CHAPTER I

INTRODUCTION

In the world, three locations where abundant species of plants are present including Southeast Asia, Tropical West Africa and Central America to the Amazon river area. Among them, only Southeast Asia did not suffer from the glacial age and has diversity in plant species. Thailand is a part of the Southeast Asia that abounds in plant species.[1] Plants have been not only the most important source of food but also essential source of chemical substances used for the direct or indirect benefits in pharmacy and agriculture.

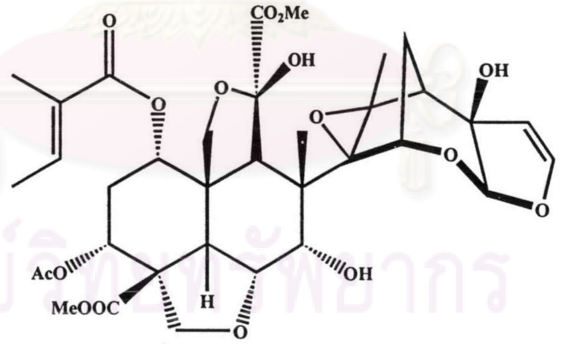
As for the agriculture, controlling the quality and quantity of crops is the most important. The crops can be damaged and reduced by pests such as weeds, fungi, insects, rodents and others.[1] These are very serious problems for all agricultural countries including Thailand. Nevertheless, chemical control agents, known as pesticide, have been the backbone of pest management and contributed a great deal in protecting crops from the damages inflicted by pests. In fact, most pesticides are obtained from synthetic agents. Most farmers usually employ synthetic agrochemicals since they are convenient, fast and easy to handle. However, they have many drawbacks. The use of synthetic pesticides has caused some concerns regarding their adverse effects on the environment. Since these compounds are often not biodegradable, thus the residues were concentrated in food chains and accumulated in soil, water and plants, so they make invariably environment pollution.[2] Moreover they are toxic to man, animal and wildlife. Besides, all of them are imported from foreign countries. Nowadays, toxicity, safety and cost-effective pesticides have been concern; a great deal of research have thus been directed towards the development of natural products. It is believed that naturally occurring compounds are environmentally friendly to nature because they are easy to decompose, low toxic to user and also reducible cost of imported pesticide.

1.1 Insect antifeedant in plants

Many phytophagous insects are equipped with taste receptor cells that are able to detect toxic compounds and to avoid such plants before being intoxicated. The feeding behavior of insects can be divided into four steps: (a) host plant recognition and orientation, (b) initiation of feeding, (c) maintenance of feeding and (d) cessation of feeding. An antifeedant is concerned with steps (b) and (c). The term antifeedant is defined as a chemical that inhibits feeding but does not kill the insect directly, *i.e.*, it remains near the treated leaves and dies through starvation. Gustatory repellent, feeding deterrent and rejectant are synonymous with antifeedant. Antifeedants could be of great value in protecting crops from noxious insects. Moreover, they are not harmful to parasites, predators or pollinators.[2]

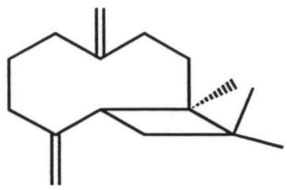
Many plant-derived terpenoids have potent insect antifeedant activity. Some examples are as follows:

The well known example for the utilization of plant terpenoids as antifeedant is azadirachtin. The neem tree, *Azadirachta indica* (Meliaceae), has long been known to contain the tetranortriterpene, azadirachtin which is the most potent natural antifeedant against a large number of insects, in both the larval and adult stages, and now commercially available in the United States.[3]

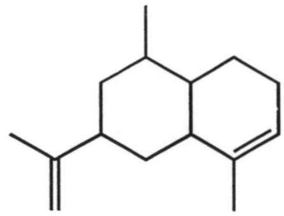


azadirachtin

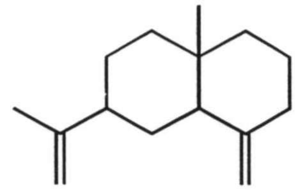
The bitter-tasting sesquiterpenoids are often antifeedants. The sesquiterpenoid caryophyllene is an aphid repellent and has antifeedant activity against cabbage butterfly larvae,[4] besides α-selinene, β-selinene and β-copaene which are also feeding deterency against *Spodoptera exigua* (beet army worm).[5] In addition, polygodial, a sesquiterpenoid from water pepper (*Polygonium hydropiper*), increased yield of barley by 36% due to its antifeedant effects on the aphid *Rhopalsoiphum padi*.[4]



