ฤทธิ์ต้านการกินของหนอนกระทู้ผัก *Spodoptera litura* Fab. จากพรรณไม้ไทยบางชนิด

นางสาวพาณี นาคทอง

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ ที่ส่งผ่านทางบัณฑิตวิทยาลัย
The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository (CUIR)
are the thesis authors' files submitted through the University Graduate School.

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

ANTIFEEDANT ACTIVITY AGAINST COMMON CUTWORM *Spodoptera litura* Fab. FROM SOME THAI PLANTS

Miss Panee Nakthong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Biotechnology
Faculty of Science
Chulalongkorn University
Academic Year 2015
Copyright of Chulalongkorn University

Thesis Title	ANTIFEEDANT ACTIVITY AGAINST COMMON CUTWORM *Spodoptera litura* Fab. FROM SOME THAI PLANTS
By	Miss Panee Nakthong
Field of Study	Biotechnology
Thesis Advisor	Assistant Professor Warinthorn Chavasiri, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Science
(Associate Professor Polkit Sangvanich, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Vudhichai Parasuk, Ph.D.)

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

.....Examiner
(Supawin Watcharamul, Ph.D.)

.....External Examiner
(Wachiraporn Phoonan, Ph.D.)

พาณี นาคทอง : ฤทธิ์ต้านการกินของหนอนกระทู้ผัก *Spodoptera litura* Fab. จากพรรณไม้ไทยบางชนิด (ANTIFEEDANT ACTIVITY AGAINST COMMON CUTWORM *Spodoptera litura* Fab. FROM SOME THAI PLANTS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. วรินทร ชวศิริ, 93 หน้า.

ได้นำน้ำมันหอมระเหย 6 ชนิดและสิ่งสกัดจากพรรณไม้ไทย 34 ชนิด มาคัดกรองเบื้องต้นเพื่อศึกษาฤทธิ์ในการยับยั้งการกินของหนอนกระทู้ผัก *Spodoptera litura* พบว่า สิ่งสกัดไดคลอโรมีเทนของเมล็ดตะบูนขาว (*Xylocarpus granatum*) น้ำมันหอมระเหยจากใบโหระพา (*Ocimum basilicum*) น้ำมันหอมระเหยจากดอกมหาหงส์ (*Hedychium coronarium*) และน้ำมันจากเมล็ดแครอท (*Daucus carota*) แสดงฤทธิ์ยับยั้งการกินดี ที่ความเข้มข้น 0.25% (น้ำหนักโดยน้ำหนัก) มีค่า EC_{50} 0.06, 0.12, 0.13 และ 0.15% (น้ำหนักโดยน้ำหนัก) ตามลำดับ ได้วิเคราะห์หาองค์ประกอบในน้ำมันหอมระเหยแต่ละชนิดด้วยวิธี GC-MS พบว่า ยูคาลิปทอลซึ่งเป็นองค์ประกอบของน้ำมันหอมระเหยจากโหระพาและมหาหงส์ แสดงฤทธิ์ในการยับยั้งการกินมากที่สุด ที่ค่า EC_{50} 0.8 มิลลิโมลาร์ ได้แยกสารจากสิ่งสกัดไดคลอโรมีเทนจากเมล็ดตะบูนขาวเพื่อหาสารยับยั้งการกินด้วยวิธีทางโครมาโทรกราฟี พบว่า xyloccensin K เป็นองค์ประกอบหลัก และสามารถยับยั้งการกินที่ค่า EC_{50} 1.3 มิลลิโมลาร์ เมื่อเปรียบเทียบความเสถียรของสิ่งสกัดไดคลอโรมีเทน, xyloccensin K และสิ่งสกัดจากสะเดา ต่อแสงอัลตราไวโอเลตและความร้อน พบว่า ฤทธิ์การยับยั้งการกินของสิ่งสกัดสะเดาลดลงมากกว่าสิ่งสกัดไดคลอโรมีเทนและ xyloccensin K นอกจากนั้นเมื่อทดสอบเปรียบเทียบระหว่างวิธีการผสมสารลงในอาหารเทียมและเคลือบสารบนใบพืช พบว่าผลที่ได้เป็นไปในทิศทางเดียวกัน ดังนั้นน้ำมันหอมระเหยทั้งสามชนิด สิ่งสกัดไดคลอโรมีเทนจากเมล็ดตะบูนขาว ยูคาลิปทอลและ xyloccensin K สามารถพัฒนาเป็นสารยับยั้งการกินที่มีประสิทธิภาพต่อแมลงศัตรูพืช

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5572061023 : MAJOR BIOTECHNOLOGY

KEYWORDS: ANTIFEEDANT ACTIVITY/ SPODOPTREA LITURA/ PLANT EXTRACTS/ ESSENTIAL OILS

PANEE NAKTHONG: ANTIFEEDANT ACTIVITY AGAINST COMMON CUTWORM *Spodoptera litura* Fab. FROM SOME THAI PLANTS. ADVISOR: ASST. PROF. WARINTHORN CHAVASIRI, Ph.D., 93 pp.

Six essential oils and thirty-four plant extracts were preliminary screened for antifeedant activity against *Spodoptera litura*. The dichloromethane extract of *Xylocarpus granatum*, the essential oils from *Ocimum basilicum*, *Hedychium coronarium* and *Daucus carota* showed high antifeedant activity at 0.25% (w/w) with EC_{50} 0.06, 0.12, 0.13 and 0.15% (w/w), respectively. The compositions of three essential oils were analyzed by GC-MS. Certain constituents were tested for antifeedant activity. Eucalyptol revealed the highest activity with EC_{50} 0.8 mM. The dichloromethane extract of *X. granatum* was separated using bioassay-guided approach to search for antifeedant compound. Xyloccensin K, the major component exhibited antifeedant activity with EC_{50} 1.3 mM. In addition, the UV light and temperature influence on the dichloromethane extract, xyloccensin K, and commercial neem extract was comparatively examined. The antifeedant activity of neem extract was significantly decreased more than the dichloromethane extract and xyloccensin K. Moreover, two methods using for antifeedant activity: mixing treatment with artificial diet and leaf disk toxicity assay were compared. Three essential oils, the dichloromethane extract and their constituents were tested for antifeedant activity with kale leave. The similar trend of antifeedant activity was found with both methods. Three essential oils, the dichloromethane extract of *X. granatum,* eucalyptol and xyloccensin K could be developed as effective antifeedant compounds against insect pests.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Field of Study: Biotechnology Student's Signature .....

Academic Year: 2015 Advisor's Signature .....

ACKNOWLEDGEMENTS

The author wishes to especially be grateful to Assistant Professor Dr. Warinthorn Chavasiri, for his generous guiding, kind help, encouragement and suggestion throughout the course of this research. Sincere thanks are extended to Associate Professor Dr. Vudhichai Parasuk, Dr. Supawin Watcharamul and Dr. Wachiraporn Phoonan serving as the chairman and members of thesis committee, for their valuable comments, discussion and suggestions. Many thanks to Natural Products Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University for the support of chemicals and laboratory facilities.

The author also acknowledges for bioassay supports from Associate Professor Dr. Vasakorn Bullangpoti (Department of Zoology, Faculty of Science, Kasetsart University) for giving an advice on bioassay. Moreover, the author would like to thanks Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok for their kind gratitude of giving information in insect rearing.

The author would like to thank Ms. Asshaima Paramita Devi, Ms. Rachsawan Mongkol, Ms. Phijaree Wikitkankosol, Ms. Patranit Charoensri, Ms. Paweena Pongpipatt and Mr. Pragatsawat Chanprapai for the advice and scientific techniques during research. Thanks are also extended to my friends and sister for their friendliness, advice helpful, cheerful attitude and encouragement.

Finally, the author wishes to express her deepest appreciation gratefulness to her parents including Mr. Niwat Nakthong and Mrs. Suwaporn Nakthong and my family member for their love, understanding, inspiration, great support and encouragement throughout the entire study.

CONTENTS

Page

Page

Page

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

LIST OF TABLES

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

LIST OF FIGURES

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

LIST OF SCHEMES

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

LIST OF ABBREVIATION

°C	=	degree Celsius
CH_2Cl_2	=	dichloromethane
CH_3OH	=	methanol
Chloroform-d_1	=	deuterated chlorofrom
cm	=	centimeter (s)
^{13}C NMR	=	carbon-13 nuclear magnetic resonance
d	=	doublet (NMR)
dd	=	doublet of doublet (NMR)
EtOAc	=	ethyl acetate
Et_2O	=	Diethyl ether
EC_{50}	=	effective concentration at 50 percent
g	=	gram
GC-MS	=	gas chromatography-mass spectrometry
Hex	=	hexane
h	=	hour
Hz	=	hertz (NMR)
^1H NMR	=	proton-1 nuclear magnetic resonance
J	=	coupling constant
L:D	=	light:dark
L	=	liter (s)
m	=	multiplet (NMR)
min	=	minute
mL	=	milliliter
mM	=	millimolar
nm	=	nanometer
no.	=	number
ppm	=	part per million
R.H.	=	relative humidity

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

R_t	=	retention time
RT	=	room temperature
s	=	singlet (NMR)
S.D.	=	standard deviation
t	=	triplet (NMR)
TLC	=	thin layer chromatography
UV	=	ultraviolet
w/v	=	weight by volume
w/w	=	weight by weight
δ	=	chemical shift
µL	=	microliter (s)

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHEPTER I

INTRODUCTION

In agriculture, controlling the quantity and quality of crops is the most important. Insect pest is one of the main problems of crops and difficult to control. Most agriculturists usually utilize synthetic insecticides since they are easy to use, fast and convenient. However, there are many flaws. The use of synthetic insecticides caused some concerns regarding their adverse effects on the environment. These compounds are often not biodegradable and the residues are concentrated in food chains and accumulated in soil, water and plants so they cause invariably environment pollution [1]. Moreover, they are toxic to human and non-target animals. Furthermore, most of them are imported from foreign countries. According to above reasons, the alternatives from natural products are likely promising to replace some synthetic chemicals. The naturally occurring compounds are environmentally friendly to nature because of they are easy to decompose, low toxic to users and inexpensive than imported insecticides [2].

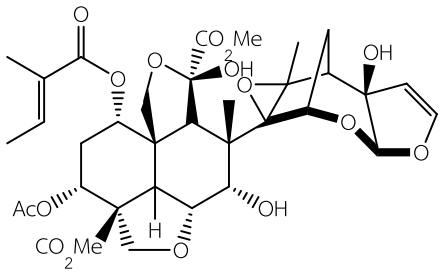
1.1 Insect antifeedants

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Insect antifeedants are defined as chemicals that inhibit feeding. They do not directly kill insect but insect will die through starvation. The chemicals that possess antifeedant activity could be found in several plants. They additionally do not damage to pollinators, predators and parasites [1].

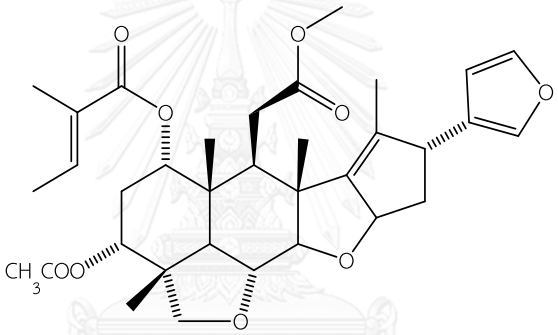
Insect antifeedants can be found amongst all major classes of secondary metabolites from plants such as limonoids, quassinoids, diterpenes, sesquiterpenes, monoterpenes, coumarins, isoflavonoids, alkaloids, ellagitannins, aristolochic acids, *etc*. However, the most potent antifeedants belong to terpenoid group, which has the greatest number and diversity of known antifeedants. In terpenoids, limonoids are well studied. The most potent example is azadirachtin from *Azadirachta indica* A. Juss (Family Meliaceae). Azadirachtin (**1**) is the most potent natural antifeedant against the large number of insects in the larvae and adult stages such as Lepidoptera, Coleoptera,

Dermaptera, Diptera, Heteroptera, Homoptera, Hymenoptera, Isoptera, Phasmida and Thysanoptera[3]. This chemical at present can be synthesized [4].



Azadirachtin(1)

Another example of a limonoid from neem showing potential antifeedant activity is salannin (**2**) which restrains feeding about 10 insect species [5].



Salannin(2)

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

The bitterness causative factor in a number of citrus species is limonin (**3**). A few other citrus limonoids, including nomilin (**4**), nomilinic acid (**5**), ichangin (**6**), and obacunoic acid (**7**) are also bitter (Figure 1.1). Amongst these, limonin (**3**) and nomilin (**4**) are known to restrain feeding in *Spodoptera, Heliothis, Choristoneura, Eldana, Maruca*, and *Leptinotarsa* species with variable efficacies [6].

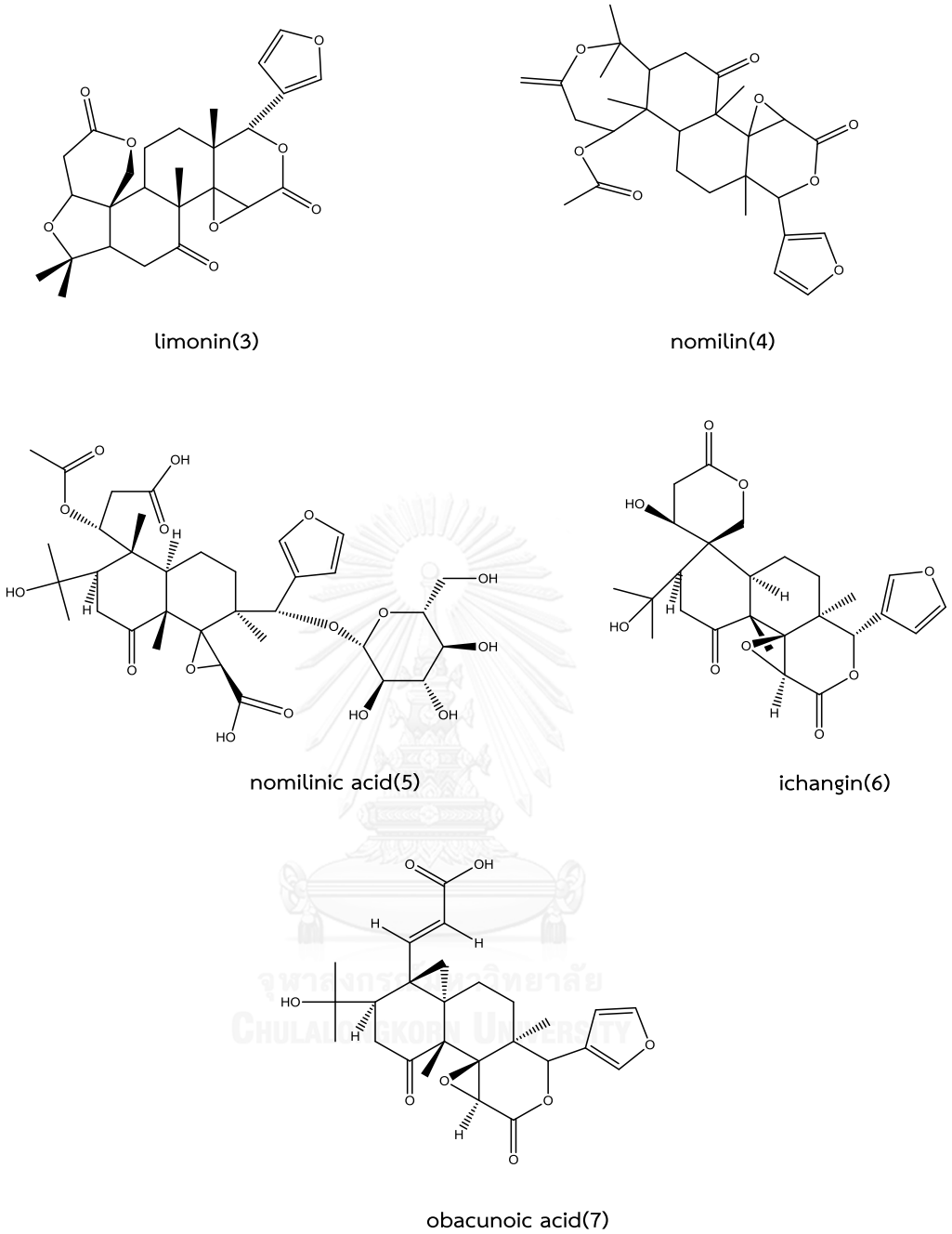


Figure 1.1 Structures of some citrus limonoids

The highly oxygenated triterpenes are quassinoids which were isolated as bitter test from the plants of Simaroubaceae family. They are more like limoniods. Those compounds including quassin (**8**) [7], isobrucein-B (**9**) [8], guineensino (**10**), pipercide (**11**) and chingchengenmind (**12**) [9] are antifeedant compounds for *Plutella xylostella* (Figure 1.2).

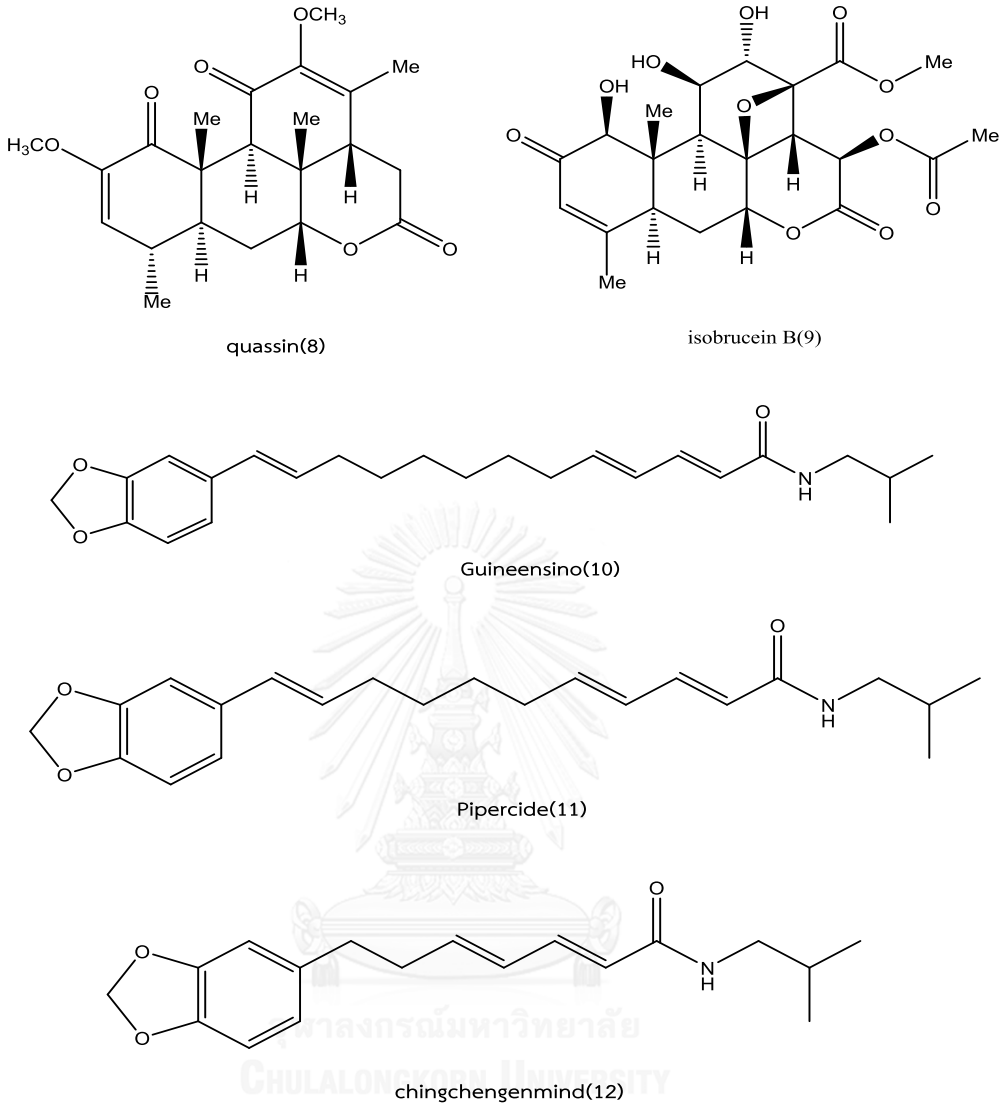


Figure 1.2 Quassinoid antifeedants

Diterpenes, especially clerodane types of diterpenes have been identified from various plant sources and exhibited to restrain feeding in various insect species [10]. Clerodin type (**13**) of compounds from Asteraceae and Lamiaceae are effective antifeedants against *Spodoptera litura* (Fab.), *Spodoptera littoralis* (Boisd.), *Ostrinia nubilalis* (Hubner), and *Euproctis subflava* (Bremer) [11]. Besides, diterpenes, ajugarin I (**14**) isolated from the bugle plant can be used to restrain feeding by Coleoptera. Other diterpenes with antifeedant activity such as clerodendrin B (15), 3-epicaryoptin (**16**),

15-hydroxyepicaryoptin (**17**), teuflin(18), 6β-acetylteuscordin (**19**) and montanin-D (**20**) showed effective antifeedant against *S. litura* [12, 13] (Figure 1.3).

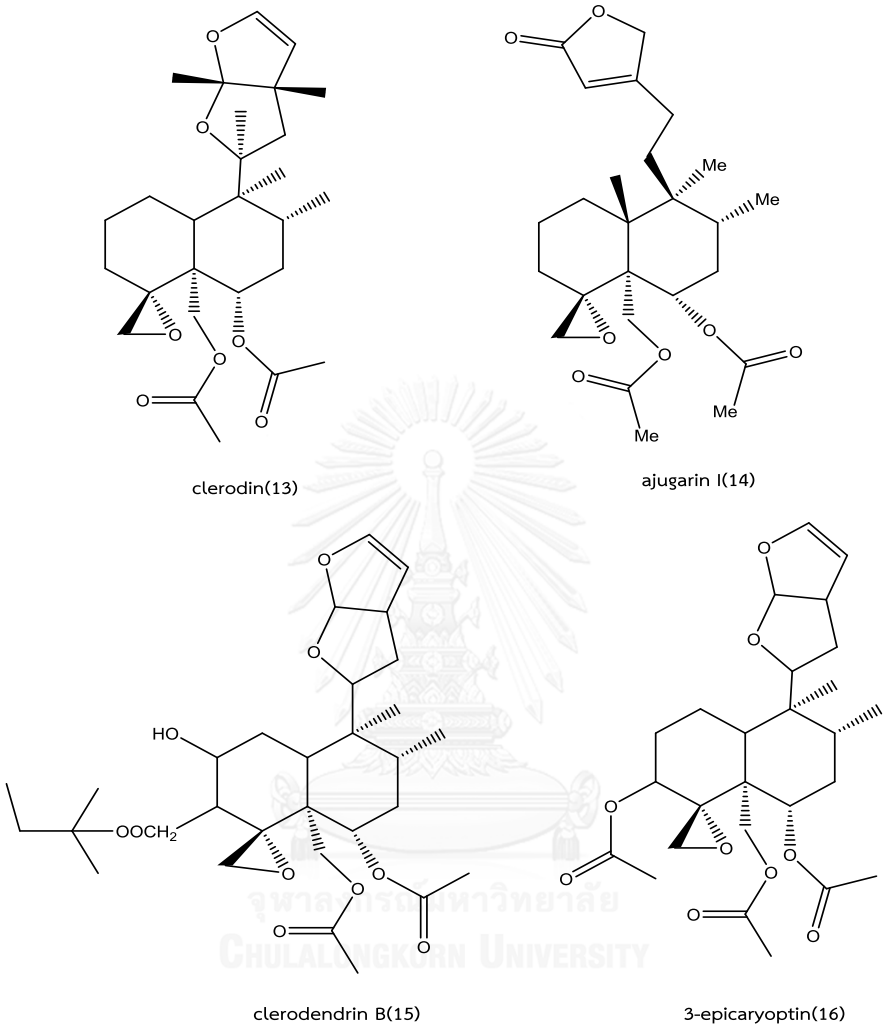
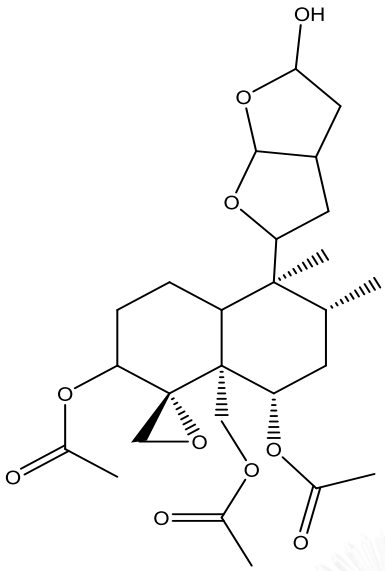
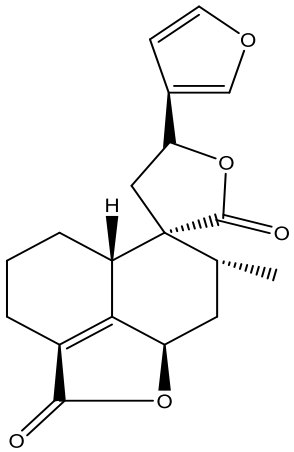


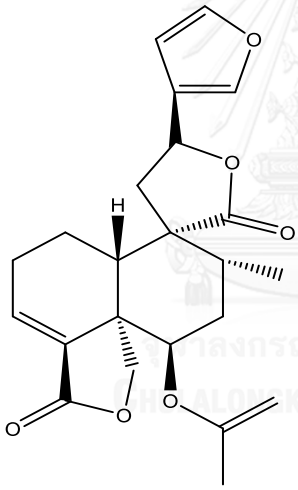
Figure 1.3 Diterpene antifeedant



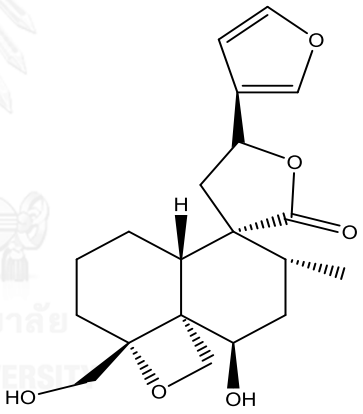
15-hydroxyepicaryoptin(17)



teuflin(18)



6beta-acetylteuscordin(19)



montanin D(20)

Figure 1.3 (cont.)

Certain sesquiterpenes such as warburganal (**21**), polygodial (**22**), muzigadial (**23**) and caryophyllene (**24**) were also known as antifeedants against cabbage butterfly larvae [1, 14]. Moreover, β-copaene (**25**), α-selinene (**26**) and β-selinene (**27**) (Figure 1.4) displayed antifeedant activity against *Spodoptera exigua* [1].

