CHAPTER I

INTRODUCTION

1.1 Allelopathy

Nobody will argue about the tremendous importance of agriculture and forestry. It furnishes making with vegetable food and feed for his domestic animals. Further, plants supply many other kinds of product which are important for normal life or which make life more agreeable such as medicines for healing, dyes for painting, pesticides for protection of crops, toxins for hunting or fishing, spices for flavoring, volatile oils for soaps, and stimulants like coffee or tea. To protect our food crops and other plants from being attacked by herbivores, various infections diseases and competition by weeds, many pesticides nowadays are used, *e.g.*, herbicides, insecticides, nematocides and fungicides. Inspite of these measures, it is estimated that 10-35 percent of all crops are lost either before harvest on the field or during stroage. Especially due to the current intensive cultivation pesticides, which are often dictated by an economy of scale, pests can have a devastating effect in the large monocultures. Although most of the synthetic pesticides are nowadays highly portent compounds, many of them possess several disadvantages, like lake of selectivity, build-up of resistance, toxicity to man, and accumulation in the environment. The development of resistance is one of the biggest problems because one has either to resort to other agents or to use more during each application thus adding to the problem of pollution. To stop this pollution process, the use of certain pesticides is there for diminished. For instance in the developing countries, may pesticides can no longer be used for all crops, some will be totally banned. One method to stop the pollution is stop the synthetic pesticides but attempt to use natural product, such as a group of tetranortriterpenoids from neem tree

(*Azadirachta indica*) (Kraus. *et.al.*) as well as insecticide of pyrethroids.(Khambay and Conner., 1993).

The term allelopathy refers to biochemical interaction between all types of plants including microorganism. In the past the term allelopathy defined as any direct or indirect harmful effect by one plant on another through production of chemical compound that escaped into the environment (Rice,1974). Allelopathy is that its effect depends on a chemical compound being added to the environment. It is thus separated from competition which involves the removal or reduction of some factor from the environment that is required by some other plant sharing the habitat. Factors that may be reduced include water, minerals, food, and light.

Both weeds and crops can produce secondary chemicals and release to environment. Various weed varieties with allelopathic properties can provide benefit for crop production. Chemical from plants may cause phytotoxic to plants but may be a growth regulator of other plants. Allelopathic mechanism can be selected in agrochemic crops and bred with good quality cultivars. Allelopathic chemicals from one crop may have selective property and can be grown with other crops as biculture.

Allelochemic substances in plants are the organic compound obtained from metabolism process of plant and they are inhibition property of plant, in a little quantity they can promote and increase growth rate of plant (Rice,1984). Allelochemic substances that proved to have allelopathic properties devided into 11 groups *e.g.*, toxic gas, organic acids and aldehydes, aromatic acid, simple unsaturated lactones, coumarins, quinones, flavonoids, tannins, alkaloids, terpenoids and steroids.

Transportation of allelochemic substances from one plant to the other plant by released in 4 methods; volatilization, root exudatation, leaching by rain and decomposition of residue.

When allelochemic substances released to the environment, some plants obtained those substances to be effecting to inhibition or stimulation the interaction of

that plant. The effecting of allelochemic substances *e.g.*, cell division and cell elongation, interaction with plant growth regulator, minerals uptake of plant, photosynthesis, respiration and protein synthesis.

1.2 Literature Search on the Allelopathy of weeds.

Chemical substances contained in weeds often play an important role as one of the environmental foctors which affect the growth of the other weeds or crops. This phenomenon is well known as an allelopathy (Premasthira,et.al.,1985). Many work on allelopathic effects has been reported with various weed species (Noda *et.al.*, 1985)

The previous comprehensive review of allelopathy (Rice,1974) covered research done primarily prior to 1984. Research has been particularly active in relation to the roles of allelopathy in agriculture, forestry, phytopathology, patterning of vegetation, and old field succession. Very little research was done on allelopathic effects of weeds on crop plants prior to about 1970.