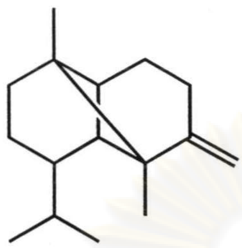
caryophyllene



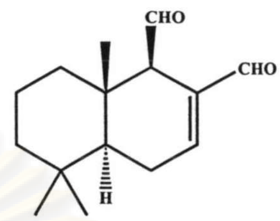
α-selinene



β-selinene



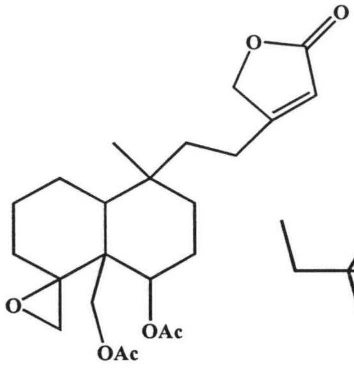
β-copaene



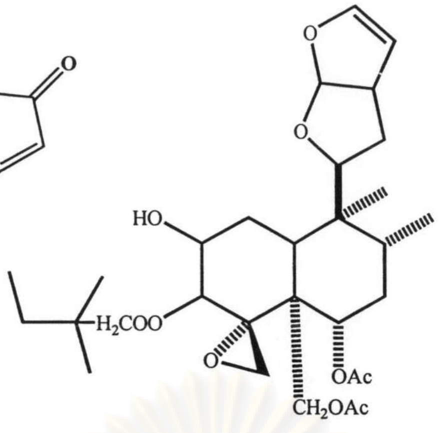
polygodial

Clerodanes are a large group of naturally occurring diterpenoids isolated mainly from Compositae and Labiatae plants. These compounds have attracted interest because of their challenging structures and their antifeedant properties against some economically important insect pests. Some of these substances are exemplified as follows:[6]

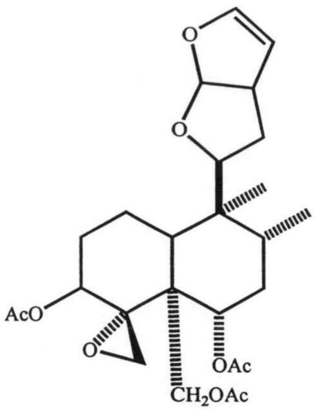
Ajugarin I, a diterpene from the bugle plant *Ajuga remota*, can be used in very low concentrations to deter feeding by Coleoptera such as the mustard beetle and also the major world pest, the Colorado potato beetle.[5] The clerodendrin B, 3-epicaryoptin, 15-hydroxyepicaryoptin and clerodin, isolated from *Clerodendron spp*., were effective antifeedants against *Earias vitella* and *Spodoptera litura*.[6] Recently, six *neo*-clerodans, teuctosin, teuflin, teucrin-H_2, 6β-hydroxy tuescordin, 6β-acetylteuscordin and montanin-D, were isolated from the aerial part of *Teucrium tomentosum* and showed effective antifeedancy against *Plutella xylostella* and *S. litura*.[7]



