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FIELD	**DEPARTMENT**

TITLE: Toxicity and Its Possible Mechanism of *Bauhinia scandens* Extract on *Plutella xylostell*a (Lepidoptera: Plutellidae)

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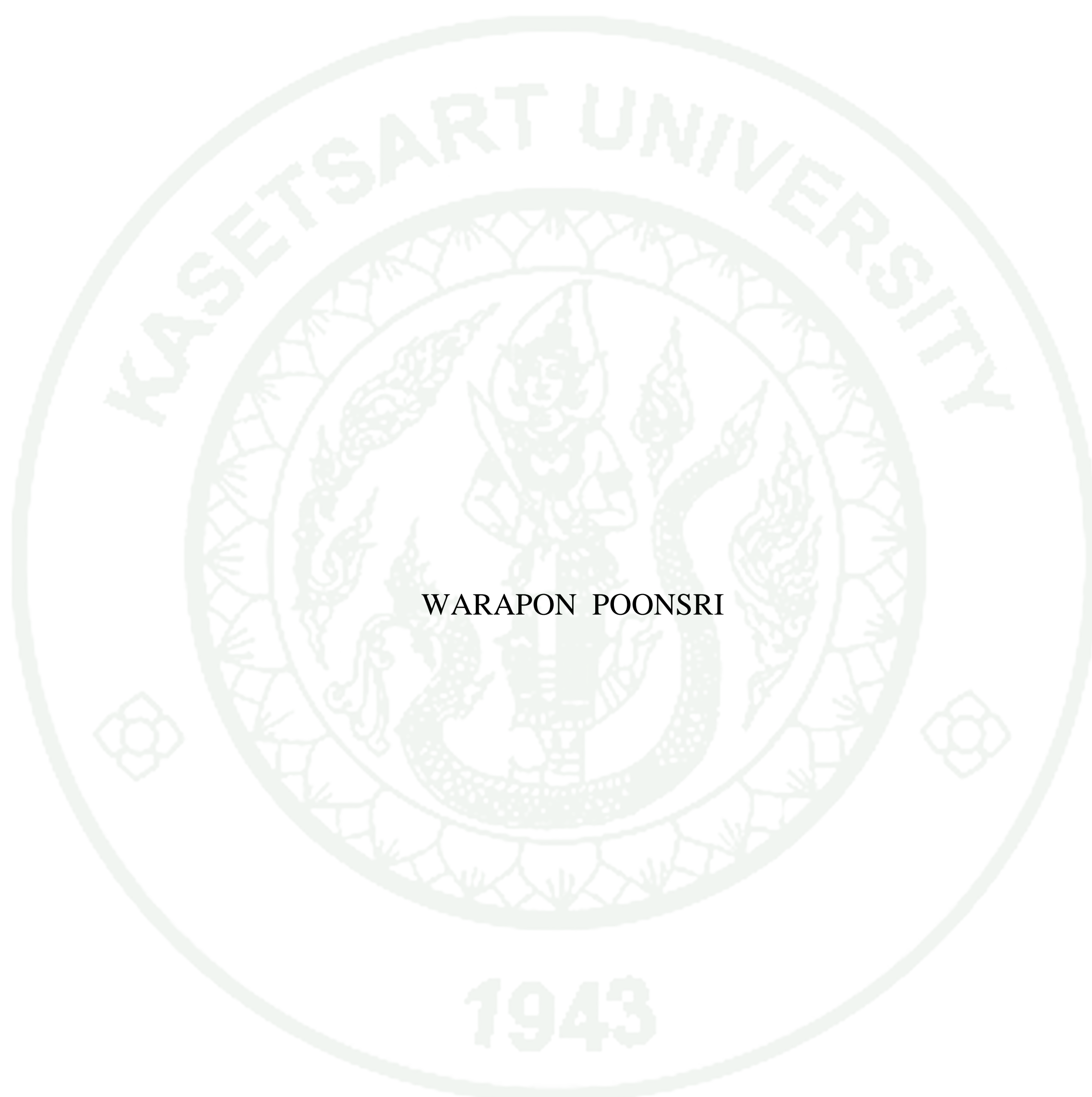
THESIS

TOXICITY AND ITS POSSIBLE MECHANISM OF *BAUHINIA SCANDENS* EXTRACT ON *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE)



WARAPON POONSRI

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Master of Science (Biology)
Graduate School, Kasetsart University
2014

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Warapon Poonsri 2014: Toxicity and Its Possible Mechanism of *Bauhinia scandens* Extract on *Plutella xylostell*a (Lepidoptera: Plutellidae). Master of Science (Biology), Major Field: Biology, Department of Zoology. Thesis Advisor: Assistant Professor Vasakorn Bullangpoti, Ph.D. 77 pages.

The insecticidal activity of materials derived from the *Bauhinia scandens* extracts against *Plutella xytostella* were examined via topical application method. The biologically active constituent was characterised as the long chain alkane, among various extracts dichloromethane based extract was the best with an LD50 of 3,778±135.48 ppm and 2,426.55±5.77 ppm at 24 and 48 hours after exposed.This extract after sequented fractionation gave Tetradecane 0.33%, Hexadecane 0.87%, Octadecane 1.26%, Nonadecane 0.28%, Eicosane 1.39%, Heneicosane 0.91%, Docasane 2.51%, Tricosane 3.52%, Tetracosane 8.28%, Pentacosane 19.01%, Hexacosane 25.88% and Heptacosane 34.67% .This alkanes mixture which caused LD50 2,925.25 ppm. Detoxification enzyme for mode of action were studied. These results indicated that *B. scandens* may be a promising naturally occurring agent for *P. xytostella* larval control.

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Waraporn Poonsri
February 2014

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LIST OF ABBREVIATIONS

α-	=	Alpha-
β-	=	Beta-
BHT	=	Butylated hydroxytoluene
cm	=	Centimeter
°C	=	Degree Celsius
CH_2Cl_2	=	Diochloromethane
EtOAc	=	Ethylacetate
EtOH	=	Ethanol
MeOH	=	Methanol
g	=	Gram
hr	=	Hours
LD_{50}	=	Median lethal dose
kg	=	Kilogram
µg	=	Microgram
µl	=	Microliter
mg	=	Milligram
min	=	Minute
mM	=	Millimolar
mol	=	Mole
nm	=	Nanometer
%	=	Percent
rpm	=	Round per minute
ppm	=	Parts per million

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TOXICITY AND ITS POSSIBLE MECHANISM OF *BAUHINIA SCANDENS* EXTRACT ON *PLUTELLA XYLOSTELLA* (LEPIDOPTERA: PLUTELLIDAE)

INTRODUCTION

Plutella xylostella is the one of serious polyphagous pest of broccoli, Brussels sprouts, cabbage, Chinese cabbage, cauliflower, collard, kale, kohlrabi, mustard, radish, turnip, and watercress causing over US $1 billion worth damage globally per annum. Outbreaks of *P. xylostella* in Southeast Asia sometimes can cause more than 90% crop loss. This pest has now been recorded from 128 countries of the world including Thailand (Saeed *et al*., 2010) and many scientists try to control this insect pest.

In Thailand, our farmers try to control this insect but it is still difficult. It is because *P. xylostella* has quick development of resistance to almost all groups of insecticides, including organochlorines, organophosphates, carbamates, pyrethroids, insect growth regulators, abamectins, pyrazoles, oxadiazines, neonicotinoids (Vanichpakorn *et al*., 2010). Accordingly, integrated Pest Movement (IPM) strategies are being used as ecofriendly control of pests and botanical insecticides are becoming important components of this strategies. There are some previous research using botanicals for control *P. xylostella* such as the ethyl acetate, ethanol, and acetone of root and rhizome *Veratrum nigrum* extracts, the ethyl acetate of *Phytolacca americana* root extract and the petroleum ether of *Pseudolarix kaempferi* Gord root bark extract which were effective against second and third instar larvae of *P. xylostella* (Vanichpakorn *et al*., 2010). In addition, hexane, chloroform and ethyl acetate extracts of *Acalypha fruticosa* Forssk showed strong antifeedant and larvicidal activities against *P. xylostella* (Lingathurai *et al*., 2010). Extracts derived from *Melia azedarach* and *Azadirachta indica* on populations of *P. xylostella* are well known (Charleston *et al*., 2006).

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Bauhinia scandens (Family Fabaceae – Caesalpiniaceae) was used in the present study because it is traditionally known in Nepal to treat virus caused diseases. In addition there are some previous researches that describe this order having insecticidal activity to some pests. The acetone root extract of *B. galpinii* has also been found to be highly cytotoxic (LD50 2.70 Ng/ml) against Vero cell lines (Samie et al., 2009).Antimicrobial activity of Combretum vendae against four bacterial pathogens (Ahmed et al., 2009) and apigenin has been isolated from the acetone leaf extract (Eloff et al., 2008), Methanol and dichloromethane leaf extracts of *B.galpinii* are reported to have antimutagenic property (Reid et al., 2006).

Therefore, present study on *Bauhinia scandens* extracts against and impact on *P. xylostella* was done to determine the toxicity detoxification enzyme like carboxyl esterase and glutathione-*S*-transferase for resistance information in the future and the changing of neuron enzyme (Acetylcholinesterase).

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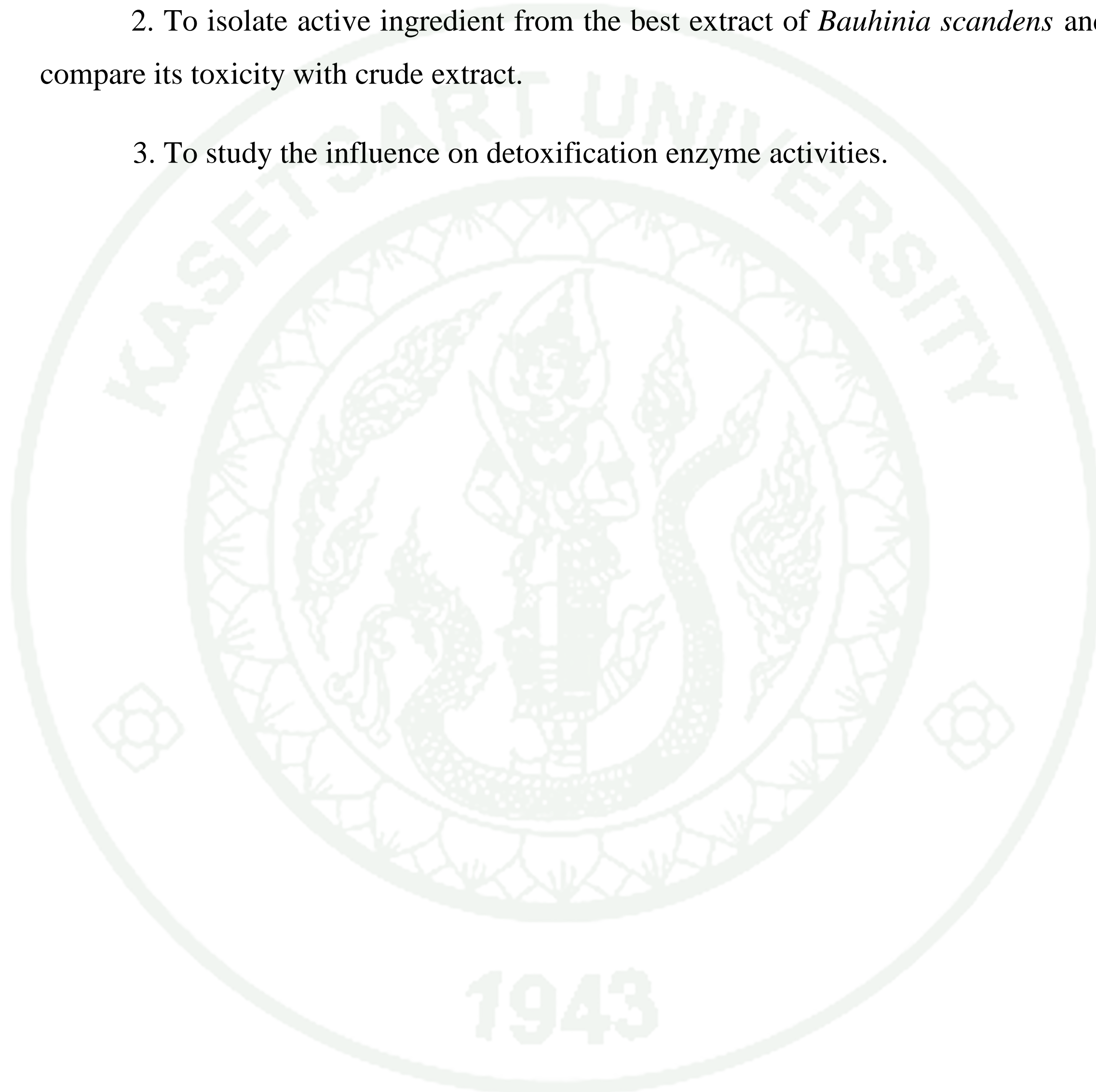
OBJECTIVES

1. To study the toxicity of *Bauhinia scandens* extracts for the control of *P. xylostella*

2. To isolate active ingredient from the best extract of *Bauhinia scandens* and compare its toxicity with crude extract.

3. To study the influence on detoxification enzyme activities.



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LITERATURE REVIEW

1. Diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae)

Diamondback moth (DBM), *Plutella xylostella* (Lepidoptera:Plutellidae), is one of the most destructive insect pests of brassicaceous crops worldwide and serious pest of cauliflower, cabbage, broccoli, mustard, radish and turnip (Lingathurai *et al*, 2010). *P. xylostella* may have originated from Europe or possibly South Africa, whatever the origin of this pest, it has now been recorded from 128 countries of the world (Saeed *et al*., 2010) including Thailand.

P.xylostella has become a very serious problem and causes great economic loss to farmers. *P.xylostella* attack is sometime so catastrophic that farmers are unable to save their crops even with the cocktails of most toxic chemicals and are compelled to uproot their cruciferous vegetables and grow other non host crops like garlic , tomatoes and onions to save themselves from complete economic wreck.

Biology and Life cycle of *P. xylostella*

Insect taxonomy of *P. xylostella*

Phylum Arthopada
Class Insecta
Order Lepidoptera
Family Plutellidae
Genus *Plutella*
Species *Plutella xylostella*

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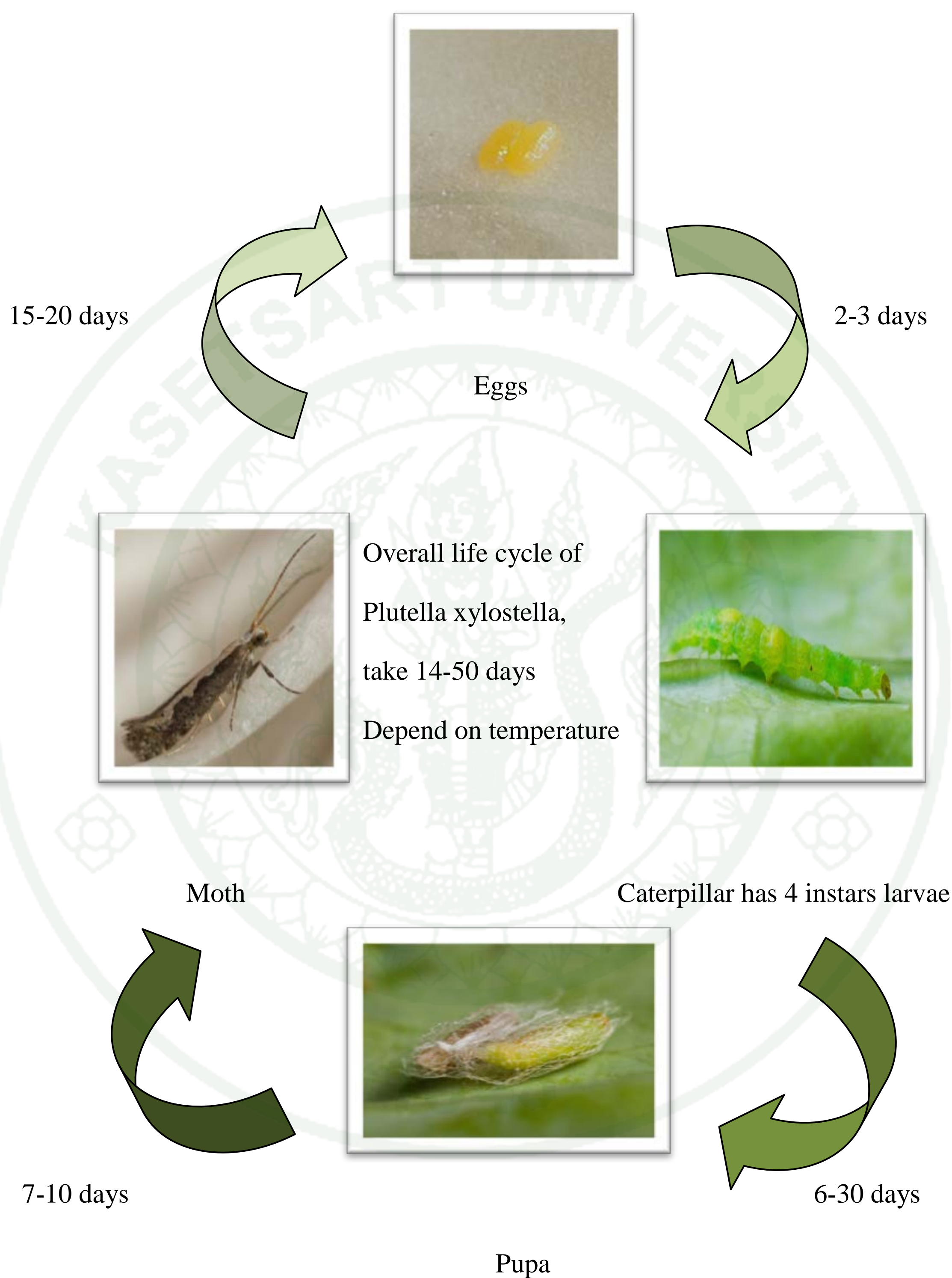

Figure 1 Life cycle of diamondback moth (Overall life cycle may take 14 – 50 days depend on temperature)

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The adult moth (Figure 1) is small and slender with very long antennae. It is grayish-brown with a broad cream or light brown band along its back. The band can have constrictions, which give it a diamond-like pattern. When viewed from the side, the wing tips appear to turn up slightly. Eggs are oval and flattened, yellow to pale green,and approximately 0.02 inches long and 0.01 inches wide.There are four larval instars. Even the oldest is quite small and very active. Larvae will wriggle violently if sturbed and will drop from the leaf suspended by a strand of silk.The body tapers at both ends and the fifth pair of prologs protrudes from the posterior (Figure 2). After the first instar, which is colorless, the larvae are green. Larvae pupate in a loose cocoon on lower or outer leaves or in the florets of cauliflower and broccoli.