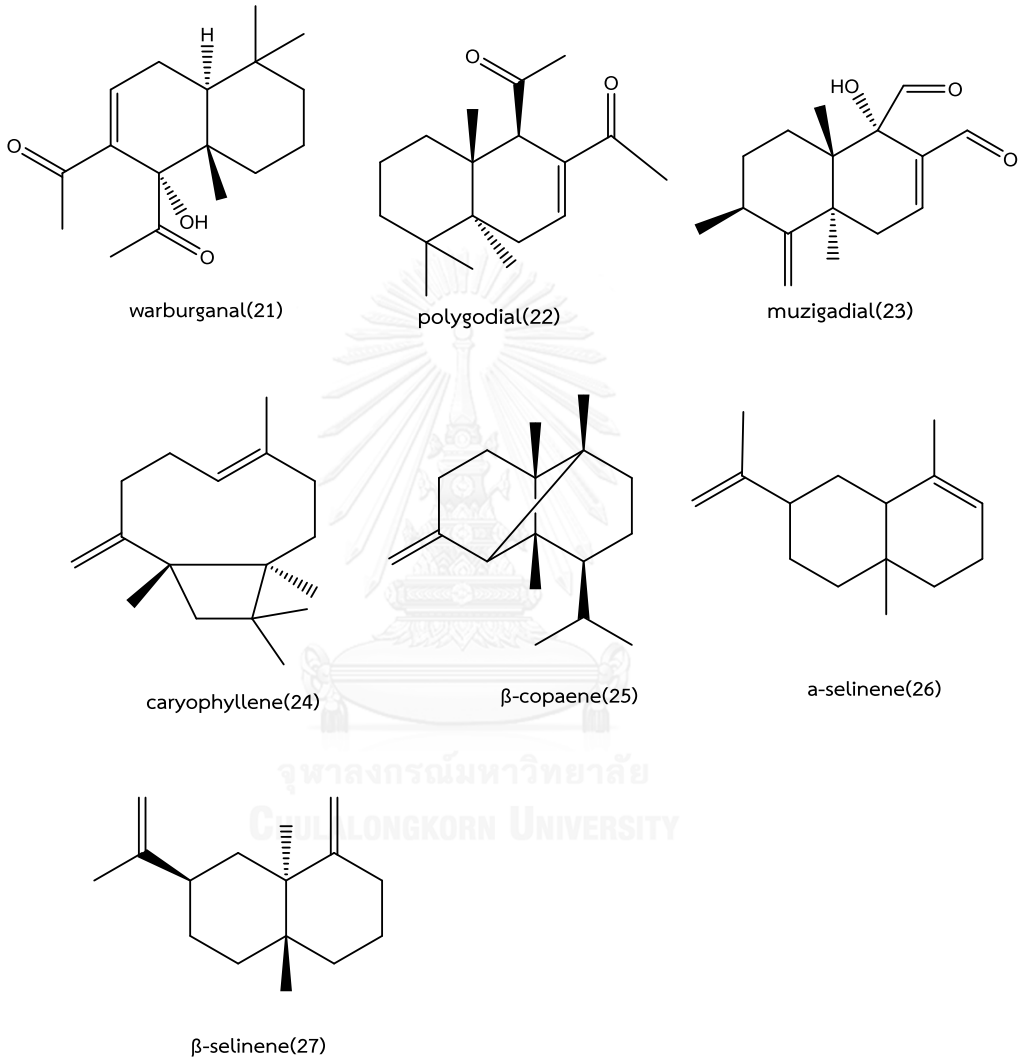
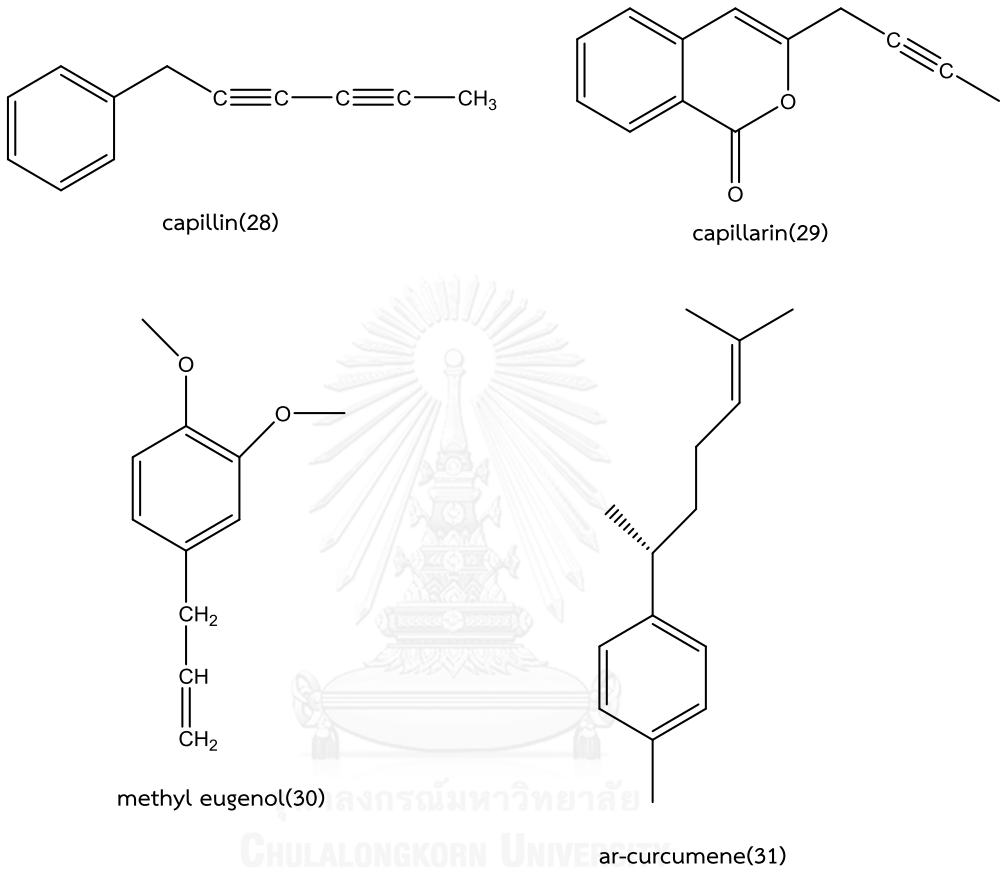


Figure 1.4 Sesquiterpene antifeedant

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Many monoterpenes from plant sources have been evaluated as feeding deterrents against insects [15]. For instance, capillin (**28**), capillarin (**29**), methyl eugenol (**30**) and ar-curcumene (**31**) (Figure 1.5) isolated from *Artemisia capillaris* revealed promising antifeedant activity against *Pieris rapae* [3].



capillin(28)

capillarin(29)

methyl eugenol(30)

ar-curcumene(31)

Figure 1.5 Monoterpene antifeedant

Some coumarins such as oxypeucedanin (**32**), xanthotoxin (**33**), isoimperatorin (**34**) and prangol (**35**) (Figure 1.6) showed antifeedant activity against *S. littoralis* larvae [16].

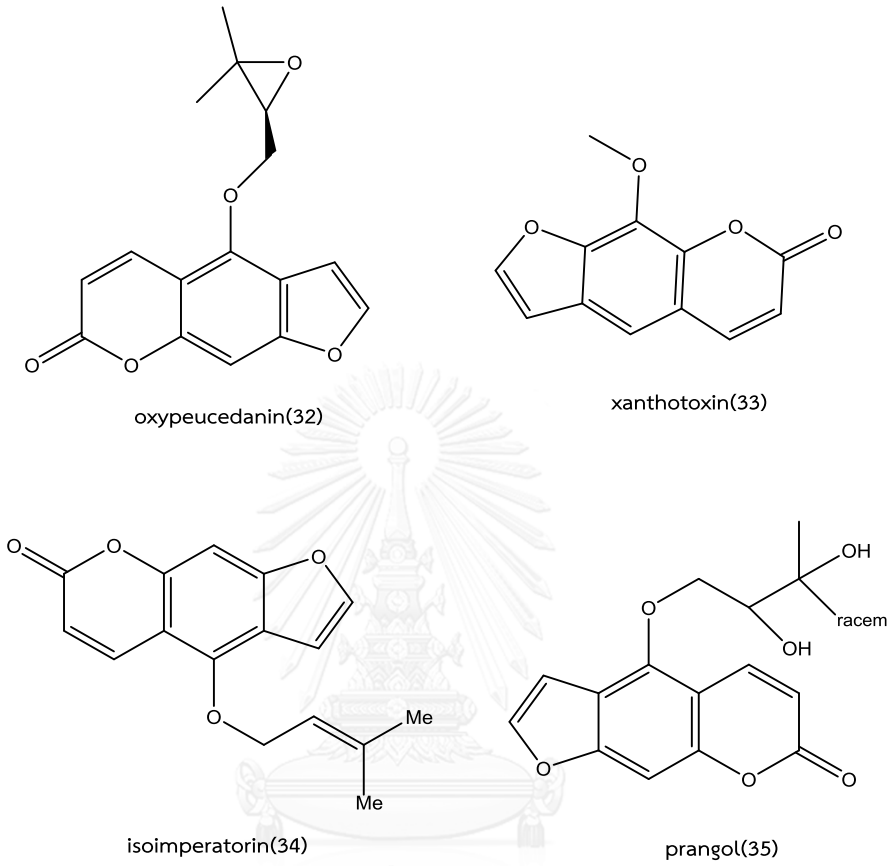


Figure 1.6 Coumarin antifeedants

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Some flavonoids have also showed antifeedant activity against *S. litura* such 5-hydroxy-3,6,7,8-tetramethoxyflavone (**36**), and 5,6-dihydroxy-3,7-dimethoxyflavone (**37**) isolated from *Gnaphalium affine* D. Don [17]. Rutin (**38**) displaying antifeedant activity against *Helicoverpa zea*, while quercetin (**39**) and phloretin (**40**) were active against *Scolytus multistriatus* [1] (Figure 1.7).

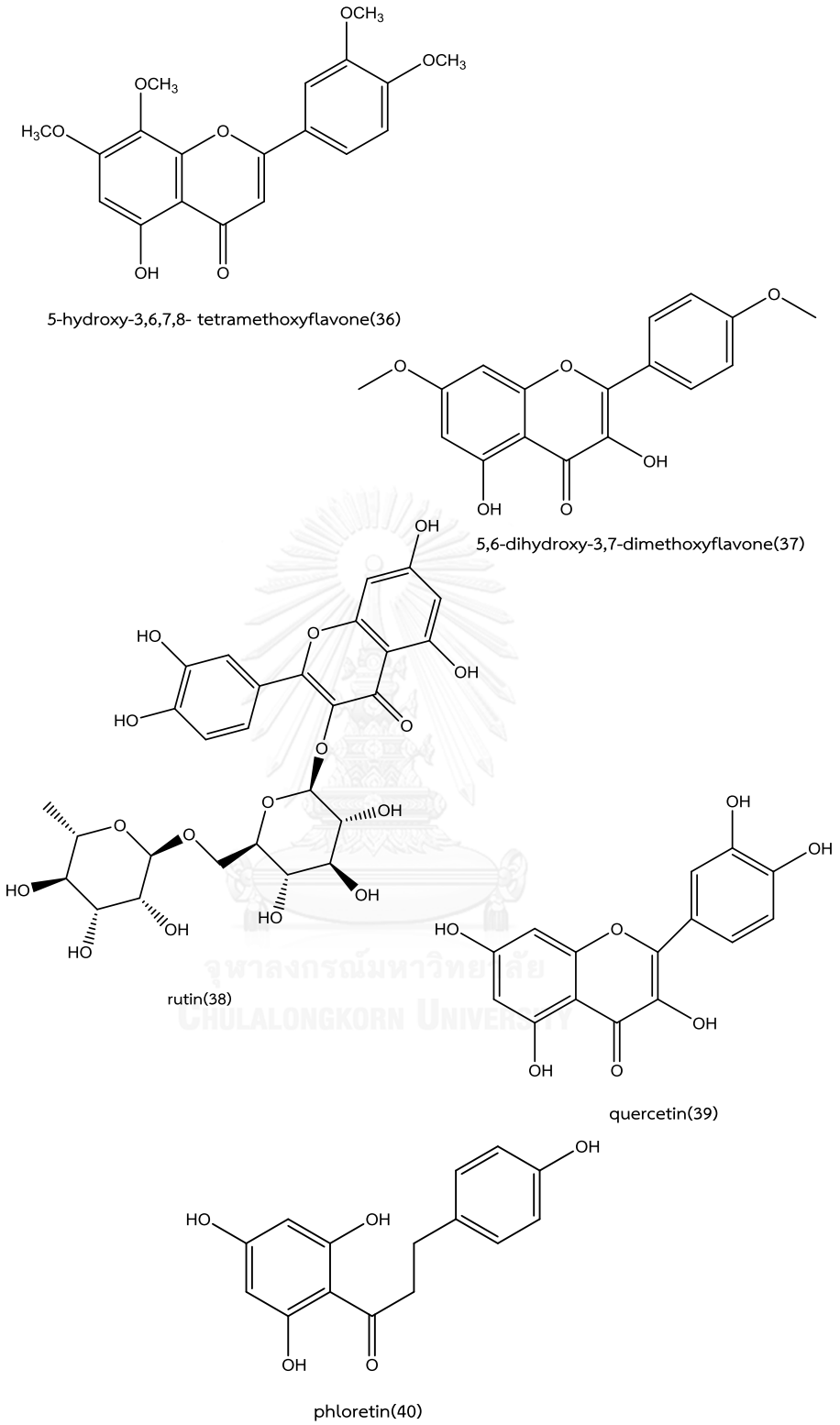
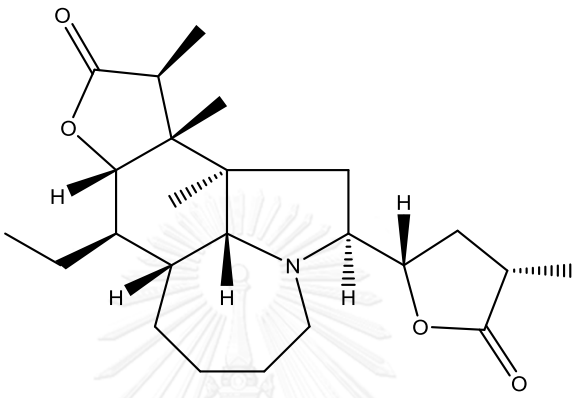


Figure 1.7 Flavonoid antifeedants

Various alkaloids have exhibited insect antifeedant activity like *Delphinium* diterpene alkaloids, 15-acetylcardiopentamine and cardiopentamine (**41**), are known to inhibit feeding of *S. littoralis* and *Leptinotarsa decemlineata* [18]. Furthermore, tuberostemonine isolated from the roots of *Stemona toerosa* displayed feeding inhibition of *S. littoralis* [19].



tuberostemonine(41)

From literature review, the use of natural compounds is a well known choice to control natural pests including *S. littoralis, S. litura* and *S. exigua.* This thesis aims to search for antifeedant compounds extracted from Thai plants.

1.2 Mode of action of insect antifeedant

Insect antifeedants may also change the activity of receptors that signal the presence of feeding stimulants, for instance when suppressing sugar receptors, and thereby act as strong antifeedants; nevertheless, it depends on chirality, functional groups, molecular size and lipophilicity of the compounds. For examples [3]:

- Alkaloids inhibit impulse generation in sugar sensitive cells in lepidopterans and competitively block sucrose responses in flesh flies. They also reduce the firing of the sugar sensitive cells.

- Terpenes stimulated the deterrent receptor cell located in the medial maxillary sensillum styloconicum and inhibition of responses of both the sugar and glycosinolate receptor cells.
- Sesquiterpenes, such as warburganal, blocked the responsiveness of the sucrose- and inositol-sensitive styloconic cell and maybe block chemoreceptors.
- Diterpenes induce greater feeding deterrency when applied to the maxillary palps as compared to the sensilla styloconica.

1.3 Botanical characteristics of *Xylocarpus granatum* Koenig.

Xylocarpus granatum (cannonball mangrove) belongs to Meliaceae family. This plant is a small to medium mangrove tree heights about 5-20 m. Leaves are oblong with a clearly round tip, size of leave about 7.5-15 cm long and 2.5-6 cm wide. Flowers are small about 4-7 cm long in axillary with white color. Fruit is distinctive, large, globose up to 15-25 cm across, heavy about 1-2 kg brown. It contains 5-20 seeds which are irregularly tetrahedral and attached to a central columella. Bark thin, smooth, scaly with irregular flakes, whitish to yellow-brown, inner bark reddish pink. The above ground root system is woody, flattened and snake-like (Figure 1.8) [20].

Figure 1.8 Botanical characteristics of *Xylocarpus granatum* Koenig.

(Source: http://frynn.com)

1.4 Chemical constituent studies on *Xylocarpus granatum* Koenig.

From literature review of chemical constituents of plants belonging to *Xylocarpus* genus revealed that many organic substrates were isolated as presented in Table 1.1.

Table 1.1 Chemical constituents of *X. granatum*.

Plant parts	Crude extract	Substance	Reference
seed	CH_2Cl_2	xylomexicanin A (**42**), xylogranatin D (**43**), hainangranatumin A (**44**), xylogranatin C (**45**), hainangranatumin C (**46**), xylocarpin H (**47**), xyloccensin K (**48**), piscidinol G (**49**), xylocarpin G (**50**), hydroxydammarenone-II (**51**) and stigmasterol (**52**) (Figure 1.9)	[21]
	MeOH	Protoxylocarpin F-H (**53**-**55**) (Figure 1.9)	
stem bark	EtOH	Xyloccensin Q-V (**56**-**61**) (Figure 1.10)	[22]
Twigs and leaves	MeOH	Xylogranatopyridine A (**62**), Xylogranato-pyridine B (**63**), Prexylogranatopyridine (**64**) (Figure 1.11)	[23]
fruit rind	EtOH	Xylocarpins A-I (**65**-**73**) (Figure 1.12)	[24]

Note: The numbers after the names indicate number of chemical structures.

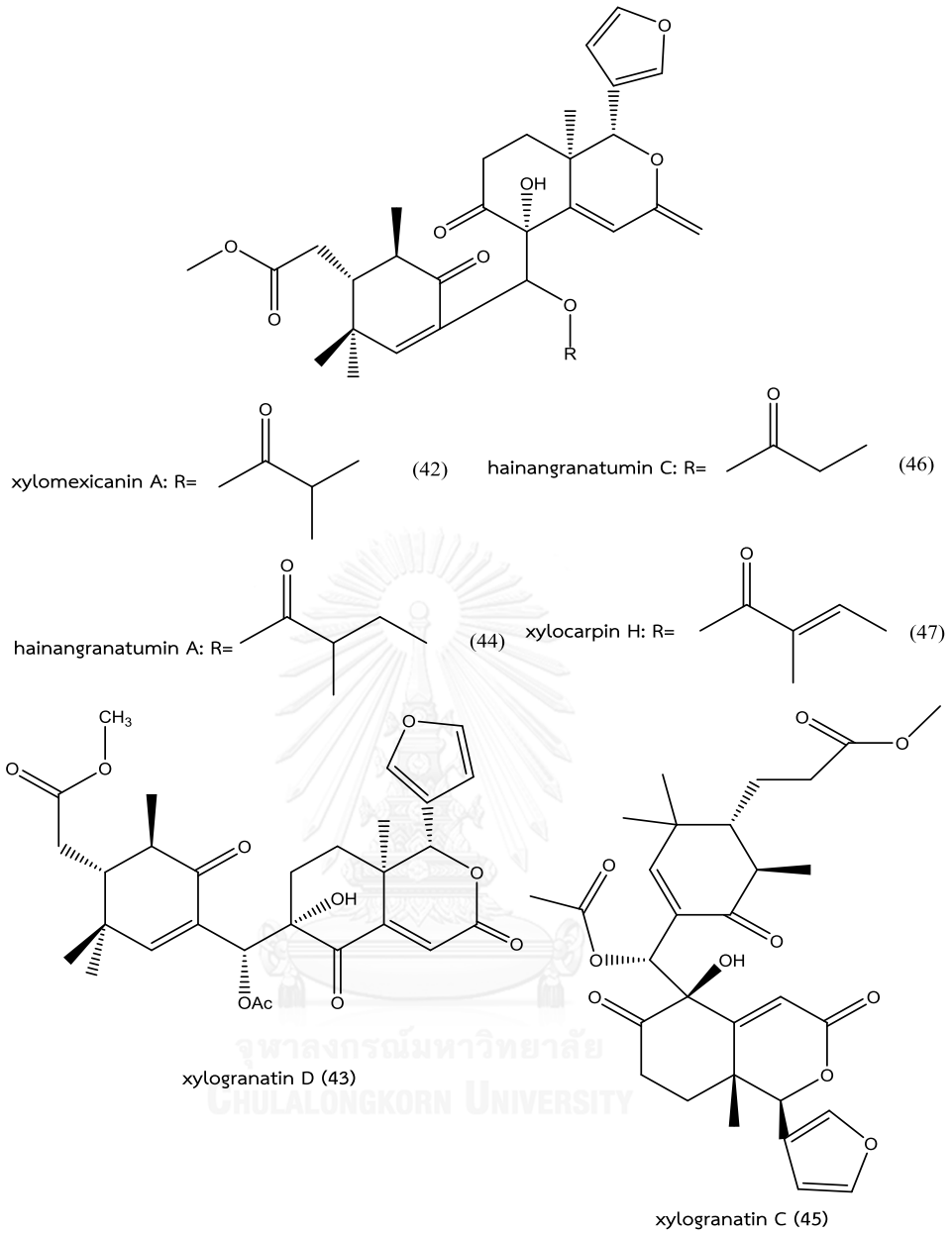
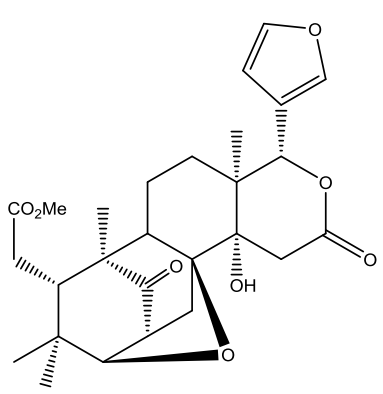
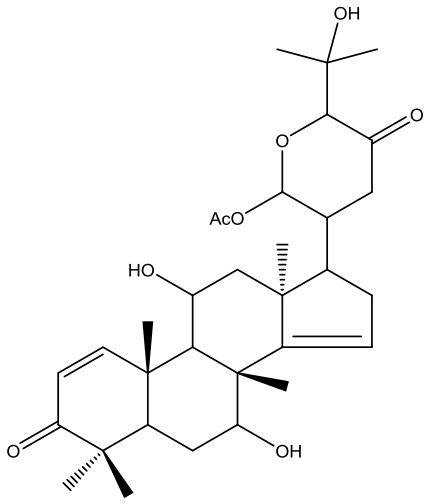


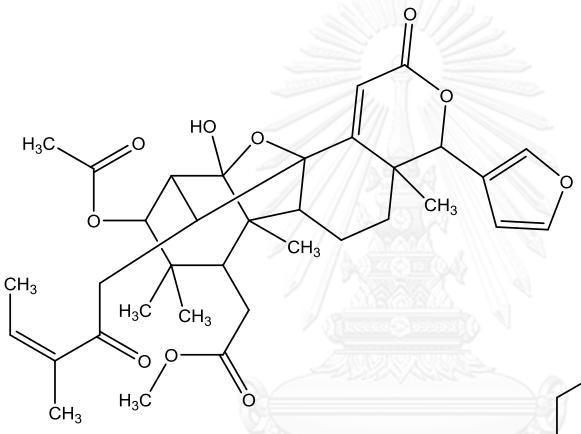
Figure 1.9 Some compounds isolated from the seeds of *X. granatum*



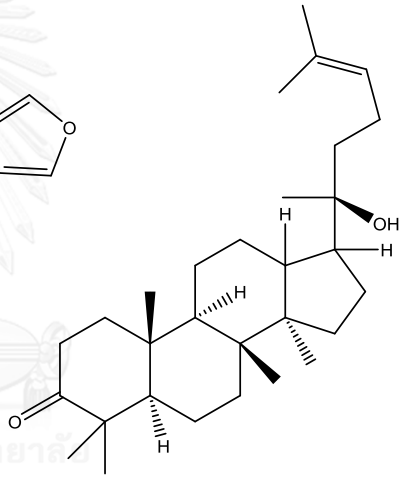
xyloccensin K (48)



piscidinol G (49)



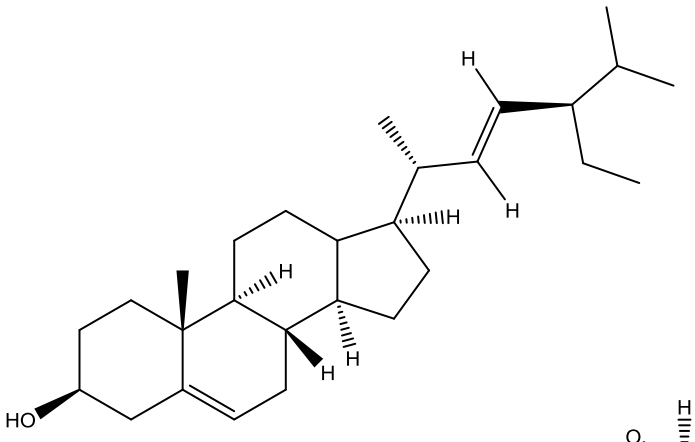
xylocarpin G (50)



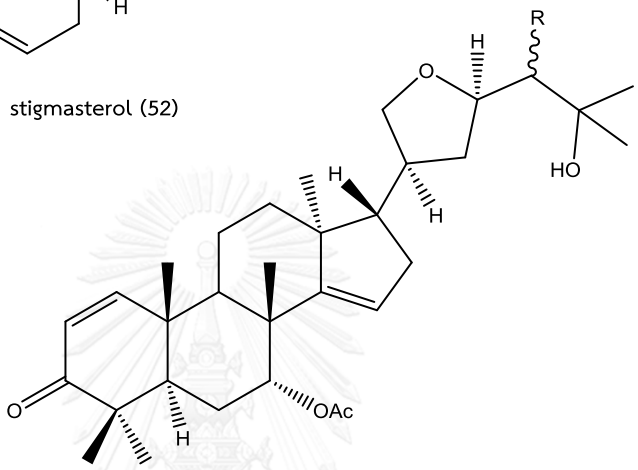
hydroxydammarenone-II (51)

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Figure 1.9 (cont.)

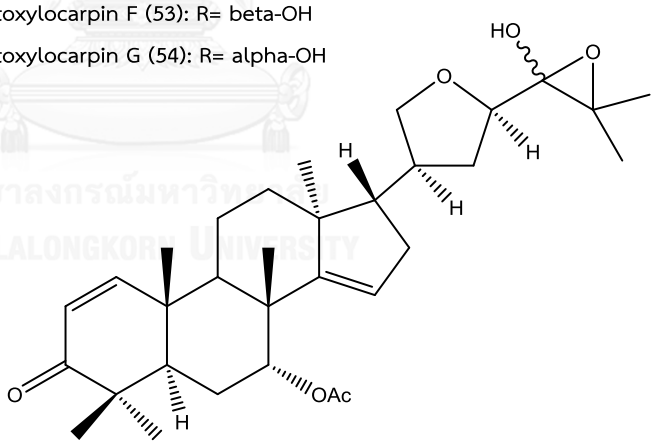


stigmasterol (52)



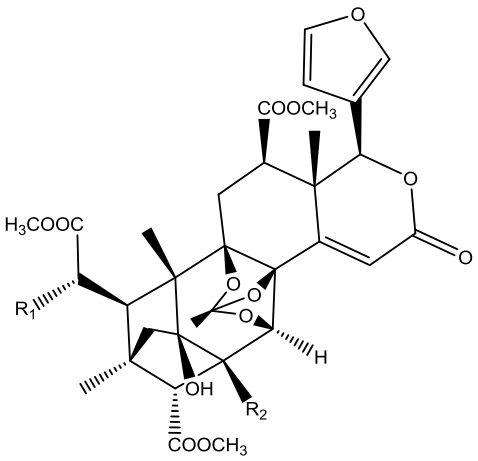
Protoxylocarpin F (53): R= beta-OH

Protoxylocarpin G (54): R= alpha-OH



Protoxylocarpin H (55)

Figure 1.9 (cont.)