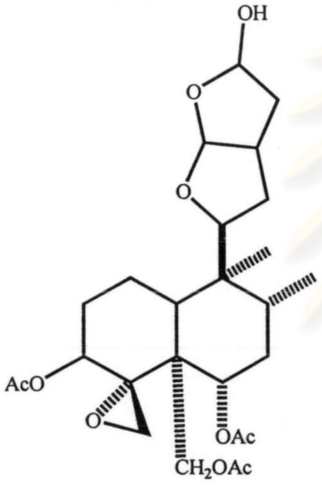
ajugarin I



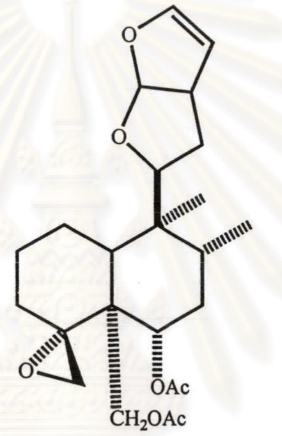
clerodendrin B



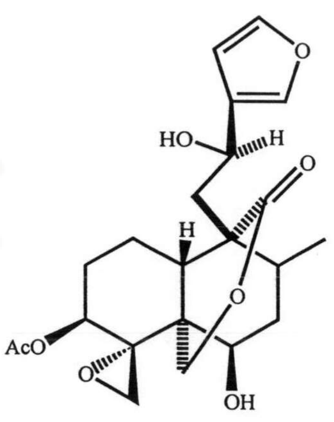
3-epicaryoptin



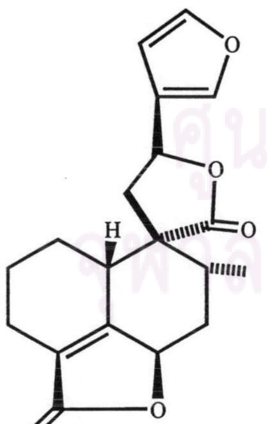
15-hydroxyepicaryoptin



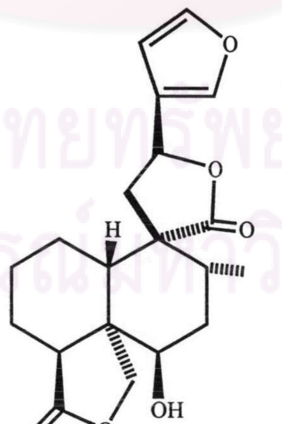
clerodin



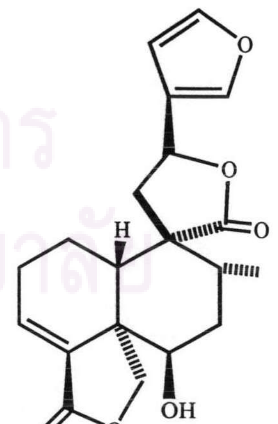
teuctosin



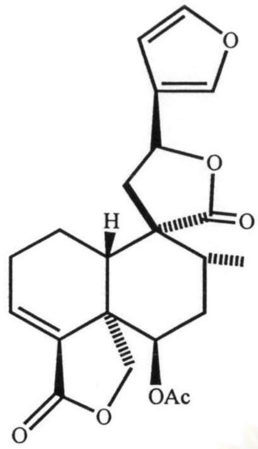
teuflin



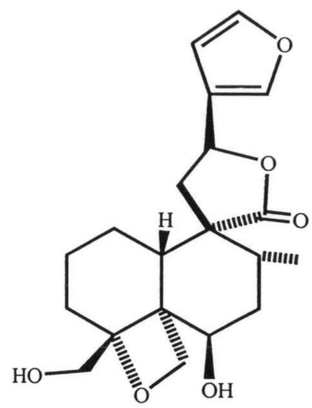
teucrin-H_2



6β-hydroxy tuescordin

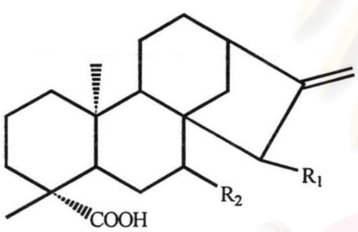


6β-acetylteuscordin



montanin-D

Some of kaurane diterpenes have also shown interesting antifeedant activity. For instance, sideroxol, isolated from *Sideritis akmanii* and *S. rubriflora* had potent antifeedant activity against *Spodoptera frugiperda*,[8] and compounds **1**, **2**, **3**, **4** have been found to inhibit feeding of lepidopteran larvae and aphids.[9]

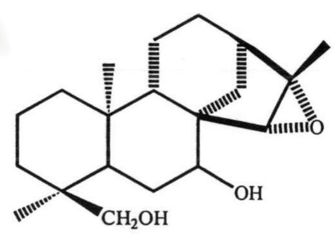


(1) R_1 = H, R_2 = OAc

(2) R_1, R_2 = H

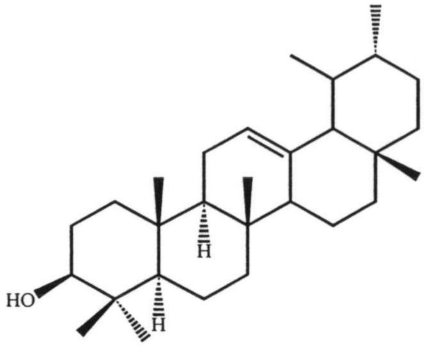
(3) R_1 = OAc, R_2 = H

(4) R_1 = O, R_2 = H

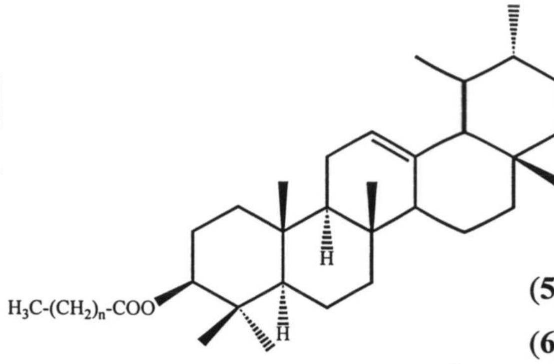


sideroxol

Among terpenoid classes, pentacyclic triterpenoids are extremely useful for the development of potent agrochemicals in view of their highly natural abundance and relatively nontoxic nature. A literature search reveals that ursolic acid, isolated from the leaves of *Diospyros melanoxylon* and its fatty acid ester analogues (**5**, **6**, **7**) exhibited potent antifeedant activity against the *S. litura*.[10] In connection with the investigation, acetyl aleuritolic acid from the roots of *Trigonostemon reidioides* Craib. also exhibited high antifeedant activity of insect antifeedant against Greater Wax Moth, *Galleria mellonella*.[11]



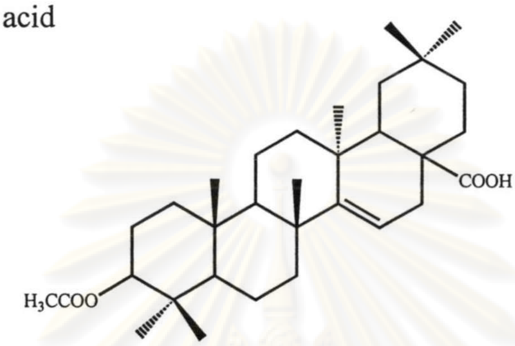
ursolic acid



(**5**) n=12

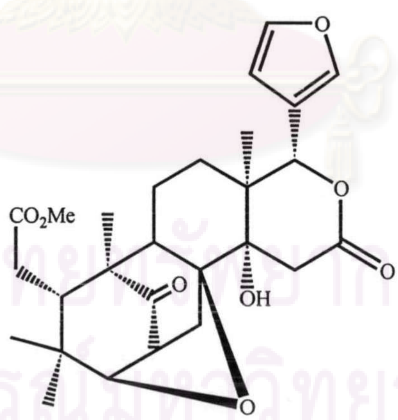
(**6**) n=14

(**7**) n=16



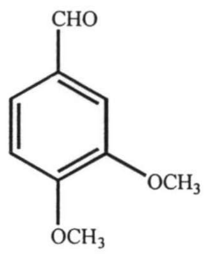
acetyl aleuritolic acid

Besides, the limonoid, xyloccensin K, isolated from the fruits and the seeds of *Xylocarpus granatum* Koen., also displayed an antifeedant activity of 81 % and 66 % against *G. mellonella* at dose level of 4.0 mg.[12]

