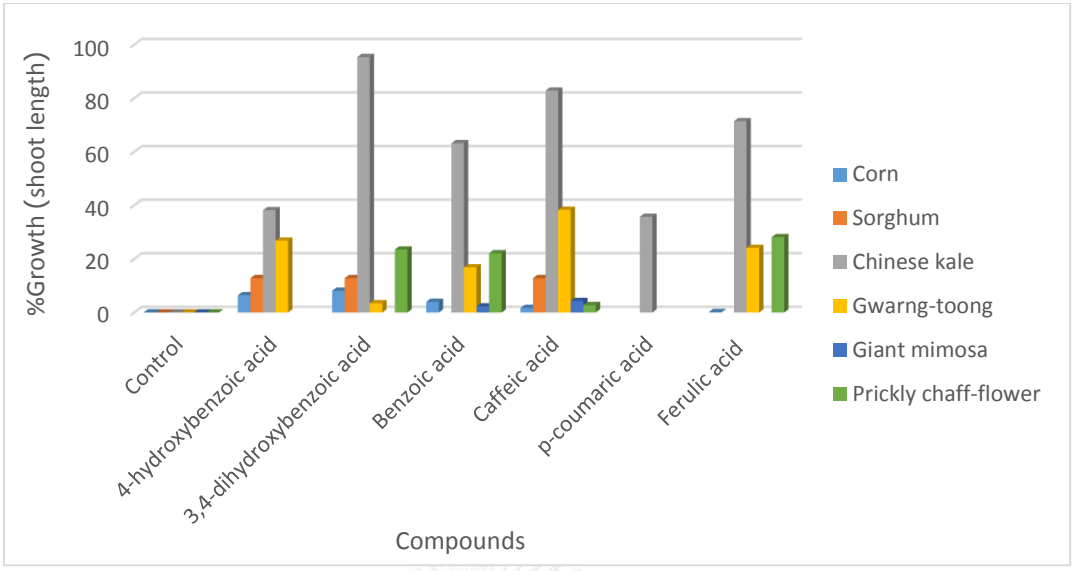


Figure 3.28 The effects of selected compounds (1 mM) on the shoot growth of selected weeds and crops

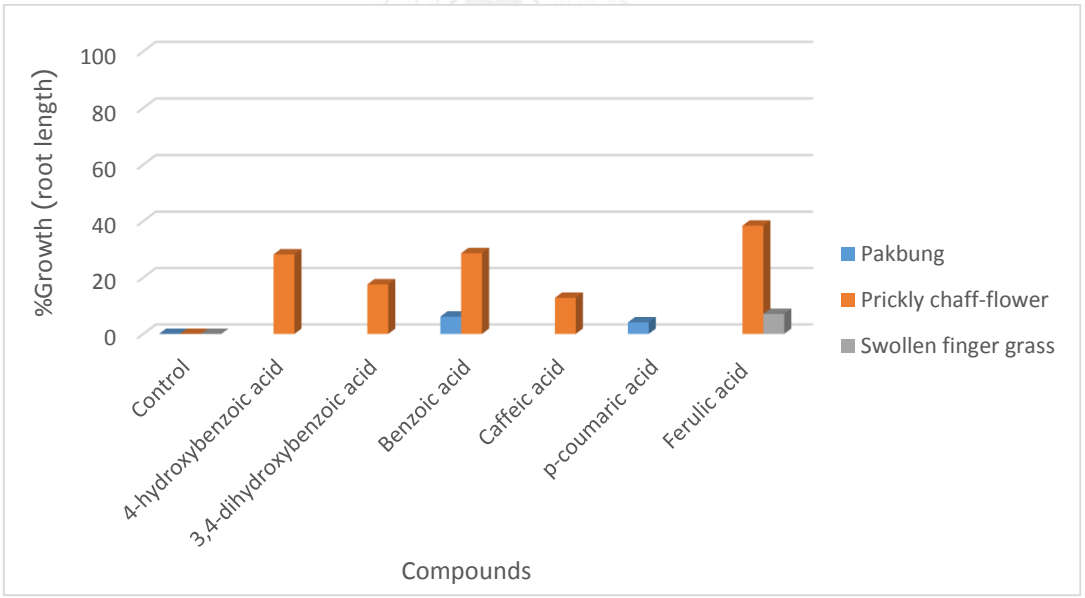


Figure 3.29 The effects of selected compounds on the root growth of weeds and crops at 1 mM

The trends on the shoot and root growth of various plants and that of the germination by 3,4-dihydroxybenzoic acid at 1mM were found to be similar. In addition, caffeic acid at the same concentration was also significantly able to promote the shoot