Xyloccensin Q (56) : R_1=$OCOCH_3$, R_2 =OH

Xyloccensin R (57) : R_1=OH, R_2 =OH

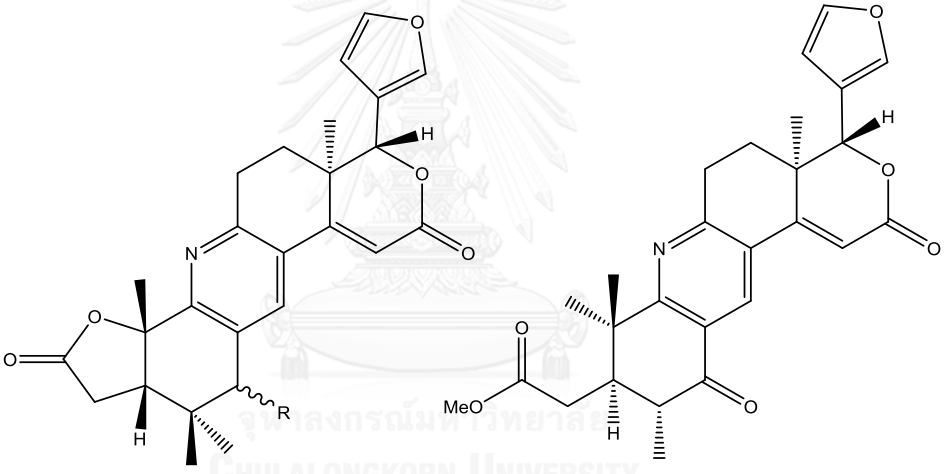
Xyloccensin S (58) : R_1=OH, R_2 =$OCOCH_3$

Xyloccensin T (59) : R_1=OH, R_2 =H

Xyloccensin U (60) : R_1=H, R_2 =OH

Xyloccensin V (61) : R_1=H, R_2 =$OCOCH_3$

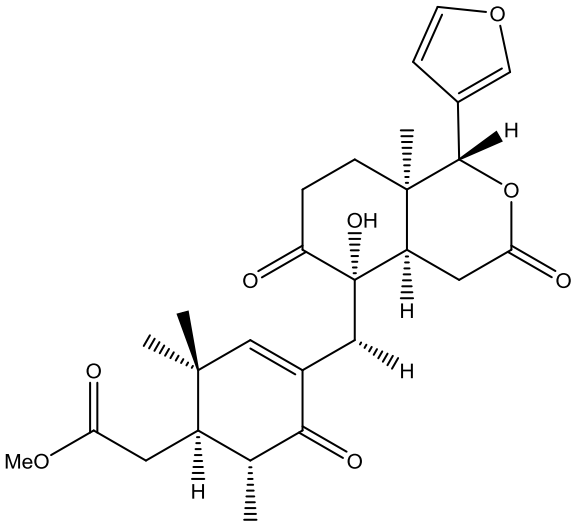
Figure 1.10 Some compounds isolated from the stem barks of *X. granatum.*



Xylogranatopyridine A (62) :R= beta-OMe

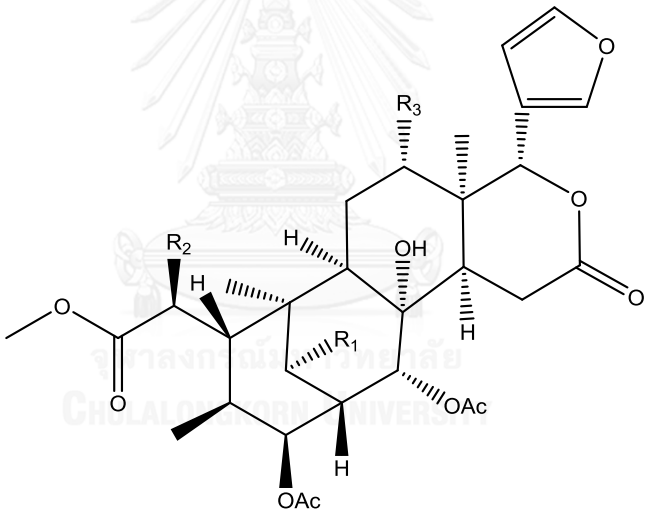
Xylogranatopyridine B (63)

Figure 1.11 Some compounds isolated from the twigs and leaves of *X. granatum.*



Prexylogranatopyridine (64)

Figure 1.11 (cont.)



Xylocarpins A (65): R_1=R_2=OAc, R_3=H

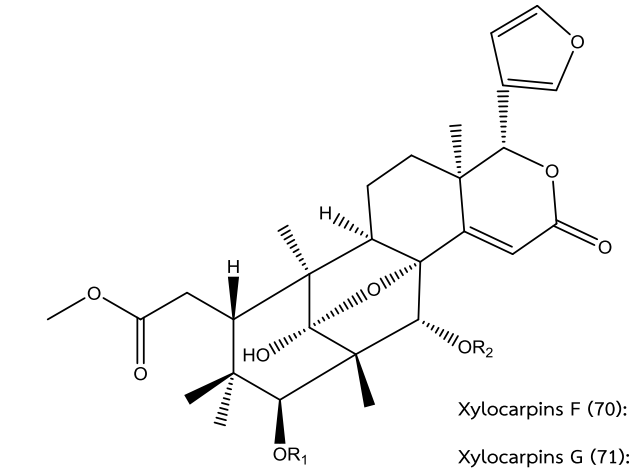
Xylocarpins B (66): R_1=OAc, R_2=R_3=H

Xylocarpins C (67): R_1=OH, R_2=H, R_3=OAc

Xylocarpins D (68): R_1=R_3=OAc, R_2=OH

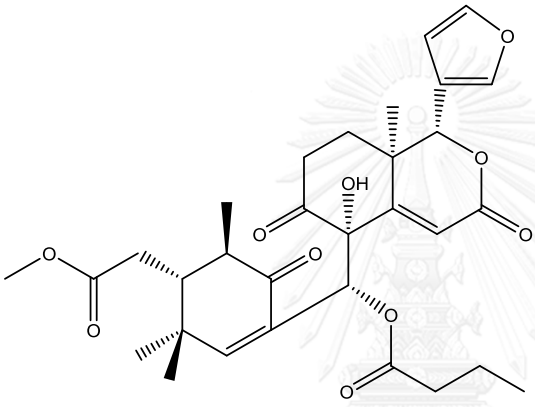
Xylocarpins E (69): R_1=R_2=OAc, R_3=OH

Figure 1.12 Some compounds isolated from the fruit rinds of *X. granatum.*

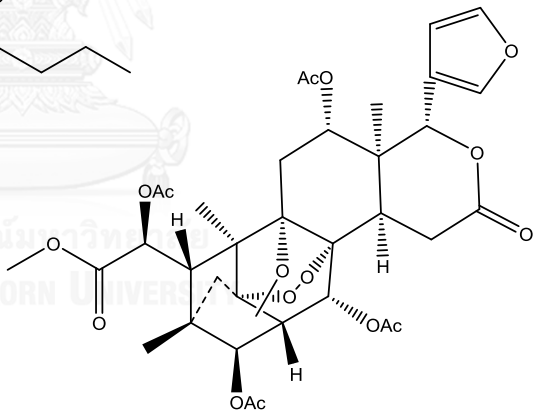


Xylocarpins F (70): R_1=R_2=Ac

Xylocarpins G (71): R_1= OAc, R_2=Tig



Xylocarpins H (72)



Xylocarpins I (73)

Figure 1.12 (cont.)

1.5 General characteristics of *Spodoptera litura* (Fabricius)

The common cutworm, *Spodoptera litura* (Fabricius), occurs worldwide because of its migration, higher reproductive rate and widely distributed in Asia and Oceania. It is the major host over 120 plant species including many vegetable, fruit and ornamental crops. Some examples are tobaccos, taros, apples, asparagus, beets, broccolis, cabbages, carrots, corns, cruciferous crops, dry beans, eggplants, grapes, lettuces, mints, orchids, potatoes, strawberries, cottons, radishs, roses, sunflowers and others [25]. The details of the species are shown as follow.

Classification

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Lepidoptera
Family	Noctuidae
Genus	Spodoptera
Species	*Spodoptera litura* (Fabricius)

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

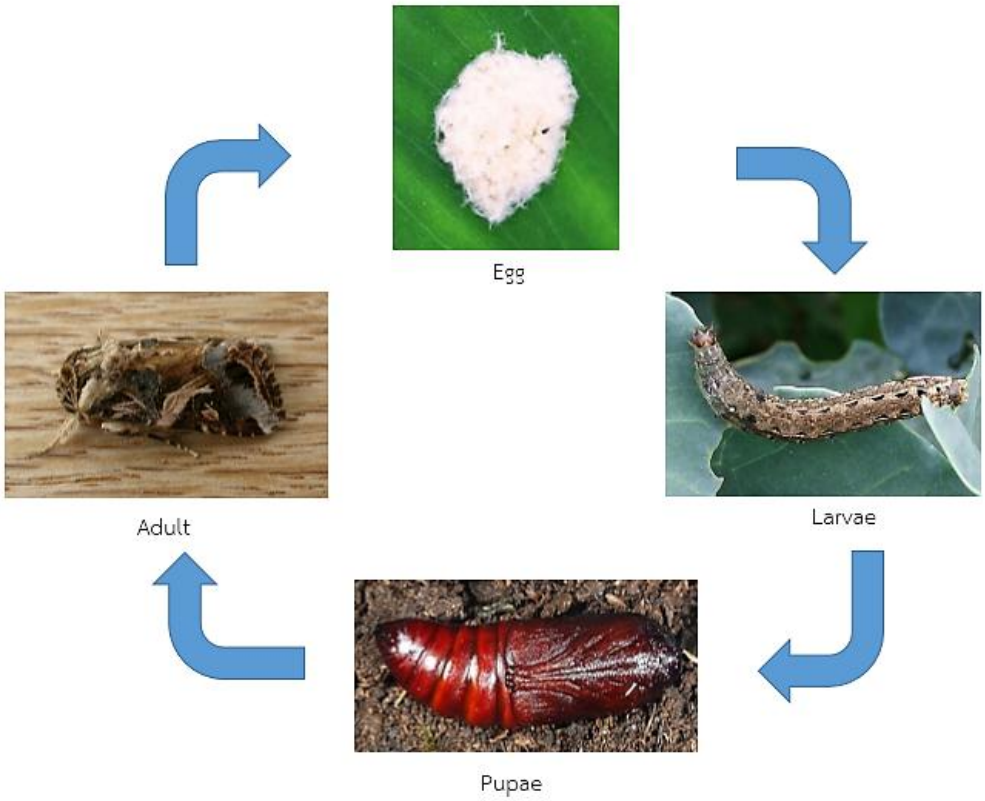


Figure 1.13 life cycle of *S. litura*

Egg: Females lay eggs in masses of 200 to 300 eggs approximately 4-7 mm in diameter and cover with brown hair. They laid on the underside of the host plant leaf. Egg usually hatches in 3 to 7 days [26].

Larvae: Young larvae or caterpillars are black head with a translucent body and 3 mm in length. They are smooth-skinned with a pattern of red, yellow, and green lines. Caterpillars eat entire leaves, and even flowers and fruits. When they mature the body color will change to green with black stripes and length of chest will increase at 3 to 4 cm [27]. The larva period lasts for 14 to 21 days.

Pupa: Common cutworm burrows into the soil 1 to 2 centimeters. The pupa is 15-20 mm long with red brown color. After 7 to 10 days the pupa molt to adult [27].

Adult: Adult or moth, with grey-brown body, 15 to 20 mm in length; wingspan 30 to 38 mm. The forewings are black-brown with strip across and dark brown spot spread all wing. Hindwings are paler with darker borders, with a light band at the wing edges [26]. After 1 to 2 days pass the moth will start breed at night. Female have life about 7 days [27]. The life cycles from egg to adult of common cutworm occupies 30 to 40 days.

Type of damage: On hatching, clusters of young larvae feed gregariously by initially scraping the surface of the leaf. When grow up they disperse and move on to other leaves and feed voraciously, producing large irregular holes and may leave only the veins. High infestation causes severe defoliation. Army worms quickly skeletonize leaves as they attack in clusters [27].

Methods of controlling *S. litura*

Chemical Control: In previous times, the control of arthropods depended mostly on inexpensive and efficient insecticides. But in recent years populations of many pests including *S. litura* have developed resistance to many commercially available pesticides [28]. For example, profenofos, cypermethrin, fenvalerate and quinalphos [9]. The control of *S. litura* is therefore becoming increasingly difficult and it is vital that all biological alternatives to insecticides need to be given greater priority, both in research and application. New chemicals have shown promising results against *S. litura* such as chlorantraniliprole, spinosad, emamectin benzoate, flubendiamide, spinosad and chlorfenapyr to be the most effective [29].

Biological control: a braconid wasp, *Microplitis bicoloratus*, is a solitary endoparasitoid of the larvae *S. litura*. They have long ovipositor for laid eggs into the worm. Immature development of the parasitoid in its host about 7 days. The development of the parasitized hosts was disrupted. When the parasitoid larvae finished development, the body weights of host larvae were significantly reduced regardless of which host instar was parasitized. Moreover, *Bacillus thuringiensis* or Bt is one of bacteria that can found

in soil, water and humus. It attacks the alimentary system then insect will die in 2 to 3 days [30].

1.6 The goal of this research

This research aims to explore the possibility to utilize the extracts of Thai plants as antifeedant agents. The goal of this research can be summarized as follows:

1. Preliminary screening test of 6 essential oils and 34 plant extracts for antifeedant activity against *S. litura*.
2. To extract and to isolate the organic compound from the plants.
3. To elucidate the structural formulae of the isolated substances.
4. To search for antifeedant compounds against second instar larvae of the common cutworm, *S. litula*.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

CHAPTER II

MATERIAL AND METHODS

2.1 Tested Specimen

Common cutworms purchased from Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok were reared on artificial diet in the plastic box under conditions of 27±2 ºC, 75±5% relative humidity (R.H.) and photoperiod of 12:12 h (L:D). Second instar larvae of common cutworm were used as tested specimen.

2.2 Plant materials

Sources of plant samples and commercial-grade essential oils used in this study are collected as shown in Table 2.1.

Table 2.1 The plant samples and essential oils used in this study

No	Family	Scientific name	Common name (Thai name)	Plant part
1	Annonaceae	*Melodorum fruticosum* lour.	White cheesewood (ลำดวน)	flower
2	Apiaceae	*Daucus carota* L.	Carrot (แครอท)	seed
3	Araceae	*Acorus calamus* L.	Sweet flag (ว่านน้ำ)	rhizome
4	Arecaceae	*Areca catechu* L.	Betel palm (หมาก)	fruit
5	Asteraceae	*Lactuca sativa* L.	Lettuce (ผักกาด)	seed
6	Cucurbitaceae	*Momordica charantia* L.	Bitter cucumber (มะระขี้นก)	leaf
7	Guttiferae	*Garcinia mangostana* L.	Mangosteen (มังคุด)	peel
8	Iridaceae	*Eleutherine americana* Merr.	Wan-hom-dang (ว่านหอมแดง)	bulb

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

9	Lamiaceae	*Ocimum basilicum* L.	Sweet basil (โหระพา)	leaf
10		*Ocimum gratissimum* L.	Tree Basil (ยี่หร่า)	seed
11	Liliaceae	*Dracaena loureiri* Gagnep.	Chan daeng (จันทน์แดง)	heart wood
12	Meliaceae	*Xylocarpus granatum* Koenig	Cannonball mangrove (ตะบูนขาว)	seed
13	Myrtaceae	*Psidium guajava* L.	Guava (ฝรั่ง)	leaf
14	Piperaceae	*Piper betle* L.	Betel (พลู)	leaf
15		*Piper sarmentosum* Roxb.	Wildbetal leafbush (ชะพลู)	fruit
16	Rutaceae	Murraya paniculata (L.) Jack	Orange jessamine (แก้ว)	leaf peel
17		*Citrus reticulate* Blanco	Mandarin orange (ส้มเขียวหวาน)	tree
18		Zanthoxylum limonella	Ma-khan (มะแข่น)	
19	Sapindaceae	Nephelium lappaceum L.	Rambutan (เงาะ)	seed
20	Sterculiaceae	*Mansonia gagei* Drumm.	Jan-Cha-Mod (จันทน์ชะมด)	heart wood
21	Zingiberaceae	*Hedychium coronarium* J.König	White ginger (มหาหงส์)	flower
22		*Kaempferia galanga* L.	Aromatic ginger (เปราะหอม)	rhizome
23		Zingiber cassumunar Roxb.	Plai (ไพล)	rhizome

Note: The essential oils Nos 3, 13, 21 and 23 were purchased from Thai-China Flavors and Fragrances industry Co., Ltd., Nontaburi and those of Nos 1-2, 5-8, 10-11, 14-15 and 18-20 were received from Natural Products Research Unit, Department of Chemistry, Chulalongkorn University. The rest crude extracts were gained by extraction using Soxhlet apparatus.

2.3 Chemicals

Merck's TLC was performed on aluminum sheet precoated with silica gel 60 F254 for the compound separation and observed the spots of compounds under UV light or other appropriate dipping reagents. All solvents used in this research were purified prior to use by standard methodology except for those which were reagent grades.

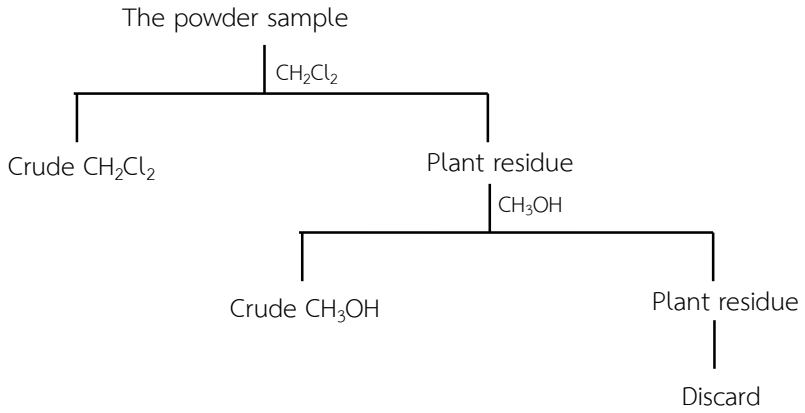
2.4 Instrument and equipment

The GC-MS was performed by Agilent 6890 gas chromatograph in electron impact (EI, 70eV) mode coupled to an HP 5973 mass selective detector and fitted with a fused silica capillary column (HP-Inowax) (30 m x 0.25 mm, film thickness 0.25 µm). Helium (1.0 mL/min) was used as a carrier gas. Samples were injected in the split mode at ratio of 1:200 and 1:300 and injection volume 0.2 µL. The injector was kept at 180°C and the transfer line at 240°C. The MS was EM mode at 2694.1 EM Voltage, in the *m/z* range 25-400. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in the literature and supplemented by the Wiley database and Natural Products GC-MS libraries.

The ^{1}H and ^{13}C spectra were recorded in chloroform-*d*1 ($CDCl_3$) on a Varian model Mercury + 400 and a Bruker Advance 400 NMR spectrometer (1H 400 MHz; 13C 100 MHz).

2.5 Extraction procedure

The dried plant (500 g) was ground to fine powder. The sample was initially extracted with CH_2Cl_2 by soxhlet apparatus. The extract was filtered and evaporated with rotatory vacuum evaporator. The plant residues were likewise extracted with CH_3OH. The extraction procedure was shown in Scheme 2.1.



Scheme 2.1 The extraction procedure

2.6 General procedure for hydrodistillation

Some essential oils were obtained by hydrodistillation[31]. The sample was chopped finely and put into a 1000 mL round bottom flask. The distilled water was added into the flask about 500 mL. The flask was connected to the Dean-stark apparatus for hydrodistillation. The hydrodistillation was carried out until no oil come out with the distillate. After that, the distillate was extracted by Et_2O. The obtained essential oil was collected and stored in the dark at 4°C to avoid the oxidation until being test for the antifeedant activity.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

2.7 Antifeedant bioassay

The antifeedant activity was estimated through a no-choice assay. The suitable solvent for each crude extract which provided good solubility, quickly volatile and non-toxic for instance CH_2Cl_2, acetone or CH_3OH was chosen. Crude extract was weighed and diluted with 1 mL of appropriate solvent, then incorporated into artificial diet to final weight 10 g (concentration: 0.1, 0.25, 0.5, 0.75 and 1% (w/w)) for crude extracts). For the control group, 1 mL of solvent was incorporated into 10 g of artificial diet. After each diet was keep at RT to release the solvent evaporate, then divided into 30 pieces and weighed. After that put the piece of diet in 24-well plates at the number of 1 piece per well, and second instar larvae were placed singly in each well after being starved for 6 h. The experiment was done under conditions of 27±2°C,

75±5% relative humidity (R.H.) and photoperiod of 12:12 h (L:D). After 24 h, the diet was weighed to record the weight loss from treatment and control [9]. Each treatment was set up with 30 larvae.

Antifeedant activity was expressed as %antifeedant calculated according to the following the equation modified from that of Hozosawa et al. (1974) [32].

$$\%\text{Antifeedant} = (1-(T/C)) \times 100$$

Where: T is the weight loss of diet in treatment

C is the weight loss of diet in control

2.8 Separation and purification of active fractions

2.8.1 Quick column chromatography

The selected plant extract was subjected to silica gel quick column using gradient solvent starting from hexane and increased polarity by mixing with EtOAc and CH_3OH. Each fraction was examined and combined by TLC.

2.8.2 Column chromatography

The fraction that showed the highest %antifeedant on common cutworm was fractionated by silica gel column using gradient solvent starting from hexane and increased polarity by mixing with EtOAc and CH_3OH. Each fraction was examined and combined by TLC.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

2.9 Antifeedant test of pure compounds

The isolated constituents from the effective plant extract were tested for antifeedant bioassay as described above at concentration of 0.5, 1, 1.5, 2 and 2.5 mM.

2.10 Stability test

2.10.1 Ultraviolet light

The crude extract of *X. granatum*, xyloccensin K and neem extract were tested for their stability by exposing to UV light (256 nm) for 12 h. After that those extracts and the compound were subjected to antifeedant activity test [33].

2.10.2 Temperature

The same set of samples used for 2.10.1 was tested for their stability by storaging at 4, RT (30ºC), 45 and 60ºC for 48 and 96 h, respectively. After that those extracts and the compounds were tested for antifeedant activity [34].

2.11 Leaf disk toxicity assay

Leaf discs (9 cm diameter) of *Brassica alboglabra* were used for bioassay tests, after washing it with water. Fifteen µL from each treatment was dropped on the leaf discs with 0.25% (w/v) for plant extract and 1 mM for constituents, air dried at RT and kept in 24 well plate. The 2^{nd} instar common cutworms were starved for 6 h. After that put the leaf disc in 24-well plates at the number of 1 leaf disc per well, and the second instar larvae were placed singly in each well. The experiment was done under conditions of 27±2ºC, 75±5% relative humidity (R.H.) and photoperiod of 12:12 h (L:D). After 24 h, the leaf discs were measured by graph paper [27]. Each treatment was set up with 30 larvae.

2.12 Statistical analysis

The percentage antifeedant activity was determined by ANOVA, and treatment means were compared and categorized by Duncan's test at *P=0.05*. The EC_{50} values were calculated by Probit analysis. All calculations were done using the SPSS program.

CHAPTER III
RESULTS AND DISCUSSION

Spodoptera litura is one of important insect pests for agricultural products. Certain agrochemicals have been continuously developed to manage this problem. This insect can resist to some synthetic insecticides. Antifeedant approach is a present important promise as components of emerging integrated pest management (IPM) because its capability to decrease feeding by insects [1]. Since Thailand has a variety of natural resources, certain natural products contain constituents possessing antifeedant compounds. During this course of research, twenty three Thai plants were selected for preliminary screening test against *S. litura* antifeedant activity.

3.1 Essential oils

3.1.1 The preparation of essential oil

The hydrodistillation of *Ocimum basilicum* and *Citrus reticulate* was conducted in accordance with the procedure described in Chapter II. The results of hydrodistillation are presented in Table 3.1.

จุฬาลงกรณ์มหาวิทยาลัย

Table 3.1 The hydrodistillation of some selected plants

Family and scientific name	Plant part	Plant weight (g)	Oil weight (g), (% w/w)
Lamiaceae			
Ocimum basilicum L.	leaf	90	1.48 (1.64%)
Rutaceae			
Citrus reticulate Blanco	peel	1,300	1.67 (0.13%)

The essential oils from *O. basilicum* and *C. reticulate* were obtained as colorless oil, 1.48 and 1.67 g (1.64 and 0.13% (w/w) of fresh weight), respectively.

3.1.2 Screening of essential oils for antifeedant activity

Two essential oils from Table 3.1 along with four commercial ones were preliminarily screened for antifeedant activity against *S. litura*. The results are presented in Table 3.2.

Table 3.2 The preliminary screening of the essential oils against *S. litura.*

Family and scientific name	Plant part	Antifeedant activity
Apiaceae		
Daucus carota L.	seed	++++
Lamiaceae		
Ocimum basilicum L.	leaf	++++
Myrtaceae		
Psidium guajava L.	leaf	+++
Rutaceae		
Citrus reticulate Blanco	peel	+++
Zingiberaceae		
Hedychium coronarium J.KÖnig	flower	++++
Zingiber cassumunar Roxb.	rhizome	+++

Note: Each treatment was set up for 30 larvae.

The data was classified and noted as + (0–20% antifeedant activity), ++ (21-40% antifeedant activity), +++ (41-60% antifeedant activity) and ++++ (61-80% antifeedant activity) and +++++ (81-100% antifeedant activity).

From Table 3.2, antifeedant activity was determined comparing with control (acetone), by mixing the selected essential oil with artificial diet at 0.25% (w/w) and %antifeedant activity was examined after 24 h treatment. The results showed that antifeedant activity was varied with species of plant materials. The essential oil of *O. basilicum* gave the highest antifeedant activity followed by *H. coronarium* and

D. carota, respectively. Thus, these three essential oils were chosen for further investigation on EC_{50}, chemical constituents and searching for insect antifeedant compounds.

3.1.3 EC_{50} values determination

Three selected essential oils were tested at five different concentrations (0.1, 0.25, 0.5, 0.75 and 1.0% w/w) to search for the effective concentration which caused 50% inhibit feeding of *S. litura* by antifeedant bioassay. EC_{50} was evaluated from Probit analysis [31]. The summary of the EC_{50} of three selected essential oils is shown in Table 3.3 (Linear regression curves shown in appendix A).

Table 3.3 EC_{50} values of three selected essential oils against *S. litura.*

Scientific name	Concentration (%w/w)					EC_{50}
	0.1	0.25	0.5	0.75	1.0	
Ocimum basilicum	46.18±9.18	68.36±7.94	78.47±8.92	81.17±9.06	91.35±9.28	0.12
Hedychium coronarium	42.23±8.14	66.44±6.22	71.44±8.47	73.37±9.77	79.05±6.40	0.13
Daucus carota	38.6±8.40	67.10±6.58	70.2±9.04	77.15±8.45	84.37±9.39	0.15

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

The EC_{50} analysis result (Table 3.3) revealed the maximal effective concentration that could inhibit insect feeding 50% of *S. litura* after 24 h with 95% of confidential limit (P=0.05). The EC_{50} of the essential oils from *O. basilicum, H. coronarium* and *D. carota* were 0.12, 0.13 and 0.15% w/w, respectively. The higher EC_{50} showed lower activity of that compound. This result revealed that the essential oil from *O. basilicum* exhibited the highest activity against *S. litura* meanwhile that of *D. carota* showed the lowest activity.

The antifeedant activity of *O. basilicum* has been reported by Devanand *et al.* (2008) that the acetone extract from the leaves of *O. basilicum* exhibited antifeedant activity against *S. litura* at EC_{50} more than 100 mg. Moreover, the methanol extract of *O. basilicum* displayed insecticidal activity against *S. littoralis* larvae at LC_{50} 0.17% (w/v)

[35]. While, the EC_{50} of the essential oil from *O. basilicum* in this study was lower than those reported [35, 36]. The reason maybe from difference assay, type of extract, strain of *S. litura* or difference in major constituents in *O. basilicum*. For *H. coronarium*, Sakhanokho *et al*. (2013) reported that *Hedychium* sp. essential oils showed insecticidal activity to *Stephanitis pyrioides*, repellent activity against *Aedes aegypti* and larvicidal activity against *Aedes aegypti* larvae [37]. For *D. carota,* Park and Park (2012) addressed the lavicidal activity against the larvae of *Culex pipiens pallens* at 0.1 mg/mL [38].

It should also be noted that the essential oils of *O. basilicum*, *H. coronarium* and *D. carota* have not been reported for antifeedant activity against *S. litura*. From aforementioned these three essential oils were chosen for further searching for antifeedant compounds against *S. litura.*

3.1.4 Essential oil analysis

Essential oils are complex mixtures with huge numbers of constituents [39]. Hence, it is considerable to analyze the active constituents for biological activity. In this research, three effective essential oils were selected to investigate for active compounds on *S. litula*. The GC-MS technique was used.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.1.4.1 The analysis of the essential oil from *O. basilicum*

The GC-MS analysis of the essential oil of *O. basilicum* was conducted. The possible components suggested from the Wiley database were collected as shown in Table 3.4 and Figures 3.1-3.7.