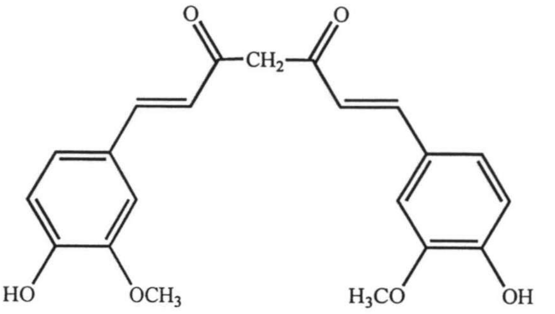


xyloccensin K

Some naturally occurring aromatic compounds have been reported to reveal potent antifeedant activity. For example, the active principles of the rhizomes of *Zingiber cassumunar* Roxb. are veratraldehyde and curcumin possessing high antifeedant activity against *G. mellonella.*[13]

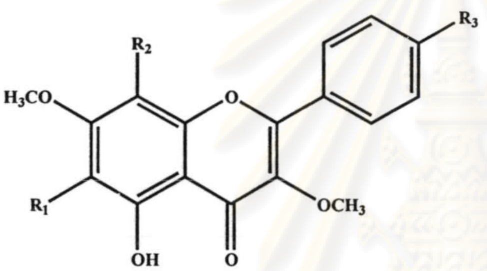


veratraldehyde

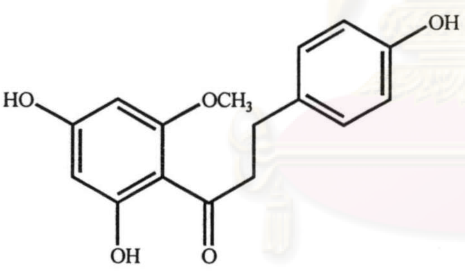


curcumin

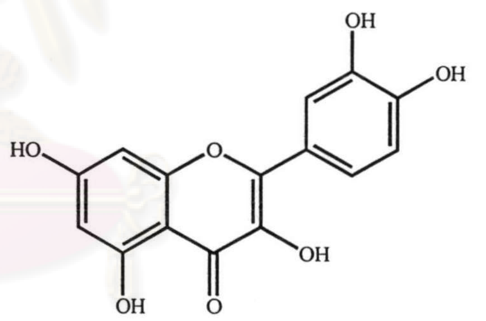
Four flavonoids (**8-11**) have been isolated from cudweed *Gnaphalium affine* D. Don, displaying strong antifeedant activity against the common cutworm, *Spodoptera litura.*[14] Quercetin and phoretin have also been shown antifeedant activity against *Scolytus multistriatus*, while rutin was active against *Helicoverpa zea.*[5]



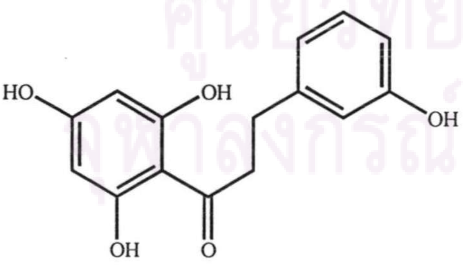
(**8**) R_1, R_2, R_3 = OCH_3
(**9**) R_1, R_2 = OCH_3, R_3 = H
(**10**) R_1 = OH, R_2, R_3 = H



11



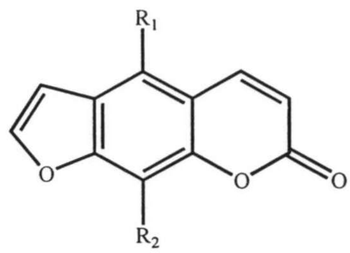
quercetin



phloretin

rutin

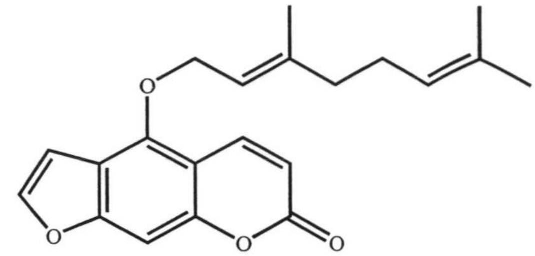
Some coumarins such as four furanocoumarin (**12**, **13**, **14**, **15**) have been isolated previously from the fruits of *Tetradium daniellii* exhibiting a potent feeding deterent effect towards larvae of *S. littoralis* and *Heliothis virescens.*[15]