Figure 2 Top of diamondback moth.

Source : Walker (2007)

Figure 3 Side of diamondback moth.

Source : Walker (2007)

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Figure 4 Diamondback moth larva.

Source : Photo courtesy the Canola Council of Canada (2014)

Figure 5 Pupa of diamondback moth.

Source : Walker, K. (2007)

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Biology

The female moth attaches eggs to the lower leaf surface either singly or in groups of two or three. Within a few days, the eggs hatch, and the larvae begin to feed on the underside of the leaf. The larval stage can last from ten days to a month depending on temperature. Diamondback moth larvae slow their feeding at temperatures below 50°F, and population growth is most rapid at temperatures greater than 80°F. The pupa stage is passed within a transparent, loose cocoon, which is usually attached to the underside of leaves. In warm weather, the pupa stage may be completed in 3 to 4 days.

In Thailand *P. xylostella* disperse well in February to April which the most optimum climate for their host plants, especially around large cities and in specializes vegetable growing area that supply vegetables to cities throughout the year. *P. xylostella* is cosmopolitan and reproduces under extremely varied climatic conditions (Paramonov 1953). Although a temperature range of 17°C to 25°C is considered optimum for this insects (Atwal 1955). It breed all year around in warm humid tropical region. In Thailand, it breeds throughout the year and can have up to 20 generations per year with considerable overlapping of generations in the field.

In southern Florida, diamondback moth is most abundant from December to February or March and can attack at any time during the crop cycle. By the end of May, moth counts in pheromone traps fall to near zero. Moth counts may rise in mid-fall through early winter, but activity is limited during that time. Populations build on winter weeds, such as wild mustard, before moving into winter and early spring plantings of cabbage and other crucifers. From mid-winter through the spring, when it is a serious pest, diamondback moth may cause losses of up to 70 percent in the absence of control. Populations may decrease after heavy rains.

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Damage

Cruciferous plants at all stages of growth may be attacked. Larvae chew small holes in leaves, with larger larvae making larger holes. Often young larvae feed on one surface of the leaf, leaving a thin layer or window of leaf epidermis. Diamondback moth larvae will also attack developing cabbage heads. The resulting damage deforms the heads and leaves entry points for decay pathogens.

Management

P. xylostella is serious pest of cruciferous crops in Thailand for a long time and only choice for control of this pest is achieved by using organochlorine, organophosphate, carbamates, pyrethroid, insect growth regulators, abamectin, pyrazole, oxadiazine, and neonicotinoid (Vanichpakorn *et al*., 2010).

Currently, botanical insecticides have been studied for control of *P.xylostella* such as Charleston *et al*, (2005) used *Melia azedarach* and commercial formulations from the neem tree for control of this insect. Extracts from the leaves of *Momordica charantia* (Bing *et al*., 2008), *Veratrum nigrum* L. root and rhizome extracts, the extract of *Phytolacca americana* L. root, and extract of *Pseudolarix kaempferi* Gord. (Vanichpakorn *et al*., 2010) have also been studied against this insect. Another biological control method to control this insect is use of Ascoviruses (AVs) that are pathogenic to lepidopteran larvae, and most commonly attack Noctuidae like *Heliothis virescens* AV (HvAV-3e) (Furlong and Asgari, 2010). Moreover there are some research on the using of natural enemies for control *P.xylostella* as *Cotesia plutellae* , the endoparasitoid wasp that parasitizes larvae of the diamondback moth (Bay and Kim, 2009).

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Botanical products have been evaluated for their toxicity (Table 1), antifeedant and larvicidal activity against *P. xylostella* such as *Mammea americana* seed extract (Kritchaya *et al*., 2011), *Derris elliptica* Benth root extract (Visetson and Milne, 2001), *Azadirachta indica* seed extract (Robert and Denis, 1993), *Annona squamosa* seed extract (Andrey and Isman, 2004 and Dadang *et al*., 2009), *Piper sarmentosum* Roxburgh leaves extract (Orratai, 2008) and *Syzygium aromaticum* flower extracts (Kumnuan, 2006).

Table 1 The median lethal dose (LD_{50}) of plants extracts on *P. xylostella* larvae after topical application in toxicity bioassay.

Scientific name	Common name	24h LD_{50} value (ppm)
Annona squamosa	Annona	0.10
Azadirachta indica	Neem	0.54
Syzygium aromaticum	Clove	1.09
Piper sarmentosum	Wild betal Leaf bush	4.34
Mammea americana	Mammey	5.90
Derris elliptica	Derris	24.25

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Bauhinia scandens L.

Plant taxonomy

Bauhinia sp. is a member of subfamily Caesalpinoideae of family Fabaceae – Caesalpiniaceae. According to Hutchinson a genus of 250 species of trees, shrubs, and climbers has gained a positive medicinal and other economic values. Many species of *Bauhinia* are used traditionally in Nepal to treat virus caused diseases.

Plants of the genus *Bauhinia* (Fabaceae), commonly known as cow's-paw or cow's hoof, are widely distributed in most tropical countries and have been used frequently in folk medicine to treat different kinds of pathologies, particularly diabetes, infections, as well as pain and inflammation. In recent years, interest in these plants has increased considerably throughout the world. The biological properties of different phytopreparations and pure metabolites have been investigated in numerous experimental *in vivo* and *in vitro* models. Although some contradicting evidence has been documented, in general, the results support the reported therapeutic properties, indicating that they are mainly due to the presence of flavonoids.

Bauhinia monandra, *Bauhinia purpurea* and *Bauhinia forficata* are used in folk medicine for the cure of diabetes have been (Albequerque et al.,2000). Antiprotozoal and antiviral activities of *Bauhinia variegata* was reported by Aswal et al.,(1984), and its root were prescribed by Charak and Susruta for treatment of snake bites (Roy et al., 1988).

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Classified of *Bauhinia scandens* L. (Larsen, K.; Larsen, S.S., 1978)

Kingdom Plantae

Division Magnoliophyta

Class Magnoliopsida

Order Fabales

Family Leguminosae - Caesalpiniaceae

Genus *Bauhenia*

Species *Bauhinia scandens*

Figure 6 *Bauhinia scandens* L.

Source : Natureman (2010)

In Thailand, *Bauhinia* species can be divided into two groups based upon their habit. The first group is comprised of trees or shrubs, while the other is tendrilled climbers. *Bauhinia scandens* L., belongs to the latter, but is clearly distinct from the other tendrilled species in having entire leaves with acuminate or caudate leaf apices, oblong or elliptic floral buds, floral bud 12–15 mm long, raceme or panicle inflorescences, 10–13 mm long hypanthium, and the anther opening by longitudinal slits.

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Characteristics and Propagation

Bauhinia is a genus of more than 200 species of flowering plants in the subfamily Caesalpinioideae of the large flowering plant family Fabaceae, with a pantropical distribution. The genus was named after the Bauhin brothers, Swiss-French botanists.

Many species are widely planted in the tropics as orchid trees, particularly in northern India, Vietnam and southeastern China. Other common names include Mountain Ebony and Kachnar (India and Pakistan). In the United States of America, the trees grow in Hawaii, coastal California, Texas, Louisiana, and Florida. *Bauhinia* is the floral emblem of Hong Kong–a stylized orchid tree flower appears on the Hong Kong flag and Hong Kong Airlines uses 'Bauhinia' as its radio callsign in air traffic communication.

Bauhinia trees typically reach a height of 6–12 m and their branches spread 3–6 m outwards. The lobed leaves usually are 10–15 cm across.The five - petaled flowers are 7.5-12.5 cm diameter, generally in shades of red, pink, purple, orange, or yellow, and are often fragrant. The tree begins flowering in late winter and often continues to flower into early summer. Depending on the species, *Bauhinia* flowers are usually in magenta, mauve, pink or white hues with crimson highlights.

Propagation of *Bauhinia* species is from seeds or cuttings. They prefer acidic soils and do not tolerate salty conditions. Full sun exposure is preferred but they can be grown under partial sun. Generous watering is needed during summer; moderate moisture required in winter.

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Chemical composition and bioactivity

B. scandens var. horsfieldii (Miq.) K. et S.S. Larsen (Leguminosae-Caesalpiniaceae) is a common tropical plant found in every part of Thailand. This plant species has been endeavored since this plant species is a part of Thai village knowledge for efficient control on mosquitoes. From our previous preliminary research, *Bauhinia scandens stem* crude extracts was shown to control *P. xylostella* (Poonsri et al, 2012). The objective of the present study was to confirm the effects of *B. scandens* and its active compound on the biological parameters of *P. xylostella* to develop the new strategies for its control. The mode of action on detoxification enzymes was also investigated.

In Brazil, plants belonging to the genus *Bauhinia* are reported by the rural population to be important antidiabetic agents; the leaves and stem-bark of these plants are used in different phytopreparations to lower blood glucose levels (da Silva and Cechinel Filho, 2002;Cavalcanti and Favoreto, 2005; Mali *et al.*, 2007). *B. forficata*, an Asiatic plant well adapted to the Brazilian climate (Miyake *et al.*, 1986), is one of the most commonly used plants against diabetes. Although it has been widely studied biologically, its chemical composition has recently attracted attention, in particular due to the isolation of kaempferol-3,7-O-(alpha)-dirhamnoside (kaempferitrin) (**1**), a flavonoid shown to occur only in the leaves of the plant and, consequently,one that can be used as a chemotaxonomical marker (da Silva *et al.*, 2000). The therapeutic potential of this plant was first confirmed in 1929, with the clinical studies of Juliani (1929) which showed the ability of *B. forficata* to lower blood sugar. Later, the antidiabetic potential of the plant was confirmed in dogs, humans and rabbits (Juliani, 1931, 1941).

The secondary metabolites and pharmacological activities of plant species in the genus *Bauhinia* were revised and compared from relevant literatures. Flavonoids were the main compounds, found in the *Bauhinia* genus plants. Pharmacological activities that have been reported in these species included hypoglycemic, antioxidant and anticancer activities, among others.

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Many compounds can be isolated from all parts of this plants including roots, stems, leaves, flowers and seeds such as 5,6,7,3',4',5'-hexamethoxyflavone (1), 5,6,7,5'-tetramethoxy-3',4'- methylenedioxyflavone from *B.championii* Benth. (Chen et al., 984) 5,6,7,3'-tetramethoxyflavone from *B.guianensis* Aubl. (Almanza et al., 2001) kaempferol (2), quercetin, kaempferol 3-O-α-rhamnoside from *B. megalandra* G. (Estrada et al.,2005) kaempferol-3-O-β-galactoside,quercetin 5,7,3',4'-tetramethyl ether from *B. racemosa* Lamk. (Jain and Srivastava, 2001) bausplendin from *B. splandens* HBK Laux (et al.,1985) and 7,3'-dimethoxy-4'-hydroxyflavan (3), 2,4'-dihydroxy-4-methoxydihydrochalcone (4) from *B. manca* standl (Achenbach et al., 1988) and there are the chemical compounds in flavonoids

Stilbenes and derivatives as preracemosol A (5), transresveratrol(6), pacharin from *B. racemosa* Lamk (Anjaneyulu et al.,1986) bauhinol A (7) from *B. saccocalyx* Perre. (Apisantiyakom et al., 2004)

Steroids and terpenoids as β –sitosterol(8) , campesterol from *B. candicans* Benth (Iribarren and Pomilio, 1983); (Iribarren and Pomilio, 1984); (Iribarren and Pomilio, 1985); (Iribarren and Pomilio, 1987); (Iribarren and Pomilio, 1989) , lupeol (9) from *B. variegata* L. (Gupta et al.,1980)

Phenolic acid derivatives as apionic acid (10), isoacteoside and lignans there are (-)-isolariciresinol 3-α-O-β -D-glucopyranoside (11), (+)-1-hydroxypinoresinol 1-O-β-D-glucopyranoside from B. tarapotensis Benth. (Braca et al.,2001) lithospermoside (12) from *B. fassoglensis* Kotschy ex Schweinf. (Fort et al., 2001) Quinones group are lapachol (13) from *B. guianensis* Aubl. (Viana et al., 1999), bauhinione from *B. variegata* L. (Zhao et al., 2005)

Benzofuran derivatives group are griffonilide (14) from *B. thonningii* schum. (Okuwute et al., 1986) and Alkaloids group are harmane (15), eleagnine From *B. ungulata* L. (Maia Neto et al., 2008)

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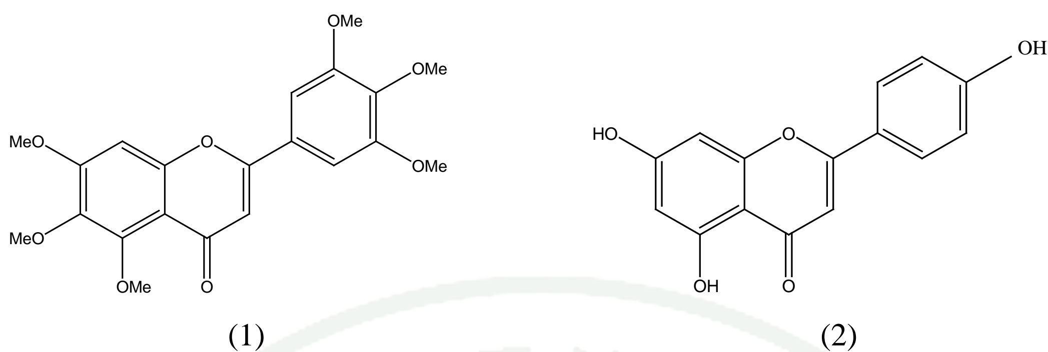

Figure 7 Structure of some secondary metabolites in *Bauhinia* genus

(1) 5,6,7,3',4',5'-hexamethoxyflavone

(2) Kaempferol

(3) 7,3'-dimethoxy-4'-hydroxyflavan

(4) 2,4'-dihydroxy-4-methoxydihydrochalcone

(5) Preracemosol A

(6) Transresveratrol

(7) Bauhinol A

(8) β –sitosterol

(9) Lupeol

(10) Apionic acid

(11) (-)-isolariciresinol 3-α-O-β -D-glucopyranoside

(12) Lithospermoside

(13) Lapachol

(14) Griffonilide

(15) Harmane

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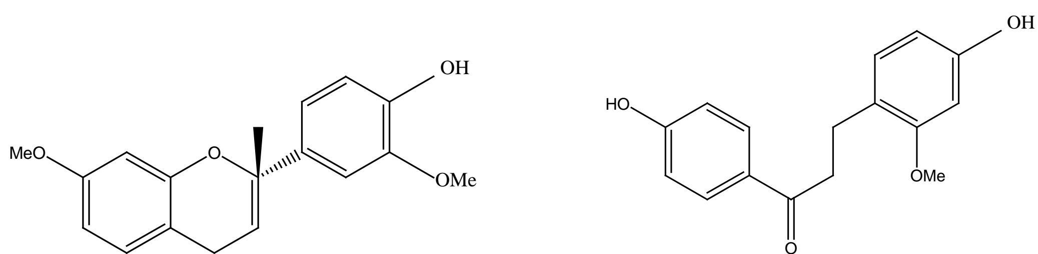
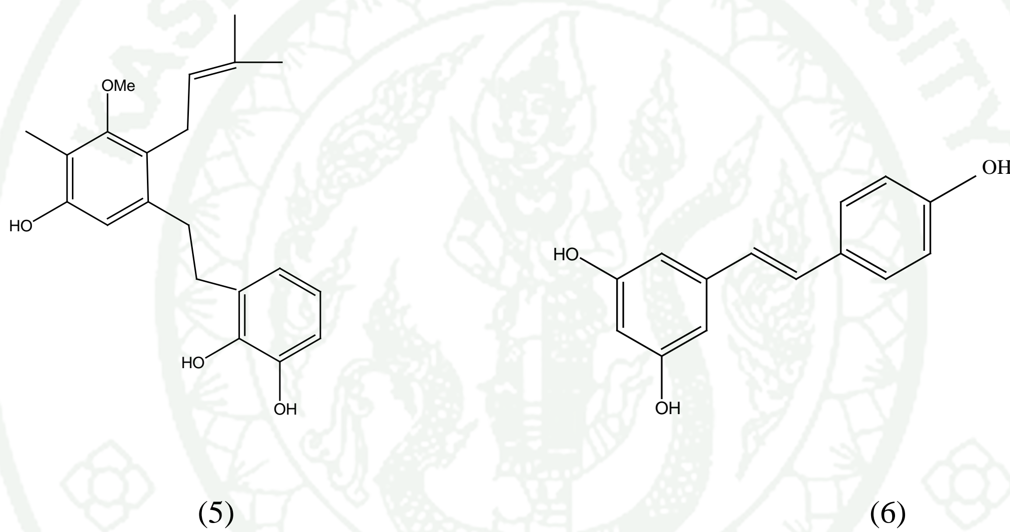


(3) (4)



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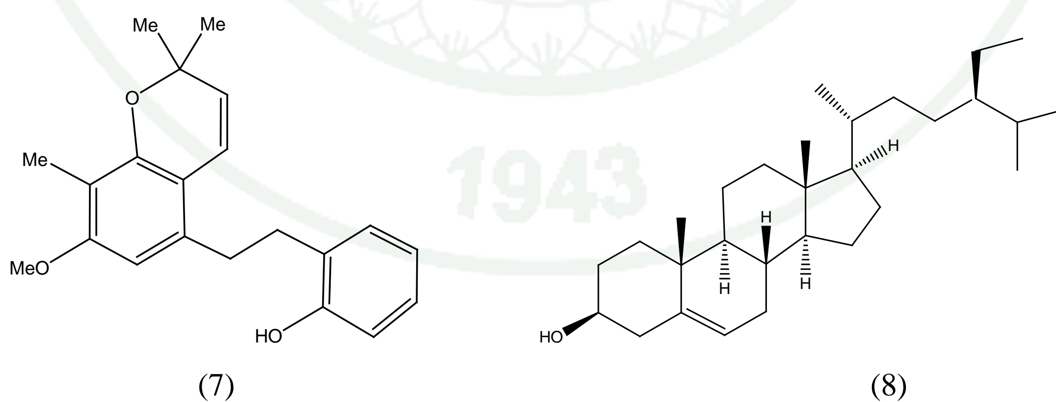

(7) (8)

Figure 7 (Continued)