Table 3.4 The GC-MS analysis of the essential oil from *O. basilicum*

No	R_t (min)	Possible compound	%Area
1	5.67	Eucalyptol	5.04
2	14.30	Linalool	10.60
3	17.74	*p*-Allylanisole	51.46
4	26.69	Methyleugenol	8.85
5	28.22	Methyl cinnamate	0.78
6	30.26	Eugenol	4.56

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

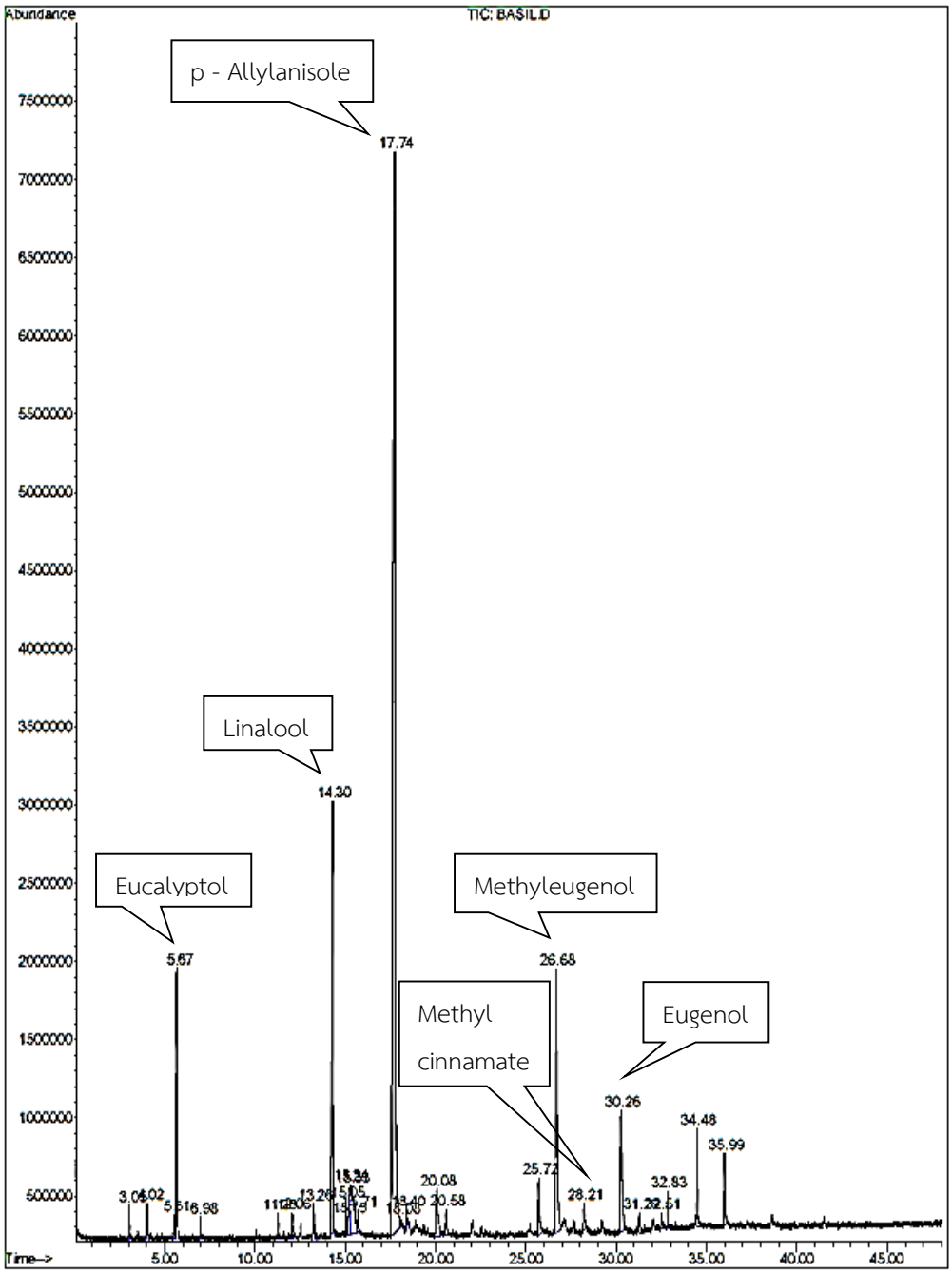


Figure 3.1 The GC-MS chromatogram of essential oil of *O. basilicum*

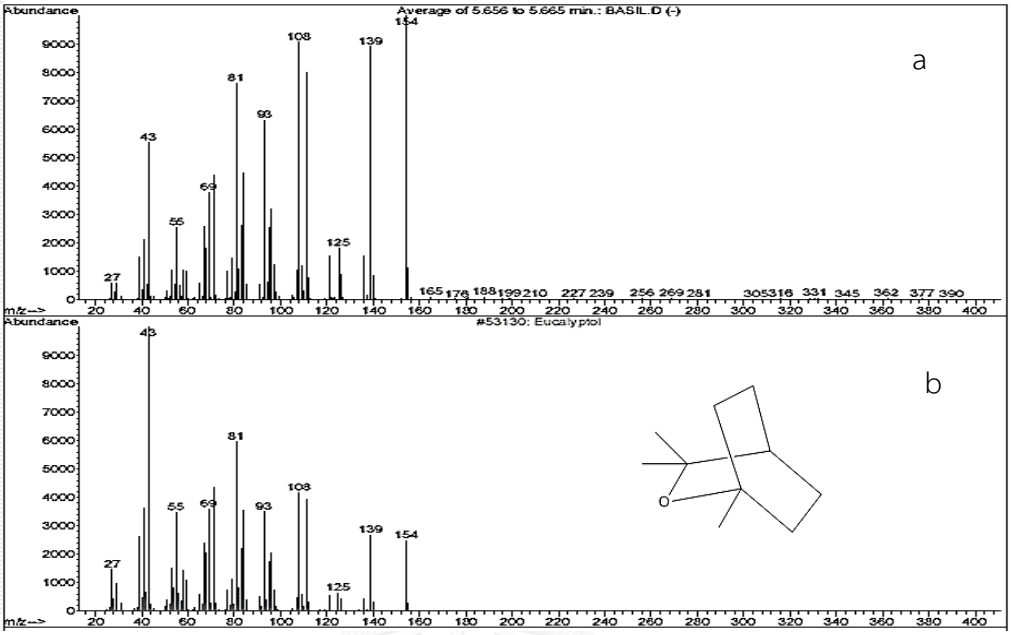


Figure 3.2 Mass spectra of a) at R_t at 5.67 min b) eucalyptol

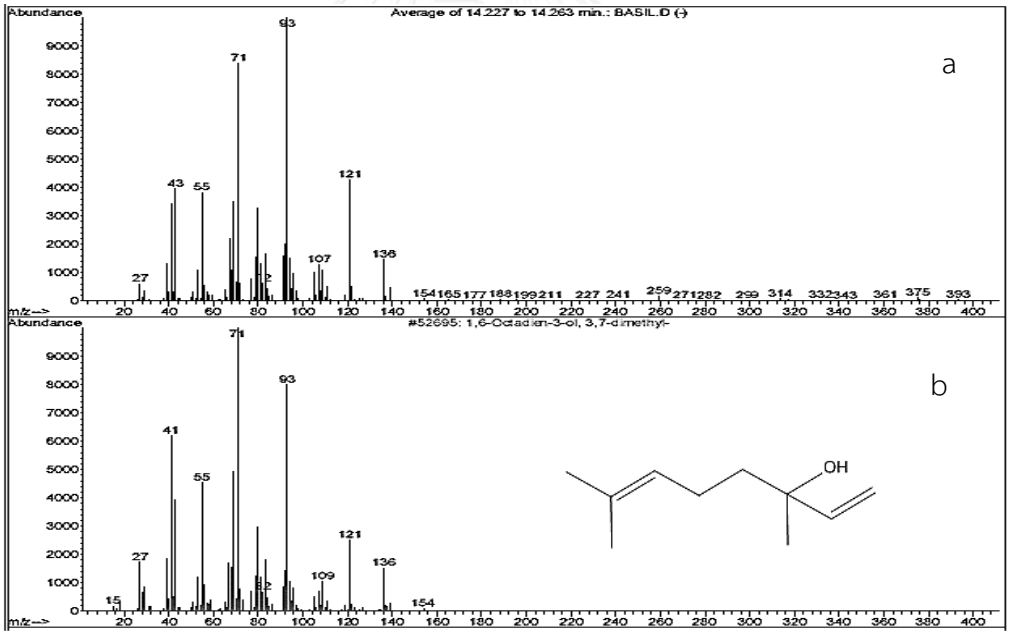


Figure 3.3 Mass spectra of a) at R_t 12.06 min b) linalool

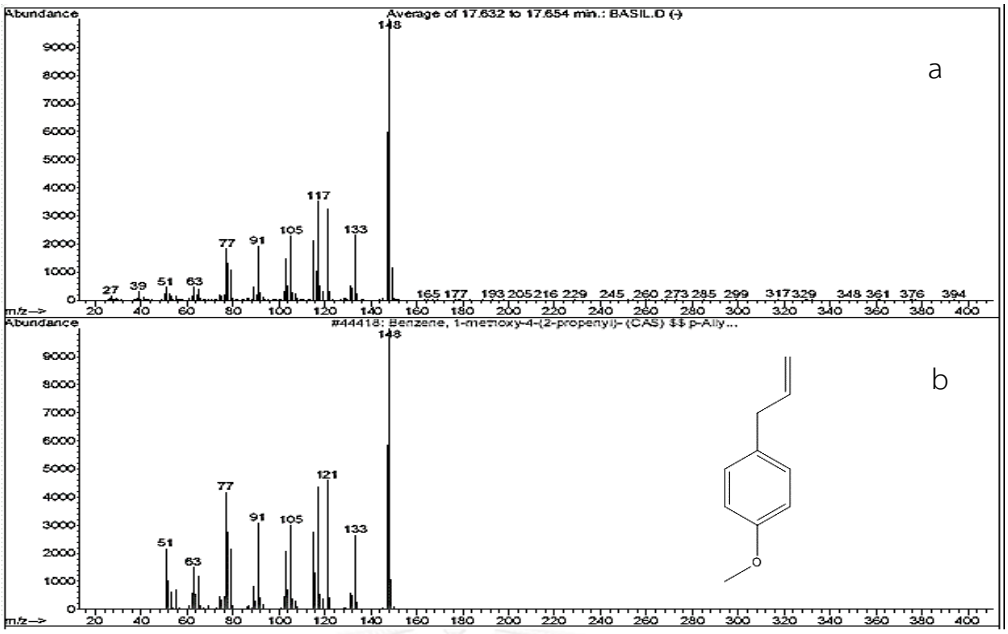


Figure 3.4 Mass spectra of a) at R_t 17.74 min b) *p*-allylanisole

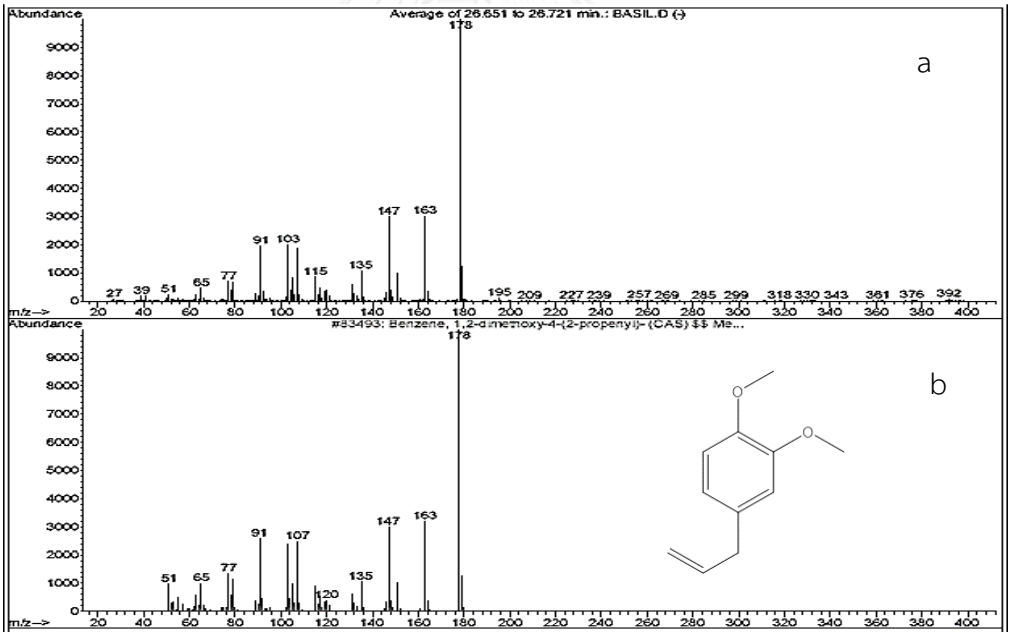


Figure 3.5 Mass spectra of a) at R_t 28.22 min b) methyleugenol

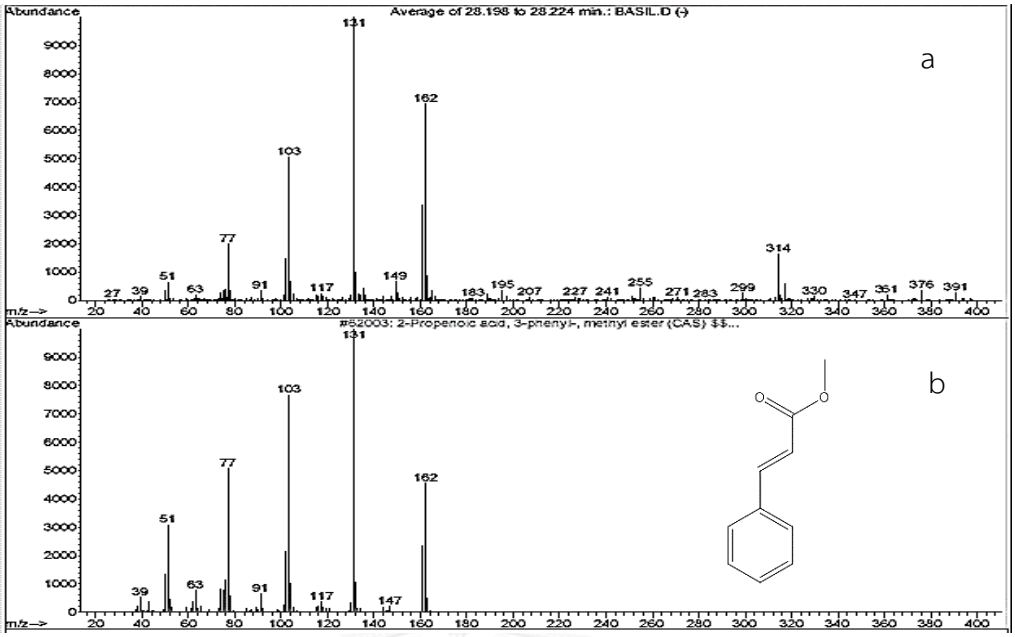


Figure 3.6 Mass spectra of a) at R_t 28.22 min b) methyl cinnamate

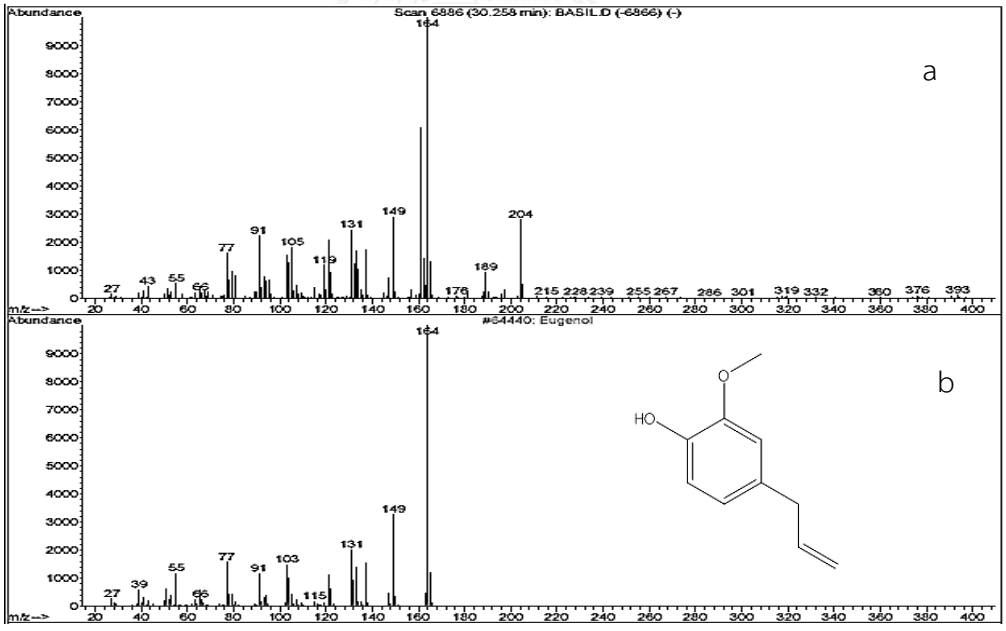


Figure 3.7 Mass spectra of a) at R_t 30.26 min b) eugenol

Each peak from the GC-MS chromatogram of the essential oil of *O. bacilicum* (Figures 3.1-3.7) was compared with the Wiley database. Most compounds were monoterpeniods. The five highest peaks were identified as *p*-allylanisole at R_t 17.74 min, linalool at R_t 14.30 min, methyleugenol at R_t 26.69 min, eucalyptol at R_t 5.67 min, eugenol at R_t 30.26 min and methyl cinnamate at R_t 28.22 min.

3.1.4.2 The analysis of the essential oil from *H. coronarium*

The GC-MS analysis of the essential oil from *H. coronarium* was conducted. The possible components suggested from the Wiley database were collected as shown in Table 3.5 and Figures 3.8-3.13.

Table 3.5 The GC-MS analysis of essential oil from *H. coronarium*

No	R_t (min)	Possible compound	%Area
1	5.66	Eucalyptol	11.02
2	13.29	Camphor	11.87
3	15.41	Elemene	10.73
4	22.90	*trans*- 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenylbenzofuran	6.08
5	31.16	Germacrone	19.56

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

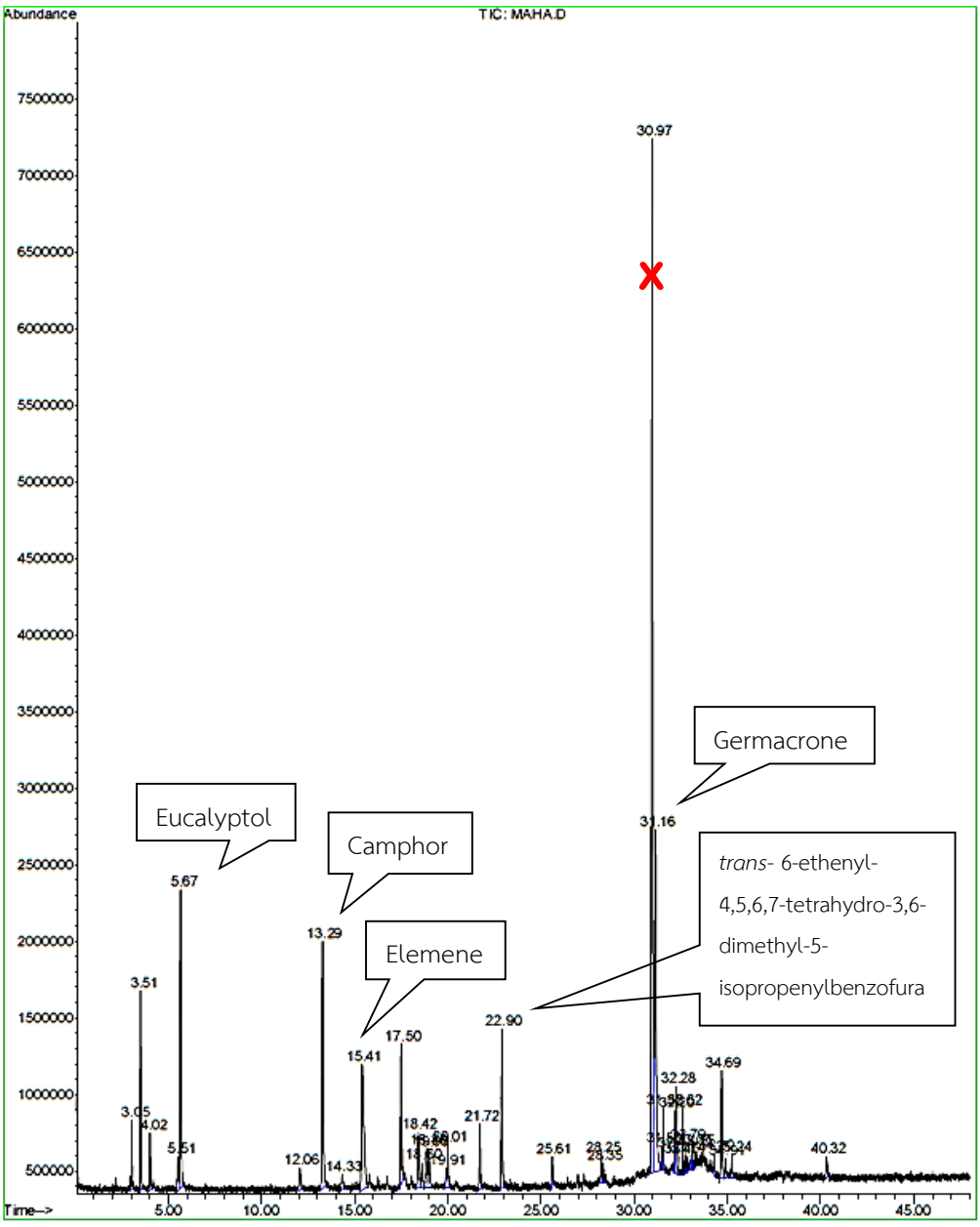


Figure 3.8 The GC-MS chromatogram of the essential oil from *H. coronarium*

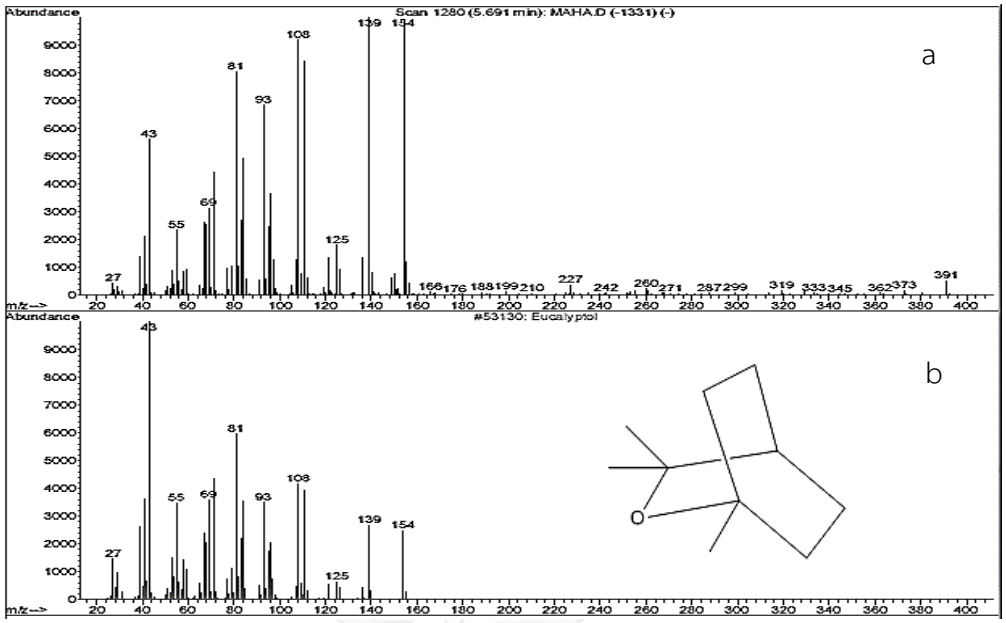


Figure 3.9 Mass spectra of a) at R_t 5.66 min b) eucalyptol

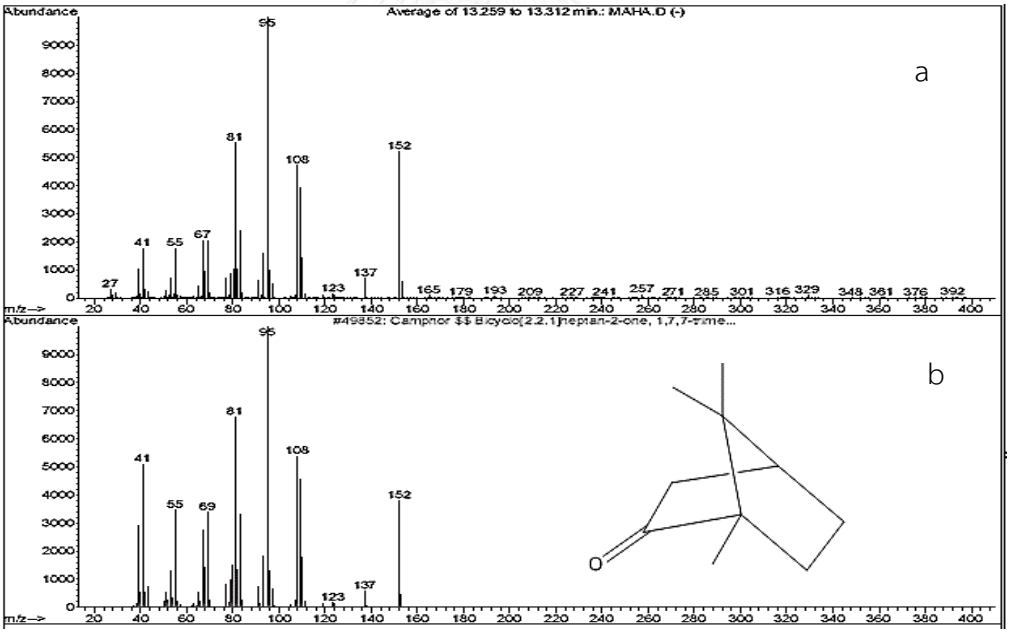


Figure 3.10 Mass spectra of a) at R_t 13.29 min b) camphor

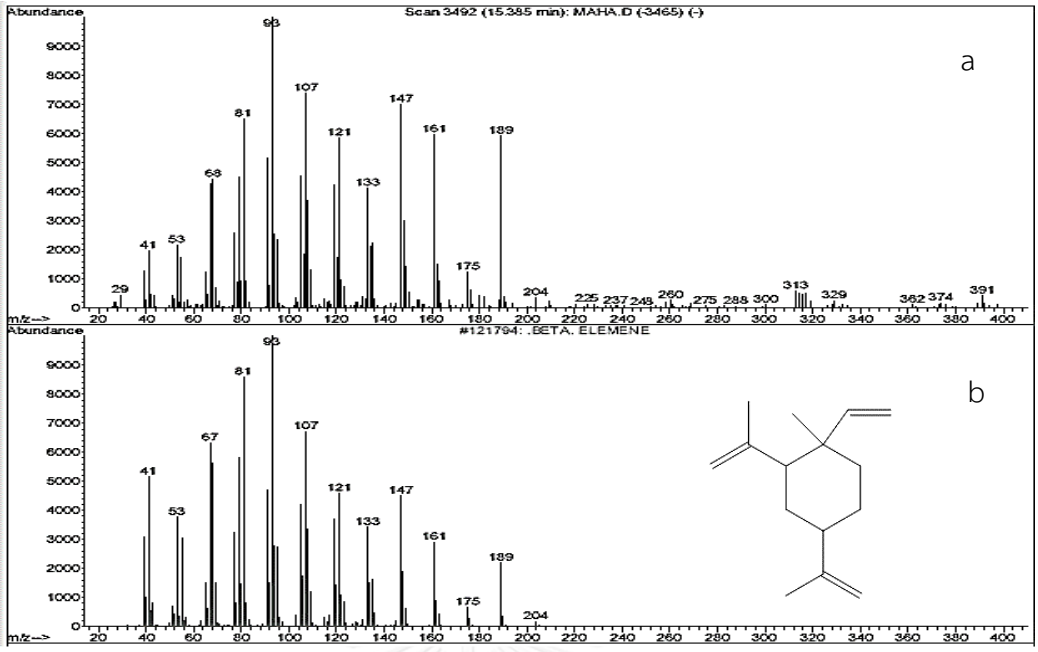


Figure 3.11 Mass spectra of a) at R_t 15.41 min b) elemene

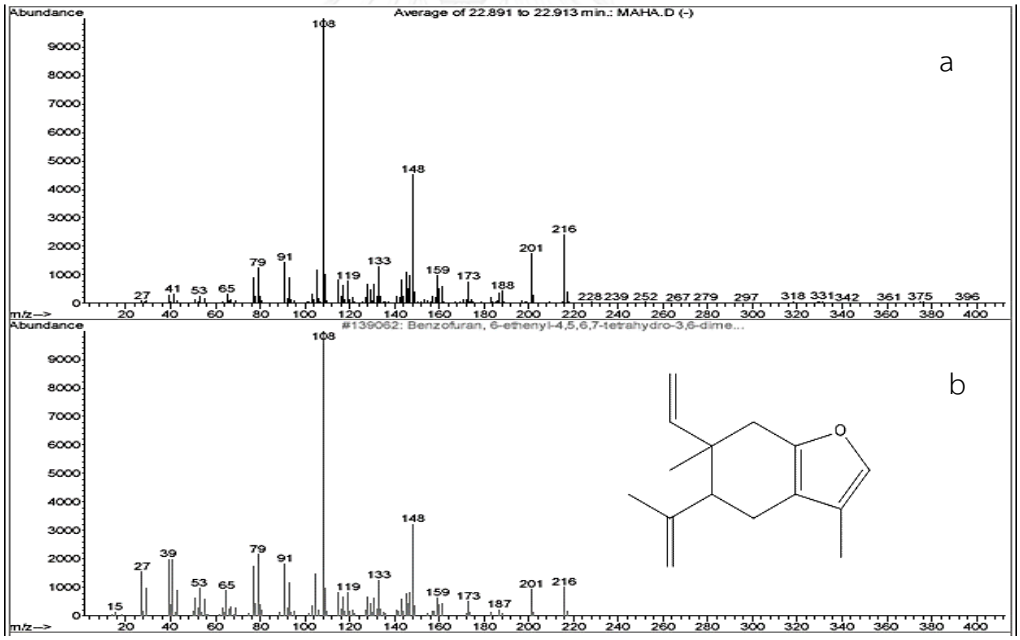


Figure 3.12 Mass spectra of a) at R_t 22.90 min b) *trans*- 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenylbenzofuran

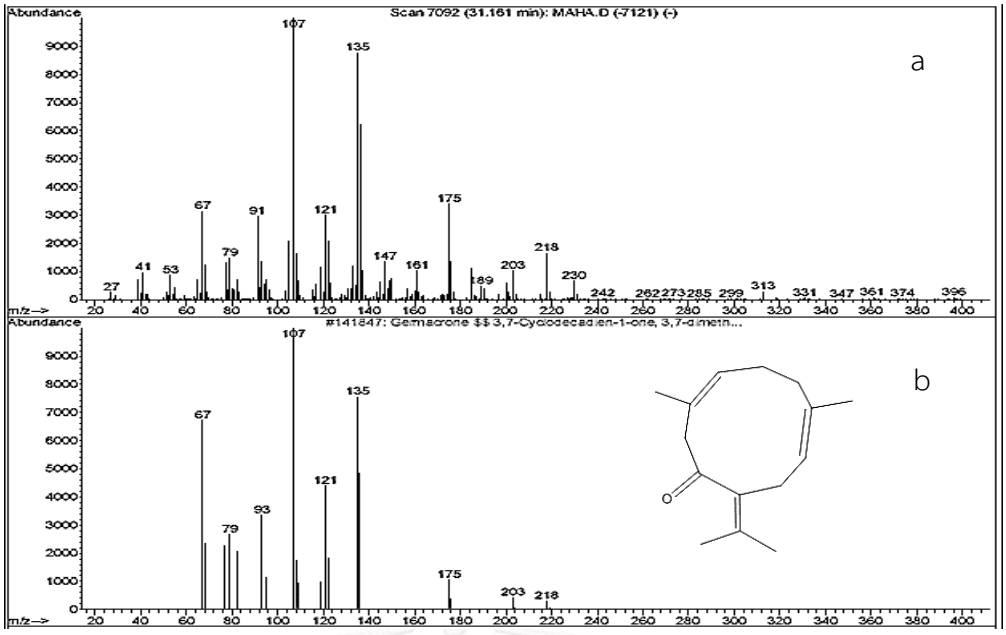


Figure 3.13 Mass spectra of a) at R_t 31.16 min b) germacrone

The GC-MS chromatogram of *H. coronarium* are showed in Figures 3.8-3.13. They were identified as germacrone at R_t 31.16 min, camphor at R_t 13.29 min, eucalyptol at R_t 5.66 min which were the same compounds in that of *O. bacilicum*. Elemene at R_t 15.41 min and *trans*- 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenylbenzofuran at R_t 22.90 min were also detected. All compounds were compared with the Wiley database.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.1.4.3 The analysis of the essential oil from *D. carota*

The GC-MS analysis of the essential oil of *D. carota* was performed and collected the data in Tables 3.6 and Figures 3.14-3.22.