(**12**) R_1= H, R_2 = OCH_3

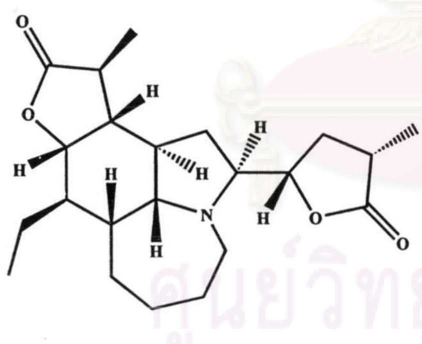
(**13**) R_1 = OCH_3, R_2= H

(**14**) R_1 = OCH_3, R_2= OCH_3

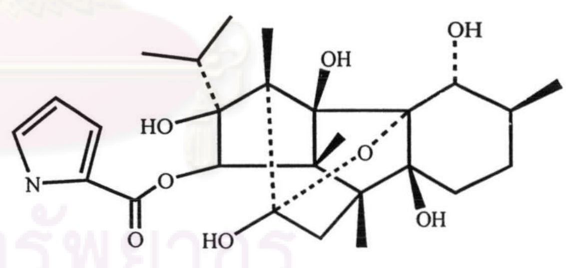


15

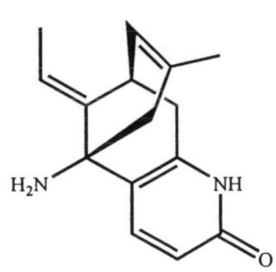
Some plant-derived alkaloids showed insect antifeedant activity, for example, tuberostemonine was the dominating alkaloid in the roots of *Stemona toberosa*, showing feeding inhibition of *Spodoptera littoralis.*[16] The natural alkaloidal ryanoid (**16**) is a very potent nonselective probe for the Ca^{2+} release channels of the house fly, cockroach, and mouse muscle membranes as well as an antifeedant against *S. litura*.[17] Recently, the isolation of huperzine A was reported as the major antifeedant and insecticidal component of the New Zealand clubmoss, *Lycopodium varium*, against the Australian carpet beetle, *Anthrenocerus australis*, the Australian sheep blow fly, *Lucilia cuprina*, and the webbing clothes moth, *Tineola bisselliella.*[18]



tuberostemonine



16



huperzine A

Based upon the information derived from literature study, the use of natural pest controlling agents is undoubtedly attracted worldwide attention. This thesis was concentrated on the isolation and identification of the insect antifeedant compounds from Thai plants. Therefore, seventeen Thai plants were accumulated for preliminary antifeedant screening study against common cutworm, *Spodoptera litura* (Fabricius). The botanical names of these plants are tabulated in Table 1.1.

Table 1.1 Selected Thai plants for screening experiment

Family	Species	Local name	Plant part
Annonaceae	*Anaxagorea luzonensis* Gray.	Kam-langwua thaloeng	Heartwood
Asclepiadaceae	*Cryptolepis buchanani* Roem, Schult.	Thao en on	Heartwood
Betulaceae	*Betula alnoides* Buch. Ham.	Kamlang suea khrong	Leaves
Cyperaceae	*Cyperus alternifolius* Roxb.	Kok rangkaa	Leaves
	Cyperus rotundus L.	Yaa haeo muu	Tubers
Lauraceae	*Cinnamomum subavenium* Miq.	Cha em	Heartwood
Leguminosae	*Pterocarpus macrocarpus* Kurz.	Pra duu	Heartwood
Leguminosae-Mimosoideae	*Xylia xylocarpa* (Roxb.) Taub var. *kerrii* (Craib&Hutch.) I.C. Nielsen	Daeng	Heartwood
Menispermaceae	*Anamirta cocculus* (L.)	Khamin khruea	Heartwood
Moraceae	*Artocarpus heterophyllus* Lam.	Khanun	Heartwood
Rhamnaceae	*Ventilago denticulata* Willd.	Raang daeng	Heartwood
Verbenaceae	*Tectona grandis* L. F.	Sak	Heartwood
Zingiberaceae	*Alpinia galanga* (Linn.) Swarts	Khaa	Rhizome
	Amomum xanthioides Walls	Reo	Rhizome
	Kaempferia galanga L.	Proh hom	Rhizome
	Zingiber cassumunar Roxb.	Phlai	Rhizome
	Zingiber zerumbet (Linn.) Smith	Ka thue	Rhizome

The screening insect bioassays were carried out at Department of Agricultural Chemistry, Kinki University, Nara, Japan under the collaboration project with Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University. It was disclosed that the dichloromethane crude extract from the heartwood of *Xylia xylocarpa* (Roxb.) Taub var. *kerrii* (Craib&Hutch.) I.C. Nielsen showed potentially interesting antifeedant activity against the common cutworm, *Spodoptera litura* (the details were presented in Chapter III). This preliminary results prompted to investigate on the chemical constituents responsible as a good template for agricultural purpose.

1.2 Botanical characteristics of *X. xylocarpa* species

Xylia represents the genus belonging to family Leguminosae-Mimosoideae. There are 12 different species in this genus mainly in Tropical Africa and Madagascar. In addition, there is only one species available in Asia. This genus can be divided into two varieties.[19]

1. *xylocarpa*:

    Botanical name: *Xylia xylocarpa* (Roxb.) Taub.

    Synonym: *Xylia dolabriformis* Benth.

    Distribution: India and Burma

2. *kerrii:*

    Botanical name: *Xylia xylocarpa* (Roxb.) Taub var. *kerrii* (Craib&Hutch.) I.C. Nielsen

    Synonym: *Xylia kerrii* Craib & Hutch.

    Distribution: Burma, Laos, Cambodia, Vietnam, Thailand

These two varieties are different in leaflets and anthers. The leaflets of *Xylocarpa* variety is glabrous to faintly puberulous beneath while the *kerrii* variety is puberulous to densely velutinous (rarely glabrescent). The anthers of the *xylocarpa* is glandular while the *kerrii* is eglandular.

In Thailand, the *kerrii* variety distributes throughout many regions of the country without the *xylocarpa* variety. The general name is known as Daeng. The other name is Khom, Kwai, Jalan, Takhom, Phan and others.[20] This plant grows in deciduous forest, dryevergreen forest, mixed deciduous forests and dry deciduous dipterocarp forests. The botanical characteristics (Fig. 1.1) can be described as follows:[21]

Tree: deciduous tree to 30 m with straight trunk and slender drooping branches.