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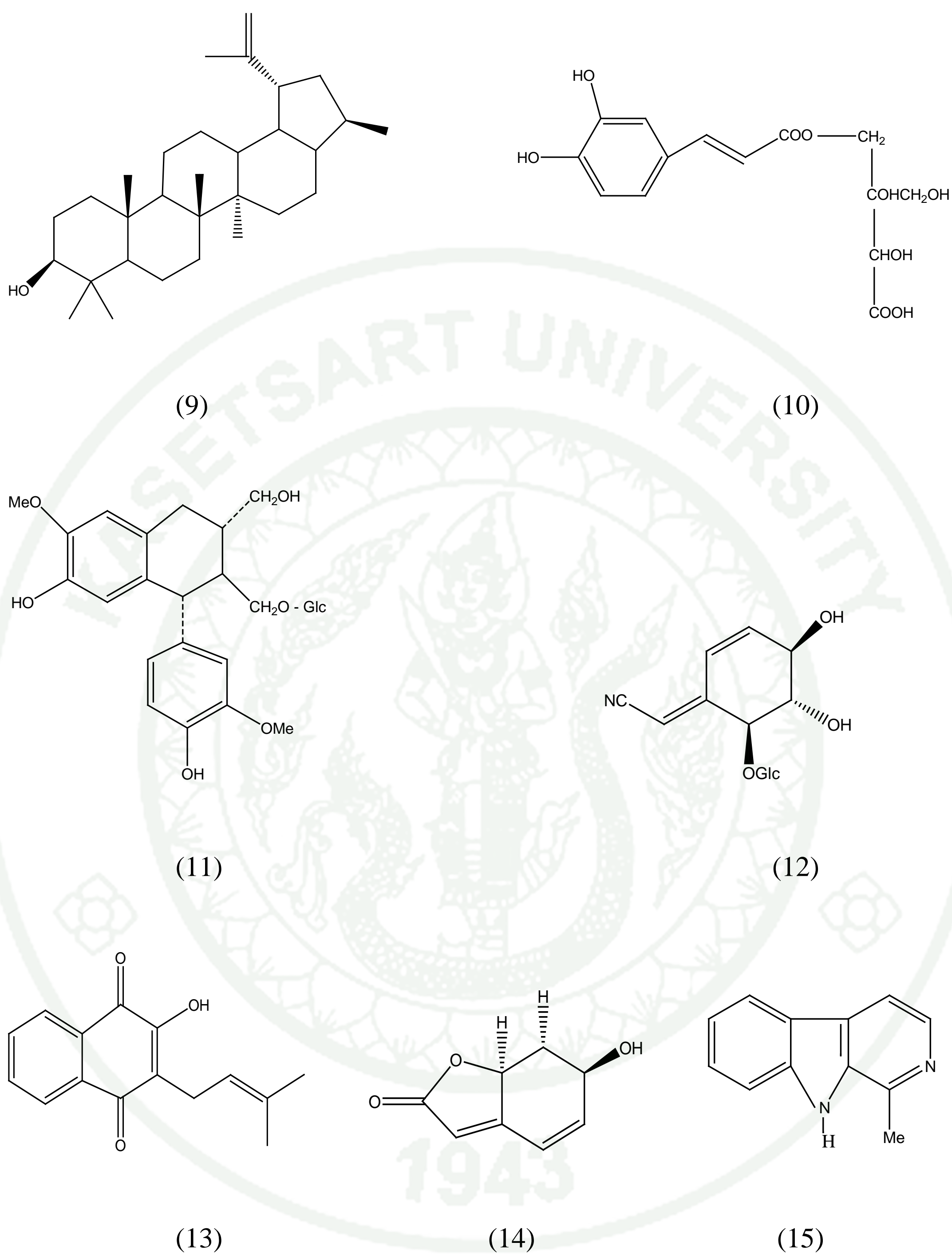

(13) (14) (15)

Figure 7 (Continued)

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Mode of action study of insect enzyme activity

General esterase activity

Esterase activity variation has been associated with scavenging action like alternative target esterase have wide range specific with substances with ester group. Or pyrethroid, organophosphate and chitin insecticide resistance protecting the target site by catalyzing and hydrolysis of insecticide (Figure 8) In insects, esterase enzymes are accumulated in intestine and adipose cells (Bullangpoti, 2007). Normally founds in nuclei, mitochondria and cytosol. The role of esterase as biochemical diagnose tool for malathion resistance in *P. xylostella* has reported significant higher frequency of esterase in zymogram study using 1-naphthyl acetate (William and Sin, 2000).

An increased rate of this detoxifying enzymes was responsible for imparting resistance in *Plutella xylostella* larvae to insecticides. These results emphasize a need for developing a location-specific insecticide resistance management.

Mohan and Gujar (2003), studies detoxification enzymes in *P.xylostella*, the carboxylesterase was reported to hydrolyze only malathion to a significant extent in *P. xylostella*. In *H. armigera* and it’s sibling species in the Americas, *Helicoverpa zea*, strains and individual insects resistant to OPs or SPs have been found with higher carboxylesterase activity compared to susceptible controls (Teese *et al*., 2010).

Carboxylesterase (CEs) metabolism shows important role in *P. xylostella* resistance. It can be utilized for enhancing toxicity of pesticides and insecticides such as permethrin and malathion by using corresponding carboxylic acid and alcohol, hydrolysis (Figure 12) of can trans-esterify fatty acids and tri-acylglycerols to fatty acid ethyl esters of variety of esterified drugs like cefpodoxime proxetil, meperidine, cocaine and heroin,cefuroximine axetil. CEs of insects are located in cytosol, microsomes; nuclei and mitochondria (Bullangpoti, 2007). The role of CEs as biochemical diagnose tool for lambdacyhalothrin and benzoate insecticide resistance

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in *P. xylostella* has reported higher level activity of CEs (Botwe *et al*., 2012). Increase of CEs is used for devising biochemical diagnostic methods of detection of insecticide resistance (Brown and Brogdon, 1987).

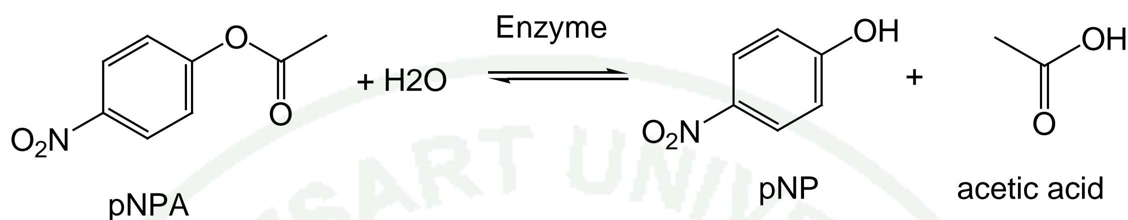

Figure 8 Reaction of pNPA assay

Source: Ganske (2009)

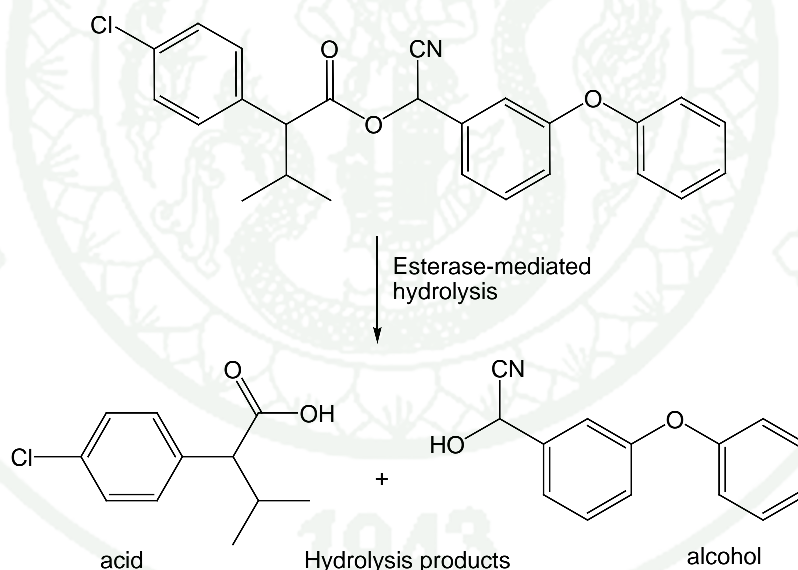

Figure 9 Esterase detoxification mechanisms by hydrolyzed pyrethroid esfenvalerate to alcohol and acid

Source: Craig (2005)

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Glutathione-S-transferase (GST) activity

It is well known that it belong in multigene family of drug metabolising enzymes which are important in phase II metabolism during cellular detoxification GSTs and it confer resistance to organochlorine, organophosphorous and pyrethroid insecticides by GSTs catalyze the conjugation of reduced glutathione (GSH) with exogenous and endogenous toxic compounds or their metabolites, rendering them more water soluble, less toxic, and easier to excrete.

In insect GSTs have been classified into six classes: delta, epsilon, omega, sigma, theta and zeta. The delta and epsilon classes of insect GSTs are insectspecific and exist as large gene clusters in insect genomes. Most of the GSTs that have been implicated to play roles in insecticide resistance belong to the delta and epsilon classes (Deng *et al*., 2009).

Glutathione-S-transferase shows important role in detoxification of oganochlorine and organophosphate by catalyzes the formation of thiol group (Figure 11) of glutathione to electrophilic xenobiotics which provides ability to scavenge toxic compound like oxidative stress. Glutathione S-transferase was present in all the developmental stages of *Plutella xylostella*. The enzyme levels increased rapidly and reached a maximum at the pupal stage and then declined towards adulthood. The role of GST as biochemical diagnose tool for methyl parathion resistance in *P. xylostella* has reported increased expression of this GST *isozyme*. (Huey *et al*., 1998).

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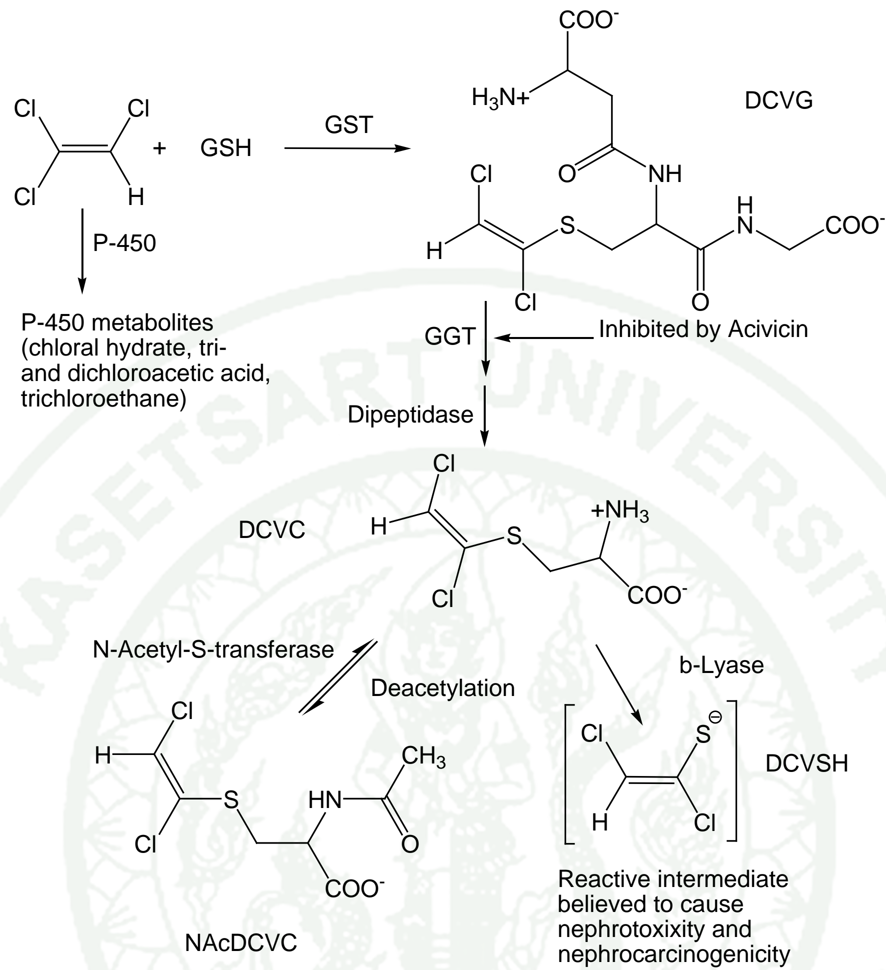

Figure 10 Scheme GSH-conjugation pathway

Source: Brian (2000)

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MATERIALS AND METHODS

Materials

1. Blender (Moulinex)
2. Hot air oven (Memmert-600)
3. Soxhlet apparatus
4. Weighing apparatus (Mettler- Toiedo)
5. Vacuum pump (Büchner)
6. Rotary evaporator (Buchi,Vacuum controller V-850)
7. Cages (24x24x24 inch)
8. Feeding boxes (20x10x4 cm)
9. Micropipette 0.5-10, 10-100 and 100-1,00 µl (DENVILLE, XL3000i™)
10. -25°C Refrigerator (Brandt)
11. Cellulose extraction thimble 28x100 mm (Whatman®)
12. pH meter (Cyberscan, 500^{PH} Eutech)
13. TLC plates (20x20cm)
14. Filter paper no.1 (Whatman®)
15. Vortex (VELP Scientifica)
16. Microplate reader (Biotek, Powerwave 340)
17. 96-well plate (Axygentechnology)
18. Centrifuge (Hettichi, Universal 32)
19. Glass column (10x10 cm)
20. pH meter (Cyberscan, 500^{PH} Eutech)

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Chemicals

1. Hexane (MERCK)
2. Dichloromethane (CH_2Cl_2) (J.T. Baker)
3. Distilled water (GIBCO^{TM})
4. Ethyl acetate (EtOAc) (LAB-Scan)
5. Ethanol (EtOH) (Mallinckrodt)
6. Hydrochloric acid (HCl) (Sigma®)
7. Sodium chloride (NaCl) (Sigma®)
8. Glycerin (Sigma®)
9. L-Glutathione reduced (GST) (Sigma®)
10. Ethylenediaminetetraacetic acid (EDTA) (UNIVAR)
11. Sodium hydroxide (NaOH) (J.T. Baker)
12. Polyvinyl poly pyrrolidone (PVPP) (ALDRICH)
13. Triton X-100 (Sigma®)
14. Ethylenediaminetetraacetic acid (EDTA) (UNIVAR)
15. Silica gel no. 1.07731 and 1.07734 (Whatman®)
16. Tris (hydroxymethyl) aminomethane (Tris-HCl) (Sigma®)Hexane (MERCK)
17. 1-Chloro-2, 4-dinitrobenzene (CDNB) (ALDRICH)
18. Potassium phosphate (KH_2PO_4) (UNIVAR)
19. Acetone (J.T. Baker)
20. Bradford solution (Sigma®)
21. Dimethylsulfoxide (DMSO) (Amresco®)
22. Chloroform-D1 ($CDCl_3$)
23. 5,5'- Dithiobis (2-nitro-benzoic acid) (DTNB) (Sigma®)
24. Bovinserum albumin (BSA) (Sigma®)
25. Disodium hydrogen phosphate (Na_2HPO_4) (Sigma®)
26. Paranitrophenyl acetate (pNPA) (Sigma®)
27. Sodium sulfate anhydrous(Garloerba)

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Methods

1. Insect rearing

P. xylostella (Figure 11) were obtained from a kale farm in Nonthaburi province, Thailand and were raised in an insect-rearing room at the Faculty of Science, Kasetsart University. *P. xylostella* larvae were reared on kale leaves that had never been exposed to pesticides in a cage (29 × 19 × 3 cm) at 26 ± 1°C, 75% RH and a photoperiod of 16:8 h (L:D). The kale leaves were changed every two days. Pupae were collected and kept in a net cage until the moths emerged. Moths were fed with a 10% sugar solution. Cabbage leaves were used for oviposition. Second instars were randomly used for the toxicity assay.

(A) (B) (C) (D)

Figure 11 (A) Eggs, (B) Larva, (C) Pupae and (D) Adult moth of *P. xylostella*

2. Plant material

B. scandens (Figure 12) stems were collected from Chachoengsao province, Thailand (60 km from Bangkok, Thailand) during the morning in the rainy season (May- August, 2012). The plant material was shade dried for three days.Plants twigs were dried in hot air oven (at 60°C) and powdered in mechanized blender. The powder stored in zip-lock bags in refrigerator at 4°C to prevent sample contamination.

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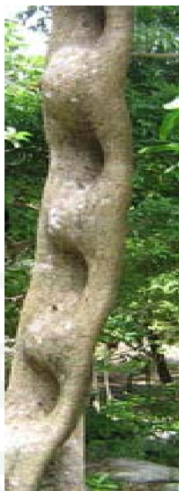

Figure 12 Sampling of *B. scandens*

3. Extraction

The dried powder of 200 g of *B. scandens* was subjected solvent extraction using Soxhlet apparatus (Figure 14). The solvent used were hexane, dichloromethane, ethyl acetate and ethanol sequentially. Each crude extract filtered with vacuum pump and was dried using a rotary evaporator, freeze dried and then stored at 4°C until further use in the toxicity experiments.

Figure 13 Blender for make *B. scandens* to powder

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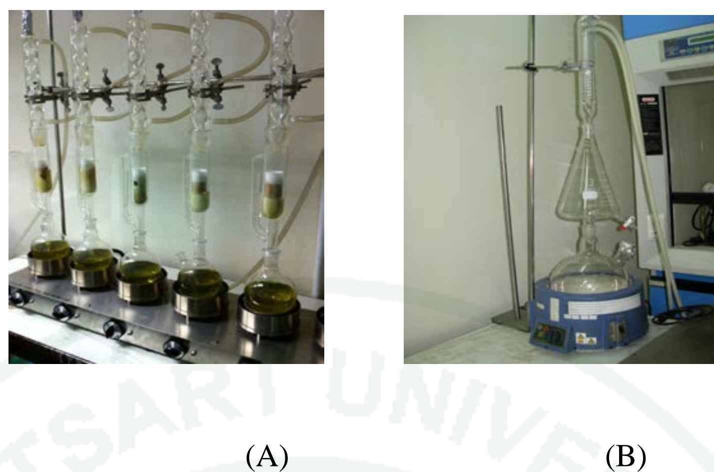

(A) (B)

Figure 14 *B. scandens* extraction by using Soxhlet's apparatus (A), Solvent Distillation Equipment (B)

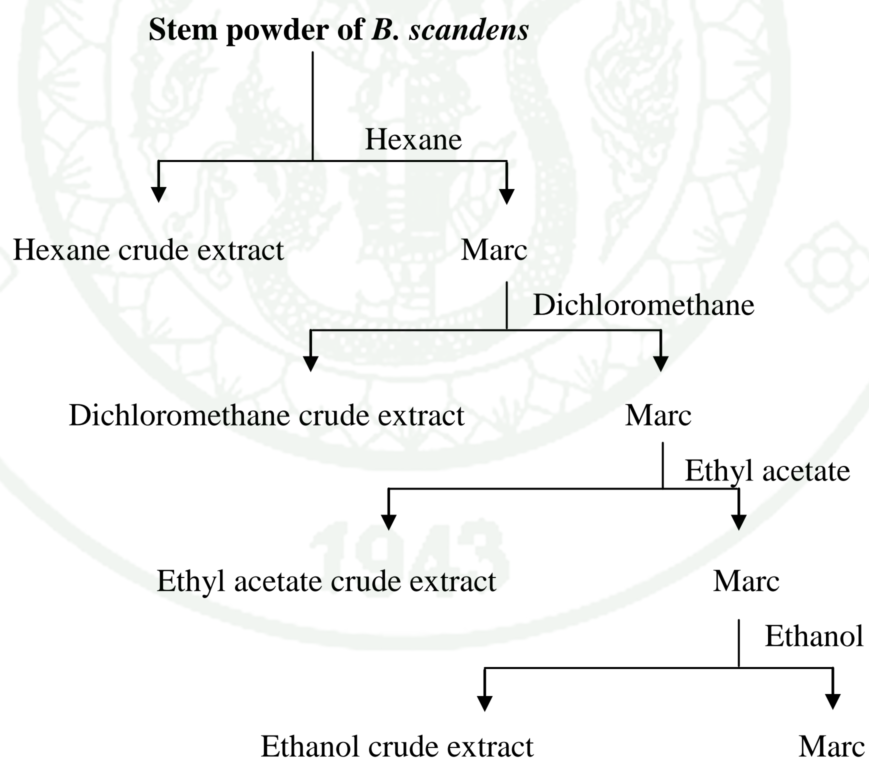

Figure 15 Extraction of *B. scandens*

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Figure 16 Evaporation

4. Isolation

The highest efficacy of *Bauhinia scandens* dichloromethane crude extracts were selected to isolate by using Column Chromatography and Preparative Thin Layer Chromatography (PTLC).