Table 3.6 The GC-MS analysis of essential oil from *D. carota*

No	R_t (min)	Possible compound	%Area
1	3.05	α-Pinene	6.67
2	4.03	β-Pinene	8.54
3	12.59	Copaene	8.59
4	14.27	Linalool	4.58
5	14.62	α-Cedrene	4.62
6	15.50	*trans*-Caryophyllene	9.44
7	20.04	(E)-3,7-dimethyl-2,6-octadien-1-ol, acetate	4.15
8	26.60	Carotol	22.12

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

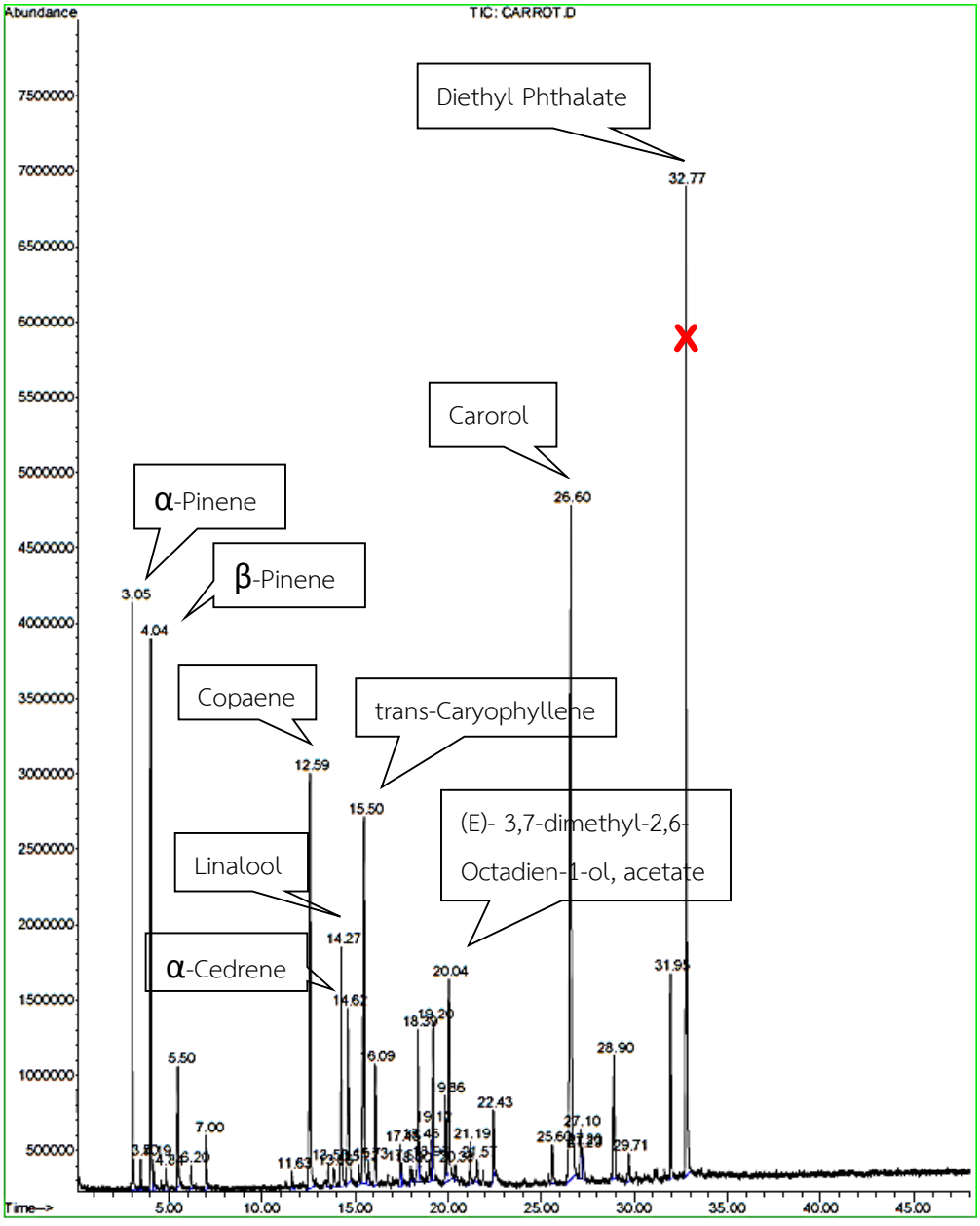


Figure 3.14 The GC-MS chromatogram of the essential oil from *D. carota*

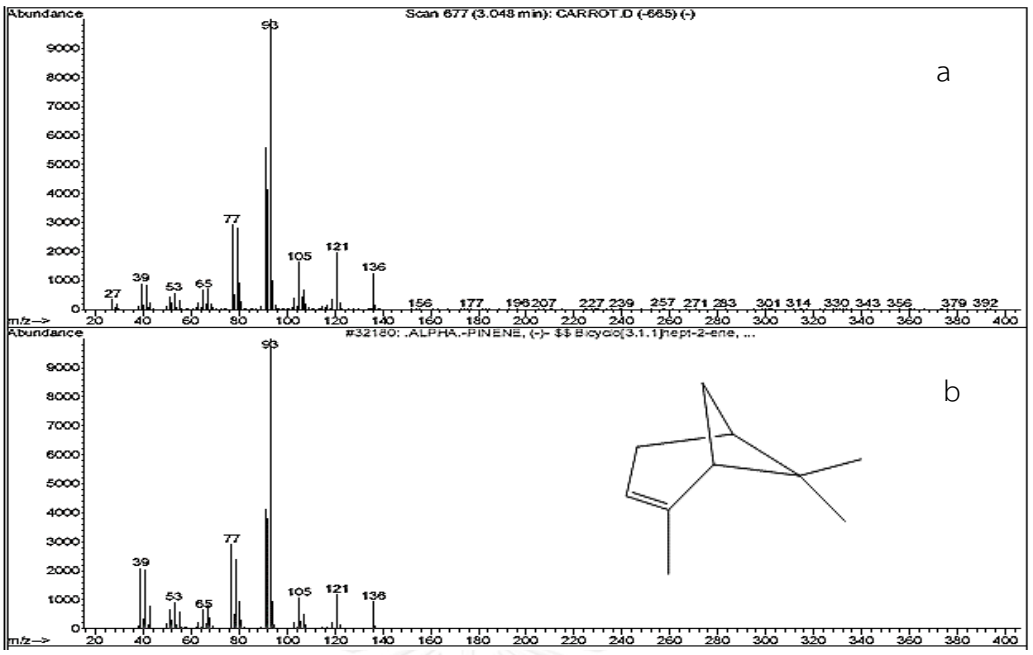


Figure 3.15 Mass spectra of a) at R_t 3.05 min b) **α**-pinene

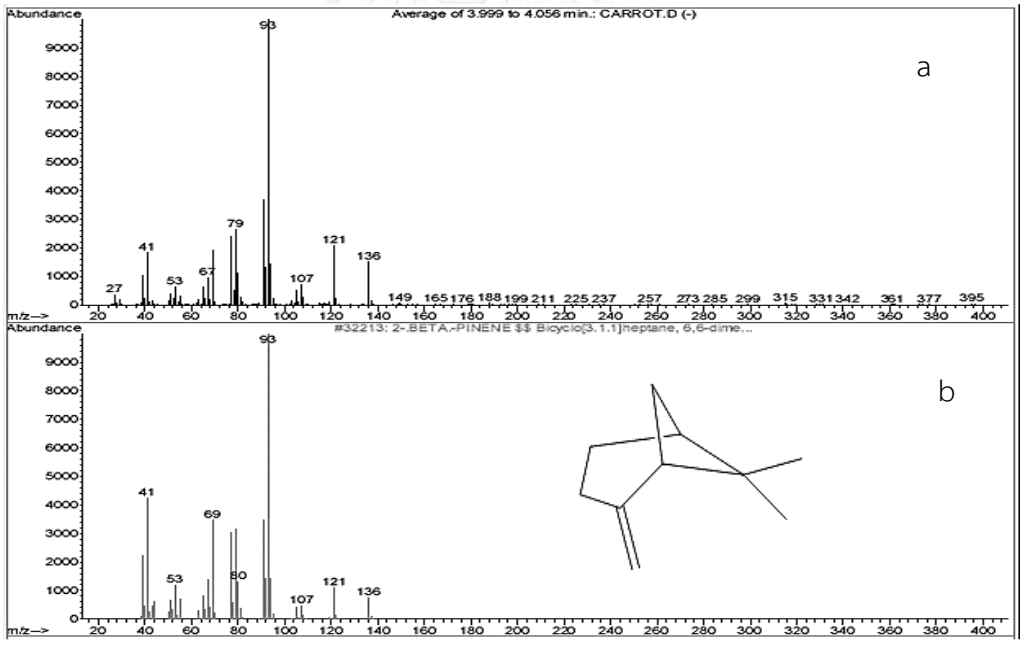


Figure 3.16 Mass spectra of a) at R_t 4.03 min b) **β**-pinene

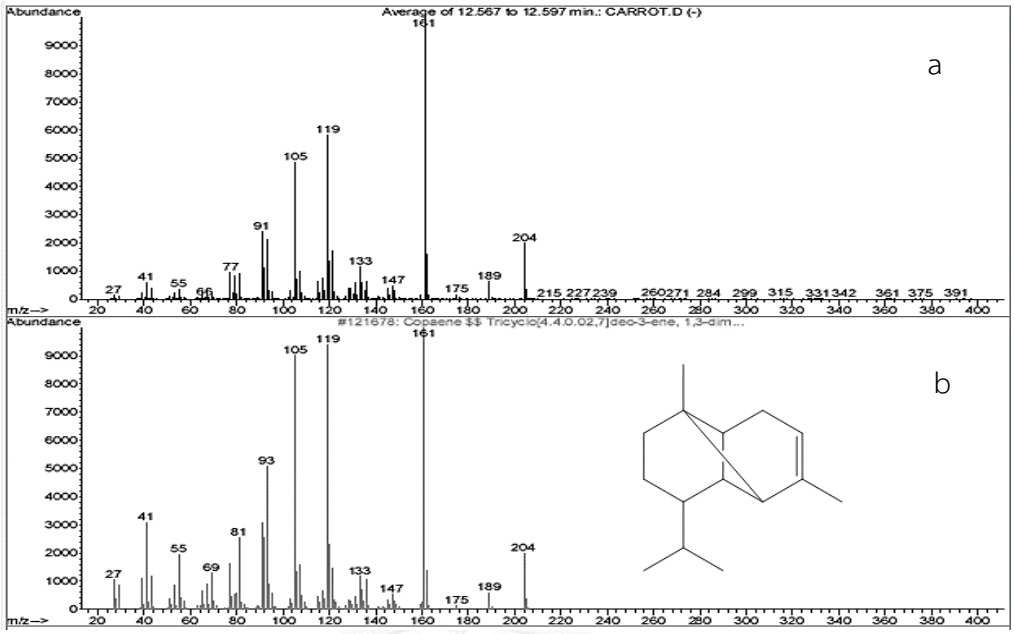


Figure 3.17 Mass spectra of a) at R_t 12.59 min b) copaene

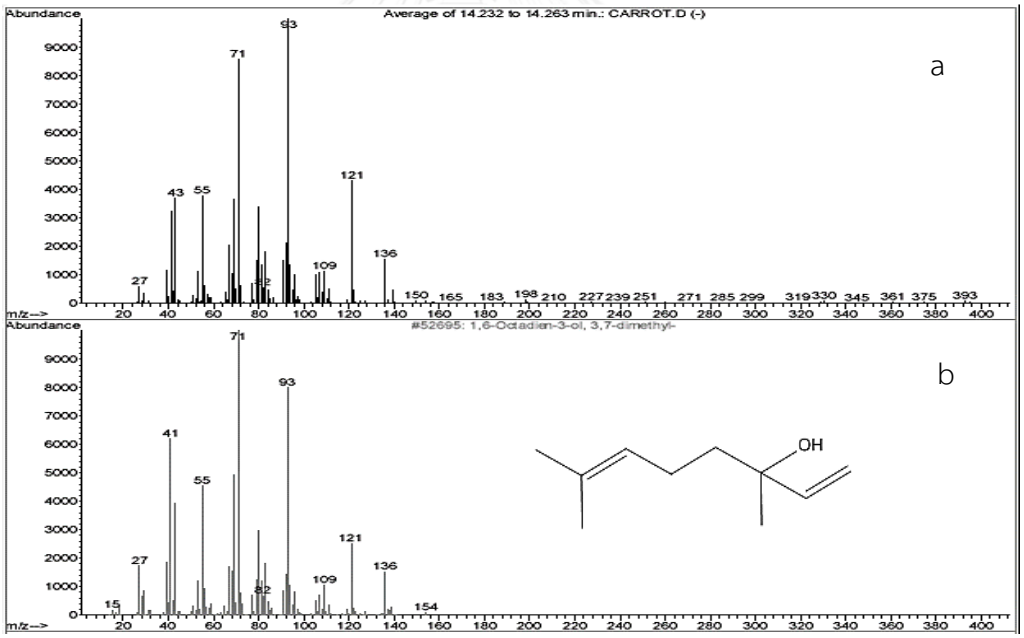


Figure 3.18 Mass spectra of a) at R_t 14.27 min b) linalool

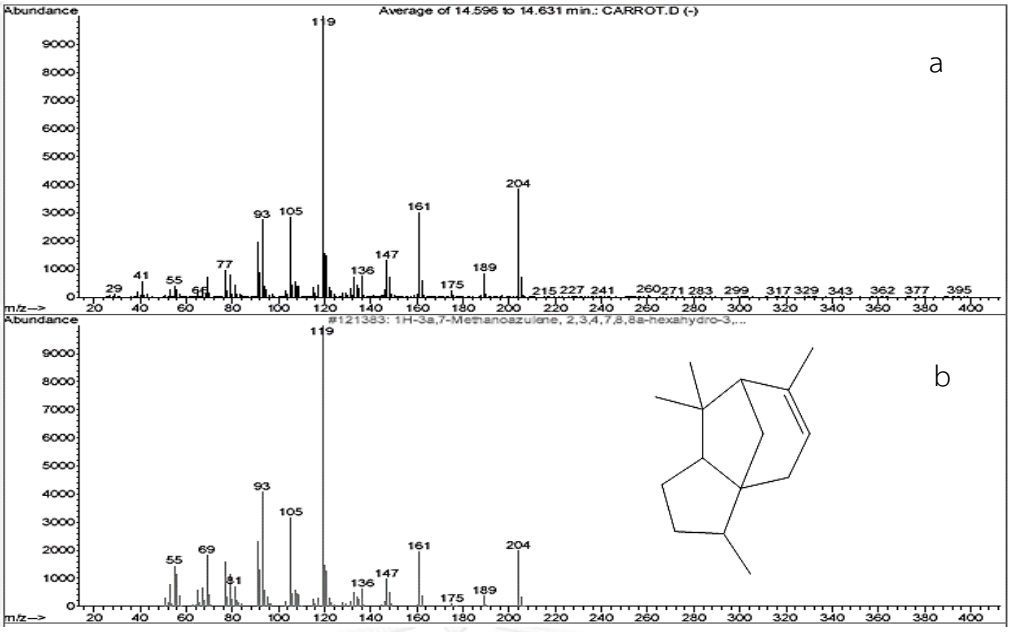


Figure 3.19 Mass spectra of a) at R_t 14.62 min b) **α**-cedrene

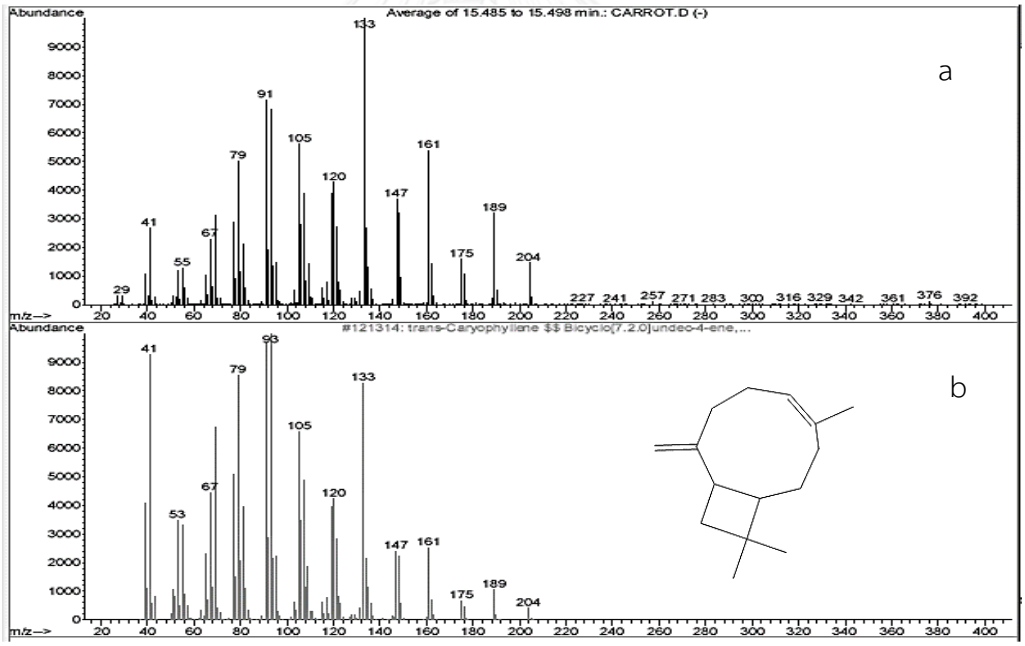


Figure 3.20 Mass spectra of a) at R_t 15.50 min b) *trans*-caryophyllene

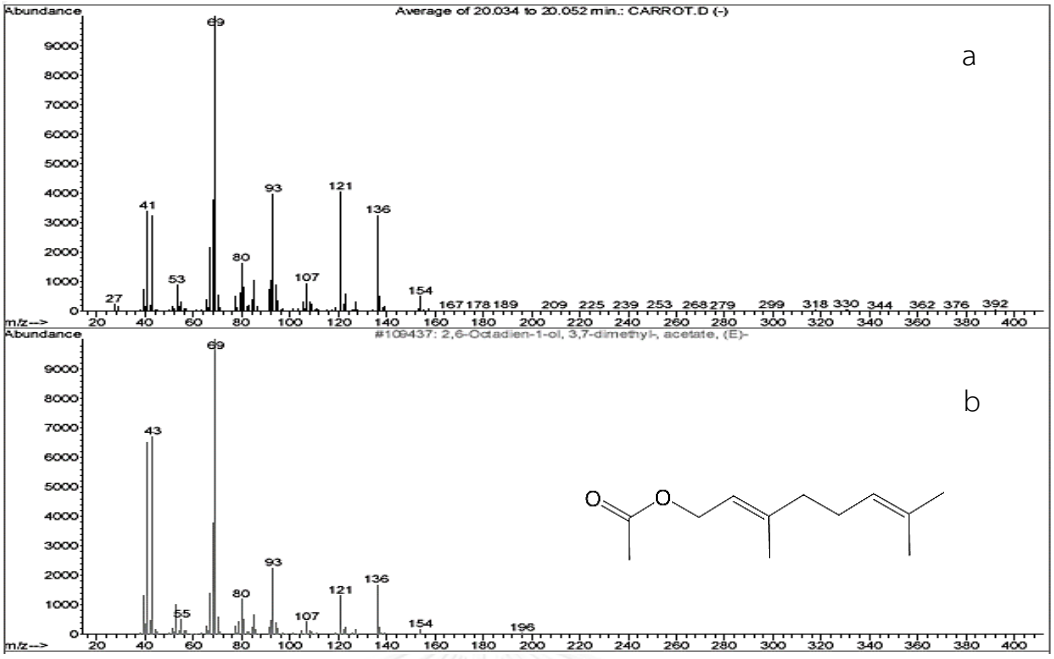


Figure 3.21 Mass spectra of a) at R_t 20.04 min b) (*E*)- 3,7-dimethyl-2,6-octadien-1-yl acetate

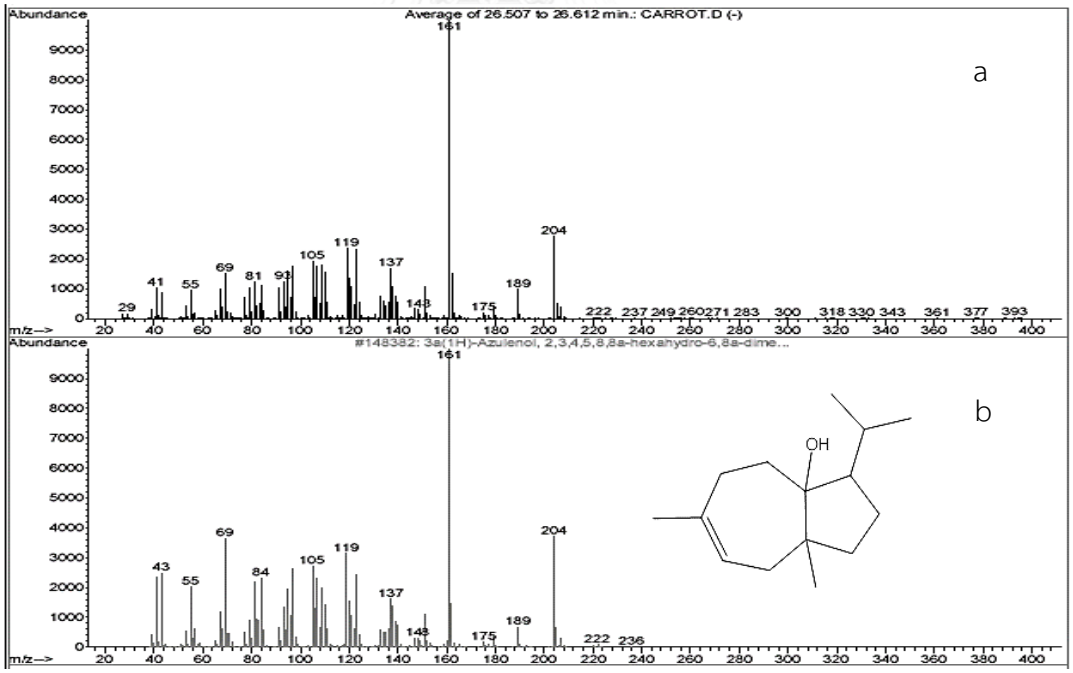


Figure 3.22 Mass spectra of a) at R_t 26.60 min b) carotol

All compounds were compared with the Wiley database and were identified as carorol at R_t 26.60 min, *trans*-caryophyllene at R_t 15.50 min, copaene at R_t 12.59 min, β-pinene at R_t 4.03 min, α-pinene at R_t 3.05 min, α-cedrene at R_t 14.62 min and linalool at R_t 14.27 min. From the GC-MS results, monoterpenoids and sesquiterpenoids were detected as main constituents. The highest peak at R_t 32.77 was identified as diethyl phthalate which was not included since it was plasticizer.

Moreover, the carrot seed oil (10 g) was separated by silica gel column chromatography. The column was eluted with CH_2Cl_2 and 1%CH_3OH in CH_2Cl_2, respectively. The yellow oil (2.26 g, 22.6% yield) as a single spot on TLC was analyzed by ^{1}H- and ^{13}C-NMR.

The ^{1}H NMR spectrum (Figure C1, appendix C) showed the important proton signals at δ_H 5.33 (m, 1H), 2.26 (s, 1H), 2.08 (m, 2H), 1.96 (m, 1H), 1.80 (m, 1H), 1.80 (m, 1H), 1.71 (s, 1H), 1.69 (s, 3H), 1.66 (s, 4H), 1.30 (m, 1H), 1.14 (s, 1H), 0.98 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 5.1 Hz, 3H) and 0.93 (s, 3H).

The ^{13}C NMR spectrum (Figure C2, appendix C) showed the important carbon signals at δ_C 139.0, 122.6, 85.0, 53.0, 49.3, 39.9, 38.7, 34.9, 28.9, 28.0, 25.4, 24.5, 23.9, 21.5 and 21.5.

From the NMR data, compound **1** was identified as carotol by comparing with those reported in literature. The tentative assignment of isolated carotol (**1**) was presented in Table 3.7 [40].