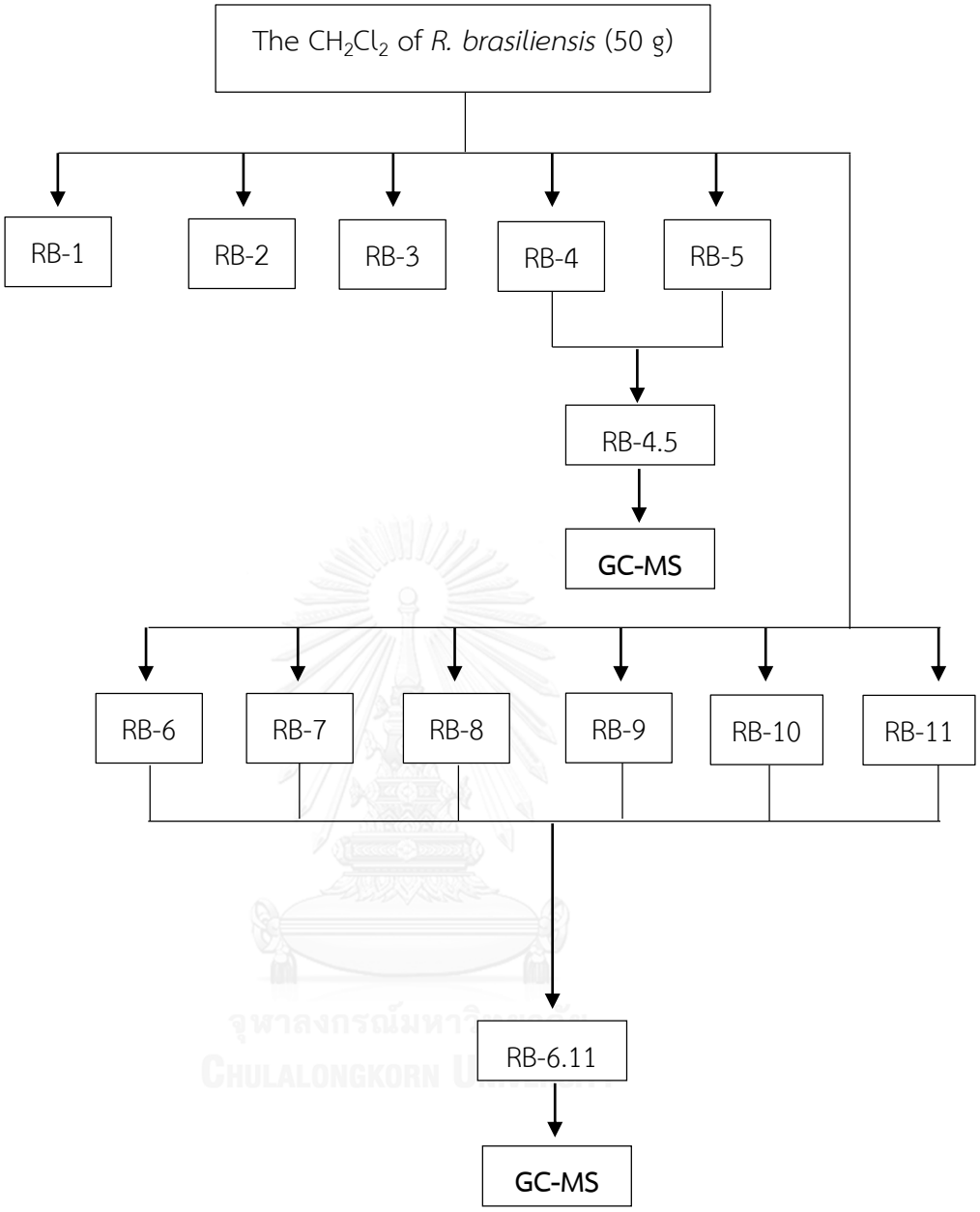
and root length (**Figures 3.28** and **3.29**). From the above experimental results, it is noteworthy that the compounds with 1,2-dihydroxyphenyl moiety promoted the growth of plants. Thus, this finding should be considered as a new alternative to be thoroughly explored and developed in order to use chemicals for stimulating the growth of plants.

3.5 Separation of the CH_2Cl_2 extract

The CH_2Cl_2 extract (RB) of the whole plant of *R. brasiliensis* (50 g) was mixed with silica gel 60 (No.7734) and separated by column chromatography using a mixture of hexane-EtOAc and CH_3OH-EtOAc. Each fraction was examined and combined by TLC. Fractions with similar chromatographic patterns were combined to yield four fractions, as shown in **Table 3.4** and **Scheme 3.2**.

Table 3.4 The separation of the CH_2Cl_2 extract by silica gel column

Fraction	Solvent system	Weight (g)
RB-1	100% Hexane	0.33
RB-2	100% Hexane	0.18
RB-3	5% EtOAc-Hexane	2.01
RB-4	5% EtOAc-Hexane	1.59
RB-5	5-15% EtOAc-Hexane	0.98
RB-6	15% EtOAc-Hexane	1.22
RB-7	15% EtOAc-Hexane	2.53
RB-8	15% EtOAc-Hexane	0.55
RB-9	15% EtOAc-Hexane	2.71
RB-10	15-40% EtOAc-Hexane	8.44
RB-11	40% EtOAc-Hexane – 10% CH_3OH-EtOAc	25.63



Scheme 3.2 The separation of the CH_2Cl_2 extract of *R. brasiliensis*

The selected fractions (**RB-4.5** and **RB-6.11**) from the CH_2Cl_2 extract were further analyzed by GC-MS.

3.5.1 Gas chromatography-mass spectrometry of RB-4.5 and RB-6.11

The GC-MS analysis of the CH_2Cl_2 extract of *R. brasiliensis* was conducted. The possible components suggested from the Wiley7n Library were collected as shown in **Tables 3.5-3.6** and **Figures 3.32-3.37**.