Bark: creamy brown or red-brown, thin, peeling in round flakes, inner bark pink, heartwood reddish brown.

Leaves: bipinnate with a single pair of side stalks, 10-30 cm, each with 3-7 pairs of opposite leaflets, top ones largest, 4-15x2.5-6 cm, narrowly ovate or elliptic with slightly pointed tips. Young shoots densely covered with yellowish hairs, mature leaves smooth above, usually with minute pale brown hairs below. Leaflet stalks 0.2-0.3 cm, main stalk 3-8 cm, all joints with rounded glands. Young leaves delicate pink, appearing in March-April just after the flowers.

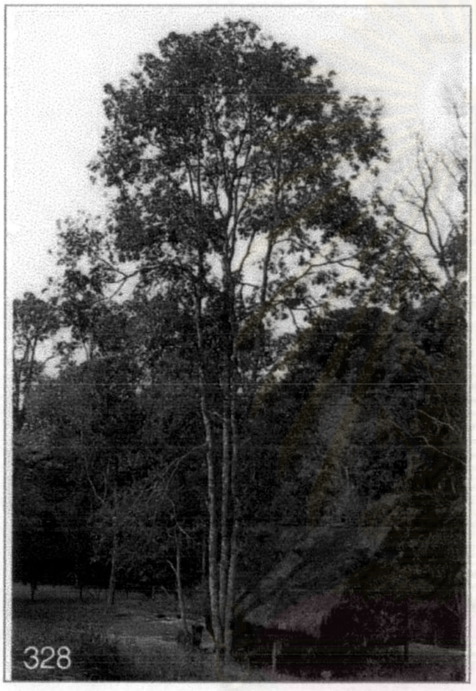
Flowers: pale yellow, in dense spherical heads, 1.5-2 cm, solitary or in very short, unbranched clusters in axils of fallen leaves. Head stalks 3.5-5 cm, individual flowers without stalks, 5 petals, 3.5-4.5 mm, slightly fused at base, hairy outside. 10-12 free stamens, 5-12 mm, much longer than petals, 5 stamens longer than others, anthers without glands.

Fruit: 10-15x5-6 cm, thick & woody, slightly curved, tapering at base, pale creamy brown, splitting suddenly into 2 parts which curl backwards, remaining on the tree for a long time.

Seed: 6-10 flat in a fruit, dark brown seeds 2x1.2 cm.

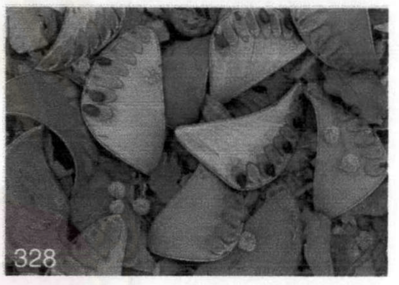
(a)

(b)



(c)

(d)



(e)

Figure 1.1 (a) anthers without glands and fruits, (b) leaves and flowers, (c) tree, (d) bark and (e) fruits and seeds

Since the wood is extremely hard, durable and rarely attacked by termites, it is used in constructions for bridges, railway crossties, harbor work, house flooring and posts.

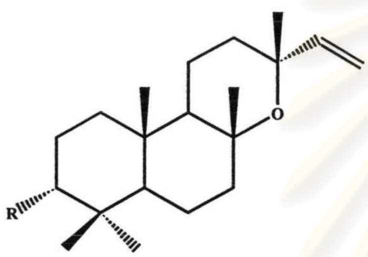
For the ancient Thai folklore, the bark is used to heal a digestive tonic and diarrhea. The heartwood can cure anaemia and diarrhoea, besides, it is used to nourish blood-body. The flower is used as antipyretic and heart nourishment.[20]

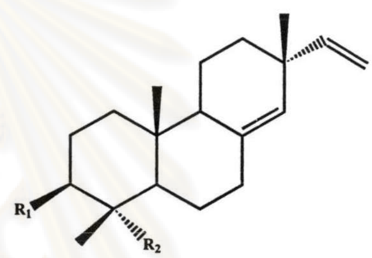
1.3 Chemical constituent and literature survey of *X. xylocarpa*

From the literature survey, there are a few reports on the chemical constituents only for var. *xylocarpa* that can be summarized as follows:

In 1951, Kudzin *et al.* reported that the composition from the heartwood of *Xylia dolabriformis* was lignin.[22]

In 1963, Laidlaw and Morgan isolated six diterpenes from light petroleum extract of the heartwood of *Xylia dolabriformis*. They are manoyl oxide (8α, 13*R*-epoxy-14-labdaen-3-ol) (**17**), 3-oxomanyl oxide (8α, 13*R*-epoxy-14-labdaen-3-one) (**18**), 8(14),15-isopimaradiene (**19**), 8(14),15-isopimaradiene-3-one (**20**), 8(14),15-isopimaradiene-3β-ol (**21**) and 8(14),15-isopimaradiene-3,18-diol (**22**).[23] The structures of these compounds are exhibited below.





