ฤทธิ์ฆ่าแมลงของสารสกัดจากสมุนไพรไทยต่อแมลงสาบสายพันธุ์เยอรมัน BLATTELLA GERMANICA

นางสาวสรญา แสนมาโนช

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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INSECTICIDAL ACTIVITY OF THAI BOTANICAL EXTRACTS AGAINST GERMAN COCKROACH, BLATTELLA GERMANICA (L.) (ORTHOPTERA: BLATTELLIDAE)

Miss Soraya Saenmanot

A Dissertation Submitted in Partial Fulfillment of the Requirements
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Thesis Title	INSECTICIDAL ACTIVITY OF THAI BOTANICAL EXTRACTS AGAINST GERMAN COCKROACH, BLATTELLA GERMANICA (L.) (ORTHOPTERA: BLATTELLIDAE)
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Field of Study	Medical Science
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สรญา แสนมาโนช : ฤทธิ์ฆ่าแมลงของสารสกัดจากสมุนไพรไทยต่อแมลงสาบสายพันธุ์เยอรมัน BLATTELLA GERMANICA (INSECTICIDAL ACTIVITY OF THAI BOTANICAL EXTRACTS AGAINST GERMAN COCKROACH, BLATTELLA GERMANICA (L.) (ORTHOPTERA: BLATTELLIDAE)) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. นพ.เผด็จ สิริยะเสถียร, 145 หน้า.

แมลงสาบสายพันธุ์เยอรมัน *Blattella germanica* มีความสำคัญทางการแพทย์และทางเศรษฐกิจที่สำคัญในประเทศไทย เนื่องจากเป็นพาหะที่สำคัญ สามารถนำเชื้อโรคต่างๆ เช่น ไวรัส แบคทีเรีย และโปรโตซัวติดต่อมาสู่มนุษย์ได้ วัตถุประสงค์ของการศึกษาครั้งนี้คือเพื่อศึกษาประสิทธิภาพของสารสกัดจากสมุนไพรไทยทั้ง 6 ชนิด ได้แก่ ดีปลี *(Piper retrofractum)*, หนอนตายอยาก *(Stemona tuberosa)*, หางไหลแดง *(Derris elliptica)*, ทองพันช่าง *(Rhinacanthus nasutus)*, กวาวเครือแดง *(Butea superba)* และเทียนข้าวเปลือก *(Foeniculum vulgare)* ต่อแมลงสาบสายพันธุ์เยอรมันในระยะต่างๆ ภายใต้สภาพห้องปฏิบัติการ

โดยสารสกัดจากสมุนไพรไทยทั้ง 6 ชนิดที่ระดับความเข้มข้นต่างๆ ได้แก่ 2, 4, 6, 8 และ 10 เปอร์เซ็นต์ (w/v) ได้นำมาทดสอบต่อแมลงสาบสายพันธุ์เยอรมันในระยะต่างๆ ได้แก่ ระยะตัวอ่อน ระยะตัวเต็มวัย และระยะตัวเมียที่มีไข่สุก โดยวิธีการหยด (Topical application method) นับจำนวนตายที่เวลา 1, 2, 4, 6 , 24 และ 48 ชั่วโมงหลังทำการทดสอบ นำข้อมูลมาวิเคราะห์ความแปรปรวนของข้อมูลโดยใช้ ANOVA และวิเคราะห์ความแตกต่างของค่าเฉลี่ยโดยใช้วิธี DMRT รวมทั้งวิเคราะห์ค่า Medium Lethal Dose (LD_{50}) โดยใช้วิธี Probit analysis นอกจากนี้ยังทำการวิเคราะห์องค์ประกอบหลักของสารสกัดจากสมุนไพรไทยที่ให้ประสิทธิภาพในการออกฤทธิ์ฆ่าแมลงสาบได้ดีที่สุด โดยการแยกสารสกัดด้วยเทคนิคคอลัมน์โครมาโตกราฟีและโครมาโตกราฟีแบบผิวบาง (TLC)

ผลการศึกษาปรากฏว่า สารสกัดจากดีปลีมีประสิทธิภาพในการออกฤทธิ์ฆ่าแมลงสาบสายพันธุ์เยอรมันในระยะต่างๆได้ดีที่สุด ในทุกช่วงเวลา โดยสารที่สกัดด้วยอะซิโตน ที่ระดับความเข้มข้นสูงสุดคือ 10 เปอร์เซ็นต์ (w/v) มีประสิทธิภาพในการกำจัดแมลงสาบสายพันธุ์เยอรมันทั้งในระยะตัวเต็มวัย (LD_{50} 2.61) และระยะตัวเมียที่มีไข่สุก (LD_{50} 1.97) ได้อย่างสมบูรณ์ (100 %) หลังการทดสอบ 6 ชั่วโมง และจากการแยกองค์ประกอบของสารสกัดดีปลีที่สกัดด้วยอะซิโตนด้วยวิธีโครมาโตกราฟีแบบผิวบาง (TLC) พบว่า Fraction ที่ 5 ซึ่งมีประสิทธิภาพในการออกฤทธิ์ฆ่าแมลงสาบสายพันธุ์เยอรมันได้ดีที่สุดนั้น มีองค์ประกอบหลักที่สำคัญคือพิเพอรีน (Piperine)