Table 3.5 The GC-MS analysis of **RB-4.5**

No	Rt (min)	Possible compound (%possibility suggested by Wiley7n library)	%Content
1	10.80	Eucalyptol (96%)	66.92
2	19.70	Epoxy-α-terpenyl acetate (82%) Hydroxy-α-terpenyl acetate (78%) *Cis*-limonene oxide (78%)	20.89

Table 3.6 The GC-MS analysis of **RB-6.11**

No	Rt (min)	Possible compound (%possibility suggested by Wiley7n library)	% Content
1	10.80	Eucalyptol (96%)	52.38
2	19.70	Epoxy-α-terpenyl acetate (82%) 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-yl acetate (82%) Hydroxyl-α-terpenyl acetate (78%) *Cis*-limonene oxide (78%) 1-methyl-4-(1-methylethenyl)-7-oxabicyclo[4.1.0]heptane (78%)	15.71
3	21.96	Methyl undecanoate (94%) Methyl dodecanoate (94%)	25.95

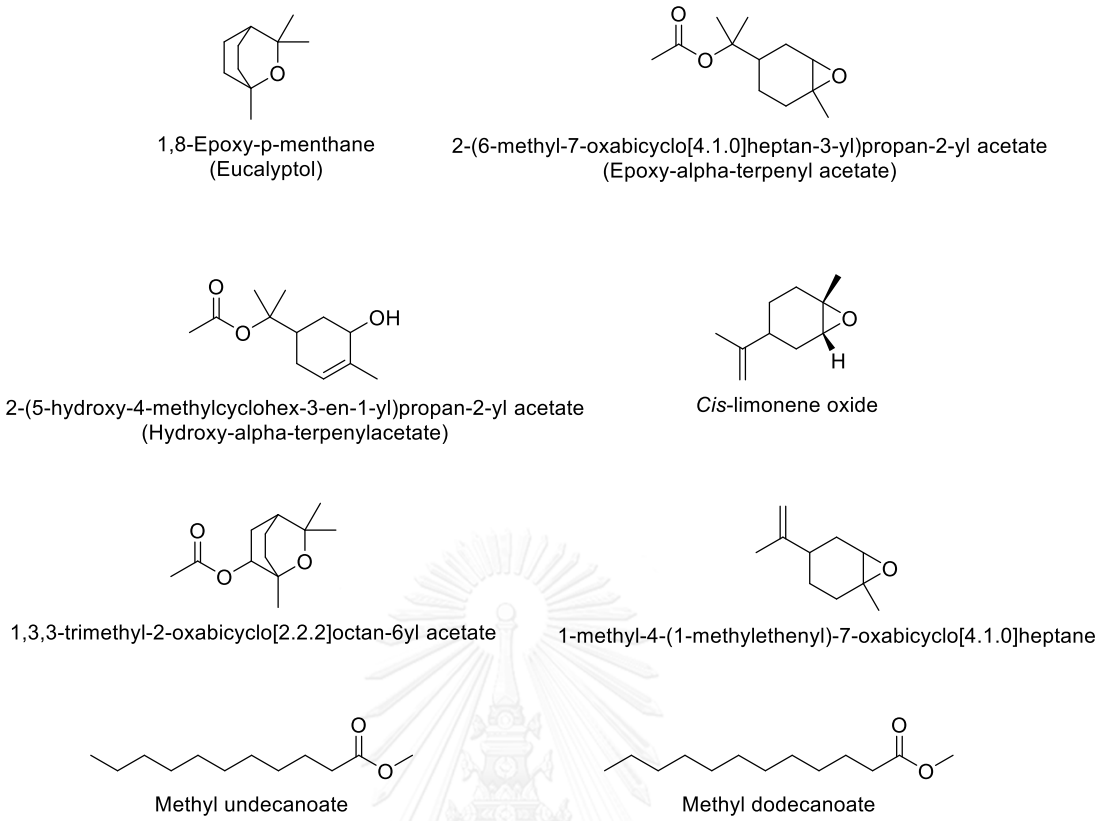