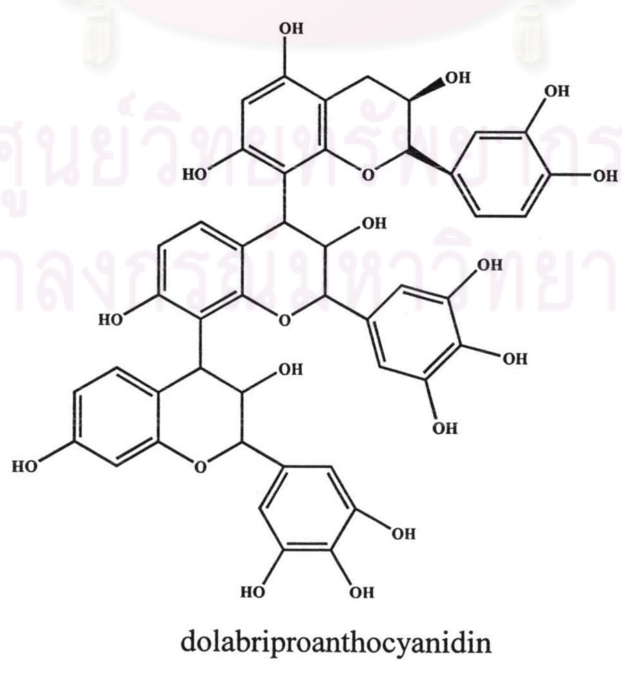
(**17**) R = OH

(**18**) R = O

(**19**) R_1 = H, R_2 = CH_3 (**21**) R_1 = OH, R_2 = CH_3

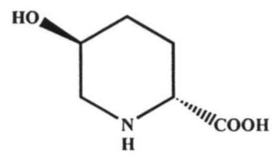
(**20**) R_1 = O, R_2 = CH_3 (**22**) R_1 = OH, R_2 = CH_2OH

In 1976, Kumar *et al.* isolated dolabriproanthocyanidin from the stem barks of *Xylia dolabriformis.*[24]



dolabriproanthocyanidin

In 1979, Mester *et al.* reported the isolation of *trans*-5-hydroxy pipecolic acid from the leaves of *Xylia dolabriformis*. This compound revealed a powerful inhibitor of platelet aggregation induced by serotonin.[25]



trans-5-hydroxy pipecolic acid

In 1984, Aswal *et al.* disclosed that 50% ethanolic extracts from the fruits and barks of *Xylia dolabriformis* exhibited toxicity (LC_{50} = 510 and 825 mg/kg, respectively) to tested mice by injection into the abdominal cavity.[26]

For the var. *kerrii*, surprisingly, there is no report concerning the chemical constituents and biological study particularly agricultural activity. Therefore, this plant has attracted interest for studying the chemical constituents and finding the lead compounds possessing antifeedant activity.

1.4 General charateristics of *Spodoptera litura* (Fabricius)

The insects named *Spodoptera litura* (Fabricius) were used for testing antifeedant activity because they are an important pest found through years and destroyed nearly any herbaeous plants such as tobacco, tomatoes, cabbage, lettuce, beetroot, peanuts, beans, cotton, strawberry, apple and others. The common name is cutworm. The details of the species are shown as follows:[27]

Life cycle: Adult moth (Fig. 1.2a) is stouts. Forewings dark with wavy white markings. Hindwings white with margins having a brown colour. Eggs are laid in clusters of 200-300 underneath the leaves covered with brown hairs. The incubation period is 4-5 days. The full grown caterpillar (Fig. 1.2b) larva is stout, cylindrical and pale greenish brown in color with dark markings. They have transverse and longitudinal gray and yellow bands. The caterpillar is nocturnal in habit, 35-40 mm in length and pupates in the soil (Fig. 1.2c, d). The larval period lasts for 2-3 weeks. Adult emerges from pupa in about two weeks. Life cycle occupies 30-40 days.

Type of damage: The common cutworm attacks the shoot of young plants and cut them. The cut portion of the shoot dries up and falls down. They also damage the leaves by feeding.

Period of occurrence: They are found through years and mostly during August to February.