ดังนั้นสารสกัดจากดีปลีสามารถใช้เป็นอีกทางเลือกหนึ่งของสารสกัดที่ได้จากธรรมชาติซึ่งมีฤทธิ์ในการฆ่าแมลงมาใช้ในการควบคุมกำจัดแมลงสาบสายพันธุ์เยอรมัน ซึ่งราคาไม่แพง และปลอดภัยต่อมนุษย์และสิ่งแวดล้อม อย่างไรก็ตามฤทธิ์ฆ่าแมลงของสารสกัดจากสมุนไพรไทยในระดับภาคสนามและรูปแบบที่เหมาะสมที่จะนำมาใช้ยังคงต้องมีการศึกษาต่อไป

สาขาวิชา วิทยาศาสตร์การแพทย์ ลายมือชื่อนิสิต ....

ปีการศึกษา 2559 ลายมือชื่อ อ.ที่ปรึกษาหลัก ....

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SORAYA SAENMANOT: INSECTICIDAL ACTIVITY OF THAI BOTANICAL EXTRACTS AGAINST GERMAN COCKROACH, BLATTELLA GERMANICA (L.) (ORTHOPTERA: BLATTELLIDAE). ADVISOR: ASSOC. PROF. DR.PADET SIRIYASATIEN, M.D., 145 pp.

German cockroach, *B. germanica* is considered an important medical and economic pest in Thailand because they can carry many pathogens, such as bacteria, virus and protozoa to humans. The objective of this study was to evaluate the efficacy of extracts derived from six Thai botanicals, which including *Piper retrofractum, Stemona tuberosa, Derris elliptica*, *Rhinacanthus nasutus, Butea superba* and *Foeniculum vulgare* against various developmental stages of *B. germanica* under laboratory conditions.

Different concentrations of extract derived from six Thai botanical (2, 4, 6, 8 and 10%) (w/v) were used to exposure to various developmental stage including nymphs, adult and gravid females stage of *B. germanica* by topical application method. The mortality was assessed at 1, 2, 4, 6, 24 and 48 hours after the exposure. Data was statistically analyzed by using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). LD_{50} were calculated by the Probit analysis program. Moreover, the major component of Thai botanical extract that had highest insecticidal activity was analyzed by column chromatography and thin layer chromatography (TLC).

The results showed that Thai botanical extracts derived from *P. retrofractum* have the highest mortality on various developmental stage of *B. germanica* at every experimental time intervals. Moreover, the acetone extract of *P. retrofractum* at highest concentration (10% w/v) showed complete insecticidal activity (100%) against both adult (LD_{50} 2.61) and gravid females stages (LD_{50} 1.97) of *B. germanica* after 6 hours post exposure. The fraction 5 of acetone extract of *P. retrofractum* obtained from TLC analysis was showed highest efficacy against *B. germanica* and the major component was identified as piperine.

Therefore, extracts of *P. retrofractum* can be used as an alternative natural insecticide for the controlling of *B. germanica* which are inexpensive and safe for humans and the environment. However, insecticidal activity of the Thai botanical extracts against *B. germanica* in field environment and appropriate formulations of this extracts should be determined.

Field of Study:	Medical Science	Student's Signature	....
Academic Year:	2016	Advisor's Signature	....

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LIST OF ABBREVIATIONS

ºC	Degree Celsius
µg	Microgram
µl	Microlitre, 10^{-6} liter
cm	Centimetre
et al. et.	alii (latin), and others
g	Grams
kg	Kilograms
L	Liter
LD_{50} and LD_{90}	Lethal dose fifty: The dose causing mortality effects in 50% and 90% of the population of treated insects, respectively.
LT_{50} and LT_{90}	Lethal time fifty: The time required for 50% and 90% of the population of insects to die after treated to botanical insecticides, respectively.
mbar	Millibar
mg	Milligram
ml	Milliliter, 10^{-3} liter
pH	The negative logarithm of the concentration of hydrogen ions
RH	Relative humidity
w/v	weight by volume
w/w	weight by weight

CHAPTER I
INTRODUCTION

1.1 Background and rationale

Cockroaches are considered an important pest of medical and public health. The insects are believed to be vital indicators of hygiene because they can carry many pathogens, such as bacteria, virus and protozoa. Moreover, cockroaches are a contributing cause of allergies and asthma [1, 2]. Cockroaches are commonly found in homes, hospitals, hotels and restaurants [3]. In Thailand, there are more than ten species of cockroaches have been described, including the American cockroach *(Periplaneta americana),* Australian cockroach (*Periplaneta australasiae*) and German cockroach (*Blattella germanica*) [4-6]. However, *B. germanica* is considered an important medical and economic pest because they have a shorter generation time and higher fecundity than other cockroaches that makes it difficult to control.