Figure 3.30 The structures of possible compounds in **RB-4.5** and **RB-6.11**

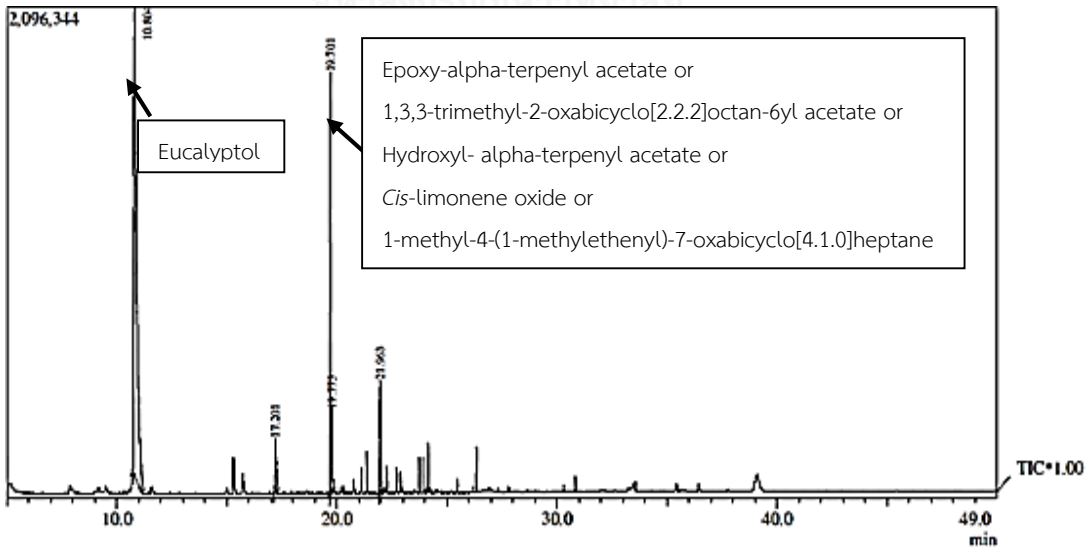


Figure 3.31 The GC-MS chromatogram of **RB-4.5**

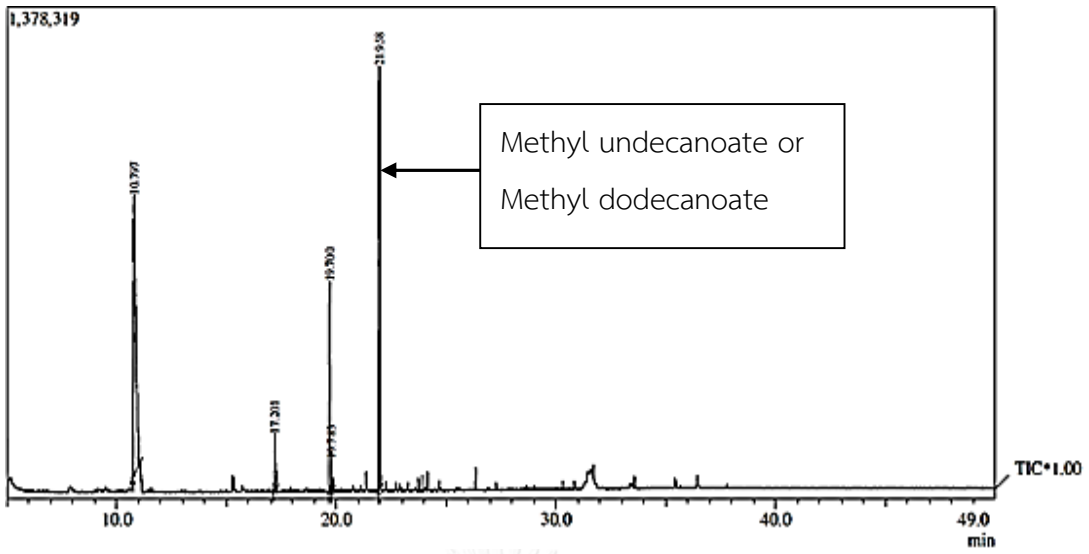


Figure 3.32 The GC-MS chromatogram of **RB-6.11**

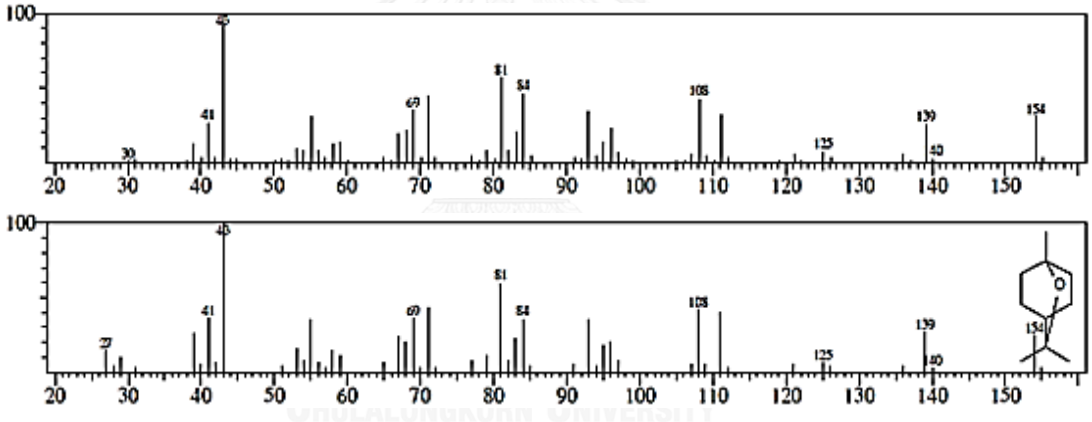


Figure 3.33 Mass spectrum of eucalyptol from **RB-4.5** and **RB-6.11**

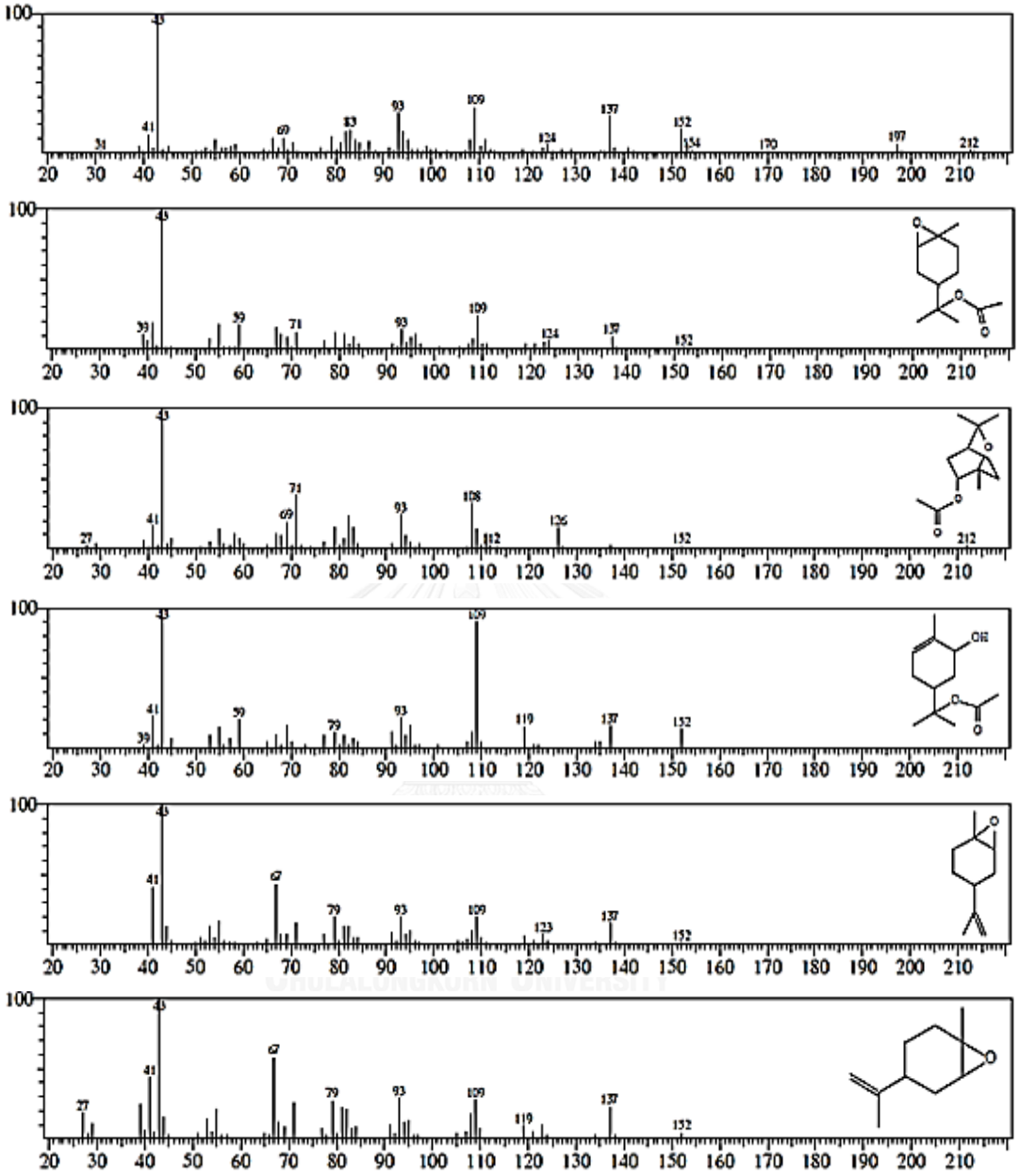


Figure 3.34 Mass spectram of epoxy-α-terpenyl acetate, 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-yl acetate, Hydroxyl-α-terpenyl acetate, *Cis*-limonene oxide, 1-methyl-4-(1-methylethenyl) -7-oxabicyclo[4.1.0]heptane from **RB-4.5** and **RB-6.11**

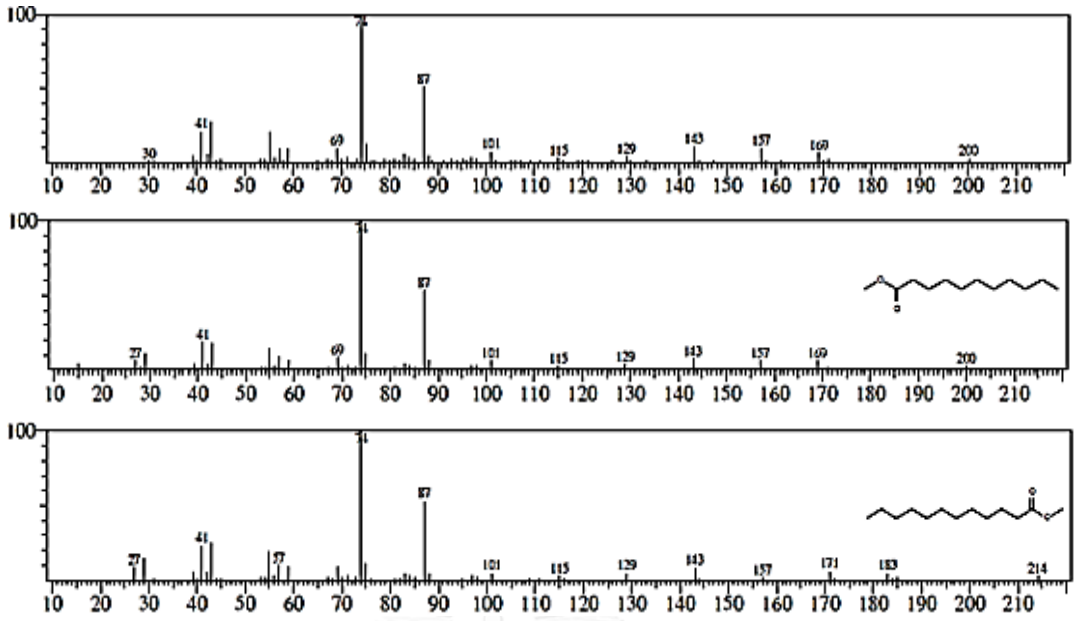


Figure 3.35 Mass spectrum (GC-MS) of methyl undecanoate and methyl dodecanoate from **RB-6.11**

The GC-MS chromatograms of **RB-4.5** and **RB-6.11** revealed two and three peaks, respectively (**Figures 3.31-3.32**). They were identified as eucalyptol at R_t 10.80 min (67%) and the other peak at R_t 19.64 min (20.89%) which could possibly be either epoxy-α-terpenyl acetate, 1,3,3-trimethyl-2-oxabicyclo[2.2.2]octan-6-yl acetate, hydroxy-α-terpenyl acetate, *cis*-limonene oxide, 1-methyl-4-(1-methylethenyl-7-oxa bicyclo[4.1.0]heptane (**Figure 3.34**). For **RB-6.11**, the third peak at R_t 21.96 min, (25.95%) could identify as either methyl undecanoate or methyl dodecanoate (**Figure 3.35**). All compounds were compared with the Wiley7n library.