Anaya and Groma-Pompa(1978) demonstrated that extract of leaves and fruits of piru (*Svhinus moble* L.) are strongly inhibitory against seed germination and seedling growth of cucumber and wheat. Aqueous extracts of the ground seeds of thirteen weed species (Gressel and Holm,1964) were assayed for their ability to inhibit the germination on the filter paper of eight crop species. Germination of some crop species was delayed when the crop seeds were surrounded by weed seeds on filter paper. The seeds of *Abutilon theophrasti* were inhibitory to tomato germinated in sterile and non-sterile soil in the laboratory. The inhibition of germination was apparent under conditions which excluded light, water and minerals as factors in the competition. Chemical studies indicated that the inhibition by *Abutilon* seeds was due to free amino acids emating from the seeds. Common milkweed (*Asclepias syriaca* L.) which is a major weed in north central and northeastern United States and in Canada, was found to

reduce the yield of grain sorghum significantly in field test (Rasmussen and Einhelling, 1975). This investigation demonstrated that aqueous extracts of milkwed leaves significantly inhibited growth of grain sorghum seedlings and reduce concentration of the extracts resulted in propotional increases in yield. Two phenolictoxin were isolated but not identified, and both inhibited seed germination of sorghum and radish. The plant growth inhibition effect of 24 weed species were examined by rice seedling bioassay (Premasthira et.al.,1985) that ***Eupatorium odoratum*** L., ***Hyptis suaveolens*** Poit., ***Dysophylla stellata*** Benth., ***Ammannia baccifera*** L., ***Heliotropium indicum*** L., ***Polygonum tomentosum*** Wild. And ***Sphenocleac Zeylanica*** Gaertn showed the strongest effect. ***Jussiaea linifolia*** Vahl., ***Euphorbia hirta*** L., ***Euphorbia Thymifolia*** L., ***Spheranthus africanus*** L. and ***Trianthema Portulacastrum*** L. showed moderate effect. ***Euphorbia geniculata*** Ort. Showed slightly effect. The allelopathic effect of croton weed (***Euphorbia adenophorum*** Spreng.) was tested on seed germination inhibition with 19 species of crops and weeds (Zungsonthiporn and Premasthira ,1994). It showed that 9 species were strongly inhibited (90-100%), 3 species were moderately inhibited (50-89%) and 5 species were slightly inhibited (5-39%). The allelopathic potential of shoot and root leachates of ***Amaranthus viridis***, ***Boerhavia diffusa***, ***Dactylon aegyptum***, ***Echinochloa colunum***,***Euphorbia hirta***, ***Parthenium hyrsterophorous*** and ***Trianthema portulacastrum***, was assessed using sorghum and blackgram (Vigna mungo) as test crops. Weeds differed significantky among themselves in their allelopathic effect. Shoot leachates of weeds, in general, showed more allelopathic effect than their root leachates. Phenolic content of weed leachates showed positive associations with the allelopathic potential of weed species (Seetha ***et.al.***,1990) Plant growth inhibiting substance contained in quackgrass (***Agropyron repens***) induced chlorosis and stunting of oats and alfafa (Ohman and Kommedahl,1964). Aqueous foliage extracts of leaf spurge (***Euphorbia esula***) inhibited the germination and seedling growth of wheat and pea. The toxic substance was

released during leaf spurge decomposition (Le Touneau et. Al., 1956). Root residues of *Setaria faberrii*, *S. gluca* and *Digitaria rangunalis* inhibited corn root growth. Among them, *S. faberrii* was the most inhibitory (Schereiber and Williams Jr., 1967). Allelopathic substances contained in *Abutilon theophrasti* inhibited tomato seed germination and they were identified as free amino acid (Gressel and Holm,1964). The competition of corn and yellow nutsedge (*Cyperus esculentus*) was not due to nutrient uptake or utilization but allelopathic activity of yellow nutsedge. Active substance of this weed were identified as phenol compounds (Drost and Doll, 1980). Emergence of Chinese cabbage and Chilli seedling was reduced with either lantana or Siam weed when exposed to all concentrations of either incorporated or soil surface treatmean (Sahid and Sugau,1993). *Euphorbia hirta* Linn. Was found to be more harmful for germination and seedling growth of sorghum and black grass (Rani *et.al.*,1990). Crude methanol extract of wild poinsettia (*Euphorbia heterophylla*) also produced significant inhibitory effects on the germination on tomato seeds. The effects were generally more severe on the early than on the final germination stage (Eniola and Fawusi,1989)

1.3 Biological Characteristic, Distribution and Chemical Constituent of Some Weeds in the Family Euphorbiaceae

Euphorbiaceae is one of the largest and most diversified family of the angiosperms and its members are distributed world wide (Prasad and Satyasree,1994).

a) *Euphorbia hirta* L.