Bauhinia scandens dichloromethane crude extracts

Column Chromatography

Preparative Thin Layer Chromatography

NMR-MS spectroscopy

Figure 17 Isolation active compounds in *Bauhinia scandens* dichloromethane crude extracts

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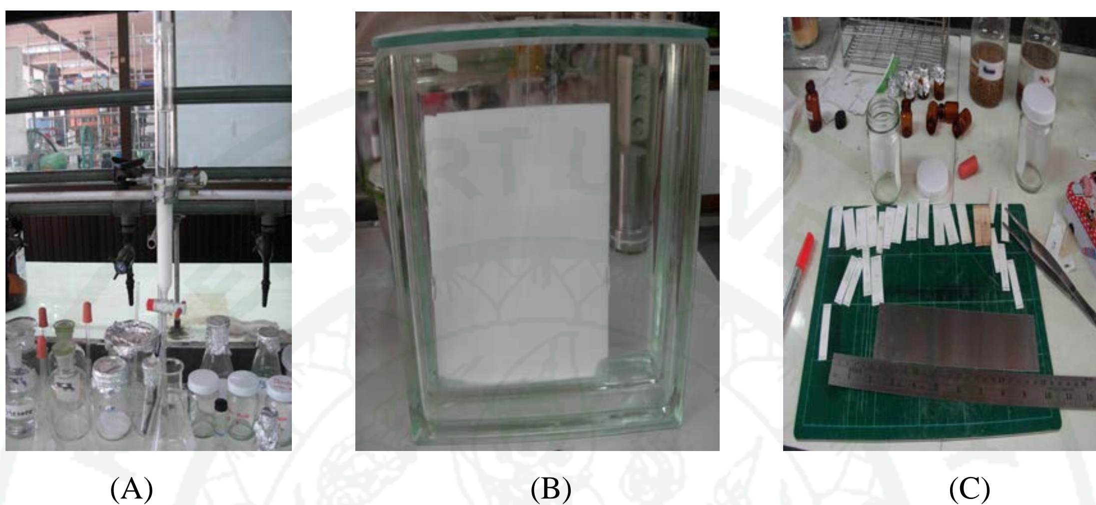
The highest efficacy was purified by using PTLC with suitable solvent system. Isolated compound was analyzed by Nuclear Magnetic Resonance spectroscopy (NMR spectroscopy) and Mass Spectroscopy (MS) for compound elucidation.



(A) (B) (C)

Figure 18 (A) Column Chromatography, (B) Preparative Thin Layer Chromatography and (C) Thin Layer Chromatography

5. Bio-efficacy against *P. xylostella*

Topical application test

5.1 Topical application method

The second instars *P.xylostella* were used to determine median lethal dose (LD_{50}) by topical method with CRD method. Five concentrations of each extract were prepared in acetone (AR grade) [0-10000 ppm]. The treatment was applied topically to the thorax region of second instar and 90 larvae were used in three replicates in each treatment. In case of controls only solvent was applied topically. The treated larvae and control larvae were then placed in Petri dishes [diameter 100 mm] and provided kale leaves for feeding. Mortality was recorded after 24 and 48 h post-treatment. The LD_{50} values were determined by Probit analysis using the StatPlus

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Program (2008 version). *P. xylostella* behavioral responses such as paralysis or knockdown were recorded.

6. Mode of action study of 2nd instars larvae of *P. xylosell*a after treated with crude extracts and active compound of *B. scandens*

6.1 Preparing insect for enzyme extraction

For *In-vivo* treatment assay, 2nd instars larvae of *P. xylosell*a were treated with leaves crude extracts and active pure compound of *B. scandens* at LD_{50} value concentration and control group were treated with 95% acetone (10 larvae/ replication). After treated 24 hours, the survival of *P. xylostella* were used for enzyme extraction. For *In-vivo* assay control and treatment groups were used non-treated *P. xylosella* 2nd instars larvae.

6.2 Extraction of enzymes activities method

This method modified from Feyereisen (2005), survival *P. xylostella* were placed micro tube and kept on ice. After that,grinded the midgut with TpA by homogenizer, after that centrifuged at 10,000g for 10 minutes and transfer supernatant in the new tube. The supernatant will continued to centrifuged at 100,000g , 4 Degree C for 60 mins. The supernatant will use for analyzed carboxylesterase and glutathione-*S*-transferase.

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Figure 19 Refrigerated centrifuge at 10,000 g, 4ºC for 5 min. was used for separation of enzyme systems.

Figure 20 Computerized Microplatereader methods modified from Feyereisen (2005) were used for Carboxylesterase and Glutathione-*S*-transferase protein concentrations in the enzyme homogenates.

6.3 Carboxylesterase activity analysis

The carboxylesterase activity of pNPA assay was modified method from Ganske (2009). For In-vivo assay, 50 mM phosphate buffer were mixed with supernatant. Then add 10 mM pNPA (paranitrophenyl acetate) and measured by kinetic mode at λ_{max} = 410nm, 37°C. For *In-vivo* assay, 50mM phosphate buffer were mixed with supernatant and treating compound then were incubated at 37°C for 30 minutes. Then added 10 mM pNPA and measured at λ_{max} = 410 nm, 37°C by kinetic

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mode using micro plate reader. The activity of pNPA was described as changing of the yellow color of paranitrophenol from hydrolysis of paranitrophenylacetate

6.4 Glutathione-*S*-transferase activity analysis

Glutathione-S-transferase activity of 1- chloro-2, 4'- dinitrobenzene (CDNB) method was follow by Spectrophotometric method. For In-vivo assay using micro plate reader, 50 mM phosphate buffer were mixed with glutathion solution, supernatant were incubated at 30°C for 30 minutes and added CDNB. Then measured at λ_{max} = 340nm.The activity was described as changing absorbance of CDNB.

6.5 Total protein analysis

The protein content of each fraction used as enzyme source was determined by the Bradford method (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin as the standard before measuring enzyme activities.

7. Statistical analysis

The data of report were expressed as mean±SD. Homogeneity of variances were calculated by Levene’s test with One-way ANOVA. Differences results significant calculated by using Duncan’s multiple range test (DMRT) and analyses median lethal dose (LD_{50}) with StatPlus 2008 before created graph with Sigma Plot 11.0.

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RESULTS

1. Bio-efficacy of *B. scandens* crude extracts

1.1 Physical properties and extraction yields of *B. scandens* extracts

All extracts were high viscosity , dark green color , semisolid properties and bad odor (Table 2, Figure 21). The percent yields were calculated by comparing the mass of crude extracts to the amount of fresh young stems. The %yield of hexane, dichloromethane, ethyl acetate and ethanol crude extracts was 3.5917%, 1.0162%, 1.1356% and 5.0243%, respectively. (Table 2)

Table 2 Information of *B. scandens* crude extracts

Treatment	Character	Yield (% W/W)
Hexane	light green viscous semisolid	3.5917^{b}
Dichloromethane	Dark green viscous semisolid	1.0161^{d}
Ethyl acetate	green viscous semisolid	1.1356^{c}
Ethanol	Dark green viscous semisolid	5.0243^{a}

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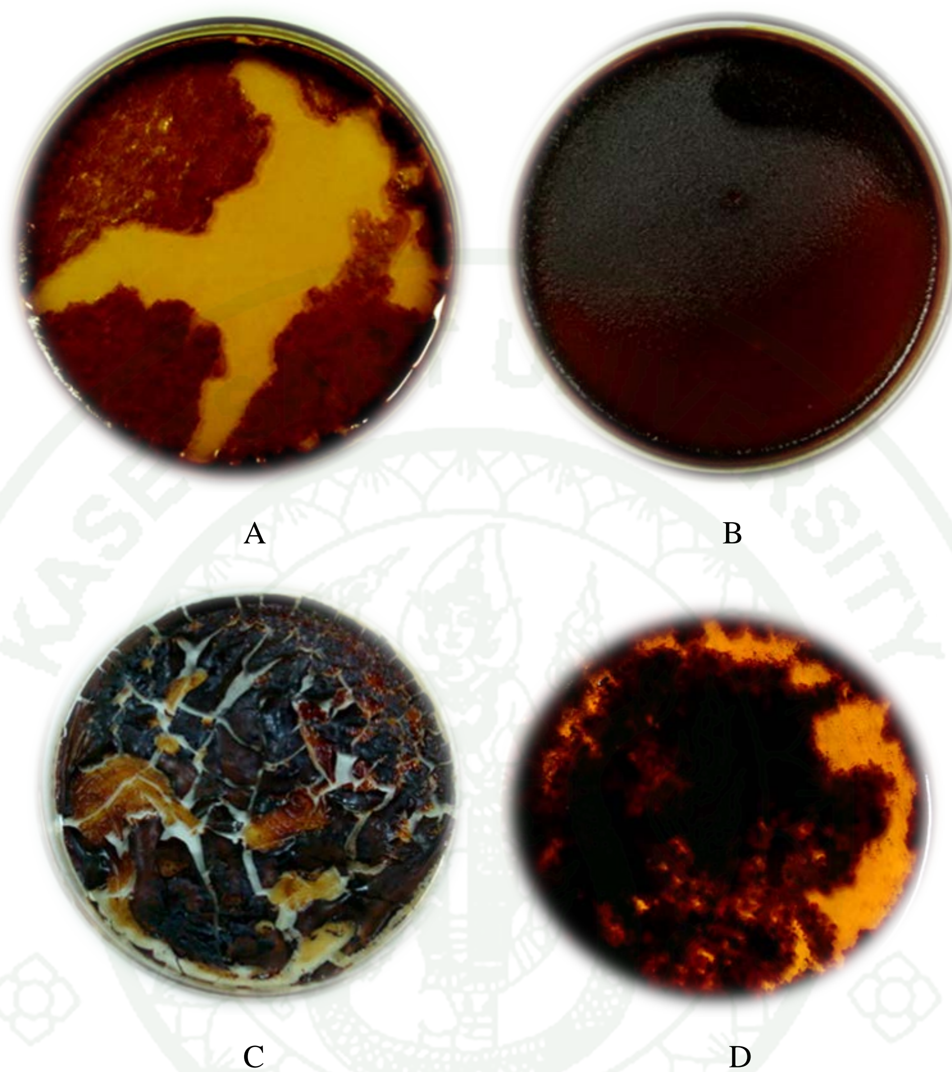

Figure 21 *B. scandens* crude extracts;

(A) Hexane crude extract

(B) Dichloromethane crude extract

(C) Ethyl acetate crude extract

(D) Ethanol crude extract

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1.2 Topical application test against *P. xylostella* 2nd instars larvae

1.2.1 Toxicity of *B. scandens* Hexane crude extracts

The 24 hours mortality percentage of 2nd instars *P. xylostella* after topical application with *B. scandens* hexane crude extracts were started 70% mortality when the dose was up to 10,000 ppm. The mortality percentage values showed significant increase (P>0.05) from Duncan's Multiple Range Test inorder to dose. LD_{50} values at 24 hours of *P. xylostella* 2nd instars after topical application test with *B. scandens* hexane crude extracts was 5,470.68 ± 5.77 ppm (Figure 22).

After 48 hours treated in same concentration mortality percentage slightly increased but not significant for time dependant. The mortality percentage values showed significant increase correlation with increasing concentration from 0 ppm to 10,000 ppm at P>0.05 from Duncan's Multiple Range Test. LD_{50} values at 48 hours of instars *P. xylostella* 2nd after topical application test with *B. scandens* hexane crude extracts was 3,141 ± 5.77 ppm (Figure 23).

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LD$_{50}$=5,470.68±5.77 ppm

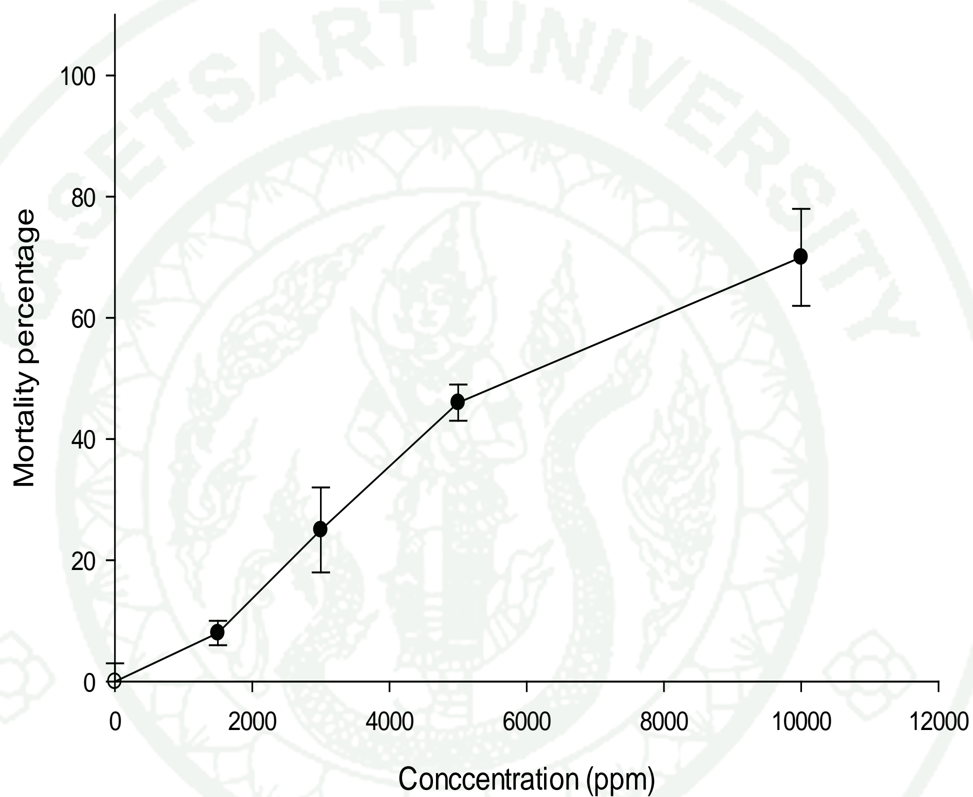

Figure 22 Mortality percentage of instars *P. xylostella* 2nd after topical ap test with *B. scandens* hexane crude extracts after 24 hours

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LD_{50}=3,141±5.77 ppm

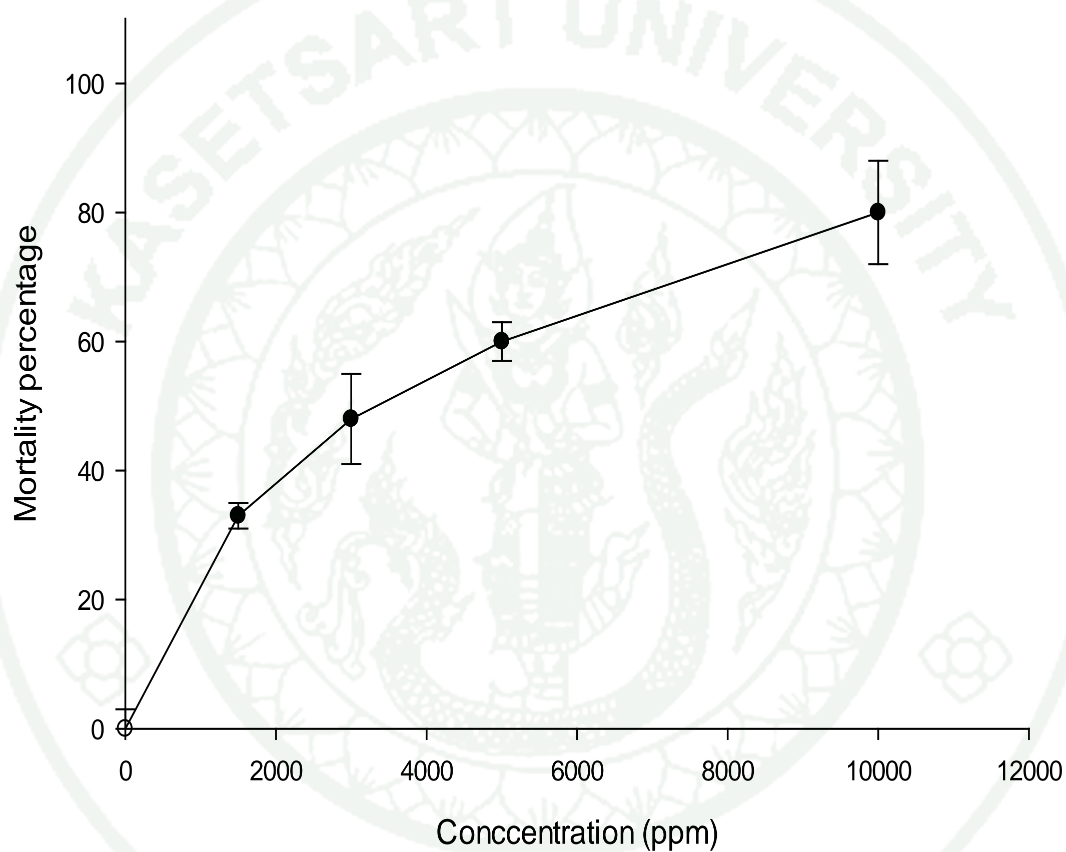

Figure 23 Mortality percentage of instars *P. xylostella* 2nd after topical application test with *B. scandens* hexane crude extracts after 48 hours

1.2.2 Toxicity of *B. scandens* Dichrolomethane crude extract

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The 24 hours mortality percentage of 2nd instars *P. xylostella* larvae after topical application with *B. scandens* dichrolomethane crude extracts were also lead 90% mortality when the dose was up to 10,000 ppm (Figure 24). The mortality percentage values showed significant increase at $P>0.05$ from Duncan's Multiple Range Test. LD_{50} values at 24 hours of 2nd instars *P. xylostella* after topical application test with *B. scandens* Dichrolomethane crude extracts was 3,778 ± 135.48 ppm.