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

Table 3.7 The tentative assignment of compound **1** and reported carotol

Position	**Carotol** [40]		**Compound 1**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		49.1		49.3
2	1.30 (*ddd*, J = 7.6, 8.1, 12.2 Hz, 1H)	39.5	1.30 (m, 1H)	39.9
3		24.4		24.5
4	1.80 (m, 1H)	52.5	1.80 (m, 1H)	53.0
5		84.6		85.0
6	1.94 (m, 1H)	34.5	1.96 (m, 1H)	34.9
7	2.08 (m, 2H)	29.5	2.08 (m, 2H)	28.9
8		138.6		139.0
9	5.32 (m, 1H)	122.1	5.33 (m, 1H)	122.6
10	2.26 (*d*, J = 16 Hz, 1H) 1.70 (m, 1H)	38.6	2.27 (*d*, J = 15.5 Hz, 1H) 1.71 (m, 1H)	38.7
11	1.80 (m, 1H)	27.6	1.80 (m, 1H)	28.0
12	1.00 (*d*, J = 6.6 Hz, 3H) 1.67 (m, 4H),	24.0	0.98 (*d*, J = 6.3, 3H) 1.66 (m, 4H)	23.9
13	0.94 (*d*, J = 6.6 Hz, 3H)	21.4	0.93 (*d*, J = 5.1 Hz, 3H)	21.5
14	1.67 (s, 3H)	25.2	1.69 (s, 3H)	25.4
15	0.95 (s, 3H)	21.5	0.93 (s, 3H)	21.5
16	1.14 (s, 1H; OH)		1.14 (s, 1H; OH)	

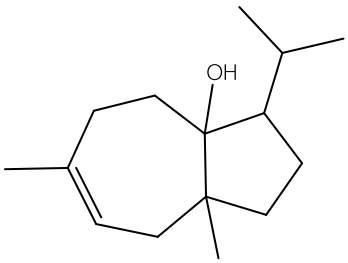


Figure 3.23 The structure of carotol

3.1.5 Antifeedant test of plant constituents

The carotol isolated from *D. carota* essential oil together with four commercial compounds present in *O. basilicum, H. coronarium* and *D. carota* essential oils as linalool, eucalyptol, eugenol and methyl cinnamate were tested with *S. litura* in antifeedant bioassay mentioned above. EC_{50} can be evaluated by Probit analysis [31]. The results of other concentrations as 0.5, 1, 1.5, 2 and 2.5 mM and Probit linear regression curve of five commercial compounds were shown in Appendix A.

Table 3.8 The antifeedant activity against *S. litura* at different concentrations of selected compounds and their EC_{50}.

Compound	Concentration (mM)					EC_{50}
	0.5	1	1.5	2	2.5	
1. Linalool	26.91±8.82	56.70±8.15	62.68±11.77	70.32±9.57	74.04±10.12	1.0
2. Eugenol	39.21±8.48	48.13±8.04	64.90±9.68	70.70±9.81	75.94±11.91	0.9
3. Eucalyptol	26.93±9.87	68.04±7.97	70.94±8.53	82.31±11.03	89.23±10.63	0.8
4. Methyl cinnamate	54.35±10.97	66.78±9.72	71.79±8.70	80.81±10.69	ND*	0.4
5. Carotol	37.53±11.73	44.53±9.79	51.87±11.18	61.20±10.71	73.77±9.25	1.1

Note: ND is no detection.

Each treatment was set up with 30 larvae.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

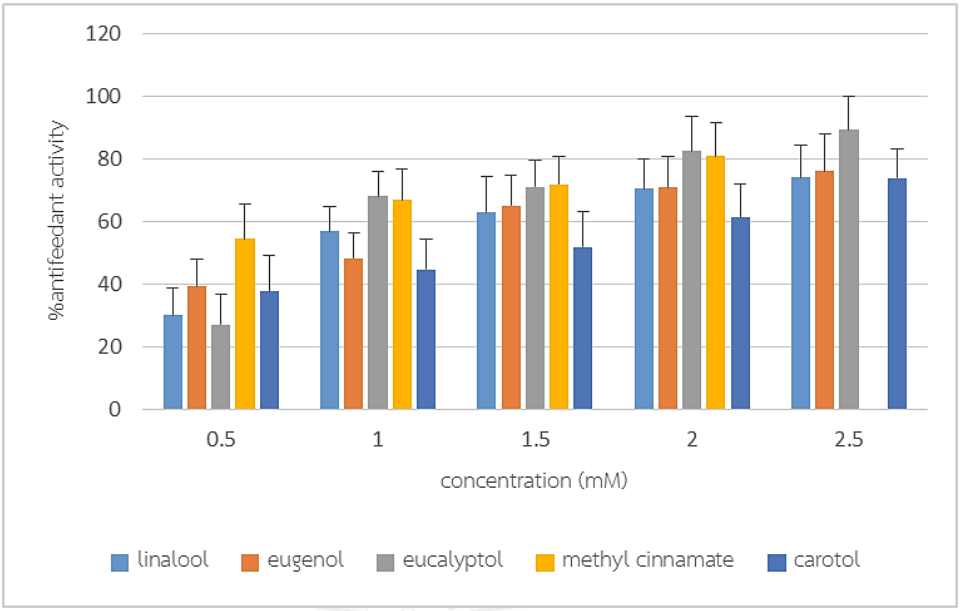
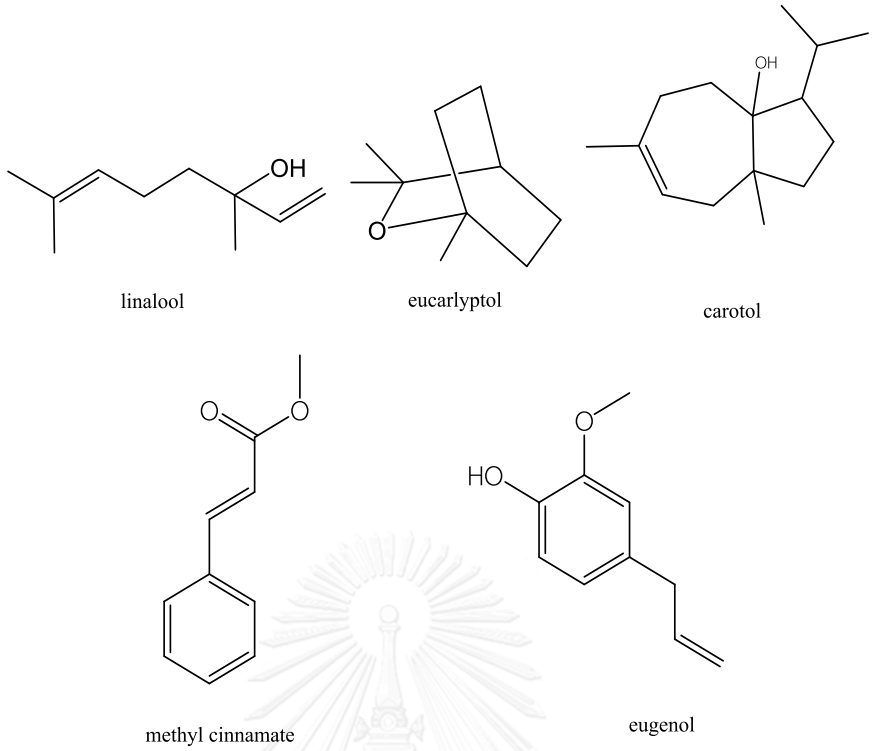


Figure 3.24 Antifeedant activity of five selected compounds

From **Table 3.8**, the compounds number 1-4 were the major constituents in the essential oils derived from *O. basilicum*, while compound number 3 was the major component in that from *H. coronarium*. Compounds number 1 and 5 were the major compounds in *D. carota*. The structures of the mentioned compounds are presented in **Figure 3.25**.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University



linalool eucarlyptol carotol

methyl cinnamate eugenol

Figure 3.25 Structures of selected compounds used in the antifeedant test.

The antifeedant activity results of five selected compounds (see in Table 3.8) revealed that all compounds showed high antifeedant activity at 2.5 mM. Methyl cinnamate exhibited the antifeedant activity more than 50% at 0.5 mM, with EC_{50} 0.4 mM. Moreover, at 2.5 mM, this compound could assassinate *S. litura*. For eucalyptol, eugenol and linalool, these three compounds showed similar antifeedant activity with EC_{50} 0.8, 0.9 and 1.0 mM, respectively. The last compound was carotol that gave displaying the lowest activity comparing with four compounds at EC_{50} 1.1 mM.

The antifeedant activity against *S. litura* of five selected compounds was compared with the report of Isman (2002) [41]. The active compounds possessing antifeedant activity were often oxygenated compounds similar to that reported by Papachristos *et al.* (2004) that oxygenated monoterpenoids showed the inhibitory activity higher than hydrocarbons [42]. Suresh *et al.* (2002) reported that the insect antifeedant activity of terpenoids has been related to the oxygenation, which may preserve sufficient polarity to allow aqueous diffusion to the taste receptor protein in the chemosensory sensilla of insect [43]. All selected compounds also contained

oxygen atom in their structures, thus they revealed good antifeedant activity. Especially methyl cinnamate expressed strong antifeedant activity against *S. litura*. Moreover, when the concentration of this compound was increased, it showed insecticidal activity. Carpinella *et al.* (2003) addressed that certain compounds that had antifeedant activity cloud display insecticidal activity when increasing the concentration [44]. Eugenol was reported to be toxic to *S. litura*, *Sitophilus granaries*, *Musca domestica* and *Diabrotica virgifera* with LD_{50} 2.5-157.6 µg/insect [45]. Similarly, in this study, eugenol exhibited high antifeedant activity at 2.5 mM. For eucalyptol and linalool, Koul *et al.* (2008) reported that these two compounds inhibited feeding against *S. litura* whereas linalool was more active than eucalyptol in topical application [3]. The similarity with this study was that both compounds gave high antifeedant activity, but eucalyptol was more active than linalool [45]. The reason maybe that when mixing the compound in hot diet, the compounds could possibly evaporate. While the topical application method dropped the compound directly onto the insect and the insect will get whole compound. Another reason may derived from different strains of *S. litura*. Carotol was reported to express strong lavicidal activity against *Aedes albopictus* [46], but no report on antifeedant activity against *S. litura*.

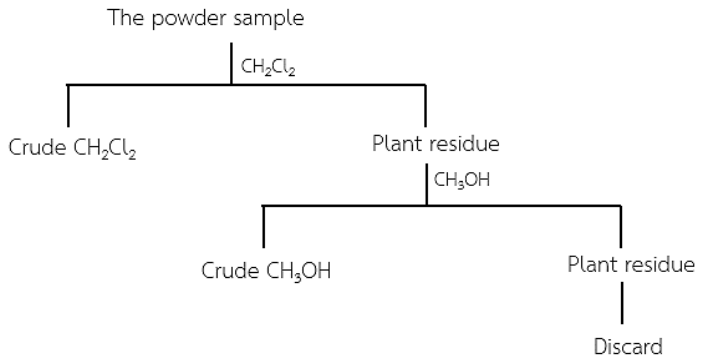
3.2 Plant extracts

3.2.1 The extraction of selected plants

The dried samples were milled to coarse powder and extracted with CH_2Cl_2 and CH_3OH for three days at RT. The process was repeated for three times. The crude extracts were evaporated with rotatory evaporator. The summary of the extraction is shown in Table 3.9 and Scheme 3.1.

Table 3.9 The extraction of selected plants

Family and scientific name	Common name (Thai name)	Plant part	solvent	Plant weight (g)	Crude extract(g) (%w/w)
Meliaceae					
1. *Xylocarpus granatum* Koenig	Cannonball Mangrove (ตะบูนขาว)	fruit	CH_2Cl_2	7000	482.30 (6.89%)
			CH_3OH	7000	726.94 (10.38%)
Rutaceae					
2. *Murraya paniculata* (L.) Jack	Orange Jessamine (แก้ว)	leaf	CH_2Cl_2	400	8.02 (2.01%)
			CH_3OH	400	19.66 (4.92%)
Zingiberaceae					
3. *Kaempferia galangal* L.	Aromatic Ginger (เปราะหอม)	rhizome	CH_2Cl_2	500	8.37 (1.67%)
			CH_3OH	500	30.35 (6.07%)



Scheme 3.1 The extraction procedure

The seeds of *X. granatum* gave the highest yield of 6.89% (w/w) CH_2Cl_2 extract and 10.38% (w/w) of CH_3OH extract, respectively, whereas *M. paniculata* gave 2.01% (w/w), 4.92% (w/w) and *K. galangal* gave 1.67% (w/w) and 6.07% (w/w) for CH_2Cl_2 and CH_3OH extracts, respectively.

3.2.2 Preliminary antifeedant bioassay results

Each crude extract was preliminarily screened for insect antifeedant activity against *S. litura* at 0.25% (w/w) for 24 h. The results are shown in Table 3.10.

Table 3.10 The preliminary screening of the crude extracts against *S. litura*.

Family and scientific name	Common name (Thai name)	Plant part	solvent	Antifeedant activity
Annonaceae				
1. *Melodorum fruticosum* Lour.*	White cheeseood (ลำดวน)	flower	CH_2Cl_2	+++
			CH_3OH	+++
Araceae				
2. *Acorus calamus* L.*	Sweet Flag (ว่านน้ำ)	rhizome	CH_2Cl_2	++++
			CH_3OH	+++
Arecaceae				
3. Areca catechu L.*	Betel palm (หมาก)	fruit	CH_2Cl_2	+++
			CH_3OH	+++
Asteraceae				
4. *Lactuca sativa* L.*	Lettuce (ผักกาด)	seed	CH_2Cl_2	+++
			CH_3OH	++++
Cucurbitaceae				
5. *Momordica charantia* L.*	Bitter Cucumber (มะระขี้นก)	leaf	CH_2Cl_2	+++
			CH_3OH	+++
Guttiferae				
6. *Garcinia mangostana* L.*	Mangosteen (มังคุด)	peel	CH_2Cl_2	++++
			CH_3OH	++++
Iridaceae				
7. *Eleutherine americana* Merr.*	Wan-hom-dang (ว่านหอมแดง)	bulb	CH_2Cl_2	+++

			CH_3OH	+++
Lamiaceae				
8. Ocimum gratissimum L.*	Tree Basil (ยี่หร่า)	seed	CH_2Cl_2	++++
			CH_3OH	+++
Liliaceae				
9. *Dracaena loureiri* Gagnep.*	Chan daeng (จันทน์แดง)	Heart wood	CH_2Cl_2	+++
			CH_3OH	+++
Meliaceae				
10. *Xylocarpus granatum* Koenig	Cannonball Mangrove (ตะบูนขาว)	fruit	CH_2Cl_2	++++
			CH_3OH	++++
Piperaceae				
11. *Piper betle* L.*	Betel (พลู)	leaf	CH_2Cl_2	+++
			CH_3OH	+++
12. *Piper sarmentosum* Roxb.*	Wildbetal Leafbush (ชะพลู)	fruit	CH_2Cl_2	++++
			CH_3OH	++++
Rutaceae				
13. *Murraya paniculata* (L.) Jack	Orange Jessamine (แก้ว)	leaf	CH_2Cl_2	++++
			CH_3OH	++++
14. *Zanthoxylum limonella* Alston.*	Ma-kan (มะแข่น)	tree	CH_2Cl_2	+++
			CH_3OH	+++
Sapindaceae				
15. *Nephelium lappaceum* L.*	Rambutan (เงาะ)	seed	CH_2Cl_2	++++
			CH_3OH	++
Sterculiaceae				
16. *Mansonia gagei* Drumm.*	Jan-Cha-Mod (จันทน์ชะมด)	wood	CH_2Cl_2	+++
			CH_3OH	+++

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Zingiberaceae				
17. *Kaempferia galanga* L.	Aromatic Ginger (เปราะหอม)	rhizome	CH_2Cl_2	+++
			CH_3OH	++++

*The crude extracts were gained from Natural Products Research Unit, Department of Chemistry, Chulalongkorn University.

**Each treatment was set up with 30 larvae.

***The data of antifeedant activity was classified and noted as + (0–20 %), ++ (21-40%), +++ (41-60%) and ++++ (61-80%) and +++++ (81-100%).

As the result of preliminary screening test compared with control (CH_2Cl_2 or CH_3OH), all seventeen plants had antifeedant activity with different results varied from species of plants material and solvent used for extraction. Most of the plants that extracted with CH_2Cl_2 displayed better antifeedant activity than those extracted with CH_3OH. Almost of the selected plants displayed antifeedant activity against *S. litura* since those plants were chosen based on previous reports on their uses against insects. In some cases, those plants belonged to the same family as the plants that revealed antifeedant activity. Nonetheless, certain plants did not show good activity such as the CH_3OH extract of *N. lappaceum, A. catechu, M. gagei* and *P. betle.* In this study the CH_2Cl_2 extracts of *X. granatum* and *A. calamus* displayed the highest antifeedant activity against *S. litura* larvae compared with other plants. Koul *et al.* (1990) reported that *A. calamus* oil from the rhizomes gave high inhibitory feeding activity against *S. litura* [47]. Two major compounds of *A. calamus* were addressed as *cis*- and *trans*-asarone. While *X. granatum* has not been previously reported about antifeedant activity against *S. litura.* Therefore, *X. granatum* was rationalized to select for further studying for antifeedant compounds against *S. litura.*

3.2.3 EC_{50} values of the CH_2Cl_2 extract of *X. granatum*

Five concentrations (0.1, 0.25, 0.5, 0.75 and 1% w/w) of the CH_2Cl_2 extract of this plant was subjected to the antifeedant assay analyzing for EC_{50} from Probit analysis [31]. The summary of the EC_{50} is shown in Table 3.11 (linear regression curves of three essential oils show in Figure A8 in Appendix A).

Table 3.11 EC_{50} of the CH_2Cl_2 extract of *X. granatum* against *S. litura*.

scientific name	concentration (%w/w)					EC_{50}
	0.1	0.25	0.5	0.75	1.0	
X. granatum	57.63±3.37	73.51±3.93	76.51±5.76	80.72±6.19	89.15±4.13	0.06%

According to Table 3.11, the concentration causing half effective antifeedant was 0.06% w/w. From the above results, *X. granatum* showed antifeedant activity although the concentration used was 0.1% (w/w). *X. granatum* belongs to the same family meliaceae as *Azadirachta indica* or neem which has been reported as an excellent example of a commercially prosperous antifeedant [41]. This implied that the CH_2Cl_2 extract of *X. granatum* should contain some active compounds. Thus, the CH_2Cl_2 extract of this plant was chosen for searching for antifeedant compounds.

Chulalongkorn University

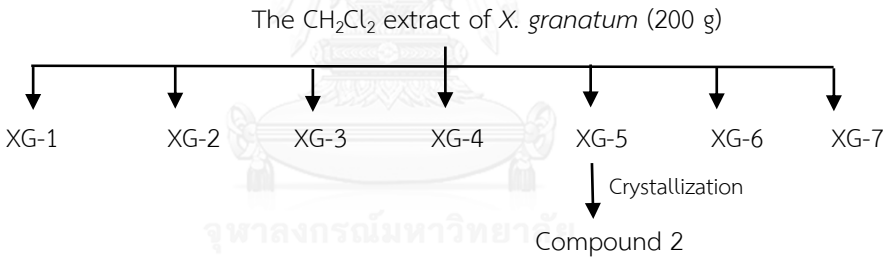
3.2.4 Separation of the CH_2Cl_2 extract from *X. granatum*

The CH_2Cl_2 extract of the seeds of *X. granatum* (200 g) was mixed with silica gel No.7734 and separated by quick column chromatography using a mixture of hexane-EtOAc as eluents and CH_3OH-EtOAc. Each fraction was examined and combined by TLC. Fractions with similar chromatographic patterns were combined to furnish seven fractions as shown in Table 3.12.

Table 3.12 The separation of the CH_2Cl_2 extract

Fraction	Eluent	Remarks	Weight (g)	%yeild
XG-1	100% Hex	Colorless oil	2.93	1.30
XG-2	5% EtOAc : Hex	Yellow-brown oil	14.20	6.29
XG-3	10% EtOAc : Hex	Brown viscosity	38.80	17.17
XG-4	15-20% EtOAc : Hex	Dark brown viscosity	71.48	31.63
XG-5	40-60% EtOAc : Hex	Yellow solid	98.19	43.45
XG-6	80% EtOAc : Hex – 100% EtOAc	Yellow powder	42.90	18.98
XG-7	100% EtOAc - 2.5% MeOH :EtOAc	Pale yellow solid	22.12	9.79

Fractions XG-5, XG-4 and XG-6 gave the high %yield as 43.45, 31.63 and 18.98%, respectively. The concentration at 0.25% (w/w) of all fractions was subjected to antifeedant activity test.



Scheme 3.2 The separation of the CH_2Cl_2 extract of *X. granatum*.

3.2.4.1 Antifeedant activity assay

The seven fractions obtained from quick column chromatography were tested for antifeedant activity against *S. litula* at 0.25% (w/w). The results are presented in Figure 3.26.

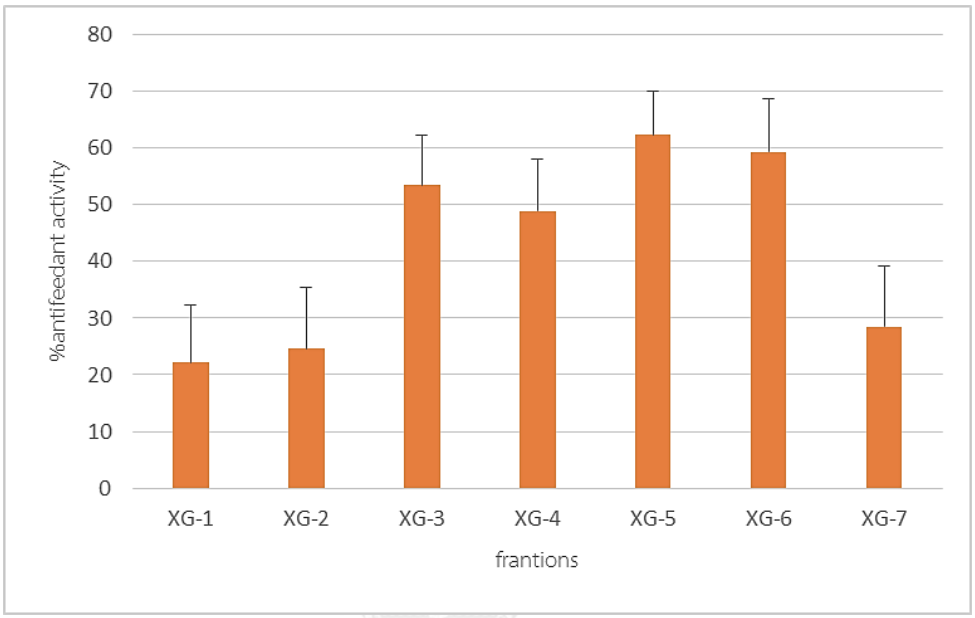


Figure 3.26 Antifeedant activity from separated seven fractions

From Figure 3.26, fractions XG-5, XG-6 and XG-3 displayed high antifeedant activity as 62, 59 and 54%, respectively. While, fractions XG-5, XG-4 and XG-6 gave the highest yield of 98.19, 71.48 and 42.90 g, respectively. Because of fraction XG-5 revealing the highest antifeedant activity and yield, it was rationalized to continue separating this fraction by column chromatography to search for its active compounds.

3.2.4.2 Separation of Fraction XG-5

Compound 2 was acquired in fraction XG-5, as the white solid in the yellow solution. The yellow solution was removed by washing with warm CH_3OH and further purified by column chromatography with sephadex eluting with 50% CH_3OH in CH_2Cl_2. The yield of cubic crystal (compound 2) was 9.69% of fraction XG-5. Compound 2 could be soluble in CH_2Cl_2 and acetone, and slightly soluble in EtOAc and CH_3OH.

Compound 2 displayed a single spot on TLC with R_f 1.80 (80% EtOAc in hexane). Dipping into vanillin strain, the spot of this compound gave a dark purple spot.

The ^{1}H NMR spectrum (Figure C3 appendix C) showed the important proton signals at δ_H 7.54 (s, 1H), 7.43 (s, 1H), 6.47 (s, 1H), 6.26 (s, 1H), 4.21 (d, *J* = 5.6 Hz, 1H), 3.68 (s, 3H), 3.12 (d, *J* = 17.8 Hz, 1H), 3.06 (d, *J* = 10.5 Hz, 1H), 2.95 (t, *J* = 6.0 Hz, 1H), 2.52 (m, 1H), 2.26 (m, 1H), 2.11 (m, 1H), 2.11 (m, 1H), 2.03 (m, 1H), 1.95 (dd, *J* = 12.4, 4.6 Hz, 1H), 1.70 (dd, *J* = 14.2, 3.4 Hz, 1H), 1.50 (d, *J* = 9.0 Hz, 1H), 1.45 (m, 1H), 1.09 (s, 3H), 0.97 (s, 3H), 0.92 (s, 3H) and 0.65 (s, 3H).

The ^{13}C NMR spectrum (Figure C4 appendix C) showed 27 signals at δ_c as: methyl carbons at 51.9, 28.1, 20.1 and 16.1, methine carbons at 143.0, 140.7, 110.0, 91.5, 76.6, 52.3, 49.0, 43.0 and 20.1, quarternary carbons at 214.9, 174.3, 170.1, 120.7, 85.4, 74.5, 51.1, 40.1 and 37.2 and methylene carbons at 42.5, 37.2, 32.7, 28.8 and 17.9. From the NMR data, this compound was identified as xyloccensin K. The ^{1}H and ^{13}C NMR assignment of compound 2 with those reported in literature are presented in Table 3.13. [20].

Table 3.13 The tentative assignment of compound 2 and reported xyloccensin K

Position	**Xyloccensin K** [20]		**Compound 2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1		215.1		215.1
2	2.97 (t, J=6.0 Hz, 1H)	49.3	2.95 (t, J=6.0 Hz, 1H)	49.2
3	4.22 (d, J=5.6 Hz, 1H)	91.7	4.21 (d, J=5.6 Hz, 1H)	91.6
4		37.3		37.4
5	3.07 (m, 1H)	43.3	3.06 (d, J=10.5 Hz, 1H)	43.2
6	2.11 (m, 1H) 2.23 (m, 1H)	32.9	2.11 (m, 1H) 2.26 (m, 1H)	32.8
7		175.0		174.3
8		85.8		85.6
9	1.95 (dd, J=12.6, 4.0 Hz, 1H)	52.4	1.95 (dd, J=12.4, 4.6 Hz, 1H)	52.4
10		51.5		51.3
11	1.47 (m, 1H) 2.11 (m, 1H)	18.0	1.45 (m, 1H) 2.11 (m, 1H)	18.1
12	1.53 (m, 1H)	29.1	1.50 (d, J=9 Hz, 1H)	29.0

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

	1.69 (m, 1H)		1.70 (dd, *J* = 14.2, 3.4 Hz, 1H)	
13		40.4		40.3
14		74.8		74.7
15	2.52 (m, 1H) 3.15 (d, J=17.7 Hz, 1H)	37.4	2.52 (m, 1H) 3.12 (d, J=17.8 Hz, 1H)	37.4
16		170.3		170.1
17	6.28 (s, 1H)	76.8	6.26 (s, 1H)	76.8
18	0.66 (s, 3H)	16.4	0.65 (s, 3H)	16.3
19	0.94 (s, 3H)	17.2	0.92 (s, 3H)	17.1
20		121.0		120.9
21	7.45 (s, 1H)	141.3	7.43 (s, 1H)	140.9
22	6.49 (s, 1H)	110.3	6.47 (s, 1H)	110.2
23	7.55 (d, J=0.5 Hz, 1H)	143.3	7.54 (s, 1H)	143.0
24	1.09 (s, 3H)	20.4	1.09 (s, 3H)	20.3
25	0.99 (s, 3H)	28.4	0.97 (s, 3H)	28.3
26	2.05 (m, 1H)	42.8	2.03 (m, 1H)	42.7
27	3.69 (s, 3H)	52.2	3.68 (s, 3H)	52.1

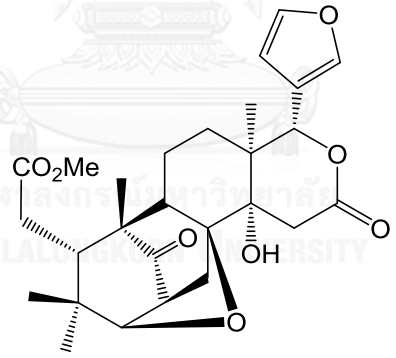


Figure 3.27 The structure of xyloccensin K.