Its Synonyms are *Chamaesyce hirta* (L.) Millsp.; *E. pilulifera* L. (Fig. 1.1) This plant is native to Central America and an annual herb which became naturalized from the tropical to the southern part of temperate zone. In Thailand, this plant was found as amajor weed along road sides, garden and upland field. The plant is prostrate in

trampled or infertile land and is erect under favorable conditions (60 cm tall). Foliage contains a milky sap. Whole plant is covered with soft hairs. Leaves with a short petiole 1 to 2 mm. Long are deformed, long ovate, opposite and serrulate. Sometimes the upper surface of the leaves shows purple mottling. Inflorescences are axillary and capitate at the end of about 2 cm stalks (AICAF,1997)

Fig. 1.1 *Euphorbia hirta* L.

The plant was used as a medicinal herb for the treatment of pneumonia, and its decoction has ben traditional used as an antidiarrheic, an antidiuretic, expoctorant, and also as a remedy for bronchitic, asthma, intestinal aliments of children and various skin

diseases in China and Indonesia(Haroda et.al.,1987). Some of phytochemical studies of this plant revealed the presence of diterpenoid, triterpenoid, flavonoids and tannins. Two new dimeric dehydroellagitannins named euphorbin A and euphorbin B, five monomeric hydrolizable tannins as well as two quinic acid ester were isolated from aerial part of *Euphorbia hirta* (Yoshida *et.al.*,1988) R.K. Baslas and R. agawal investigated some anticancer plants of Euphorbia genus and isolated several terpenes, anthocyanins, alcohols and steroids from *Euphorbia hirta* Linn., *E. nerifolia* Linn. And *E. thymifolia* as showed in Table 1 and concluded that the co-carcinogenic activity of these plants are due to the presence of 12-deoxyphorbal-13,20-diacetate, ingenoltriacetate and resiniferonol derivative (Baslas and Agawal,1980). J.Galvez and coworkers reported the antidirrholic activity of *E. hirta* extract and isolation of an active flavonoid (Galvez *et.al.*,1993), geritin. Nineteen Euphorbia species including *E. hirta* L., were studies for their flavonoid patterns (Kawashty *et.al.*,1990). Flavonoids were identified as kaempferol, quercetin, apigenin and luteolin.

b) *Euphorbia thymifolia* L.

. Its Synonym is Syn.Chamaesyce thymifolia (L.) Millsp. (Fig. 1.2). This plant is native of Central America up to the West Indies and an annual herb which became widely naturalized in the tropical regions of the world. Stems are branched, prostrate and about 30 cm long. Foliage contains a milky sap. Leaves are 1 cm long, 0.5 cm wide, densely opposite, glabrous or with sparse hairs and not spotted on the upper side. Axillary stalks bear inflorescences with a small number of flowers. Styles split into two tips. Fruits have 3 ridges, 1 to 1.5 mm long, with short hairs. Seeds are red.

Fig. 1.2 ***Euphorbia thymifolia*** **L.**

The plant grows on dry soil along road sides and in upland fields. It is said that the plant is effective for the treatment of diarrhea, skin diseases and eczema (Morita,1990).

B.P. Pradnam and H.N. Khastgir (1969) isolated 1-hexacosanol from petroleum ether extract. Evan and coworkers reported the isolation of 12-deoxy-β-hydroxylphorphol-13-dodecanoate-20-acetate (Kinghorn and Evan,1975) and 12-deoxyphorphol-13,20-diacetate(Evan and Schmidt,1976).

c) *Euphorbia heterophylla* Linn.

Its Synonym is *E. geniculata* Ort. The common name of *E. heterophylla* Linn. is wild pionsettia, in Thailand is "Yaa-Yaang" or "Pak-Yang" (Smitinand,1980). (Fig. 1.3). It was originally a native plant of tropical and sub-tropical America but is now widespread in the tropics as a weed of cultivated land and waste placed (Hutchinson and Daziel,1958).

E. heterophylla Linn. It is native to the tropics from North America to Argentina and is an annual herb which became naturalized in the tropical and temporate regions of the world. The plant is glarbrous or hairly. Stems are branched dichotomously and become 1 m long. Leaves have a short petiole and are alternate. Leaf blades are long ovate, acuminate. Leaf edges are serrate, lobate or entire. Foliage is poisonous and a white milk sap. Internodes at the tip of stem are short and clustered leaves become ornamental leaves with a reddish purple leaf edge or sometime white leaf blades.