CHAPTER IV
CONCLUSION

The extraction of the whole plant of *R. brasiliensis* by hexane, CH_2Cl_2, EtOAc and CH_3OH, respectively was investigated on the germination and growth inhibition of *M. pigra* at 0.1, 0.5, 1.0 2.5 and 5.0 gE. The CH_3OH extract exhibited higher germination and growth inhibitory effect than those of hexane, CH_2Cl_2 and EtOAc extracts. The CH_3OH extract could also inhibit the germination and growth of weeds and other crops such as prickly chaff-flower, phak sian phee, barnyard grass, swollen finger grass, corn, rice, sorghum, pakbung, gwarng-toong and Chinese kale at 1 gE. This confirms the effectiveness and the possibility to use this extract as weed inhibitor and to develop as natural herbicide. Interestingly, the CH_3OH extract inhibited the germination and growth of weeds while it did not affect on the crops.

The separation of the CH_3OH extract displayed higher germination and growth inhibition activity. Quick column chromatography of this fraction furnished four fractions, namely **RBM-1**, **RBM-2**, **RBM-3** and **RBM-4**. Each fraction was tested to observe the germination and growth inhibition on *M. pigra* at 0.1, 0.5, 1.0 2.5 and 5.0 gE. All four fractions did not inhibit the seed germination and shoot length, except for **RBM-4** at 2.5 and 5.0 gE. For the root length of *M. pigra*, all four CH_3OH fractionations revealed the inhibitory with different extents depending on the concentration used. The bioassays clearly indicated that this extract should contain some useful bioactive compounds. The HPLC analysis of the promising fractions displayed that there were 4 phenolic compounds, namely 4-hydroxybenzoic acid, *p*-coumaric acid, benzoic acid and caffeic acid. *R. brasiliensis* should contain growth inhibitory substances, possess allelopathic potentials and be a promising candidate for developing as natural herbicides.

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APPENDIX

Table A1. Analysis of variance of total germination of *M. pigra*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	98563.428[a]	19	5187.549	353.543	.000
Intercept	40473.206	1	40473.206	2758.338	.000
Extract	44834.464	3	14944.821	1018.522	.000
Concentration	25617.519	4	6404.380	436.473	.000
Extract * Concentration	28111.445	12	2342.620	159.655	.000
Error	586.922	40	14.673		
Total	139623.556	60			
Corrected Total	99150.350	59			

a. R Squared = .994 (Adjusted R Squared = .991)

Table A2. Effect of extracts on germination inhibition of *M. pigra* according to Duncant's Multiple Range Test

Type of data	**Extracts**	**Average**	**DMRT (P<0.05)**
Germination inhibition (%)	CH_3OH	66.39	a
	EtOAc	35.69	b
	CH_2Cl_2	2.36	c
	Hexane	-0.55	d

Average of germination calculated from 3 replications. (each replication about 50 seedling)

Table A3. Effect of concentrations on germination inhibition of *M. pigra* according to Duncant's Multiple Range Test

Type of data	Concentrations	Average	DMRT (P<0.05)
Germination inhibition (%)	5.0 gE	53.82	a
	2.5 gE	43.92	b
	1.0 gE	25.34	c
	0.5 gE	6.25	d
	0.1 gE	0.52	e

Average of germination calculated from 3 replications. (each replication about 50 seedling)

Table A4. Effect of extracts on growth inhibtion of *M. pigra* according to Duncant's Multiple Range Test

Type of data	Extracts	Average	DMRT (P<0.05)
Growth inhibition (%)	CH_3OH	37.93	a
	EtOAc	31.43	a
	CH_2Cl_2	11.64	b
	Hexane	8.02	b

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

Table A5. Effect of concentrations on growth inhibition of *M. pigra* according to Duncant's Multiple Range Test

Type of data	Concentrations	Average	DMRT (P<0.05)
Growth inhibition (%)	5.0 gE	46.06	a
	2.5 gE	30.43	b
	1.0 gE	22.44	bc
	0.5 gE	16.68	c
	0.1 gE	-4.33	d

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

Table A6. Inhibitory effect of *R. brasiliensis* extracts on the germination inhibition of *M. pigra*

Maceration solvents	%Germination inhibition				
	Concentration (gE)				
	0.1	0.5	1.0	2.5	5.0
Hexane	-0.68	0	-2.08	-0.68	0.7
CH_2Cl_2	-1.37	-3.45	-1.37	3.47	14.58
EtOAc	-0.68	1.39	4.87	72.91	100
CH_3OH	4.87	27.08	100	100	100

Table A7. Inhibitory effect of *R. brasiliensis* extracts on the growth inhibition of *M. pigra*

Maceration solvents	%Growth inhibition (shoot length)				
	Concentration (gE)				
	0.1	**0.5**	**1.0**	**2.5**	**5.0**
Hexane	-33.33	6.66	6.66	26.66	28.88
CH_2Cl_2	-26.66	0	6.66	13.33	46.66
EtOAc	19.99	37.77	28.88	28.88	57.77
CH_3OH	-9.52	-14.28	42.85	65.07	75.39
Control	0	0	0	0	0

Table A7. Inhibitory effect of *R. brasiliensis* extracts on the growth inhibition of *M. pigra* (continue)

Maceration solvents	%Growth inhibition (root length)				
	Concentration (gE)				
	0.1	**0.5**	**1.0**	**2.5**	**5.0**
Hexane	6.66	20	20	25.63	23.07
CH_2Cl_2	-7.69	23.07	15.38	15.38	43.58
EtOAc	12.81	30.76	30.76	23.07	43.58
CH_3OH	22.21	49.2	47.61	47.61	53.96
Control	0	0	0	0	0