3.2.4.3 EC_{50} of xyloccensin K from *X. granatum* against *S. litura*

Xyloccensin K was tested for antifeedant activity at concentrations of 0.5, 1, 1.5, 2 and 2.5 mM and calculated for EC_{50}. The antifeedant results are presented in Table 3.14 and linear regression is shown in Figure A9 in Appendix A.

Table 3.14 The antifeedant activity result of xyloccensin K at different concentrations.

Treatment	Concentration (mM)					EC_{50}
	0.5	1	1.5	2	2.5	
xyloccensin K	25.34±9.92	45.77±8.25	52.72±9.02	60.62±8.89	70.29±9.49	1.3

Xyloccensin K revealed antifeedant activity more than 50% at 1.5 mM and the activity was increased when the concentration increased. At the concentration of 2.5 mM, xyloccensin K could inhibit almost 70% antifeedant. The results clearly showed that xyloccensin K displayed inhibitory feeding effect on *S. litura*. According to the EC_{50} analysis curve (see in appendix A) and at 95% confidential limit (P=0.05), the concentration of xyloccensin K causing 50% antifeedant of *S. litura* was 1.3 mM.

Compounds with insect antifeedant activity normally have a more oxidized or unsaturated structure. However, molecular size and shape including functional group and stereochemistry also affected the antifeedant activity [3]. Xyloccensin K was in limonoid group that well studied and could inhibit feeding in a variety of insect species [3] such as azadireachtin that well known in antifeedant activity. In addition, Pinjinda, 1996 revealed that xyloccensin K had antifeedant activity against Greater wax moth, *Galleria mellonella* at dose level 4.0 mg [20]. This could be concluded that the xyloccensin K was one active compound in *X. granatum* against *S. litura*.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

3.3 Stability test

The stability of the CH_2Cl_2 extract of *X. granatum* and xyloccensin K was tested compared with neem extract that has been well known to use for controlling insect pests. Two conditions: temperature and UV light were investigated

3.3.1 The effects of temperature

Three treatments were kept at four different temperatures including 4ºC, room temperature (30ºC), 45 and 60ºC, respectively for 48 and 96 h. Each treatment was used the same concentration that tested in normal conditions as the CH_2Cl_2 extract of *X. granatum* [0.25% (w/w)], and xyloccensin K (2 mM). For neem extract containing

azadirachthin 0.1% w/v, the commercial product was used as such. The antifeedant activity results at different temperatures for 48 and 96 h are presented in Figures 3.28 and 3.29, respectively.

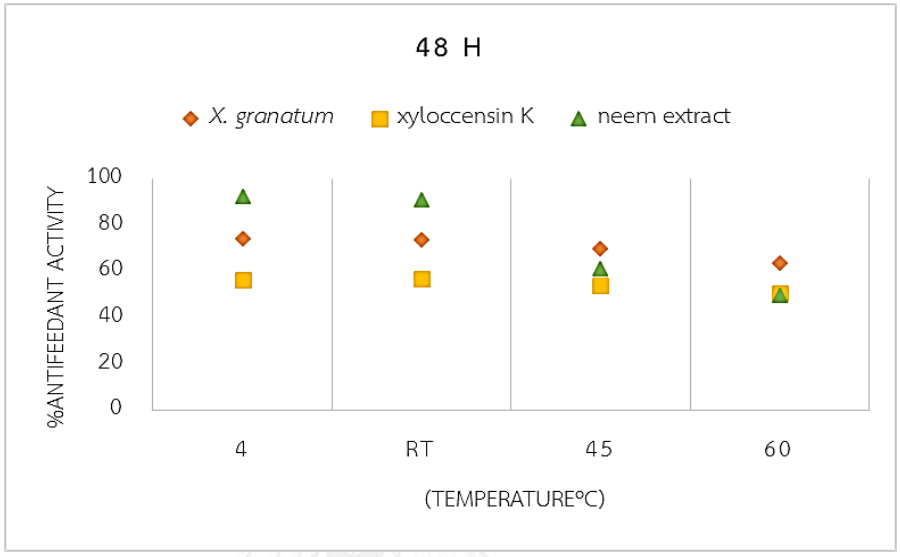


Figure 3.28 The antifeedant activity of three treatments at different temperatures after 48 hours.

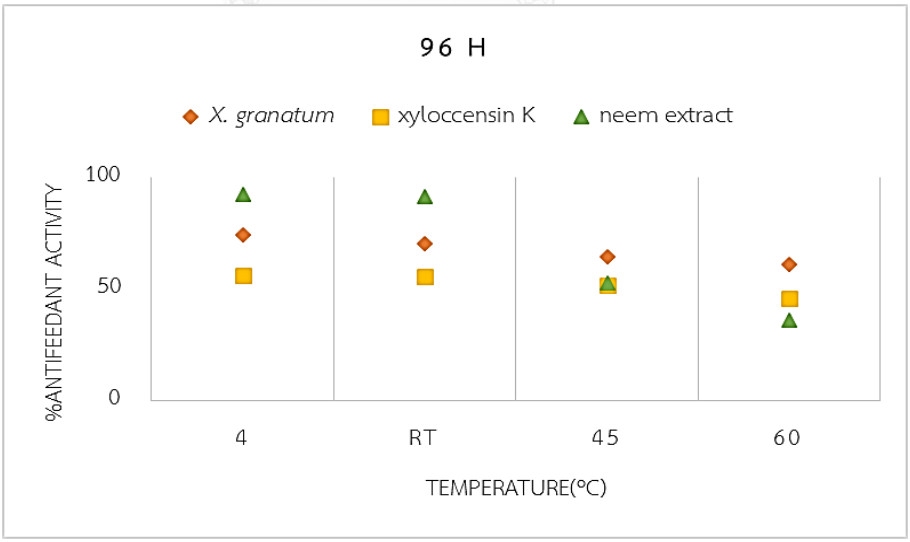


Figure 3.29 The antifeedant activity of three treatments at different temperatures after 96 hours.

The influence of temperature on the stability of treatment was examined. Figure 3.28 shows the antifeedant activity at 48 h. At four different temperatures, the CH_2Cl_2 extract of *X. granatum* and xyloccensin K revealed similar antifeedant activity (within 6-11%), whereas approximately 43% antifeedant activity of the neem extract was dramatically decreased especially at 45 and 60 °C. The similar result was observed in Figure 3.29 (96 h). The CH_2Cl_2 extract of *X. granatum* and xyloccensin K were decreased antifeedant activity in range 10-13%. While, neem extract was decreased 55%.

From the work of Madaki (2015), the activity of azadirechtin in neem extract which was stored at room temperature (28 °C) significantly decreased more than the sample that stored in refrigerator [48].

3.3.2 The effects of UV light

The CH_2Cl_2 extract of *X. granatum*, xyloccensin K and commercial neem extract were tested for their stability by exposing to UV light (256 nm) for 12 h. The results are presented in Figure 3.30.

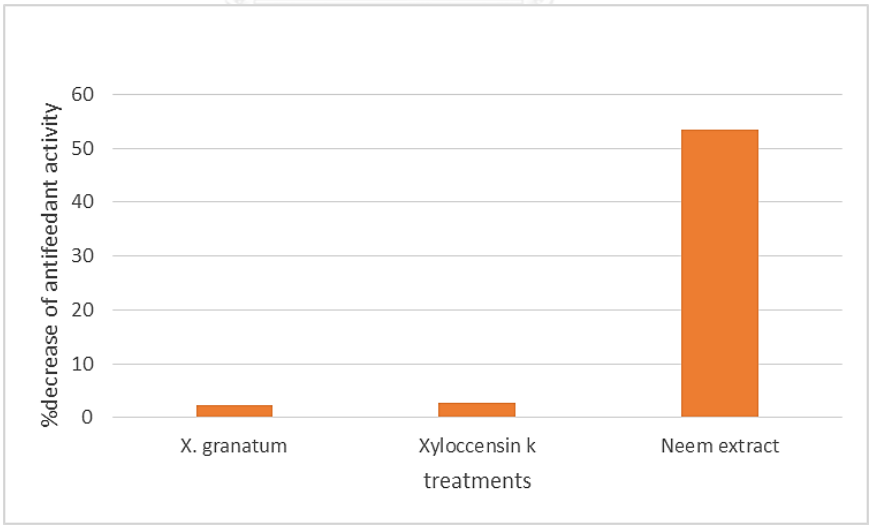


Figure 3.30 %decrese of antifeedant activity of three treatments after exposing to UV light for 12 hours.

Figure 3.30 shows %decrease of antifeedant activity compared between the sample in normal conditions and those exposing to UV light for 12 h at the same concentration in previous antifeedant assay. The antifeedant activity of the neem extract decreased significantly when exposing to UV light (256 nm) as 54%, whereas only 2-3% decreasing activity of the CH_2Cl_2 extract of *X. granatum* and xyloccensin K were detected. Madaki (2015) reported that when the neem extract exposed to UV, the concentration of azadirachtin decreases from 47.31 to 31.04 μg/mL [48].

From the above results, it could be concluded that the neem extract needed to keep away from light and heat, while those extracts of *X. granatum* and xyloccensin K were more stable under these explored conditions.

3.4 Leaf disk toxicity assay

3.4.1 Three essential oils and *X. granatum*.

Three essential oils of *D. carota*, *O. basilicum* and *H.coronarium*, and the CH_2Cl_2 extract of *X. granatum* showed high antifeedant activity when tested with artificial diet. Next experiment was performed using kale leaf. Neem extract was used as positive control. The results are collected as shown in Table 3.15.

Table 3.15 Antifeedant activity of three essential oils and *X. granatum* extract by leaf disk toxicity assay

treatments	% antifeedant
D. carota	57.47±10.27
O. basilicum	87.79±8.89
H. coronarium	73.64±5.58
X. granatum (CH_2Cl_2)	69.90±5.96

O. bacilicum gave 88% antifeedant activity which was the highest result compared with other extracts followed by *H. coronarium* (74%), *X. granatum* (70%) and *D. carota* (58%), respectively. This result revealed the similar trend with previous tests in artificial diet.

3.4.2 Six compounds from three essential oils and *X. granatum*

The selected compounds as carotol, eucalyptol, linalool, eugenol, methyl cinnamate and xyloccensin K were used to test for antifeedant activity with kale leave at 1 mM. The results are revealed in Table 3.16.

Table 3.16 %antifeedant activity of selected compounds at 1 mM

compounds	% antifeedant
Eucalyptol	66.08±3.45
linalool	45.35±3.89
Eugenol	34.6±2.79
Methyl cinnamate	ND*
Carotol	41.53±8.04
Xyloccensin K	51.48±9.53

*ND = no detection

The results in Table 3.16 displayed interesting information. Methyl cinnamate exhibited insecticidal activity at 1 mM that made *S. litura* died when tested with kale leaf. While, eucalyptol gave the highest antifeedant activity as 66% at 1 mM followed by xyloccensin K, linalool, carotol and eugenol: 52, 45, 42 and 35%, respectively. Compared with previous results using artificial diet, similar results except methyl cinnamate gave only antifeedant activity at 1 mM when tested with artificial diet, but showed insecticidal activity when test with leave at the same concentration. The reason maybe when testing with kale leaf, *S. litura* will be fed only the compound,

while testing with artificial diet, the compounds were mixed with the ingredients of diet. Perhaps some ingredients rendered antifeedant activity of the compounds.

As aforementioned, essential oils, plant extract and selected compounds gave antifeedant activity when tested with both artificial diet and kale leave. Moreover, acetone was a good solvent because it had no effect on kale leave.

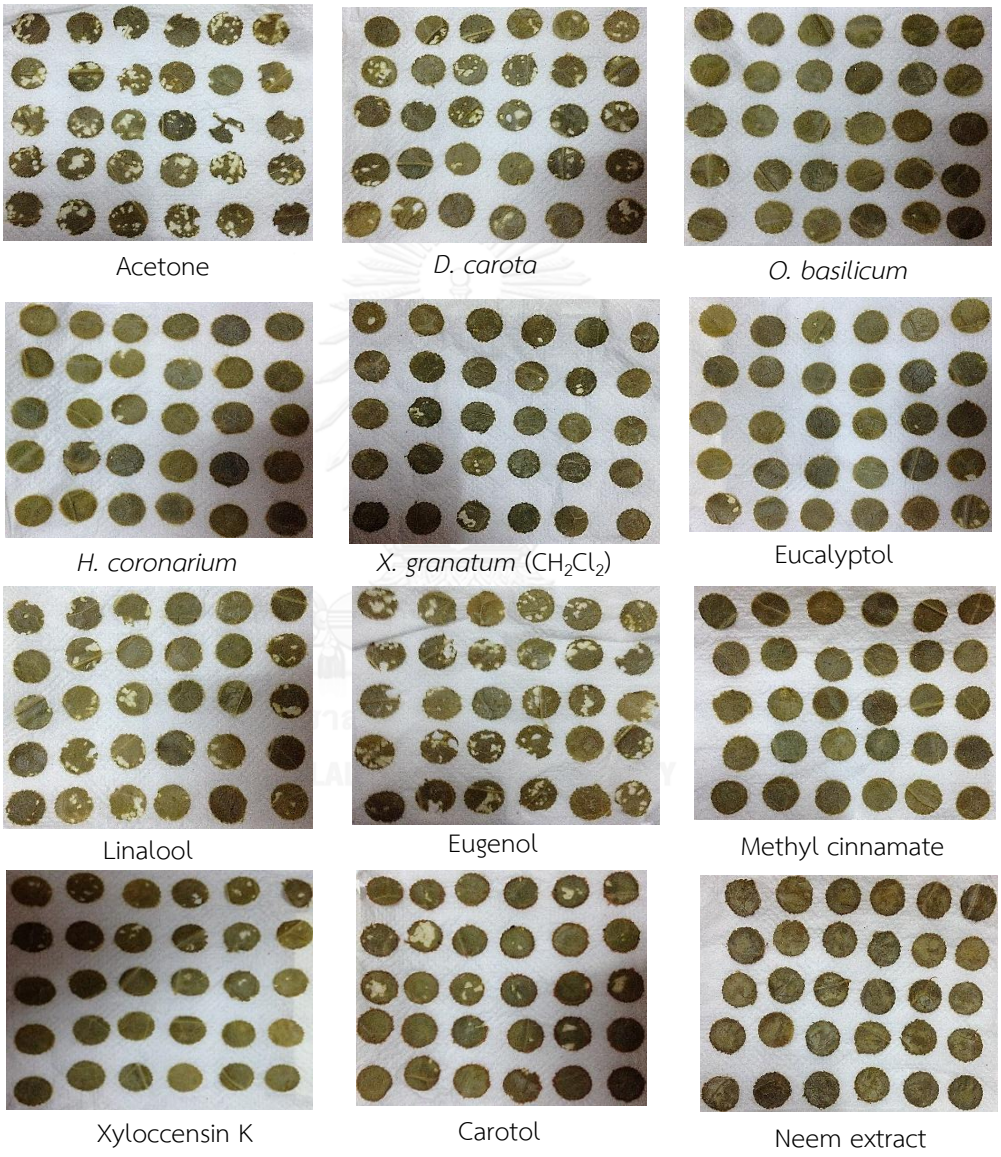


Figure 3.31 Antifeedant activity by leaf disk toxicity assay

CHAPTER IV
CONCLUSION

The antifeedant activity of six essential oils and thirty-four plant extracts were screened against the insect pest, *Spodoptera litura* (common cutworm). Three essential oils from *Ocimum basilicum* (sweet basil), *Hedychium coronarium* (white ginger), *Daucus carota* (carrot) and the dichloromethane extract from *Xylocarpus granatum* (cannonball mangrove) exhibited the highest antifeedant activity against *S. litura*. Their EC_{50} ranged from 0.06 to 0.15% (w/w).

In addition, chemical constituents and antifeedant activity of each active essential oil were investigated. The essential oils from *O. basilicum* and *H. coronarium* were found to be rich in eucalyptol while that of *D. carota* contained carotol as a main constituent. Eucalyptol revealed the highest antifeedant activity against *S. litura* with EC_{50} 0.8 mM. Moreover, the separation of the CH_2Cl_2 extracted of *X. granatum* using quick column chromatography gave seven fractions. Each fraction was tested for antifeedant activity at 0.25% (w/w). The XG-5 can highly inhibit the feeding of *S. litura* and the major compound from this was white crystal, namely xyloccensin K. This compound cloud inhibit the feeding of *S. litura* with EC_{50} 1.3 mM. The CH_2Cl_2 extract of *X. granatum* and xyloccensin K were stabled to UV light and high temperature more than the neem extract. Three essential oils, the CH_2Cl_2 extract of *X. granatum* and their constituents also showed good results when tested with kale leave.

In summary, three essential oils as *O. basilicum*, *H. coronarium* and *D. carota*, the CH_2Cl_2 extract of *X. granatum* and their constituents disclosed as a promising alternative as natural antifeedant compounds.

REFERENCES

[1] Sittiwong, W. Insect control agents from heartwood of *Xylia xylocarpa* Taub. Master of Science, Chemistry Chulalongkorn University, 2003.

[2] Lee, B.-H.; Choi, W.-S.; Lee, S.-E., and Park, B.-S. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Prot. 20 (2001): 317-320.

[3] Koul, O. Phytochemicals and insect control: An antifeedant approach. Crit. Rev. Plant Sci. 27 (2008): 1-24.

[4] Veitch, G. E.; Beckmann, E.; Burke, B. J.; Boyer, A.; Maslen, S. L., and Ley, S. V. Synthesis of azadirachtin: A long but successful journey. Angew. Chem., Int. Ed. Engl. 46 (2007): 7629-32.

[5] Koul, O.; Singh, G.; Singh, R., and Singh, J. Bioefficacy and Mode-of-Action of Aglaroxin B and Aglaroxin C from *Aglaia elaeagnoidea* (syn. A. roxburghiana) against *Helicoverpa armigera* and *Spodoptera litura*. Biopestic. Int. 1 (2005): 54-64.

[6] Champagne, D. E.; Koul, O.; Isman, M. B.; Scudder, G. G. E., and Neil Towers, G. H. Biological activity of limonoids from the rutales. Phytochemistry 31 (1992): 377-394.

[7] Daido, M.; Fukamiya, N.; Okano, M.; Taoahara, K.; Hatakoshi, M., and Yamazaki, H. Antifeedant and insecticidal activity of quassinoids against diamondback moth (*Plutella xylostella*). Biosci. Biotechnol. Biochem. 57 (1993): 244-246.

[8] Daido, M., *et al.* Antifeedant and insecticidal activity of quassinoids against the diamondback moth (*Plutella xylostella*) and structure-activity relationships. Biosci. Biotechnol. Biochem. 59 (1995): 974-979.

[9] Khaiprapai, P. Searching for antifeedant compounds against greater wax moth *Galleria mellonella* larvae from some Thai plants. Master of Science, Biotechnology Chulalongkorn University, 1997.

[10] Giordano, O. S.; Sosa, M. E., and Tonn, C. E. Biological activity of plant secondary metabolites towards *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). An. Acad. Nac. Cienc. Exactas. Fis. Nat. B. Aires 52 (2000): 163-181.

[11] Enriz, R. D., *et al.* Structure–antifeedant activity relationship of clerodane diterpenoids. Comparative study with withanolides and azadirachtin. J. Agric. Food Chem. 48 (2000): 1384-1392.

[12] Krishna Kumari, G. N., *et al.* Antifeedant neo-clerodanes from *Teucrium tomentosum* Heyne. (Labiatae). Phytochemistry 64 (2003): 1119-1123.

[13] Krishna Kumari, G. N.; Balachandran, J.; Aravind, S., and Ganesh, M. R. Antifeedant and growth inhibitory effects of some neo-clerodane diterpenoids isolated from clerodendron species (verbenaceae) on *Earias vitella* and *Spodoptera litura.* J. Agric. Food Chem. 51 (2003): 1555-1559.

[14] Blaney, W. M.; Simmonds, M. S. J.; Ley, S. V., and Katz, R. B. An electrophysiological and behavioural study of insect antifeedant properties of natural and synthetic drimane-related compounds. Physiological Entomology 12 (1987): 281-291.

[15] Koul, O. Feeding deterrence induced by plant limonoids in the larvae of *Spodoptera litura* (F.) (Lepidoptera, Noctuidae). Z. Angew. Entomol. 95 (1983): 166-171.

[16] Ballesta-Acosta, M. C.; Pascual-Villalobos, M. J., and Rodríguez, B. The antifeedant activity of natural plant products towards the larvae of *Spodoptera littoralis.* Span. J. Agric. Res. 6 (2008): 85-91.

[17] Morimoto, M.; Tanimoto, K.; Nakano, S.; Ozaki, T.; Nakano, A., and Komai, K. Insect antifeedant activity of flavones and chromones against *Spodoptera litura.* J. Agric. Food Chem. 51 (2003): 389-393.

[18] Gonzalez-Coloma, A., *et al.* Silphinene sesquiterpenes as model insect antifeedants. J. Chem. Ecol. 28 (2002): 117-29.

[19] Brem, B.; Seger, C.; Pacher, T.; Hofer, O.; Vajrodaya, S., and Greger, H. Feeding deterrence and contact toxicity of stemona alkaloids-a source of potent natural insecticides. J. Agric. Food Chem. 50 (2002): 6383-6388.

[20] Pinjinda, S. Chemical constituents and biological activity of the fruits of *Xylocarpus granatum* Koen. Master of Science, Chemistry Chulalongkorn University, 1996.

[21] Wu, Y., *et al.* Chemical constituents of *Xylocarpus granatum.* Chem. Nat. Compd. 50 (2014): 549-551.

[22] Wu, J., *et al.* Xyloccensins Q–V, six new 8,9,30-phragmalin ortho ester antifeedants from the Chinese mangrove *Xylocarpus granatum.* Tetrahedron 61 (2005): 8382-8389.

[23] Zhou, Z.-F., *et al.* Bioactive rearranged limonoids from the Chinese mangrove *Xylocarpus granatum* Koenig. Tetrahedron 70 (2014): 6444-6449.

[24] Cui, J.; Wu, J.; Deng, Z.; Proksch, P., and Lin, W. Xylocarpins A-I, limonoids from the Chinese mangrove plant *Xylocarpus granatum.* J. Nat. Prod. 70 (2007): 772-8.

[25] Baskar, K. and Ignacimuthu, S. Antifeedant, larvicidal and growth inhibitory effects of ononitol monohydrate isolated from *Cassia tora* L. against *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). Chemosphere 88 (2012): 384-388.

[26] Wheeler, D. A.; Isman, M. B.; Sanchez-Vindas, P. E., and Arnason, J. T. Screening of Costa Rican Trichilia species for biological activity against the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). Biochem. Syst. Ecol. 29 (2001): 347-358.

[27] Nhuseedam, A. Efficacy of *Embelia ribes* burm. f. leaf extract to control common cutworm cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Master of Science, Entomology Kasetsart University, 2007.

[28] Abbas, N.; Samiullah; Shad, S. A.; Razaq, M.; Waheed, A., and Aslam, M. Resistance of *Spodoptera litura* (Lepidoptera: Noctuidae) to profenofos: Relative fitness and cross resistance. Crop Prot. 58 (2014): 49-54.

[29] GADHIYA, H. A.; BORAD, P. K., and BHUT, J. B. Effectiveness of synthetic insecticides against *Helicoverpa armigera* (hubner) hardwick and *Spodoptera litura* (fabricius) infesting groundnut. Int. Q. J. Life Sci. 9 (2014): 23-26.

[30] Walker, G. P.; Cameron, P. J.; MacDonald, F. M.; Madhusudhan, V. V., and Wallace, A. R. Impacts of *Bacillus thuringiensis* toxins on parasitoids (Hymenoptera: Braconidae) of *Spodoptera litura* and *Helicoverpa armigera* (Lepidoptera: Noctuidae). Biol. Control 40 (2007): 142-151.

[31] Phoonan, W. Essential oils as fumigant against rice weevils, *Sitophilus oryzae* (linnaeus). Master of Science, Biotechnology Chulalongkorn University, 2006.

[32] Hosozawa S., K. N., Munakana K. and Chen Y.L. Antifeedant substance for insects in plant. . Agricultural and Biological Chemistry 38 (1974): 1045-1048.

[33] Madaki, F. M. Studies on the Effect of Temperature, Light and Storage on the Stability of Neem (Azadirachta Indica A. Juss) Seeds Oil Extract. (2015).

[34] Jarvis, A. P.; Johnson, S., and Morgan, E. D. Stability of the natural insecticide azadirachtin in aqueous and organic solvents. Pestic. Sci. 53 (1998): 217-222.

[35] Pavela, R. Insecticidal activity of certain medicinal plants. Fitoterapia 75 (2004): 745-749.

[36] Devanand, P. and Rani, P. U. Biological potency of certain plant extracts in management of two lepidopteran pests of *Ricinus communis* L. J. Biopest. 1 (2008): 170-176.

[37] Sakhanokho, H., *et al.* Chemical composition, antifungal and insecticidal activities of hedychium essential oils. Molecules 18 (2013): 4308.

[38] Park, H.-M. and Park, I.-K. Larvicidal activity of *Amyris balsamifera*, *Daucus carota* and *Pogostemon cablin* essential oils and their components against *Culex pipiens* pallens. J. Asia Pac. Entomol. 15 (2012): 631-634.

[39] Tak, J.-H.; Jovel, E., and Isman, M. Contact, fumigant, and cytotoxic activities of thyme and lemongrass essential oils against larvae and an ovarian cell line of the cabbage looper, *Trichoplusia ni.* J. Pest Sci. (2015): 1-11.

[40] Bülow, N. and König, W. A. The role of germacrene D as a precursor in sesquiterpene biosynthesis: Investigations of acid catalyzed, photochemically and thermally induced rearrangements. Phytochemistry 55 (2000): 141-168.

[41] Isman, M. Insect antifeedants. Pestic Outlook 13 (2002): 152-157.

[42] Papachristos, D. P.; Karamanoli, K. I.; Stamopoulos, D. C., and Menkissoglu-Spiroudi, U. The relationship between the chemical composition of three

essential oils and their insecticidal activity against *Acanthoscelides obtectus* (Say). Pest Manage. Sci. 60 (2004): 514-520.

[43] Suresh, G.; Gopalakrishnan, G.; Wesley, S. D.; Pradeep Singh, N. D.; Malathi, R., and Rajan, S. S. Insect Antifeedant Activity of Tetranortriterpenoids from the Rutales. A Perusal of Structural Relations. J. Agric. Food Chem. 50 (2002): 4484-4490.

[44] Carpinella, M. C.; Defago, M. T.; Valladares, G., and Palacios, S. M. Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. J. Agric. Food Chem. 51 (2003): 369-374.

[45] Koul, O.; Walia, S., and Dhaliwal, G. Essential oils as green pesticides: Potential and constraints. Biopestic. Int. 4 (2008): 63-84.

[46] Seo, S.-M., *et al.* Larvicidal and acetylcholinesterase inhibitory activities of apiaceae plant essential oils and their constituents against *Aedes albopictus* and formulation development. J. Agric. Food Chem. 63 (2015): 9977-9986.

[47] Koul, O.; Smirle, M. J., and Isman, M. B. Asarones from *Acorus calamus* L. Oil. J. Chem. Ecol. 16 (1990): 1911-1920.

[48] Madaki, F. M. Studies on the effect of temperature, light and storage on the stability of neem (*Azadirachta indica* A. Juss) seeds oil extract. IOSR J. Pharm. Biol. Sci. 10 (2015): 46-50.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

APPENDIX

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Appendix A

Additional results

Table A1. The preliminary screening of the essential oils at 0.25% w/w against *S. litura.*

Family and scientific name	Common name (Thai name)	Plant part	% Antifeedant after 24 hours
Apiaceae			
Daucus carota L.	Carrot (แครอท)	seed	67.10±6.58[a]
Lamiaceae			
Ocimum basilicum L.	Sweet Basil (โหระพา)	leaf	68.16±12.80[a]
Myrtaceae			
Psidium guajava L.	Guava (ฝรั่ง)	leaf	44.97±9.37[c]
Citrus reticulata Blanco.	Mandarin orange (ส้มเขียวหวาน)	peel	61.04±9.89[b]
Zingiberaceae			
Hedychium coronarium J.KÖnig	White Ginger (มหาหงส์)	flower	66.91±7.23[a]
Zingiber cassumunar Roxb.	Phai (ไพล)	rhizome	65.82±8.61[b]

Note: Means in the column that had same letter are not significantly different at *P=0.05* (Duncan's test).