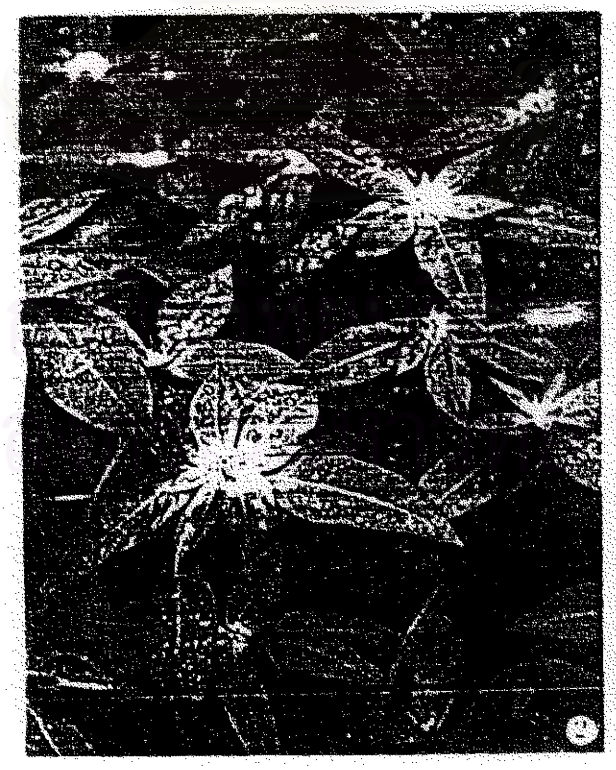


Fig. 1.3 ***Euphorbia heterophylla*** **L.**

The inflorescence consists of clusters of numerous small short-stallked flowers, laking petals or sepals but with conspicuous glands surrounds by radiating leaf-like bracts.

The fruit is hard-coated, three - lobed capsule with reddish blotches, containing three seeds. These are shed by an explosive mechanism of the capsule, dispersing them some distance from the parent plant (Wilson,1981).

β-amyrin, euphyl acetate and a ketone, $C_{28}H_{56}O$ were isolated from the leaves of *E. heterophylla* (Tiwari *et.al.*,1981). A phytochemical study of *E heterophylla* L. cultivated in Egypt yielded β-amyrin, β-sitosterol and β-sitosterol glucoside were isolated from the ether extract of the root. In addition, quercetin, 3-methylquercetin and kaempferol-7-o-glucoside were isolated from the ethyl acetate extract of the herb (El-Amary *et.al.*,1990)

Table 1.1 Substances isolated from Euphorbia plants

Plant	Part	Solvent for extraction	Compounds isolated
E. hirta	aerial	pet. ether	24-methyleneartenol, cycloartenol, β-sitosterol, eupholbol hexacosoate, β-amyrin acetate, 1-hexacosanol, tinyatoxin,12-deoxy-4β-hydroxyphorphol-13-phenylacetate-20-acetate, ingenol triacetate
	roots	pet. ether	24-methyleneartenol,cycloartenol, cyanidin-3,5-diglucoside, pelargonidin-3,5-diglucoside 20-O-acetylreseniderenol-9,13,14-orthophenyl acetate
	pet.ther defatte roots	EtOH	12-deoxy-4β-hydroxy-phorphol-13-odecano-ate-20-acetate, 12-deoxyphorphol-13-phenyl-acetate-16-O-a-methylbutyrate-20-acetate, ingenol triacetate, taraxerone
E. nerifolia	bark	pet.ether	24-methyleneartenol, euphol, euphol hexaco-zoate, 1-hexacosanol, 12-deoxy-4β-hydroxy-phorphol-13-odecanoate-20-acetate, tulipanin -3,5-diglucoside, pelargonin-3,5-diglucoside
	roots	pet.ether	24-methyleneartenol, cycloartenol, ingenol triacetate, euphorpol, 12-deoxyphorphol-13, 20-diacetate, delphinidin-3,5-diglucoside, tulipanin-3,5-diglucoside

Table 1.1 (cont.)