After 48 hours treated in same concentration mortality percentage slightly increased which no significant different for time dependent (Figure 25). The mortality percentage values showed significant increase correlation with increasing concentration from 0 ppm to 10,000 ppm at $P>0.05$ from Duncan's Multiple Range Test. LD_{50} values at 48 hours of 2nd instars *P. xylostella* after topical application test with *B. scandens* dichrolomethane crude extract was 2,426.55 ± 5.77 ppm.

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LD_{50}=3,778 ± 135.48 ppm

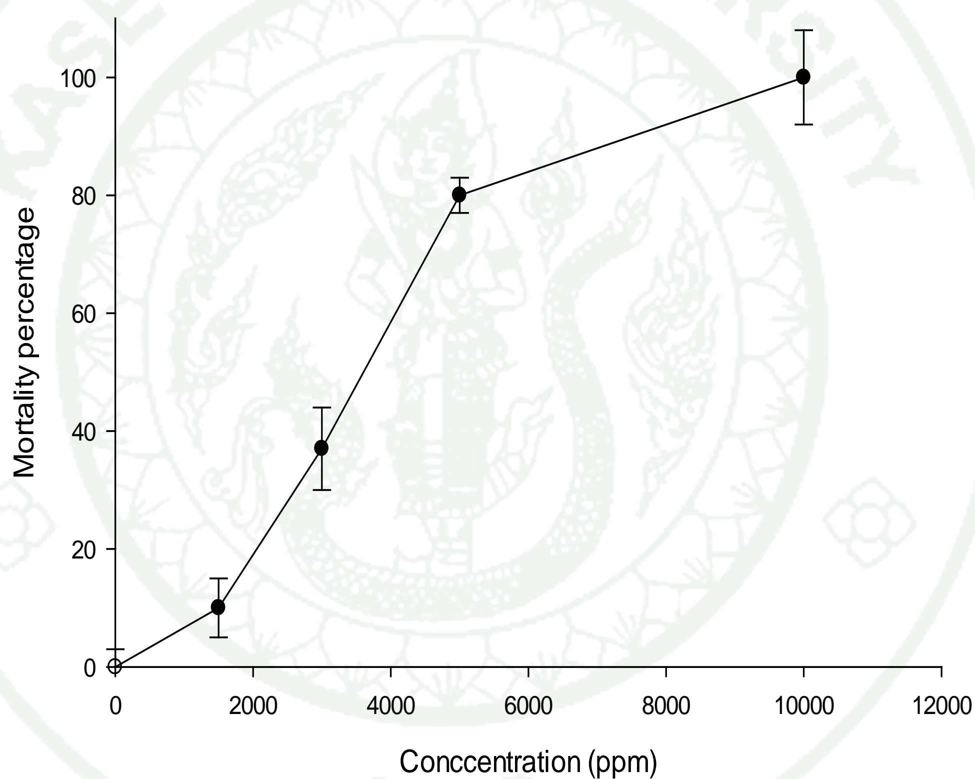

Figure 24 Mortality percentage of instars *P. xylostella* 2nd after topical application test with *B. scandens* dichrolomethane crude extracts after 24 hours

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LD_{50}=2,426.55 ± 5.77 ppm

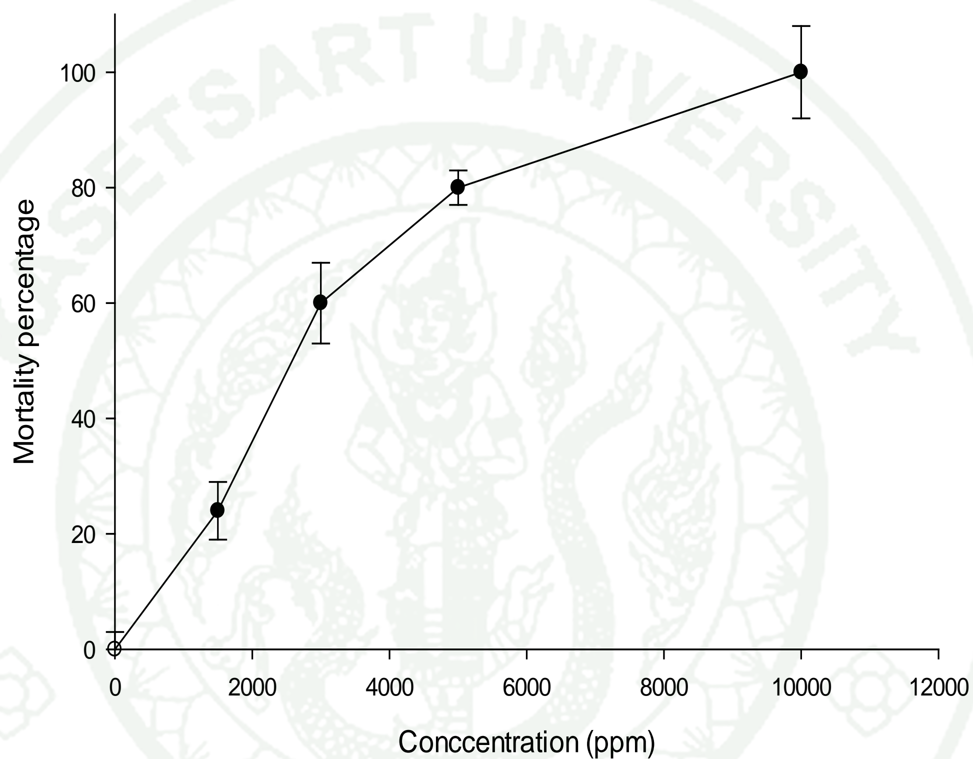

Figure 25 Mortality percentage of instars *P. xylostella* 2nd after topical application test with *B. scandens* dichrolomethane crude extracts after 48 hours

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1.2.3 Toxicity of *B. scandens* ethyl acetate crude extract

The 24 hours mortality percentage of 2nd instars *P. xylostella* larvae after topical application with *B. scandens* ethyl acetate crude extracts were also lead 70% mortality when the dose was up to 10,000 ppm (Figure 26). The mortality percentage values showed significant increase at P>0.05 from Duncan's Multiple Range Test. LD_{50} values at 24 hours of 2nd instars *P. xylostella* after topical application test with *B. scandens* ethyl acetate crude extract was 7,902.83 ± 5.77 ppm.

After 48 hours treated in same concentration mortality percentage slightly increased which no significant different for time dependent (Figure 27). The mortality percentage values showed significant increase correlation with increasing concentration from 0 ppm to 10,000 ppm at P>0.05 from Duncan's Multiple Range Test. LD_{50} values at 48 hours of 2nd instars *P. xylostella* after topical application test with *B. scandens* ethyl acetate crude extracts was 4,114.01 ± 5.77 ppm.

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LD_{50}=7,902.83 ± 5.77 ppm

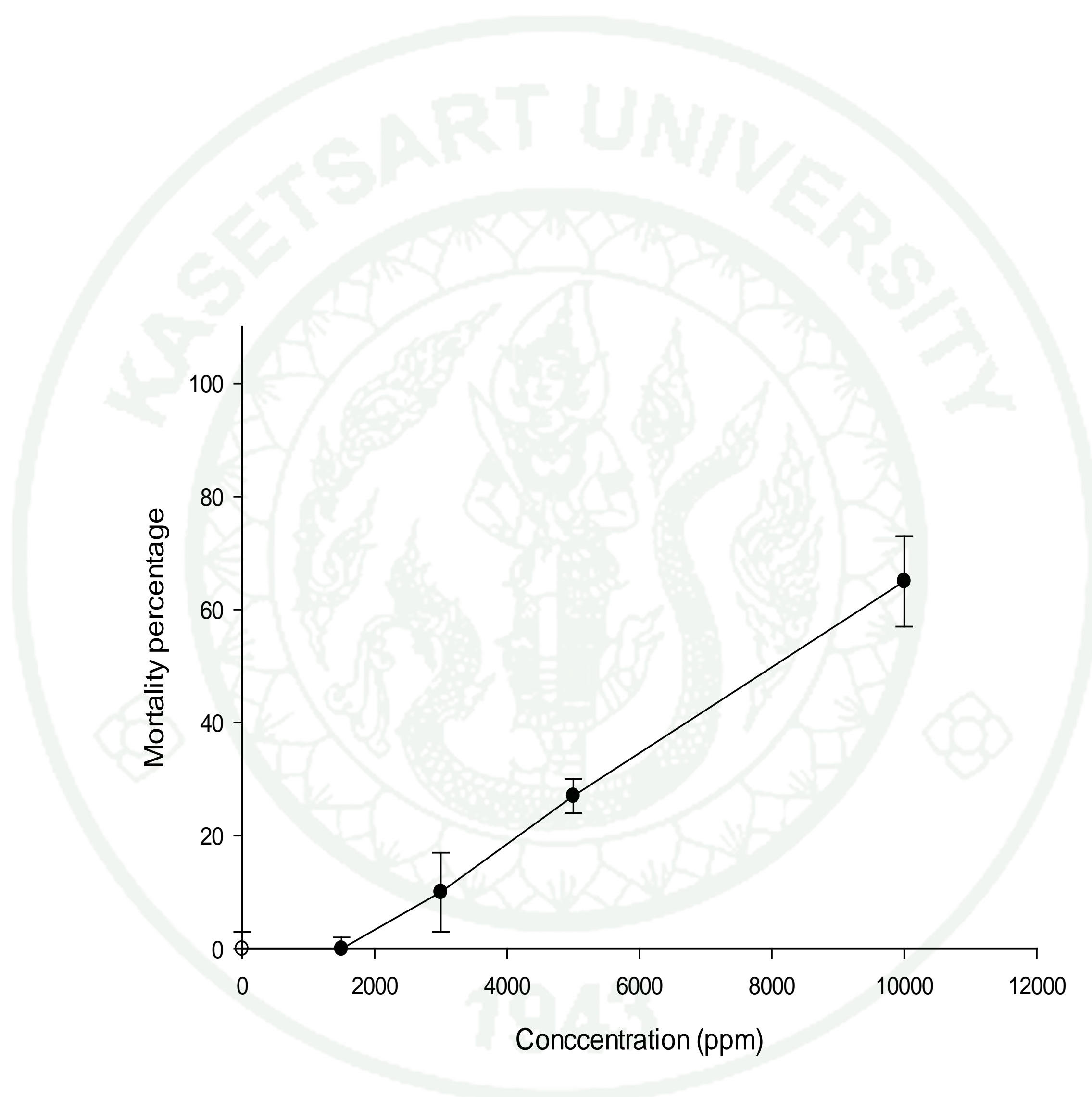

Figure 26 Mortality percentage of instars *P. xylostella* 2nd after topical application test with *B. scandens* ethyl acetate crude extracts after 24 hours

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LD_{50}=4,114.01 ± 5.77 ppm

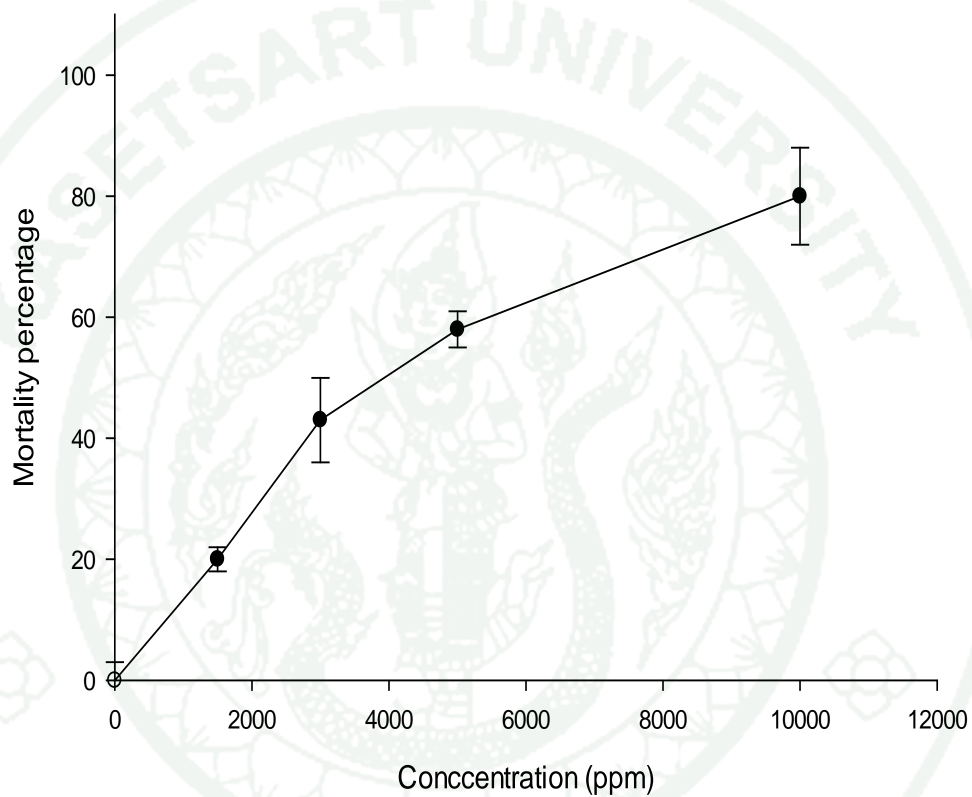

Figure 27 Mortality percentage of instars *P. xylostella* 2nd after topical application test with *B. scandens* ethyl acetate crude extracts after 48 hours

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1.2.4 Toxicity of *B. scandens* Ethanol crude extracts

The 24 hours mortality percentage of 2nd instars *P. xylostella* after topical application with *B. scandens* ethanol crude extracts were also lead 70% mortality when the dose was up to 30,000 ppm (Figure 28). The mortality percentage values showed significant increase at P>0.05 from Duncan's Multiple Range Test. LD_{50} values at 24 hours of 2nd instars *P. xylostella* after topical application test with *B. scandens* ethanol crude extracts was 1,5947.72 ± 5.77 ppm.

After 48 hours treated in same concentration, mortality percentage slightly increased which no significant differant as time dependent (Figure 29). The mortality percentage values showed significant increase correlation with increasing concentration from 0 ppm to 30,000 ppm at P>0.05 from Duncan's Multiple Range Test. LD_{50} values at 48 hours of 2nd instars *P. xylostella* after topical application test with *B. scandens* ethanol crude extracts was 4,555.56 ± 5.77 ppm.

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LD_{50}=1,5947.72±5.77 ppm

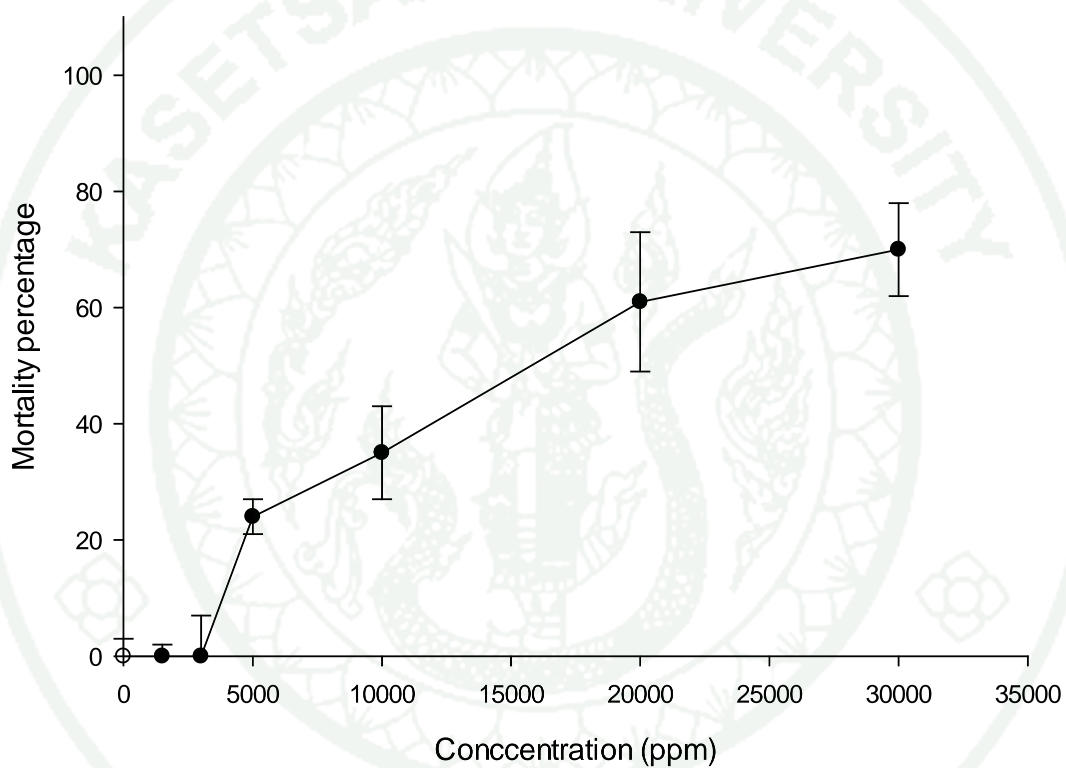

Figure 28 Mortality percentage of instars *P. xylostella* 2nd after topical application test with *B. scandens* ethanol crude extracts after 24 hours

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LD_{50}=4,555.56± 5.77 ppm

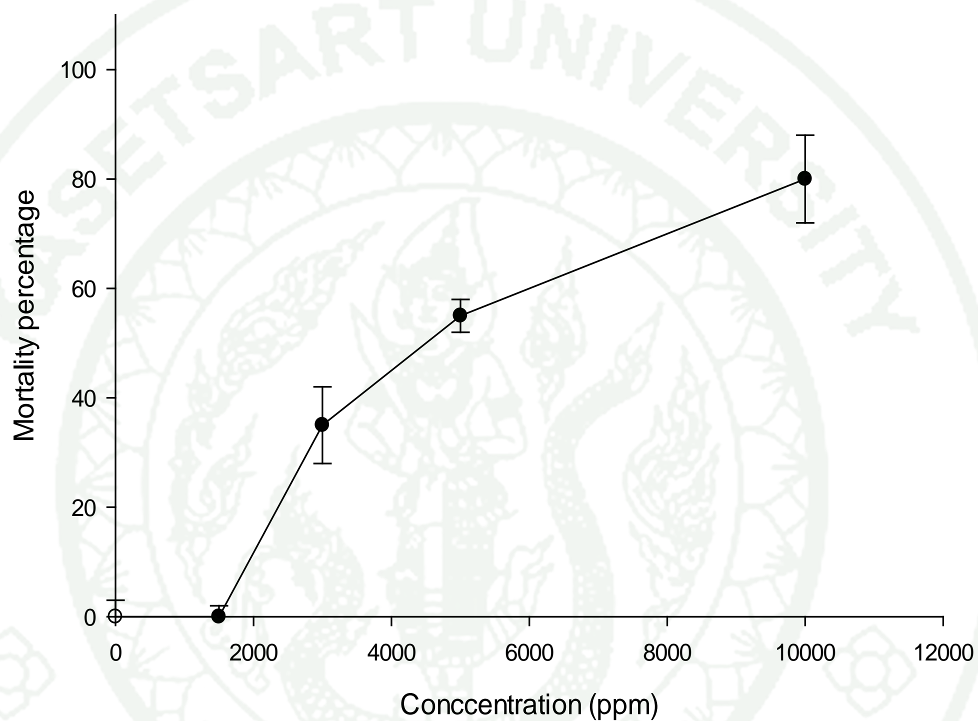

Figure 29 Mortality percentage of instars *P. xylostella* 2nd after topical application test with *B. scandens* ethanol crude extracts after 48 hours

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Comparing LD_{50} value by topical application on *P. xylostella* 2nd insters larvae of *B. scandens* four crude extracts; hexane, dichrolomethane, ethyl acetate and ethanol (Figure 31). The LD_{50} values after 24 hours exposed were 5470.68 ± 5.77, 3778 ± 135.48, 7902.83 ± 5.77 and 15947.72 ± 5.77 ppm. For 48 hours after exposed, the LD_{50} values were 3141 ± 5.77, 2426.55 ± 5.77, 4.114.01 ± 5.77 and 4555.56 ± 5.77 ppm, respectively. Dichloromethane crude extract show the most control efficiency against 2nd instar *P. xylostella* (Table 2).