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Table A2. The preliminary screening of plant extracts at 0.25% w/w against *S. litura.*

Family and scientific name	Common name (Thai name)	Plant part	solvent	% Antifeedant after 24 hours
Annonaceae				
Melodorum fruticosum lour.	White cheeseood (ลำดวน)	flower	CH_2Cl_2	$58.08 \pm 10.14^{f,g,h,l}$
			CH_3OH	$60.65 \pm 9.706^{e,f,g}$
Araceae				
Acorus calamus L.	Sweet Flag (ว่านน้ำ)	rhizome	CH_2Cl_2	$73.05 \pm 9.56^{a,b}$
			CH_3OH	$65.18 \pm 9.81^{g,h,l,j}$
Arecaceae				
Areca catechu L.	Betel palm (หมาก)	fruit	CH_2Cl_2	$51.65 \pm 12.84^{g,h,l,j}$
			CH_3OH	$46.95 \pm 13.89^{j,k,l}$
Asteraceae				
Lactuca sativa L.	Lettuce (ผักกาด)	seed	CH_2Cl_2	$57.82 \pm 10.92^{e,f,g,h}$
			CH_3OH	$66.65 \pm 8.39^{b,c}$
Cucurbitaceae				
Momordica charantia L.	Bitter Cucumber (มะระขี้นก)	leaf	CH_2Cl_2	$54.46 \pm 8.62^{g,h,i,j}$
			CH_3OH	$50.47 \pm 11.15^{i,j,k,l}$
Guttiferae				
Garcinia mangostana L.	Mangosteen (มังคุด)	peel	CH_2Cl_2	$62.19 \pm 11.23^{c,d,e}$
			CH_3OH	$62.46 \pm 9.41^{c,d,e}$
Iridaceae				
Eleutherine americana Merr.*	Wan-hom-dang (ว่านหอมแดง)	bulb	CH_2Cl_2	$58.86 \pm 8.98^{e,f,g}$
			CH_3OH	$53.81 \pm 7.78^{g,h,l,j}$
Lamiaceae				
Ocimum gratissimum	Tree Basil (ยี่หร่า)	seed	CH_2Cl_2	$62.27 \pm 8.48^{c,d,e}$
			CH_3OH	$58.95 \pm 11.08^{e,f,g}$

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Liliaceae				
Dracaena loureiri Gagnep.	Jan-Dang (จันทน์แดง)	heart wood	CH_2Cl_2	51. 40± 5.81^{i,j,k}
			CH_3OH	54.77 ± 8.26^{g,h,l,j}
Meliaceae				
Xylocarpus granatum Koenig	Chinese Mangrove (ตะบูนขาว)	fruit	CH_2Cl_2	73.51 ± 3.93^{a}
			CH_3OH	66.42 ± 6.83 g,h,i
G,hPiperaceae				
Piper betle L.	Betel (พลู)	leaf	CH_2Cl_2	54.63 ± 11.22^{g,h,l,j}
			CH_3OH	49.16 ± 9.72^{j,k,l}
Piper sarmentosum Roxb.	Wildbetal Leafbush (ชะพลู)	fruit	CH_2Cl_2	65.35 ± 7.33^{g,h,i}
			CH_3OH	61.44 ± 7.85^{c,d,e}
Rutaceae				
Murraya paniculata (L.) Jack	Orange Jessamine (แก้ว)	leaf	CH_2Cl_2	70.50 ± 5.84^{a,b}
			CH_3OH	70.11 ± 5.10 ^{a,b}
Zanthoxylum limonella	Ma-Kan (มะแข่น)	tree	CH_2Cl_2	54.01 ± 10.53 ^{g,h,l,l}
			CH_3OH	54.56 ± 9.52^{f,g,h,i}
Sapindaceae				
Nephelium lappaceum L.	Rambutan (เงาะ)	seed	CH_2Cl_2	62.30 ± 8.74^{c,d,e}
			CH_3OH	40.35 ± 18.44^{m}
Sterculiaceae				
Mansonia gagei Drumm	Jan-Cha-Mod (จันทน์ชะมด)	heart wood	CH_2Cl_2	46.15± 46.17^{k,l}
			CH_3OH	52.71±11.69^{h,l,j}
Zingiberaceae				
Kaempferia galanga L.	Aromatic Ginger (เปราะหอม)	rhizome	CH_2Cl_2	64.86 ± 8.50^{b,c,d}
			CH_3OH	63.79 ± 8.43^{c,d,e}

Note: Means in the column that had same letter are not significantly different at *P=0.05* (Duncan's test).

Table A3 Antifeedant activity from separated seven fractions at 0.25% (w/w)

Fractions No.	% Antifeedant activity
XG-1	22.2±10.15
XG-2	24.59±10.91
XG-3	53.51±8.79
XG-4	48.96±9.08
XG-5	62.31±7.79
XG-6	59.34±9.32
XG-7	28.46±10.82

Table A4 The antifeedant activity of three compounds at different temperature after kept in 48 hours.

Temperature (°C)	*X. granatum* (CH_2Cl_2) at 0.25% (w/w)	xyloccensin K at 2 mM	Neem extract
4	74.31±7.78	56.46±9.93	92.42±3.76
RT	73.97±10.03	57.06±11.08	91.08±6.53
45	69.97±7.95	54.51±9.86	62.43±11.33
60	63.64±9.55	50.69±10.95	49.81±9.76

Table A5 The antifeedant activity of three compounds at different temperature after kept in 96 hour.

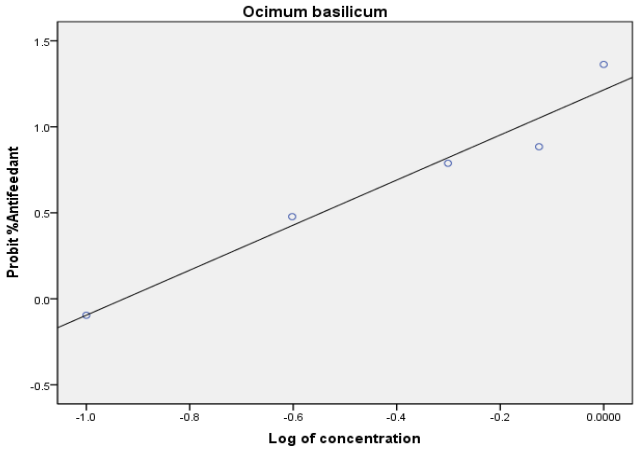
Temperature (°C)	*X. granatum* (CH_2Cl_2) at 0.25% (w/w)	xyloccensin K at 2 mM	Neem extract
4	73.59±8.69	55.50±6.04	91.92±9.87
RT	70.01±9.67	54.95±9.21	91.28±10.5
45	64.03±7.97	50.88±10.81	51.96±8.32
60	60.52±10.35	45.09±9.41	34.76±10.69

Table A6 % antifeedant activity decrease of three compounds after exposing to UV light 12 h.

Treatments	concentration	%antifeedant activity in dark	%antifeedant activity after exposing to UV light 12 h	%decrease
X. granatum (CH_2Cl_2)	0.25% (w/w)	75.18±7.88	73.39±4.84	2.38%
xyloccensin k	2 mM	58.07±8.37	56.52±7.27	2.67%
Neem extract	-	95.60±6.75	45.40±9.98	53.56%

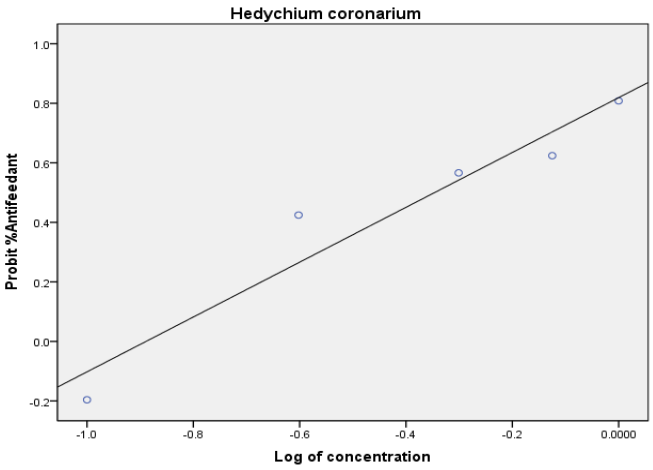
y = 2x + 1.5

R^2 = 0.954



y = 1.2x + 1

R^2 = 0.930



y = 1.5x + 1.2

R^2 = 0.942

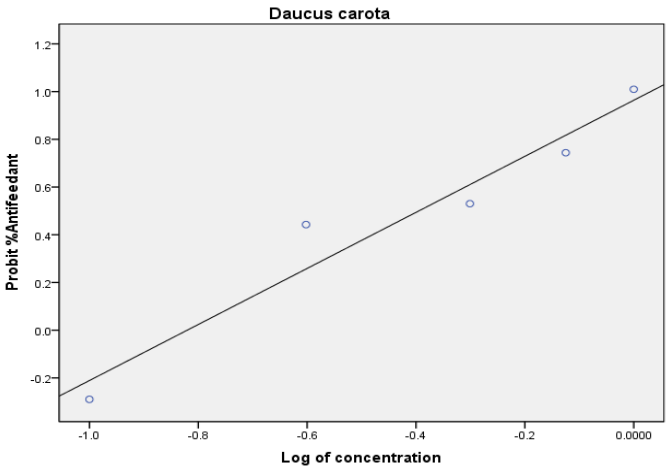
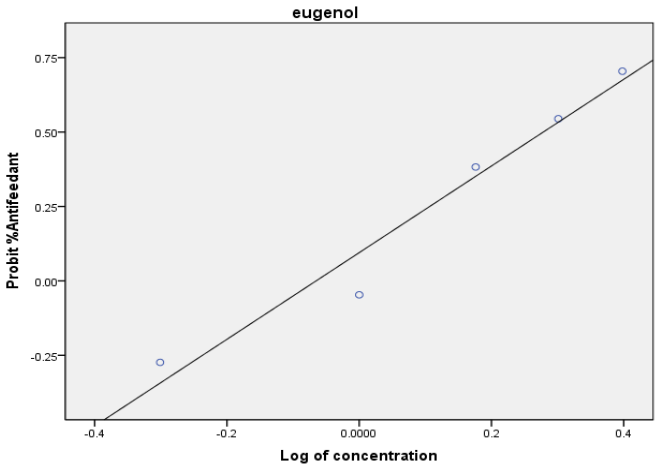


Figure A6 Linear regression of concentrations of three highest potent essential oils against *S. litura* after 24 h.

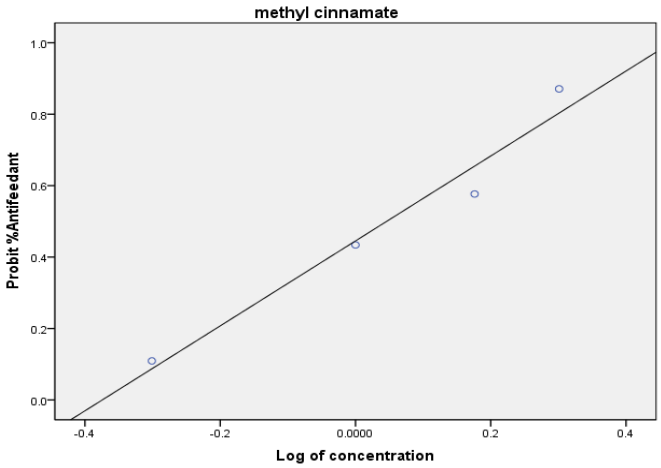
y = 1.5x + 0.2

R^2 = 0.960



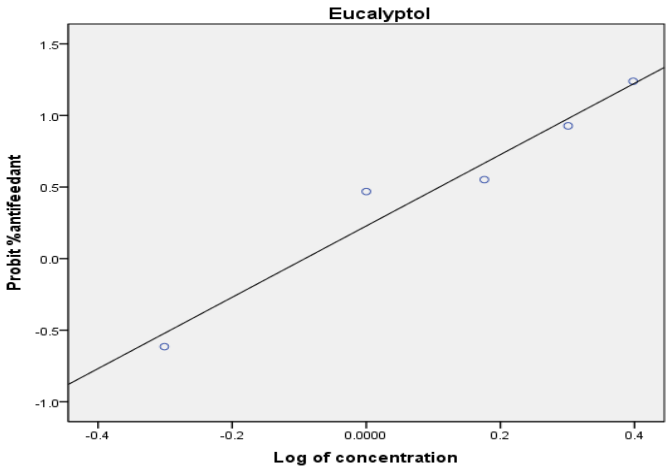
y = 1.25x + 0.5

R^2 = 0.962



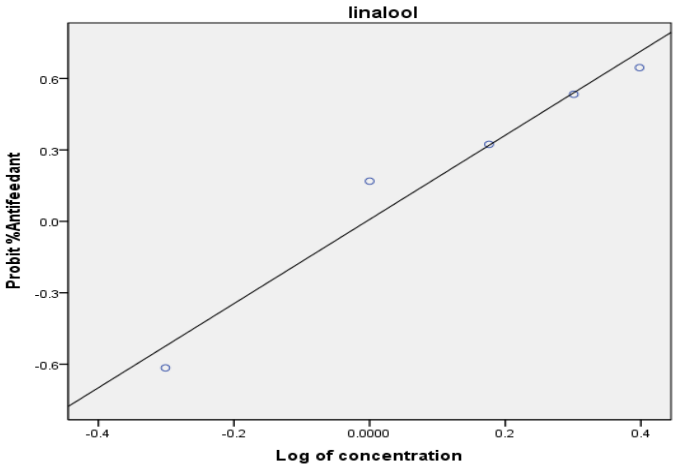
y = 3.125x + 0.25

R^2 = 0.958



y = 1.875x

R^2 = 0.961



y = 1.5x + 0.2

R^2 = 0.868

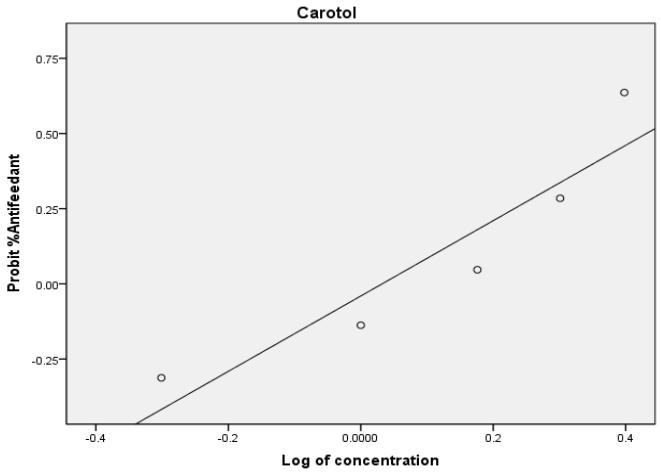


Figure A7 linear regression of concentrations of five commercial compound against *S. litura*

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

y = 1.25x + 1.25

R^2 = 0.915

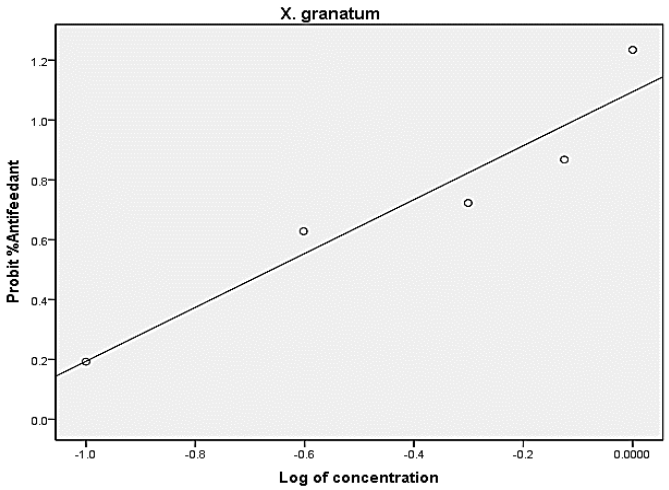


Figure A8 Linear regression of concentrations of *X. granatum* against S. litura after 24 h

y = 1.75x-0.1

R^2 = 0.985

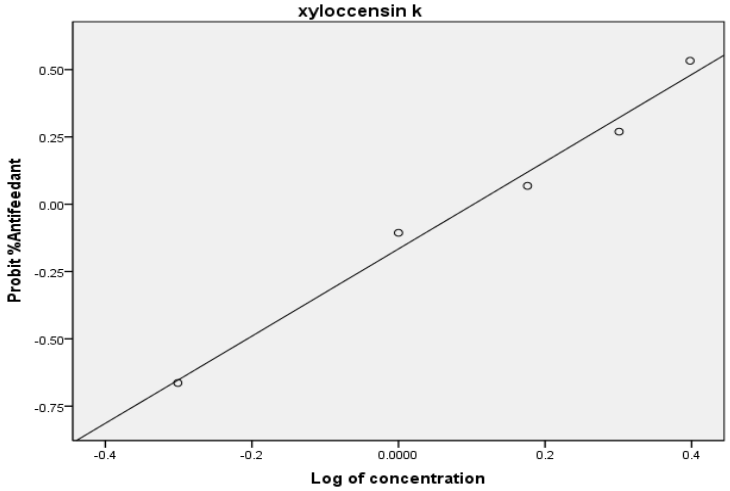


Figure A9 Linear regression of concentrations of xyloccensin K against *S. litura* after 24 h.

Appendix B

The preparing of artificial diet

Artificial diet formula for *S. litura*:

mung bean	150	g
Dried brewer's yeast	10	g
Methyl parahydroxy benzoic acid	2.5	g
Sorbic acid	1.5	g
Ascorbic acid	3	g
Casein	3	g
Choline chloride	0.5	g
Agar	14	g
40% Formalin	2	mL
Vitamin stock	10	mL
Distill water	750	mL

Vitamin stock formula:

Niacin	6	g
Calcium panthothenate	6	g
Thiamine (B1)	3	g
Riboflavin (B2)	3	g
Pyridoxine monohydrochloride	1.5	g
Folic acid	1.5	g
Biotin	120	mg
Vitamin B12 (Cyanocobalamin)	12	mg
Inositol	10	g
Choline chloride	25	g
Distill water	1	L

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Method:

- Soaked mung bean for 3-4 hour until mung bean be soft.
- Weight the chemical follow above mentioned.
- Put soaked mung bean, distill water 350 mL and all chemical except 40% Formalin and vitamin stock into the moulinex to blend for 10 minute. At the same time dissolve agar in distill water 400 mL that stand on hot plate.
- Add hot agar in the moulinex that have the mixed chemical and blend after that add 40% Formalin 2 mL and vitamin stock 10 mL. Then, pour the artificial diet into the box and leave it cool and harden. Keep the artificial diet in refrigerator.

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Appendix C

The NMR spectra

Nuclear magnetic resonance (NMR) of Compound 1 (Carotol) from *D. carota*

^{1}H and ^{13}C NMR analysis of compound 1 was performed in figure C1 and C2, respectively.

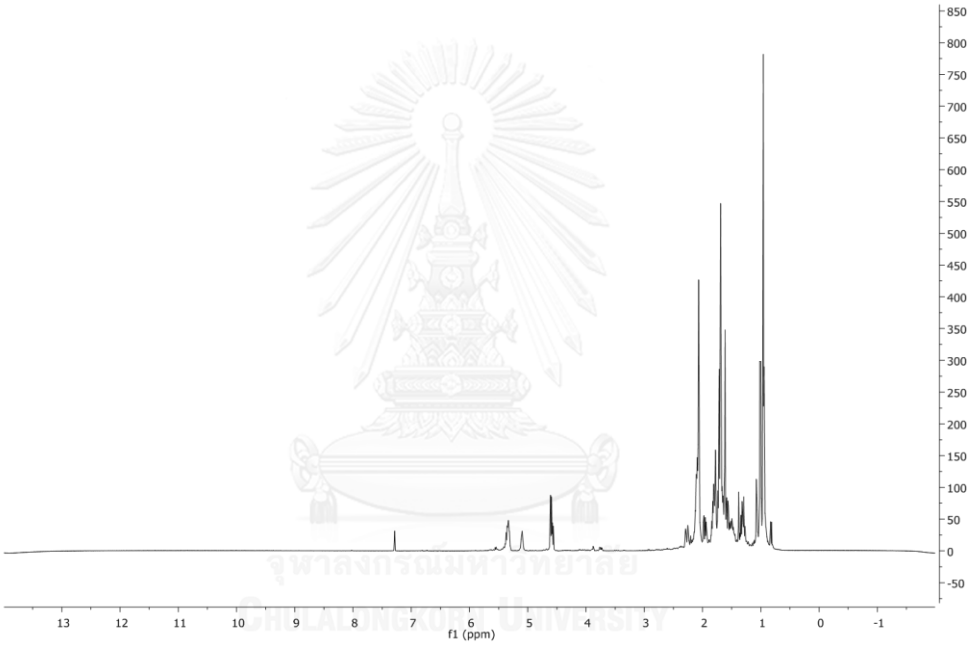


Figure C1 ^{1}H NMR analysis of carotol

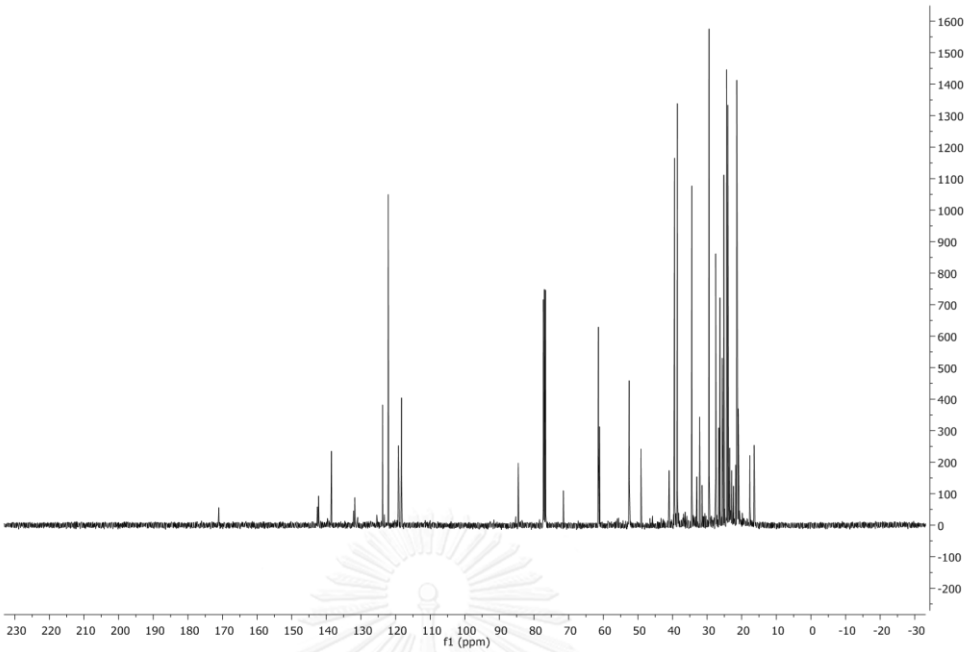


Figure C2 ^{13}C NMR analysis of carotol

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

Nuclear magnetic resonance (NMR) of Compound 2 (xyloccensin K) from *X. granatum*

^{1}H and ^{13}C NMR analysis of compound 2 was performed in figure C3 and C4, respectively.

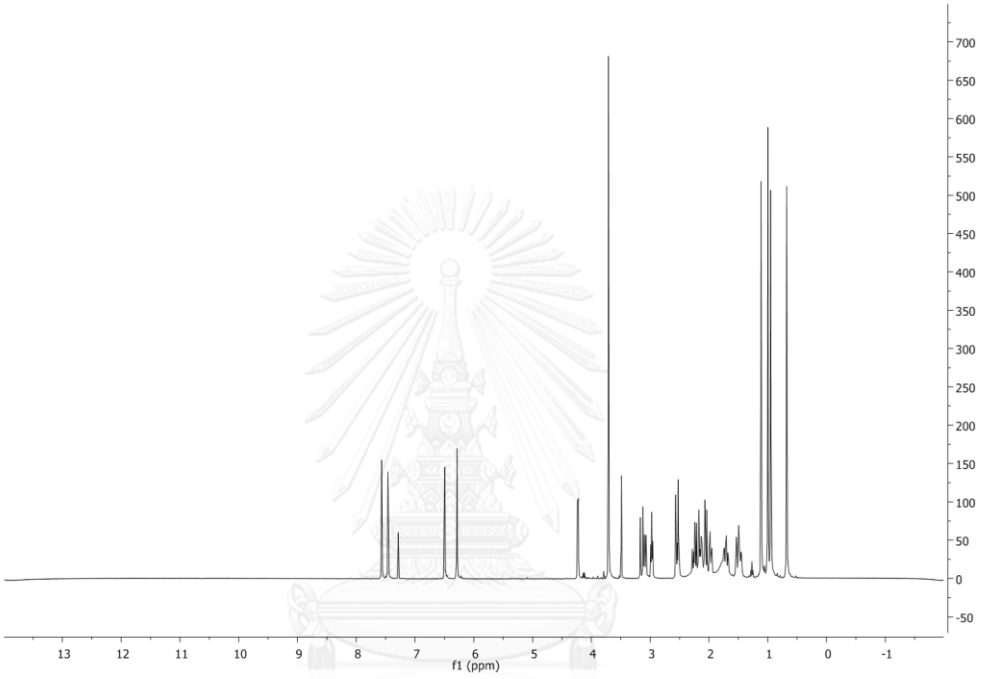


Figure C3 ^{1}H NMR analysis of xyloccensin K

Chulalongkorn University

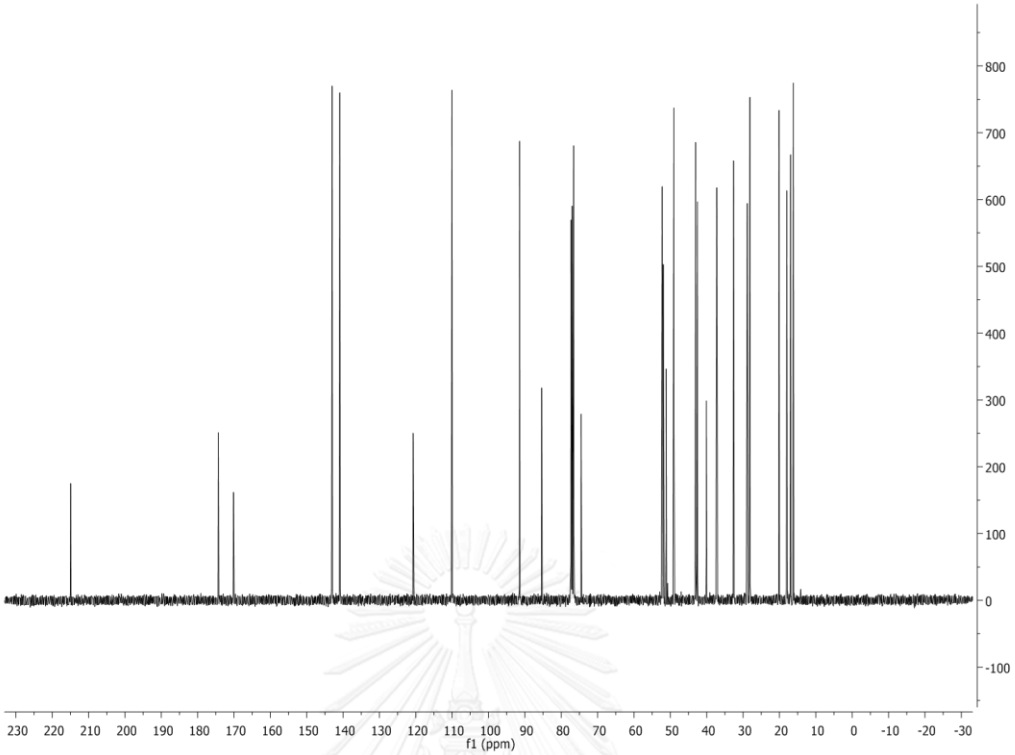


Figure C4 ^{13}C NMR analysis of xyloccensin K

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

VITA

Ms. Panee Nakthong was born on February 16, 1990 in Chonburi province, Thailand. She graduated with Degree of Bachelor of Science (Biology) from Faculty of Science, Chulalongkorn University, in 2012. She graduated a Master Degree of Science in Biotechnology in 2015 from program in Biotechnology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.