Plant	Part	Solvent for extraction	Compounds isolated
E thymifo- lia	whole plant	pet.ether	12-deoxyphorphol-13,20-diacetate, 12-deoxy -β-hydroxyphorbol-13-decanoate-20-acetate 1-hexacosanol

1.4 Chemical Constituents Studies on *Euphorbia heterophylla* Linn.

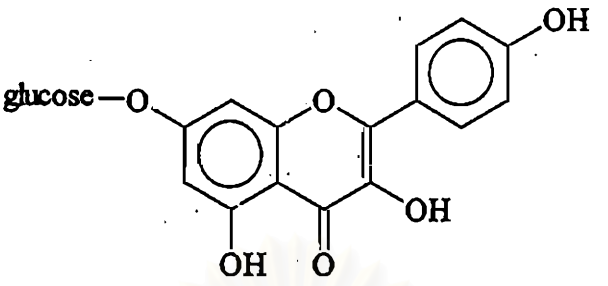
Literature surveys of chemical constituents of the plants belonging to *Euphorbia heterophylla* revealed that there have been a variety of organic substrates isolated (Table 1.2).

Table 1.2 Chemical constituents of *E. heterophylla*

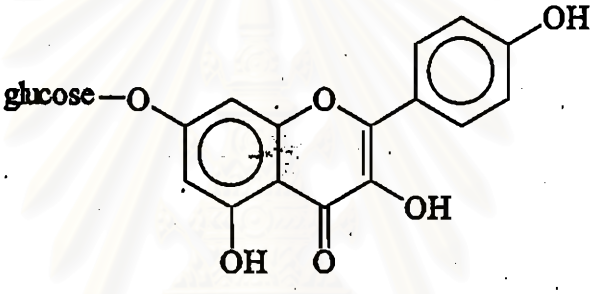
Plant parts	crude extract	Isolated compound	reference
whole plant	Pet. ether	10,10-dimethyl hexacosane-2-one	Tiwari and Bajpal, 1982
		taraxasterol acetate	Tanaka and Matsunaga,1988

Table 1.2 (cont)

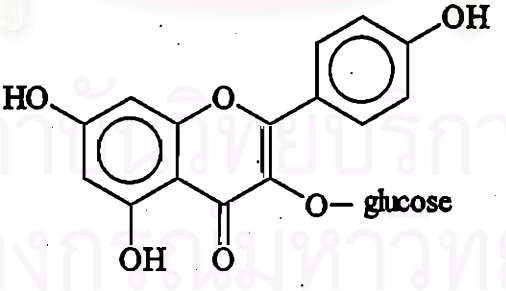
Plant parts	crude extract	Isolated compound	reference
Whole plant	Pet. ether	β-amyrin	EL-Emary et.al 1990
		β-sitosterol	
		β-sitosterol glucoside	Sekula and Nes 1980
Whole plant	Ethyl acetate	quercetin	EL-Emary et.al 1990
		3-methyl quercetin	
		Kaempferol-3-0-arabinoside	
		Kaempferol-7-0-glucoside	
		Kaempferol-3-0-glucoside	
Root	Ether	lupeol acetate	Sahai et. al,1981
		taraxasterol	Tanaka and Matsunaga,1988
		β-sitosterol	EL-Emary et.al 1990



Kaempferol-3-0-arabinioside

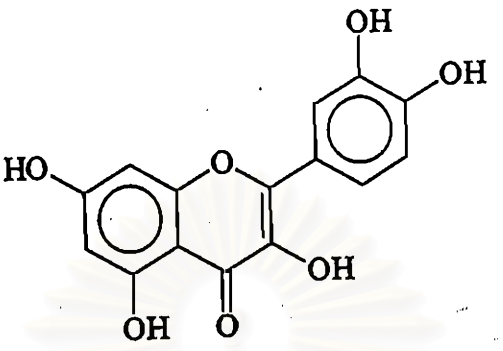


Kaempferol-7-0-glucoside

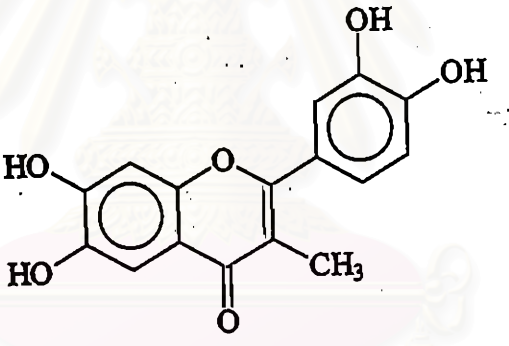


Kaempferol-3-0-glucoside

Fig. 1.4 Some Flavonoids Isolated from ***E. heterophylla*** L.