Table A8. The percentage germination inhibition of **RBM-1** to **RBM-4** on *M. pigra*

Concentration (gE)	%Germination inhibition			
	RBM-1	RBM-2	RBM-3	RBM-4
0.1	6.08±1.52	12.83±6.24	2.70±1.73	2.02±1.52
0.5	1.35±0.57	1.35±2.30	4.05±1.15	12.83±3.46
1.0	2.70±1.00	4.05±1.52	6.08±2.08	6.08±0.57
2.5	20.94±6.24	7.43±2.88	3.37±2.08	100±0
5.0	27.02±1.73	18.91±3.00	7.43±2.51	100±0
Control	0±0	0±0	0±0	0±0

Table A9. The percentage growth inhibition (shoot length) of **RBM-1** to **RBM-4** on *M. pigra*.

Concentration (gE)	%Growth inhibition (shoot length)			
	RBM-1	RBM-2	RBM-3	RBM-4
0.1	-114.88±1.20	-125.28±1.59	-137.92±0.89	-119.38±1.13
0.5	-153.09±0.57	-96.34±1.32	-153.37±0.64	33.14±1.11
1.0	-96.06±1.36	-96.34±1.28	-69.66±1.25	63.48±0.94
2.5	-101.96±0.56	-103.09±1.20	-61.23±0.72	100±0
5.0	-34.26±0.85	-55.61±1.04	-25.56±1.02	100±0
Control	0±0	0±0	0±0	0±0

Table A10. The percentage growth inhibition (root length) of **RBM-1** to **RBM-4** on *M. pigra*.

Concentration (gE)	**%Growth inhibition (root length)**			
	RBM-1	**RBM-2**	**RBM-3**	**RBM-4**
0.1	60.86±0.66	56.54±0.75	64.74±0.47	80.14±0.36
0.5	50.50±0.68	72.37±0.38	74.67±0.39	94.38±0.21
1.0	62.44±0.61	79.28±0.39	84.31±0.32	94.38±0.19
2.5	66.47±1.19	85.46±0.24	88.20±0.19	100±0
5.0	90.93±0.22	91.51±0.12	91.51±0.16	100±0
Control	0±0	0±0	0±0	0±0

Table A11. The percentage germination inhibition of phenolic compound on crop plant at 1 mM

Compounds	**%Germination inhibition**		
	Corn	**Rice**	**Sorghum**
4-hydroxybenzoic acid	2.70±3.60	1.13±1.00	-19.04±3.05
3,4-dihydroxybenzoic acid	16.21±2.30	4.54±0.00	-23.80±2.51
Benzoic acid	5.40±2.08	3.40±0.57	0.00±1.00
Caffeic acid	1.35±0.57	2.27±1.52	33.33±4.04
p-coumaric acid	13.51±2.88	2.27±0.57	-7.14±1.00
Ferulic acid	8.10±1.15	1.13±1.00	-21.42±1.73
Control	0±0	0±0	0±0

Table A11. The percentage germination inhibition of phenolic compound on crop plant at 1 mM (continue)

Compounds	%Germination inhibition		
	Pakbung	Chinese kale	Gwarng-toong
4-hydroxybenzoic acid	4.61±2.51	5.07±0.57	0±3.60
3,4-dihydroxybenzoic acid	7.69±1.73	-5.79±1.15	-0.79±2.88
Benzoic acid	9.99±7.81	5.07±4.04	11.90±5.29
Caffeic acid	13.07±1.52	5.07±1.52	9.52±2.00
p-coumaric acid	6.92±5.68	1.44±1.15	25.39±6.50
Ferulic acid	10.76±3.51	-0.72±1.15	0±1
Control	0±0	0±0	0±0

Table A12. The percentage germination inhibition of phenolic compound on weeds at 1 mM

Compounds	%Germination inhibition		
	Giant mimosa	Prickly chaff-flower	Phak sian phee
4-hydroxybenzoic acid	7.14±6.42	-12.16±4.72	42.10±2.30
3,4-dihydroxybenzoic acid	12.85±4.16	-18.91±8.38	-31.57±4.93
Benzoic acid	7.85±2.64	-14.86±11.01	21.05±1.73
Caffeic acid	12.85±5.50	-13.51±3.60	-63.15±2.88
p-coumaric acid	1.42±1.73	-8.10±8.73	47.36±0.57
Ferulic acid	8.57±5.13	8.10±2.08	10.52±2.30
Control	0±0	0±0	0±0

Table A12. The percentage germination inhibition of phenolic compound on weed at 1 mM (continue)

Compounds	%Germination inhibition	
	Barnyard grass	Swollen finger grass
4-hydroxybenzoic acid	20.68±4.16	-42.85±3.78
3,4-dihydroxybenzoic acid	6.89±4.00	-28.57±3.46
Benzoic acid	27.58±1.00	-57.14±1.52
Caffeic acid	17.24±2.00	-28.57±3.60
p-coumaric acid	24.13±5.03	7.14±3.78
Ferulic acid	55.17±2.08	21.42±3.05
Control	0±0	0±0

Table A13. The percentage growth inhibition (shoot length) of phenolic compound on crop plant at 1 mM