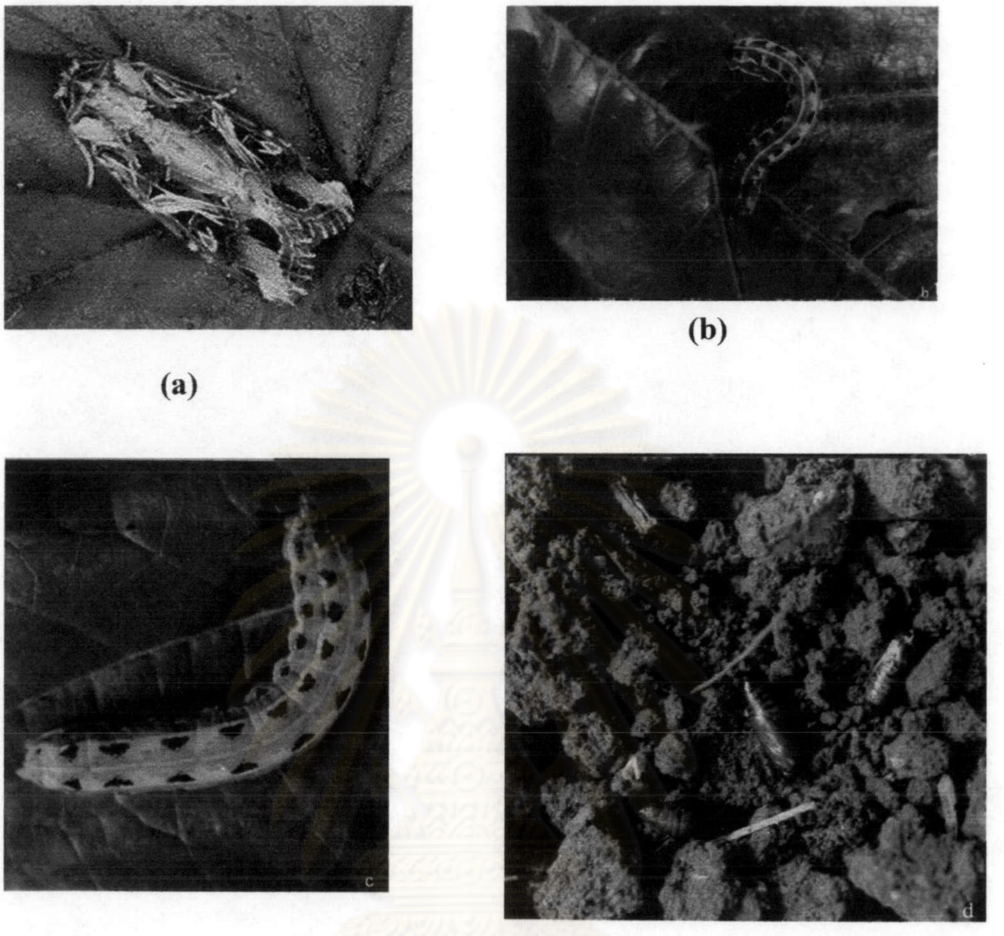

(a) **(b)** **(c)** **(d)**

Figure 1.2 (a) adult (b) young cutworm (c) late age cutworm and (d) pupae in the soil

1.5 General charateristics of termite (*Reticulitermes speratus*)

The termite used in this study, *Reticulitermes speratus* belongs to family Rhinotermitinae which is a major pest in America, Europe and Asia. They can cause damage to crops, buildings, pasture and forestry as well as to non-cellulose materials such as dam linings and electrical cables.[28]

Termites, just as some ants, have castes such as workers, soldiers and winged reproductives (Figure 1.3).

Workers are wingless, not sexually mature and blind. As with other social insects, termite workers have an important role in the nest. Their tunnelling and food collection make some of them pests. Other jobs include building and maintaining the nest, looking after and feeding the young and other non-wood feeding castes such as soldiers and the royal pair. Workers also groom and clean other castes.

Soldiers possess a larger head that is longer and wider than that of the worker. Size may also be related to sex or different developmental pathways in some species. The role of soldiers is for defence.

Reproductives have wings of equal size, which can produce new kings and queens.

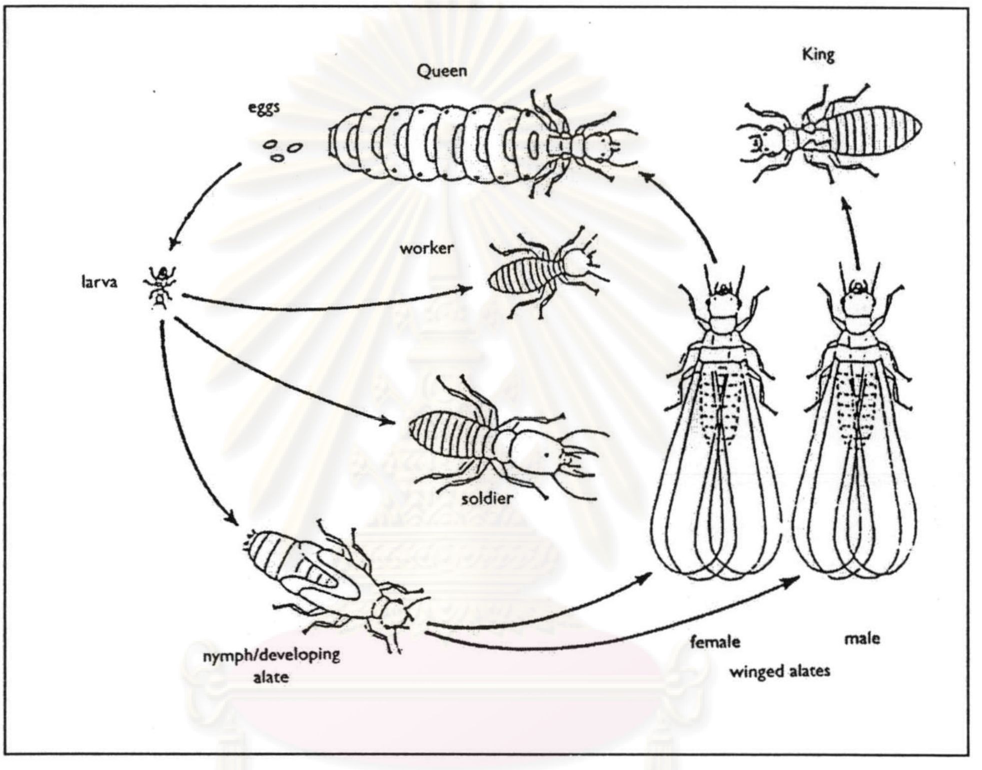


Figure 1.3 The castes of termites

1.6 The goal of this research

From the primarily biological screening result, the extracts of *X. xylocarpa* showed the excellent antifeedant activity. Therefore, the goal of this research could be summarized as follows:

1. To extract the heartwood of *X. xylocarpa*.
2. To preliminarily screen for antifeedant activity of crude extracts.
3. To separate and isolate organic constituents from some crude extracts and elucidate the structures of isolated substances.
4. To perform agricultural-based activity such as antifeedant activity of isolated substances.