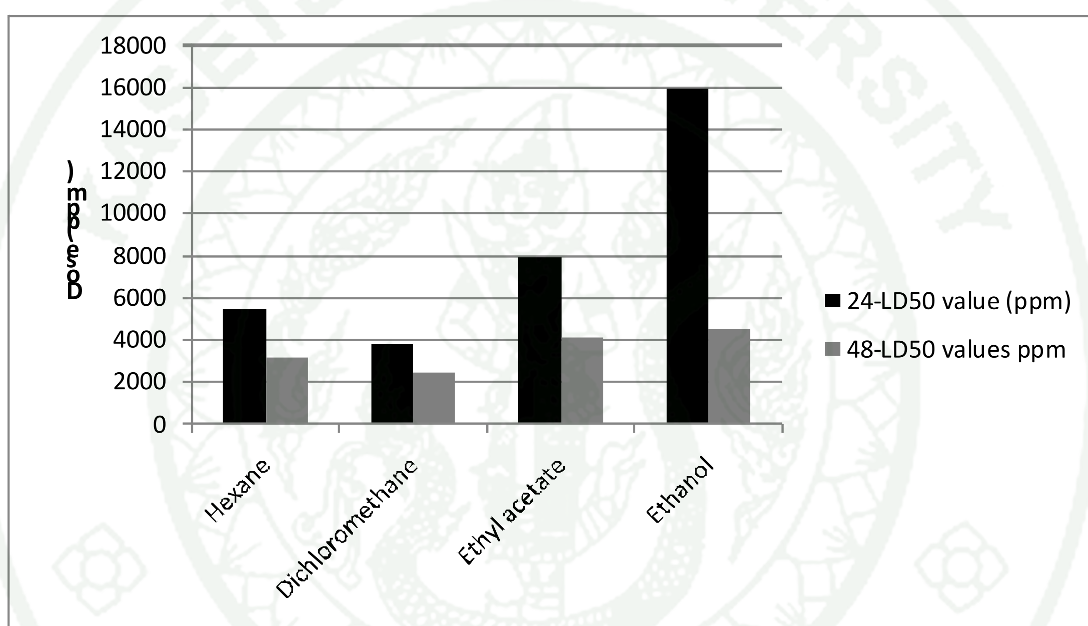

Figure 30 Compared LD_{50} values of *B. scandens* stems extracts by topical application test on 2nd instars *P. xylostella* at 24 and 48 hours

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Table 3 LD_{50} value (ppm)[1)] of *P. xylostella* after treated with *B. scandens* four crude extract at 24 and 48 hours

Extract	24-LD_{50} value (ppm)	48-LD_{50} values
Hexane	5,470.68 ± 5.77 c	3,141.00 ± 5.77 c
Dichloromethane	3,778.00 ± 135.48 d	2,426.55 ± 5.77 d
Ethyl acetate	7,902.83 ± 5.77 b	4,114.01 ± 5.77 b
Ethanol	15,947.72 ± 5.77 a	4,555.56 ± 5.77 a

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2. Bio-efficacy of alkanes mixture in *B. scandens* crude extracts

2.1 Chemical Structure Elucidation

The highest efficacy of crude dichloromethane extract of *B. scandens* dried stems (1.05 g) was subjected column chromatography with hexane/ethyl acetate (10:1) to give fraction 1. This fraction was purified further by Preparative Thin Layer Chromatography with hexane to obtain subfraction 1-1 as a colorless oil (46.5 mg) with 4.29 % W/W yield respectively, which was subsequently subjected to structural analysis using ^{1}H NMR and GC-MS. It was found that subfraction 1-1 was identified as a mixture of long chain alkanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15-1.37 (br m), 0.76-0.95 (br m). Gas Chromatography- Mass Spectrometry (GC-MS) analysis data of subfraction 1-1 was carried out on an Agilent 6890N column Hp-5 and Agilent 5973 Mass Selective Detector.and elucidated by compared with the literature data (Figure 31).

The subfraction 1-1 was alkane mixture of long-chain saturated aliphatic hydrocarbon consisting of Tetradecane 0.33%, Hexadecane 0.87%, Octadecane 1.26%, Nonadecane 0.28%, Eicosane 1.39%, Heneicosane 0.91%, Docasane 2.51%, Tricosane 3.52%, Tetracosane 8.28%, Pentacosane 19.01%, Hexacosane 25.88% and Heptacosane 34.67% (Table 4) It had colorless oil viscous semisolid properties and there are five 99% match compound and their structure (Figure 32).

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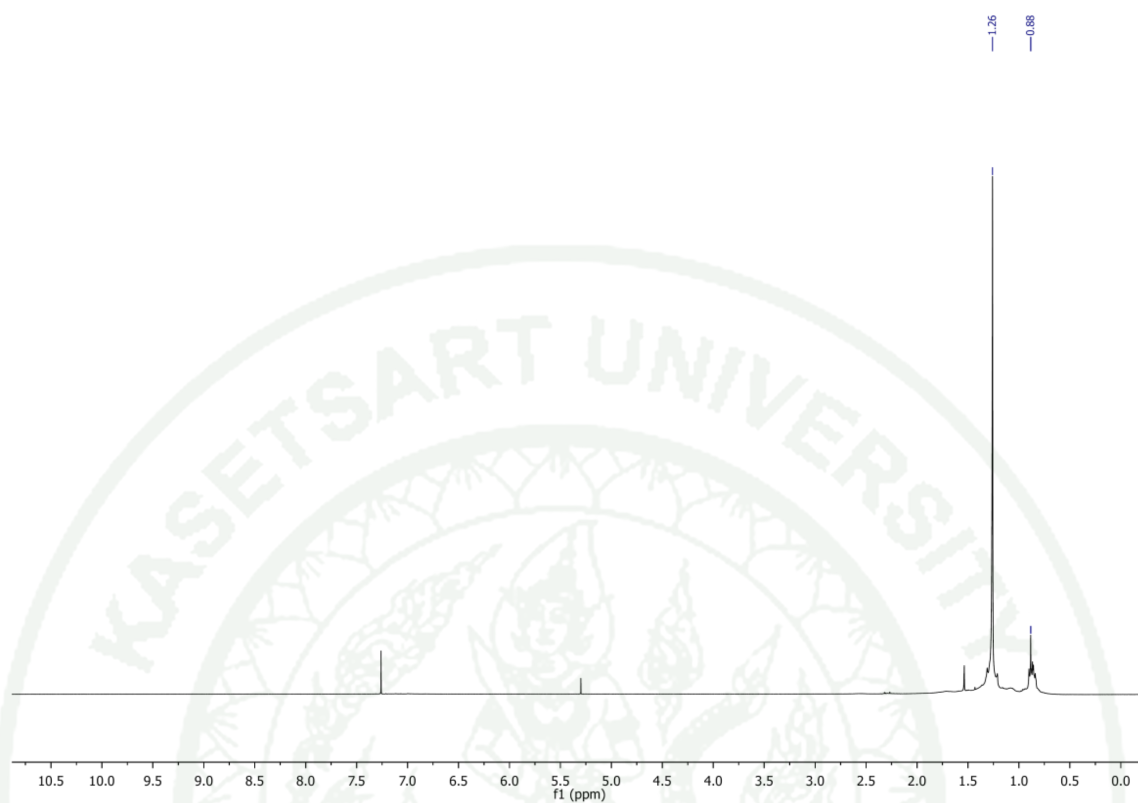

Figure 31 The ^{1}H NMR spectrum of subfraction 1-1

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Table 4 GC-MS analysis data of Subfraction 1-1

Compound	Results[a]		
	Retention time	% Area	% Match
Tetradecane	24.13	0.33	94
Hexadecane	39.74	0.87	98
Octadecane	46.78	1.26	99
Nonadecane	49.28	0.28	95
Eicosane	51.49	1.39	98
Heneicosane	53.49	0.91	98
Docosane	55.37	2.51	99
Tricosane	57.15	3.52	98
Tetracosane	59.01	8.28	99
Pentacosane	61.35	19.01	98
Hexacosane	64.28	25.88	99
Heptacosane	68.11	34.67	99

a) In house method based on GC-MS

From table 4 there are interested five compound had 99% match (Table 5), octadecane (A), docosane(B) , tetracosane (C), hexacosane (D), heptacosane (E) (Figure 32).

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Table 5 GC-MS analysis data of subfraction1-1 99% match compound

Compound	Results[a]		
	Retention time	% Area	% Match
Octadecane	46.78	1.26	99
Docosane	55.37	2.51	99
Tetracosane	59.01	8.28	99
Hexacosane	64.28	25.88	99
Heptacosane	68.11	34.67	99

(A) Octadecane ($CH_3(CH_2)_{16}CH_3$)

(B) Docosane ($CH_3(CH_2)_{20}CH_3$)

Figure 32 Five 99% match compound and their structure

(A) Octadecane ($CH_3(CH_2)_{16}CH_3$)

(B) Docosane ($CH_3(CH_2)_{20}CH_3$)

(C) Tetracosane (CH_3-$[CH_2]_{22}$-CH_3)

(D) Hexacosane (CH_3-$[CH_2]_{24}$-CH_3)

(E) Heptacosane (CH_3-$[CH_2]_{25}$-CH_3)

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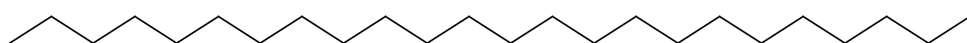
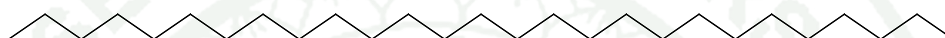


(C) Tetracosane (CH_3-$[CH_2]_{22}$-CH_3)

(D) Hexacosane (CH_3-$[CH_2]_{24}$-CH_3)



(E) Heptacosane (CH_3-$[CH_2]_{25}$-CH_3)

Figure 32 (Continued)

2.2 Topical application test of alkanes mixture

After analysis by ^{1}H-NMR white flake wax from subfraction 1-1 had the lowest impurity spectrums. Mixture of alkane did show toxicity against 2nd instars *P. xylostella*. The alkanes mixture was prepared with Acetone (AR grade) at concentration 0 ppm - 10,000 ppm follow LD_{50} value of dichloromethane crude extracts.

The results of LD_{50} value (ppm) of alkanes mixture was 2,903.15 ± 0.425 and 2,993.67 ± 0. 105 at 24 and 48 hours after exposed (Table 6). The toxicity showed relative close to crude extract from dichloromethane crude extracts (Table 3).

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Table 6 LD_{50} value (ppm) of *P. xylostella* 2nd instars larvae after topical application with alkanes mixture after 24 and 48 hours

Fraction	LD_{50} value (ppm) [1)]	
	24-LD_{50} value (ppm)	48-LD_{50} value (ppm)
alkanes mixture	2903.15 ± 0.425	2993.67 ± 0. 105

3. Mode of action study

There were two methods using to determine insects enzyme activity including Carboxylesterase activity ,Glutathione-S-transferase activity and Protein concentration by using microplate reader techniques.

1) *In-vivo* assay determined variation amount of enzyme in survival 2nd instars *P. xylostella* after treated for 24 hours with *B.* scandens stems dichloromethane crude extracts. comparing with Control group ,which treated with acetone (AR grade).

3.1 Enzyme activity of 2nd instars *P. xylostella* after treated with *B. scandens* dichloromethane crude extracts 24 hours

3.1.1 Carboxyl esterase enzyme activity after treated with *B.scandens* dichloromethane crude extracts

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The results of Carboxylesterase activity showed enzyme was inhibited.

The results shows carboxyl esterase enzyme activities of *P. xylostella* after 24 hours exposures treated by *B. scandens* crude and alkane mixture at LD_{50} dose were 0.013 ± 0.0025, 0.017 ± 0.0013, 0.017 ± 0.0012, 0.020 ± 0.0049 and 0.015 ± 0.0013 from hexane crude extract, dichloromethane crude extract, ethylaceate crude extract, ethanol crude extract and alkane mixture (Table 7).

3.1.2 Glutathione-S-transferase (GST) activity after treated with *B.scandens* dichloromethane crude extracts

The Glutathione-S-transferase activity results of *B.scandens* dichloromethane crude extracts in assay showed the enzyme was inhibited (Table 6). The results showed significant decrease from control at $P < 0.05$ by Duncan's Multiple Rang Test. The results shows glutathione-S-transferase (GST) enzymes activities of *P. xylostella* after 24 hours exposures treated by *B. scandens* crude and alkane mixture at LD_{50} dose were 1.64 ± 0.73, 1.04 ± 0.68, 1.11 ± 0.36 , 1.53 ± 0.67, 0.422 ± 0.12 from hexane crude extract, dichloromethane crude extract, ethylaceate crude extract, ethanol crude extract and alkane mixture (Table 7).

As dicholoromethane extract contained the possible active compounds (Alkane mixture), this extract was used to determine their activity against the detoxification enzymes *in vivo*. These experiments revealed that *B.scandens* dichloromethane crude extracts and alkane mixture inhibited both detoxification enzymes, glutathione-s-transferase and carboxylesterase (Table 7)

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Table 7 Detoxification enzymes activities of *P. xylostella* after 24 hours exposures treated by *B. scandens* crude and alkane mixture at LD_{50} dose.

Treatment	carboxylesterase (nM p-nitrophenol/min/mg protein) (T/C)[2)]	glutathione-s-transferase (glutathione conjugated product/ min/mg protein[)] (T/C)[2)]
Control[1)]	0.040 ± 0.004 a	1.14 ± 0.45 c
Hexane crude extract	0.013 ± 0.0025 d (0.325)	1.64 ± 0.73 a (0.699)
Dichloromethane crude extract	0.017 ± 0.0013 c (0.425)	1.04 ± 0.68 c (1.095)
Ethylaceate crude extract	0.017 ± 0.0012 c (0.425)	1.11 ± 0.36 c (0.971)
Ethanol crude extract	0.020 ± 0.0049 b (0.500)	1.53 ± 0.67 b (1.339)
Alkane mixture	0.015 ± 0.0013 a (2.67)	0.422 ± 0.12 b (2.70)

1) Control = 20% acetone in distilled water

2) T/C = the ration of the enzyme activity of treated group over the control group

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DISCUSSION

1. Bi-oefficacy of *B. scandens* crude extracts

Bauhenia genus has a few reports in pest management. *Bauhinia vahlii* which have chemical closely relating to *B.* manca (Renée J *et al.,* 1994). Toxic petroleum ether extracts were obtained from *Bauhinia vahlii* Wight and Am. These extracts gave 100% larval mortality for the corn borer (Valdir Cechinel Filho *et al.,* 2009).

According to the result toxicity of *B. scandens* crude extracts can be order from lowest to highest efficacy against *P. xylostella* after exposed by topical application test at LD_{50} values at ethanol, ethyl acetate, hexane and dichloromethane crude extracts respectively. LD_{50} values at 24 hours after exposed were 15,947.72, 7,902.83, 5,470.68 and 3,778 ppm respectively and 48 hours LD_{50} values were 4,555.56, 4,114.01, 3,141.00 and 2,426.55 ppm, respectively (Table 3). The LD_{50} values decreased correlatively the higher concentration that *P. xylostella* probably not enhance detoxification enzyme at higher concentration or other detoxification mechanism for eliminate toxicity and cause death. The LD_{50} values show significant time dependent.

As table 3, *B. scandens* had quite a high toxic effect on larvae of *P. xylostella* by contact via the topical application method, with the dichlromethane extract showing the highest toxicity. The acute toxicity levels of all crude *B. scandens* stems crude extract show lower efficacy against *P. xylostella* than *Annona squamosa* seed extract (LD_{50} value ≈ 0.10 ppm; Andrey and Isman, 2004), *Azadirachta indica* seed extract (LD_{50} value ≈ 0.54 ppm; Robert and Denis, 1993), *Syzygium aromaticum* flower extracts (LD_{50} value ≈ 1.09 ppm; Kumnuan, 2006), *Piper sarmentosum* Roxburgh leaves extract (LD_{50} value ≈ 4.34 ppm; Orratai, 2008), *Mammea americana* seed extract (LD_{50} value ≈ 5.90 ppm; Kritchaya *et al.*, 2011) and *Derris elliptica* Benth root extract (LD_{50} value ≈ 24.25 ppm; Visetson and Milne, 2001) , (Table 1).

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Our test results, also studied the effect of the different extraction methods used for each solvent and indicated that dichloromethane crude extract of *B. scandens* registered the best effectiveness while the ethanolic extract gave the highest yield.