Compounds	**%Growth inhibition (shoot length)**		
	Corn	**Rice**	**Sorghum**
4-hydroxybenzoic acid	-6.44±0.58	2.53±0.38	-12.82±0.47
3,4-dihydroxybenzoic acid	-8.12±0.75	0.31±0.54	-12.82±0.53
Benzoic acid	-3.98±0.94	3.79±0.37	16.84±0.55
Caffeic acid	-1.68±0.91	8.86±0.31	-12.82±0.38
p-coumaric acid	3.22±0.92	5.06±0.41	17.21±0.63
Ferulic acid	-0.15±1.02	4.74±0.45	14.65±0.60
Control	0±0	0±0	0±0

Table A13. The percentage growth inhibition (shoot length) of phenolic compound on crop plant at 1mM (continue)

Compounds	%Germination inhibition (shoot length)		
	Pakbung	Chinese kale	Gwarng-toong
4-hydroxybenzoic acid	18.58±0.96	-38.21±0.67	-26.81±0.48
3,4-dihydroxybenzoic acid	31.75±0.90	-95.35±0.90	-3.44±0.36
Benzoic acid	9.45±1.01	-63.21±0.53	-16.85±0.41
Caffeic acid	53.04±0.70	-82.85±0.79	-38.31±0.42
p-coumaric acid	7.77±0.60	-35.71±0.80	0±0.38
Ferulic acid	23.64±0.71	-71.42±0.76	-24.13±0.42
Control	0±0	0±0	0±0

Table A14. The percentage growth inhibition (shoot length) of phenolic compound on weeds at 1 mM

Compounds	**%Growth inhibition (shoot length)**		
	Giant mimosa	**Prickly chaff-flower**	**Phak sian phee**
4-hydroxybenzoic acid	7.08±0.29	1.38±0.25	48.96±0.33
3,4-dihydroxybenzoic acid	13.92±0.29	-23.50±0.48	43.56±0.30
Benzoic acid	-2.27±0.20	-22.11±0.23	50.62±0.25
Caffeic acid	-4.30±0.29	-2.76±0.29	39.00±0.25
p-coumaric acid	12.15±0.27	1.84±0.44	63.07±0.32
Ferulic acid	8.86±0.42	-28.11±0.27	61.82±0.34
Control	0±0	0±0	0±0

Table A14. The percentage growth inhibition (shoot length) of phenolic compound on weeds at 1 mM (continue)

Compounds	%Growth inhibition (shoot length)	
	Barnyard grass	Swollen finger grass
4-hydroxybenzoic acid	80.27±0.07	7.95±0.09
3,4-dihydroxybenzoic acid	82.99±0.06	30.68±0.12
Benzoic acid	72.78±0.14	28.40±0.10
Caffeic acid	74.82±0.11	32.95±0.12
p-coumaric acid	46.93±0.08	29.54±0.10
Ferulic acid	53.74±0.45	50.00±0.12
Control	0±0	0±0

Table A15. The percentage growth inhibition (root length) of phenolic compound on crop plant at 1mM

Compounds	**%Growth inhibition (root length)**		
	Corn	**Rice**	**Sorghum**
4-hydroxybenzoic acid	36.02±4.65	72.72±0.72	32.25±2.75
3,4-dihydroxybenzoic acid	29.22±6.68	69.42±0.69	48.82±1.68
Benzoic acid	34.45±5.42	73.41±0.58	59.11±1.68
Caffeic acid	28.39±4.50	62.80±0.64	48.03±1.59
p-coumaric acid	28.78±6.73	7.85±1.25	47.94±2.43
Ferulic acid	44.92±4.50	68.45±0.48	68.33±1.43
Control	0±0	0±0	0±0

Table A15. The percentage growth inhibition (root length) of phenolic compound on crop plant at 1mM (continue)

Compounds	%Growth inhibition (root length)		
	Pakbung	Chinese kale	Gwarng-toong
4-hydroxybenzoic acid	3.06±0.51	39.18±2.14	62.42±0.98
3,4-dihydroxybenzoic acid	20.40±0.53	13.94±2.17	67.28±0.96
Benzoic acid	-6.12±0.68	14.38±1.92	73.22±0.74
Caffeic acid	28.57±0.42	21.35±1.73	69.29±0.82
p-coumaric acid	-4.08±0.57	28.77±2.11	33.25±1.81
Ferulic acid	23.46±0.36	26.47±2.12	58.48±1.21
Control	0±0	0±0	0±0

Table A16. The percentage growth inhibition (root length) of phenolic compound on weeds at 1mM

Compounds	%Growth inhibition (root length)		
	Giant mimosa	Prickly chaff-flower	Phak sian phee
4-hydroxybenzoic acid	45.17±0.50	-28.19±0.52	87.84±0.23
3,4-dihydroxybenzoic acid	61.15±0.49	-17.57±1.10	27.37±1.89
Benzoic acid	54.82±0.67	-28.63±0.68	71.01±0.96
Caffeic acid	53.52±0.64	-12.79±0.81	34.40±1.54
p-coumaric acid	62.44±0.34	19.73±1.50	96.04±0.09
Ferulic acid	49.06±0.61	-38.39±1.02	93.99±0.21
Control	0±0	0±0	0±0

Table A16. The percentage growth inhibition (root length) of phenolic compound on weeds at 1mM (continue)

Compounds	%Growth inhibition (root length)	
	Barnyard grass	Swollen finger grass
4-hydroxybenzoic acid	88.09±0.39	1.11±0.49
3,4-dihydroxybenzoic acid	85.46±0.29	18.95±0.82
Benzoic acid	85.36±0.55	37.17±0.67
Caffeic acid	85.06±0.31	16.72±0.77
p-coumaric acid	33.19±1.85	16.35±0.96
Ferulic acid	73.96±0.69	-7.06±1.17
Control	0±0	0±0

VITA

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