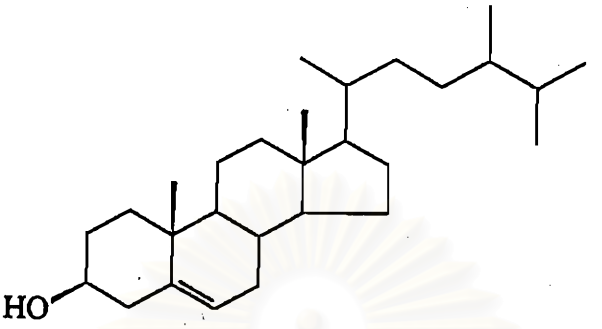


Quercetin

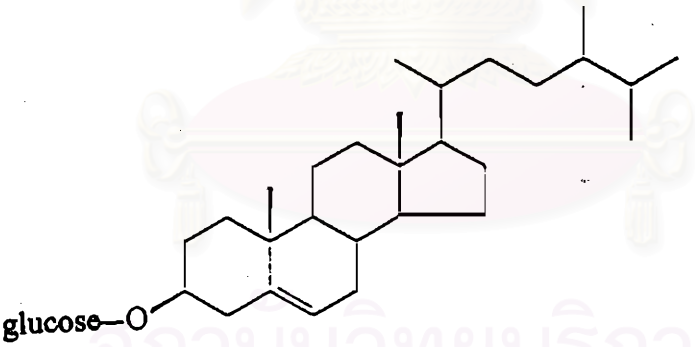


3-Methyl quercetin

Fig. 1.4 (Cont.)

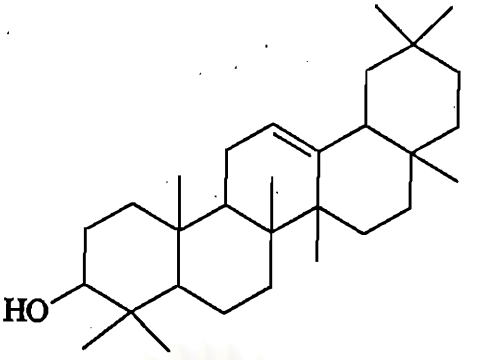


β-Sitosterol

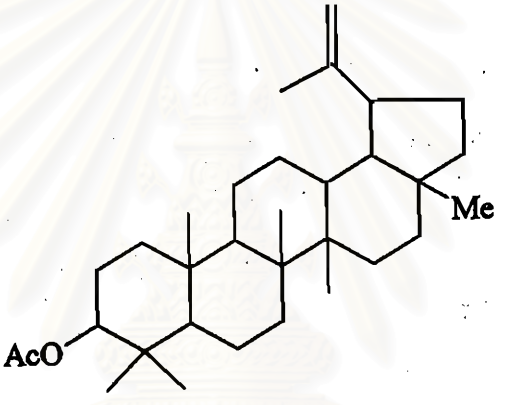


β-Sitosterol glucoside

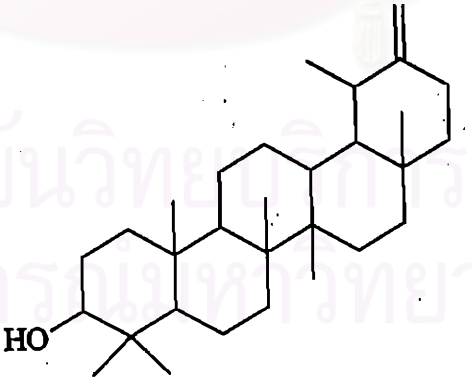
Fig. 1.5 Some Steroid from ***Euphorbia heterophylla*** L.



β-Amyrin



Lupeol acetate



Taraxsterol

Fig. 1.6 Some terpenoids Isolated from *Euphorbia heterophylla* L.

1.5 Goal of This Research.

The goal of this research could be summarized as follows :

1. Preliminary screening test for rice growth inhibition activity of 3 species weeds from the family Euphorbiaceae.

2. To extract and fractionation the organic compound from the plant.

3. To elucidate the structural formula of the isolation substances from the most attractive crude extract.

4. To search for rice growth inhibition compound.