In addition, from Table 3, all extracts except for the dichloromethane extract were significantly correlated ($P < 0.05$). Thus, one characteristic of the extract active ingredient group of *B. scandens* should be that it is slightly hydrophilic compared to *B. scandens*. These results support the earlier findings ofArannilewa et al. (2006)who observed potential insecticidal agents for the control of the larvae of *Anticarsia gemmatalis* in acetone and ethanol extracts of *A. pubescens* plants which exhibited larval mortality against *Sitophulus zeamais*; and the findings of Khumrungsee et al. (2010) who observed potential insecticidal agents for the control of *Spodoptera exigua* by ethyl aceate crude extract, and the findings of Bullangpoti et al. (2012) for the control of *Spodoptera frugiperda*. Thus, most crude solvent extracts which had acidic metabolites (like aristolic acid, aristolochic acid, aristoloctam and aristolone) largely exhibited pest control efficiency.

While *P. xylostella* exposed to *B. scandens* crude extracts, their behavior related to behavior avoidance to decrease expose to chemical (Bullangpoti, 2007) After that, paralyzed and died within 4-48 hours because they change behavior to stop feeding within 0- 2 hours and avoid extracts by moving immediately.

Nonetheless, *B. scandens* has lower efficacy than previous report extracts but we can also recommend agriculturist use *B. scandens* extract to control pest. This plant species has been endeavored since this plant species is a part of Thai village knowledge how as an efficient control on mosquitoes. From our previous preliminary research, *Bauhinia scandens stem* crude extracts show bioefficiency to control *P. xylostella* (Poonsri et al, 2012).Thus, it may have some active compounds in their stem as pest control agent. The objective of the present study was to confirm the effects of *B. scandens* crude and searching its active compound on the biological parameters of *P. xylostella* for develop the new strategies for *P.xylostella* control. The mode of action on detoxification enzymes also investigated.

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2. Bio-efficacy of alkanes mixture in *B. scandens* crude extracts

We treated the *P. xylostella 2nd* instars larvae with alkanes mixture in *B. scandens* crude extracts , after exposed to alkanes mixture by topical on *P. xylostella* *2nd* instars larvae, treated larvae immediatly stop feeding and decrease moving, indoxacarb against *P. xylostella* with the appearance of neurotoxic symptom (Wing *et al*., 2000)., then paralyzed and died. This result is the same as when apply alkane alone which can significantly reduce movement rate of *P. xylostella* (Joseph L. *et al*., 1999) , *Spodoptera fugiperda*, *Drosophila melanogaster* and *Tenebrio molitor* during treatment contract (Spencer *et al*., 1999)

LD_{50} value (ppm) of *P. xylostella* after treated with *B. scandens*crude extract isolated from Preparative Thin Layer Chromatography 24 and 48 hours were 2,925.25 ± 0.3433 and 2,334.59 ± 0.135, respectively. Alkane mixture shown lower efficacy than long chain n- alkanes compounds against *Tenebrio molitor* ($LD_{50} \approx 5.2$ ppm) , *Drosophila melanogaster* ($LD_{50} \approx 3.23$ ppm) and *Spodoptera frugiperda* ($LD_{50} \approx 3.89$ ppm), respectively (Cespedes et al., 2013). Moreover, this compound show lower efficacy than indoxacarb and chlorantraniliprole, commercial insecticide for control several lepidopteran that reported LD_{50} value against *Plutella xylostella* was 18.53 ppm (Nehare et al., 2010) and 0.015 ppm (Silva *et al*., 2012), respectively.

Our interested five compound had 99% match (Table 5) were octadecane, docosane , tetracosane , hexacosane and heptacosane. Those molecules have been reported to possess pesticidal activity (Mansour *et al*., 2000, Duke, 1992, Abdolhamid *et al*., 2009, Murugesan *et al*.,2012, Sathya *et al*., 2012, Shailesh, 2012). Such as the Stems of *Salvadora oleoides* aqueous and organic solvent extracts contain high concentration of alkane mixture ,2-methoxy-4-vinylphenol (21.6%), phytol (12.9%), n- hexadecanoic acid (3.6%), octacosane (7.9%), nonacosane (7.3%), 1-octadecene (5.8%), heptacosane (5.9%), hexacosane(4.5%), pentacosane (3.4%), squalene (3.9%) and transs-damascenone (2.3%) their antimicrobial potential against eight bacterial strains*:Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus vulgaris, Proteus mirabilis, Klebsiella pneumoniae* and three fungal strains*: Aspergillus fumigatus, Candida albicans* and

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Aspergillus niger (Akash Garg et al., 2013). The epicuticular wax on the leaves of sweet corn, *Zea mays vars . saccharata* which comprised long-chain n-alkane hydrocarbon, such as n-hexacosane ($C_{26}H_{54}$) and n-heptacosane ($C_{27}H_{56}$), acts as an oviposition stimulant for the European corn borer, Ostrinia nubilalis (James R.Hanson *et al*.,2011)

Previous reported stated that the compounds such as Octadecane and Heptadecane were found in *Spirulina platensis* algae shows potent antioxidant, anticancer and antimicrobial activity (Lee *et al*., 2007; Mishra and shree 2007). In present study, Octadecane and heptadecane were identified in ethanol, hexane, chloroform and dichloromethane extract of *Spirulina*. The methanol and acetone extract of *Spirulina platensis* and identified the hexadecane, heptadecane,Eicosane, octadecane, phytol and pentadecane by GC-MS and these compounds shows antibacterial activity against *Staphylococcus aureus* and *Salmonella typhimurium* (Vinaykumar et al., 2011). The ethyl acetate extract of anabaena *variabilis, oscillatoriaanustissima* and *anabaena flosaquae* shows potent antimicrobial activity against gram positive, gram negative,yeast and fungi and GCMS analysis of ethyl extract reveals that heptadecane, octadecane,hexadeconic acid, docosane (Hanan M *et al*., 2010).

Saturated hydrocarbons (SHC) of five cruciferous host plants viz., cabbage, cauliflower, broccoli, knol khol and Brussels sprout and the larvae of diamondback moth (DBM), *Plutella xylostella* reared on these host plants were identified through gas-chromatography. The hydrocarbon profile of those plants and larval of body extract of DBM reared on respective host plants revealed a wide variation in quantity as well as quality. Long chain hydrocarbon C_{26}-C_{30} were detected in all the extracts. In electroantennogram (EAG) studies, SHCs at 10^{-3} g does elicited differential EAG response in the antennal receptors of gravid *Cotesia plutellae* females. Tricosane (C_{23}) and hexacosane (C_{26}) elicited 10-fold increased EAG response compared to control stimulus. Long chain hydrocarbon C_{27}, C_{28} and C_{29} elicited, 6-7 fold increased response. The sensitivity of antenna was 4-5 fold for C_{25}, C_{14}, C_{24}, C_{15} and C_{30}, while the short chain hydrocarbons elicited 2-3 fold increased EAG responses. Doul choice

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flight orientation experiments in a wind tunnel revealed the gravid *Cotesia plutellae* females preferred the odour of C16, C26, C29, C15, C21, C23, C30, C27, C24 and C22 as 60-70 % females oriented and landed on SHC treated substrate compared to control odour, while the odour of eicosane (C20), pentacosane (C25) and octacosane (C28) were not preferred by the females (Seenivasagan and Paul, *et al* .,2010).

Although the alkane mixture has no report in toxicological information (TCI Americana material safety data sheet) but their oil compound have been use as pesticides for centuries and it well known as ingredient base of commercial synthetic insecticide and fungicide (Bogran *et al*., 1914) which carrier active compound move though into insect cuticle. Furthermore alkanes has been found in many insecticides including moth insecticide (Jurenka and Subchev, 2000; Subchev and Jurenka, 2001) as DDT (Dichloro diphenyl trichoroethane) and dicofal.

Moreover, alkanes mixture could be considered to use as bio-insecticide in class of oil pesticide which active for a short time , low toxicity to human, insect and mites have not developed resistance (Bogran *et al*., 1914).

3. Mode of action study from dichloromethane *B. scandens* extract

According to detoxification enzyme activity results of *P. xylostella* after topical 24 hours carboxylesterase activity was significant inhibited by *B. scandens* dichloromethane crude extracts (Table 7) for *In-vivo* assay of this result has tended to be decrease related to carboxylesterase for malathion on *P. xylostella* (Maa and Liao, 2000). Glutathion-s-transferase activity of *In- vivo* assay was significant inhibited by dichloromethane crude extracts. The *In-vivo* result is same as described by Kao *et al*. (1989) that enzymes such as glutathione-S-transferase was degraded by parathion in *P. xylostella*.

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Although, alkane mixture showed significant inhibited acetylcholinesterase (AchE) *In-vivo* assay. Alkane mixture not showed significant in carboxylesterase esterase, and glutathione-s-transferase enzyme activity correlated to indoxacarb resistance in *P. xylostella* that not associated with esterase and glutathione-s-transferase (Toshio *et al*., 2004).

Toxicity mechanism of alkanes could be inhibition of proteinase and other polyphenol oxidases that could bind to alkanes (Cespedes *et al.*, 2013) or effectively of hydrophobic environmental presents the action of morphogenesis enzyme of insect which require an aqumeous medium for its action (Jung and Deetz, 1993) Moreover alkane could produce white substance accumulation (deposits) invacuoles (Delgado *et al.,* 2011).

In fact, only other reports to show that inhibition of detoxification enzymes do occur by crude plant extracts is that of *Melia, Amaranthus,* and *Derris* against *Spopdoptera exigua* (Hu˙bner) (Rachokarn *et al*. 2008, Rattanapan 2009) and *Melia toosendan* Sieb. et Zucc. Pron. against *Spodoptera litura* (F.) and *Mel- anoplus sanguinipes* (F.) (Feng *et al*. 1995).

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CONCLUSION AND RECOMMENDATIONS

Conclusion

Bauhenia scandens crudes extracts yield in hexane, dichloromethane, ethyl acetate and ethanol was 3.5917%, 1.0161%, 1.1356% and 5.0243% w/w, respectively. LD_{50} value for 2nd instars *P. xylostella* larvae after topical application with hexane, dichrolomethane, ethylacetate and ethanol after 24 hours exposed showed 5,470.68, 3,778.00 , 7,902.83 and 15,947.72 ppm, after 48 hours showed 3,141.00, 2,426.55, 4,114.01 and 4,555.56 ppm, respectively.

The results of alkanes mixture which could be a consist of five 99% match compound, there were tetradecane, hexadecane , octadecane, docasane and heptacosane which % Area 8.28 %, 25.88 %, 1.26 %, 2.51 % and 34.67 % . LD_{50} value (ppm) of 2nd instars *P. xylostella* larvae after 24 hours topical application as 2,925.25 ppm and 48 hours as 2,334.59 ppm,respectively.

Mode of action study of insect enzyme activity after exposed 24 hours of dichloromethane crude extract showed inhibition on *In-vivo* assay of carboxylesterase activity and glutathiol-s-transferase activity. Alkanes mixture showed inhibition on both of Gluutathiol-s-transferase and carboxylesterase,respectively.

In addition, according to the results, the present results demonstrate the potential of *B. scandens* extracts or its active compounds (Alkanes mixture) to control *P.xylostella,* but they also show that the inhibition of carboxylesterase has been established. Given that carboxylesterase can mediate insecticide resistance via their induction (Li et al. 2007), inhibition of these enzymes by plant allelo- chemicals may constitute a useful alternative approach for pest management. Our ongoing studies with ethanol extracts should give us more insight into these allelochemicals for pest control.

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Recommendations

1. It should be studied and analysis in case of using in the real field.

2. Isolate other active compounds in *Bauhenia scandens* must be done.

3. The active compounds remaining chemical toxicity which directly effects on environmental especially benefit insects must be tested.

4. The stability and degradable times of compounds must be analysis to further developing into the commercial product.

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LITURATURE CITED

Achenbach,H, M.Stocker and M.A. Constenla.1988. Flavonoid and others constituents of *Bauhinia manca*. **Phytochemistry** 27:1835–1841.

Aderogba, M.A., L.J. Mcgaw, A.O. Ogundaini and Eloff JN. 2007. Antioxidant activity and cytotoxicity study of the flavonol glycosides from *Bauhinia galpinii*. **Nat Prod Res** 21: 591–599.

Ahmad, I. and F. Aqil. 2007. In vitro efficacy of bioactivie extracts of 15 medicinal plants against ESbL producing multi drug resistant bacteria. **Micobiol. Res**. 162, 264.

Akhtar, A.H. and K.U. Ahmad. 1995. Anti-ulcerogenic evaluation of the methanol extract of some indigenous medicinal plants of Pakistan in aspirin ulcerated rats. **J Ethnopharmacol** 46:1–6.

Alencar, D.C.K.M., L.A. Paiva, F.A. Santos, N.V. Gramosa, E.R. Silveira and V.S. Rao. 2003. Smooth muscle relaxant effect of kaurenoic acid, a diterpene from *Copaifera langsdorffii* on the rat uterus *In-vitro*. **Phytother.** 17: 320-324.

Ali M.S., I. Azhar, Z. Amtul, V.U. Ahmad and K.U. smanghani. 1995.Antimicrobial screening of some Caesalpiniaceae. **Fitoterapia** 70: 299–304.

Almeida, E.R., M.C. Guedes, J.F. Albuquerque and H. Xavier. 2006.Hypoglycemic effect of *Bauhinia cheilandra* in rats.**Fitoterapia** 77: 276–278.

Andrey, J. and B. Murray. 2004. Toxicity and antifeedant activity of clued seed extracts of Annona squamosa (Annonaceae) against lepidopteran pests and natural enemies. **International Journal of Tropical Insect Science**. 24: 150-158.

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Atwal, A.S., 1976. **Agricultural pests of the India and Southeast Asia**. second Ed. Kalyani Publisher, Delhi, 502.

Barata, L. 2005. Empirismo e ciência: fonte de novos medicamentos. **Ciên Cult** 57: 1–4.

Baskar, K., R. Maheswaran, S. Kingsley and S. Ignacimuthu. 2010.Bioefficacy of Couroupita guianensis (Aubl) against Helicoverpa armigera (Hub) (Lepidoptera : Noctuidae) larvae. **Spanish J. Agric. Res**. 8, 135–141.

Bentley, M.D., D.E. Leonard, W.F. Stoddard and L.H. Zalkow. 1984.Pyrrolizidine alkaloids as larval feeding deterrents for spruce budworm, Choristoneura fumiferana (Lepidoptera: Tortricidae).**Annal. Entomol. Soc. America.** 77, 393–397.

Bianco, E.M. and C.A.M. Santos. 2003. Substâncias isoladas das folhas de *Bauhinia microstachya* (Raddi) Macbr. (Caesalpinaceae).**Rev Bras Farmacog** 13: 93–99.

Bodakhe, S.H. and A. Ram. 2007. Hepatoprotective properties of *Bauhinia variegata* bark extract. **Yakugaku Zassi** 127: 1503 –1507.

Bompart, G.J., D.S. Prevot and J.L. Bascands. 1990. Rapid automated analysis of glutathione reductase, peroxidase, and S-transferase activity : Application to cisplatin-induced toxicity. **Clin Biochem**. 23: 501-504.

Braca, A., N. De Tommasi, L. Di Bari, C. Pizza, M. Politi and I. Morelli.2001. Antioxidant principles from *Bauhinia tarapotensis*.**J Nat Prod** 64: 892–895.

Brian, S.C., C.P. Jean and H.L. Lawrence. 2000. Role of cytochrome P450 and glutathione *S*-transferase in the metabolism and cytotoxicity of trichloroethylene in rat kidney. **Biochemical Phamacology**. 59(5): 531-543.

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Bresciania, L., R. Yunesa, C. Burgerb, L. Oliveirab, K. Leal and V. Filho. 2004. Seasonal Variation of Kaurenoic Acid, a Hypoglycemic Diterpene Present in *Wedelia paludosa* (*Acmela brasiliensis)* (Asteraceae). **Verlag der Zeitschrift für Naturforschung, Tübingen**. 0939 -5075.

Bullangpoti, V., S. Visetson, J. Milne, M. Milne, C. Sudthongkong and S. Pornbanlualap, . 2007. Effects of alpha-mangostin from mangosteen pericarp extract and imidacloprid on *Nilaparvata lugens* (Stal.) and non-target organisms: Toxicity and detoxification mechanisms. **Comm. Appl. Biol. Sci**., 72:431–423.

Carvalho, J.C.T., L.S. Santos and E.P. Viana. 1999. Anti-inflammatory and analgesic activities of the crude extracts from stem bark of *Bauhinia guianensis*. **Pharm Biol** 37: 281–284.

Capinera, J.L., 2001. **Handbook of vegetable pest**. Academic press,SanDiego, pp.729.

Charleston, D.S., K.W. Rami, D. Marcel and E.M. Louise. 2005. Impact of botanical pesticides derived from *Melia azedarach* and *Azadirachta indica* on the biology of two parasitoid species of the diamondback moth. Biological control. 12: 131-142.

Cipak, L., L. Grausova, E. Miadokova, L. Novotny and P. Rauko. 2006. Dual activity of triterpenoids: Apoptotic versus antidifferentiation effects. **Arch Toxicol** 80: 429–435.

Coimbra Teixeira, C., F.D. Danni, R.M. Blotta, D.G Mussnich, P.P. da Costa, and G.G Ranquetat. 1992. Plants employed in the treatment of diabetes mellitus: results of an ethnopharmacological survey in Porto Alegre, Brazil. **Fitoterapia** 63: 320–323.

Copyright by Kasetsart University All rights reserved

da Silva, K.L., M.W Biavatti, S.N. Leite, R.A. Yunes, F. Delle Monache and V. Cechinel Filho. 2000. Phytochemical and pharmacognostic investigation of *Bauhinia forficata* Link. **Z Naturforsch C** 55:478–480.

da Silva, K.L. and V. Cechinel Filho. 2002. Plantas do gênero Bauhinia:composição química e potencial farmacológico. **Quim Nova** 25: 449–454.

Damasceno, D.C., G.T. Volpato, M. Calderon Ide, R. Aguilar and M.V. Rudge. 2004. Effect of *Bauhinia forficata* extract in diabetic pregnant rats: maternal repercussions. **Phytomedicine** 11: 196–201.

Dhar, M.L., M.M. Dhar, B.N. Dhawan, B.N. Mehrotra and C. Roy. 1968.Screening of Indian lants for biological activity. **Indian J Exp Biol** 6: 232–247.

Duarte-Almeida, J.M., G. Negri and A. Salatino. 2004. Volatile oils leaves of *Bauhinia* (Fabaceae Caesalpinioideae). **Biochem Syst Ecol** 32: 747–753.

El-Kathiba, A.S. and A.E. Khaeel. 1995. **Evaluation of some pharmacological properties of different extracts of *Bauhinia muricata* whole plant**. Bull. Fac. Pharm. Cairo Univ. 33: 59–65.

Estrada, O., M. Hasegawa and F. Gonzalez-Mujíca. 2005. Evaluation of flavonoids from *Bauhinia megalandra* leaves as inhibitors of glucose-6-phosphatase system. **Phytother Res** 19: 859–863.

Feyereisen, R. 2005. Insect Cytochrome P450 in: Comprehensive Molecular Insect Science. **Elsevier**. 4: 1-77.

Freedman, B., L. Nowak, J. Kwolek, W. F. Berry, E. C. and W. D. A. Guthrie. 1979. Bioassay for Plant-Derived Pest Control Agents Using the European Corn Borer. **Journal of Economic Entomology**, 72. 541-545(5)

Copyright by Kasetsart University All rights reserved

Fuentes, O. and J. Alarcón. 2006. *Bauhinia candicans* stimulation of glucose uptake in isolated gastric glands of normal and diabetic rabbits. **Fitoterapia** 77: 271–275.

Gadotti, V.M., L.O. Schmeling, C. Machado. 2005. Antinociceptive action of the extract and the flavonoid quercitrin isolated from *Bauhinia microstachya* leaves. **J Pharm Pharmacol** 57: 1345–1351.

Gupta, M,, U.K. Mazumder, T. Siva Kumar, R. Sambath Kumar and P. Gomathi. 2004. Antioxidant and hepatoprotective effect of *Bauhinia racemosa* against paracetamol and carbon tetrachloride induced liver damage in rats. **Iran J Pharmacol Ther** 3: 12–20.

Hazra, A.G. and P. Chatterjee. 2008. A nontoxic antitumour compound from the leaves of Bauhinia scandens characterized as 1-O-alkyl glycerol by gas-liquid chromatography and evaluation of its antitumour property by Brine Shrimp bioassay. **Industrial Crops and Products**. 27 (1), pp. 39-43

Jiwajinda, S., N. Hirai, K. Watanabe, V. Santisopasri, N. Chuengsamarnyart, K. Koshimizu, and H. Ohigashi. 2000. Occurrence of the insecticidal 16, 17-didehydro-16(E)-stemofoline in *Stemona collinsae*. **Phytochemistry** 56, 693–695

Jorge, A.P., H. Horst, E. de Sousa, M.G. Pizzolatti and F.R. Silva. 2004. Insulinomimetic effects of kaempferitrin on glycaemia and on 14C-glucose uptake in rat soleus muscle. **Chem Biol Interact** 149: 89–96.

Juliani, C. 1929. Ação hipoglicemiante da unha-de-vaca. **Rev Med Pharm Chim Phys** 2: 165–169.

Juliani, C. 1931. Ação hipoglicemiante da *Bauhinia forficata*.Novos estudos experimentais. **Rev Sudamer Endocrinol Imunol Quimioter** 14: 326–334.

Copyright by Kasetsart University All rights reserved

Juliani, C. 1941. Hypoglycemic action of bauintrato (*Bauhinia forficata* preparation): new clinical and experimental study. **J Clin** 22: 17.

Kittakoop, P., K. Kirtikara, M. Tanticharoen and Y. Thebtaranonth. 2000. Antimalarial preracemosols A and B, possible biogenetic precursors of racemosol from *Bauhinia malabarica* Roxb.**Phytochemistry** 55: 349–352.

Koul, O. and S. Walia, 2009. Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. CAB Reviews: Perspcetives in Agriuculture, Veterinary Science, **Nutrition and Natural Resource**. 4 (049), 1–30.

Krebsfänger, N. Characterization and enantioselectivity of a recombinant esterase from *Pseudomonas fluorescens.* **Enzyme Microb. Tech**., 1998. 22: p. 641-646.

Kumar, R.S., T. Sivakumar, R.S. Sunderam. 2005. **Antioxidant and antimicrobial activities of *Bauhinia racemosa* L**. stem bark. Braz J Med Biol Res 38: 1015–1024.

Lee, H.S., W.K. Shin, C. Song, K.Y. Cho and Y.J. Ahn. 2001. Insecticidal activities of arturmerone identified in Curcuma longa rhizome against *Nilaparvata lugens* (Homoptera: Delphacidae) and *Plutella xylostella* (Lepidoptera: Yponomeutidae). **J. Asia-Pac. Entomol**. 4, 181–185.

Lemus, I., R. García, E. Delvillar and G. Knop. 1999. Hypoglycaemic activity of four plants used in Chilean popular medicine.**Phytother Res** 13: 91– 94.

Li, X., M.A. Schuler and M.R. Berenbaum, 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. **Entomol**. 52: 231–253.

Copyright by Kasetsart University All rights reserved

Lim, H., M.K. Kim, Y. Lim, Y.H. Cho and C.H. Lee. 2006. Inhibition of cell-cycle progression in HeLa cells by HY52, a novel cyclindependent kinase inhibitor isolated from *Bauhinia forficata*.**Cancer Lett** 233: 89–97.

Lingathurai, S., S.E. Vendan, M.G. Paulraj and S. Ignacimuthu. 2010. **Antifeedant and larvicidal activities of Acalypha fruticosa Forssk. (Euphorbiaceae) against *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) larvae**. King Saud University.

Meyre-Silva, C., R.A. Yunes, F. Delle Monache . 2001. Phytochemical and pharmacological analysis of *Bauhinia microstachya* (Raddi) Macbr. (Leguminosae). **Z Naturforsch C** 56: 939–942.

Mohan, M. and G.T. Gujar. 2003. Local variation in susceptibility of the diamondbackmoth, *Plutella xylostella* (Linnaeus) to insecticides and role of detoxification enzymes. **Crop Protec**. 22: 495–504.

Morallo-Rejesus, B., 1986. Botanical insecticides against the diamondback moth. In: Talekar, N.S. (Ed.), Diamondback moth management. Proceedings of the first international workshop, 11–15 March 1985. **Asian Vegetable Research and Development Center**,Tainan, Taiwan, pp. 241–255.

Muñoz, V., M. Sauvain, G. Bourdy. 2000. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. **J Ethnopharmacol** 69: 127–137.

Neto, M.M., M.A. Neto, R. Braz Filho, M.A.S. Lima and E.R. Silveira. 2008. Flavonoids and alkaloids from leaves of *Bauhinia ungulate* L. **Biochem Syst Ecol** 36: 227–229.

Copyright by Kasetsart University All rights reserved

Ooi, P.A.C., 1992. **Role of parasitoids in managing diamondback moth in the Cameron Highlands, Malaysia. In: Moth, Diamondback.,Crucifer**, Other., Talekar, N.S. (Eds.), Pests: Proceedings of the Second International Workshop. Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp. 255–262.

Patcharaporn Vanichpakorn, Wei Ding and Cen Xiao-Xi. 2010. Insecticidal activity of five Chinese medicinal plants against *Plutella xylostella* L. larvae. **Journal of Asia-Pacific Entomology**. 13: 169–173

Pettit, G.R., A. Numata and C. Iwamoto. 2006. Antineoplastic agents.551. Isolation of structures of bauhiniastatins 1-4 from *Bauhinia purpurea***. J Nat Prod** 69: 323–327.

Pinheiro, T.S.D.B., L.A.P. Johansson, M.G. Pizzolatti and M.W. BIavatti. 2006. Comparative assessment of kaempferitrin from medicinal extracts of *Bauhinia forficata* Link. **J Pharm Biomed Anal** 41: 431–436.

Pipithsangchan, S., S. Subhadhirasakul, T. Butpha, N. Phadoongsombat, and K. Chantrapromma. 2004. Effects of extracts from thiem seeds on diamondback moth (*Plutella xyllostella* Linn.). Songklanakarin. **J. Sci. Technol**. 26: 221–232.

Pokhrel, N.R., R.P. Adhikari and M.P. Baral. 2002. *In vitro* evaluation of the antimicrobial activity of *Bauhinia variegata*, locally known as Koiralo. **World J Microbiol Biotechnol** 18: 69–71.

Rajkapoor, B., B. Jayakar, N. Murugesh and D. Sakthisekaran. 2006.Chemoprevention and cytotoxic effect of *Bauhinia variegate* against *N*-nitrosodiethylamine induced liver tumors and human cancer cell lines. **J Ethnopharmacol** 104: 407–409.

Copyright by Kasetsart University All rights reserved

Ramsey, J.S., D.S. Rider, T.K. Walsh, M. DeVos, K.H. Gordon, L. Ponnala, S.L. Macmil, B.A. Roe and G. Jander, 2010. Comparative analysis of detoxification enzymes in *Acyrthosiphon pisum* and *Myzus persicae*. **Insect Mol. Biol.** 19: 155–164.

Riddiford, L.M., K. Hiruma, X. Zhou and C.A. Nelson, 2003. Insights into the molecular basis of the hormonal control of molting and metamorphosis from *Manduca sexta* and *Drosophila melanogaster*. **Insect Biochem. Mol. Biol**. 33: 1327–1338.

Rowell, B., N. Bunsong, K. Sathaporn and S.S. Doungsa-Ard. 2005. Hymenoptera parasitoids of Diamondback moth (Lepidoptera:Ypeunomutidae) in Northern Thailand. **J. Econ. Entomol**. 98: 449–456.

Saeed, R., H. Sayyed, A. Sarfraz and S.M. Zaka. 2010. Effect of different host plants on the fitness of diamond-back moth, *Plutella xylostella* (Lepidoptera: Plutellidae). **Crop protection**. 4: 178-182.

Sayyed, A.H., D.J. Wright. 2002. **Genetic diversity of Bt resistance.Implications for resistance management.** Pak. J. Biol. Sci. 5: 1330–1344.

Shelton, A.M., J.A. Wyman, N.L. Cushing, K. Apfelbeck, T.J. Dennehy, S.E.R. Mahr and S.D. Eigenbrode, 1993. Insecticide resistance of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) in North America. **J. Econ. Entomol.** 86: 11–19.

Silva, F.R., B. Szpoganicz, M.G. Pizzolatti, M.A. Willrich and E. de Sousa. 2002. Acute effect of *Bauhinia forficata* on serum glucose levels in normal and alloxan-induced diabetic rats. **J Ethnopharmacol** 83: 33–37.

Songarsa, S., S. Rajviroongit, D. Sae-Tang, S. Hannongbua, K. Kirtikara and P. Kittakoop. 2005. New racemosol derivatives as potent cyclooxygenase (COX) inhibitors. **Chem Biodivers** 2: 1635–1647.

Copyright by Kasetsart University All rights reserved

Sosa, S., A. Braca, G. Altinier, R. Della Loggia, I. Morelli and A. Tubaro. 2002. Topical anti-inflammatory activity of *Bauhinia tarapotensis* leaves. **Phytomedicine** 9: 646–653.

Sparg, S.G., M.E. Light and J.V. Staden. 2004. Biological activities and distribution of plant saponins. **J. Ethnopharmacol**. 94, 219–243.

Sripathi, S.K. and S. Uma. 2010. Ethnobotanical documentation of a few medicinal plants in the Agasthiayamalai region of Tirunelveli district India. **Ethnobot. Leaflet**. 14, 173–181.

Tabashnik, B.E., 1985. Deterrence of diamondback moth (Lepidoptera:Plutellidae) oviposition by plant compounds. **Environ**. **Entomol**.14, 575–578.

Taylor, P. and Z. Radic, 1994. The cholinesterases: From genes to proteins. *Annu. Rev.* **Pharmacol. Toxicol**. 34: 281–320.

Varasutpisal, O. 2008. Insecticidal Efficacy of Essential Oil from Wildbetal Leafbush (*Piper sarmentosum* Roxburgh.) Leaf Against the Daimondback Moth (*Plutella xylostella* Linnaeus.) larvae. **Master thesis**. Kasetsart University, Bangkok Thailand.

Verkerk, R.H.J. and D.J. Wright. 1996. Multitrophic interactions and management of the diamondback moth: a review. **Bull**. **Entomol. Res**. 86, 205–216.

Volpato, G.T., D.C. Damasceno, M.V. Rudge, C.R. Padovani and I.M. Calderon. 2008. Effect of *Bauhinia forficata* aqueous extract on the maternal-fetal outcome and oxidative stress biomarkers of streptozotocin-induced diabetic rats. **J Ethnopharmacol** 116: 131–137.

Wang, C. 2000. Luteolin beneficial. **Drugs of the future**. 25(2): 146-149.

Copyright by Kasetsart University All rights reserved

Watson, L. and M.J. Dallwitz. 2000. The Families of Flowering Plants: Descriptions, illustrations, Identification and Information Retrieval. **Ecology and Evolutionary Biology Conservatory**.

Whistler, W.A. 1995. Wayside plants of the islands: A guide to the lowland flora of the Pacifiic Islands including Hawai'i, Samoa, Tonga, Tahiti, Fiji, Guam and Belau. **Isle Botanical**. Honolulu.

Waterhouse, D.F. and K.R. Norris, 1987. Biological control: Pacific prospects. Inkata Press, Melbourne, Australia.**Fitoterapia** 75: 527–532.

Wel, Hui., Juan. Wang, Hong-shan. Li. Hua-guo. Dai, and Xiao-jun. Gu. 2010. Sub-Lethal Effects of Fenvalerate on the Development, Fecundity and Juvenile Hormone Esterase Activity of Diamondback Moth,*Plutella xylostella*(L.). **Agriculture Science in China** 9(11): 1612–1622.

Willain Filho, A., E. Breviglieri, V. Cechinel Filho and A.R.Santos 1997.Antinociceptive effect of the hydroalcoholic extract of *Bauhinia splendens* stems in mice. **J Pharm Pharmacol** 49: 823–827.

Wing, K.D., M. Sacher, Y. Kagaya, Y. Tsurubushi, L. Mulderig, M. Connair and M. Schnee. 2000. Bioactivation and mode of action of oxadiazine indoxacarb in insects. **Crop Protection**. 19: 537-545.

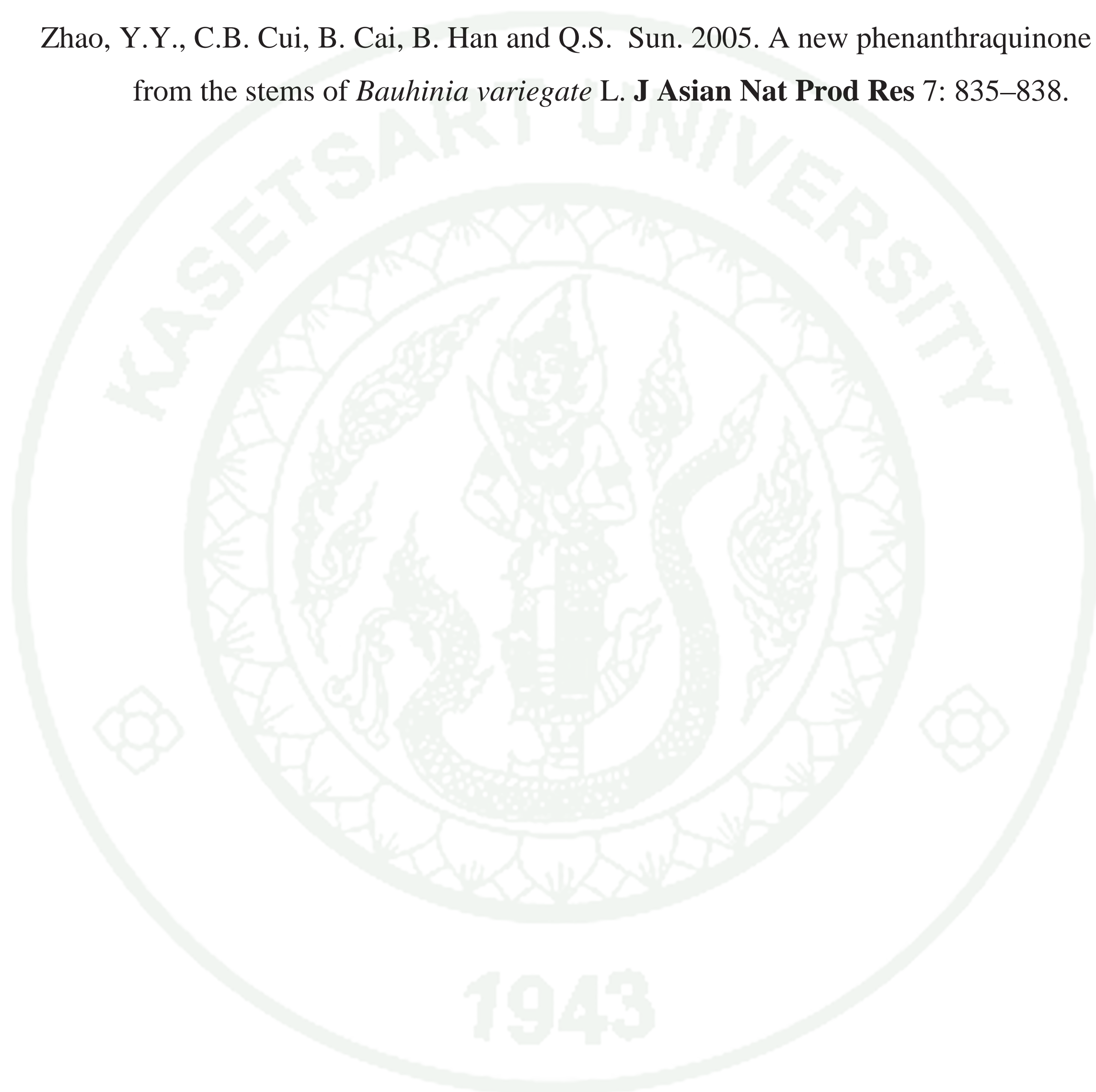
Xiaodun, He. 2003. A Continous Spectrophotometric Assay for the Determination of Diamondblack Moth Esterase Activity. **Archives of Insect Biochemistry and Physiology** 54: 68–76.

Yadava, R.N. and V.M. Reddy. 2003. Anti-inflammatory activity of a novel flavonol glycoside from the *Bauhinia variegata* Linn.**Nat Prod Res** 17: 165–169.

Copyright by Kasetsart University All rights reserved

Zakaria, Z.A., L.Y. Wen, N.I. Abdul Rahman, A.H. Abdul Ayub, M.R. Sulaiman and H.K. Gopalan. 2007. Antinociceptive, anti-inflammatory and antipyretic properties of the aqueous extract of *Bauhinia purpurea* leaves in experimental animals.**Med Princ Pract** 16: 443–449.

Zhao, Y.Y., C.B. Cui, B. Cai, B. Han and Q.S. Sun. 2005. A new phenanthraquinone from the stems of *Bauhinia variegate* L. **J Asian Nat Prod Res** 7: 835–838.



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