In the past, naphthalene was a common chemical used as a cockroach repellent. However, it is hazardous to humans, it absorbed through inhalation, ingestion and dermal contact. Long term exposure to naphthalene can cause hemolytic anemia, liver damage and neurological disorder especially in infants [7]. Furthermore, conventional synthetic insecticides, such as pyrethroids, carbamates, and organophosphates are highly popular methods used for controlling cockroaches [8, 9]. However, these insecticides have several adverse effects including acute or chronic toxicity to humans and animals. They can also have an impact on food chain in the ecosystem. Moreover, the cockroach can develop resistance to these insecticides [10].

Due to the problems described previously, an alternative approach is to search for effective botanical extracts which are safe to humans and the environment.

Thailand is one of the Southeast Asian countries where plants are abundant in many parts. Several Thai botanicals have been used as natural insecticides for pest and insect control for a long time [11-13]. Advantages of using botanical extracts include the fact that they are more environmental friendly and less toxic compared to chemical products. Some plant species contain insecticidal or repellent substances, such as pyrethrin from *Chrysanthemum cinerariaefolium* (Pyrethrum), azadirachtin from *Azadirachta indica* (Neem) and nicotine from *Nicotiana tabacum* (Tobacco) [14-16]. Furthermore, there have been no reports of resistance by pests and insects against botanicals, therefore botanicals may be an alternative way for controlling cockroaches [17].

There are several reports that have described the effects of botanical extracts against cockroaches. For example, Arthur *et al.* (2001) and Peterson *et al*. (2002) showed that the essential oils extracted from *Mentha arvensis* (Mint) and *Nepeta cataria* (Catnip) had a repellency effect against *B. germanica* in laboratory conditions [18, 19]. In 2007, Thavara *et al*. demonstrated that the essential oils of *Citrus hystrix* (Kaffir lime) exhibited complete repellency (100%) against *P. americana* and *B. germanica* and also showed a high repellency of about 87.5% against the Harlequin cockroach (*Neostylopyga rhombifolia)* under laboratory conditions [20]. In addition, Ling *et al*. (2009) has stated that the essential oils of *Piper aduncum* (Spiked pepper) can have insecticidal activity against adults and nymphs stage of *P. americana* [21].

Moreover, Liu *et al*. (2011) has pointed out that the eight essential oils of Chinese medicinal herbs, *Angelica sinensis* (Dang gui), *Curuma aeruginosa* (Pink and blue ginger), *Cyperus rotundus* (Nut grass), *Eucalyptus robusta* (Swamp mahogany), *Illicium verum* (Star anise), *Lindera aggregate* (Chinese spice bush), *Ocimum basilicum* (Sweet basil) and *Zanthoxylum bungeanum* (Chinese prickly-ash) have shown repellency effect to nymphs of *B. germanica*. Essential oils from *I. verum* have shown strong repellence to the *B. germanica* [22]. Chang and Ahn (2001) reported that the methanol extracts of the fruit from *I. verum* also had insecticidal activity against adults of *B. germanica* by fumigation method [23]. In 2011, Tine *et al.* revealed that azadirachtin from the kernels of the neem tree, *A. indica* had insecticidal effect against newly emerged adults of the oriental cockroach (*Blatta orientalis)* under laboratory conditions [24].

Furthermore, the report by Yeom *et al*. (2012) showed that the essential oil from *Anethum graveolens* (Dill), *Carum carvi* (Caraway) and *Cuminum cyminum* (Cumin) had fumigant activity against the adult male *B. germanica*, while a contact toxicity testing the essential oils from *A. graveolens*, *C. carvi*, *C. cyminum* and *Trachyspermum ammi* (Ajwain) showed strong insecticidal activity against adult male and female *B. germanica* [3].

According to Sittichok *et al*. (2013), the essential oils derived from Thai plants, such as *Cymbopogon citatus* (Lemon grass), *C. nardus* (Citronella grass) and *Syzygium aromaticum* (Clove) had a repellency effect on the adult *P. americana*. Finally, the essential oils from *C. citatus* in ethyl alcohol showed the highest repellency (100%) among the tested repellents [25].

In addition, the fumigant toxicity of essential oils, such as *Allium sativum* (Garlic), *A. cepa* (Onion), *Thymus vulgaris* (Thyme), *Oregano dubium* (Carvacrol), *Rosemarinus officinalis* (Rosemary) and *Chenopodium ambrosioides* (Mexican tea) have tested positively insecticidal activity against adult *B. germanica* [26, 27].

Integrating all the evidence, several reports indicate that some botanicals contain insecticidal or repellent substances that could be used as alternative strategies for controlling cockroaches. However, most of the previous reports showed the repellent efficacy of essential oil against cockroaches, while only few indicated insecticidal efficacy of botanical crude extracts against cockroaches. Therefore, the present study aimed to determine the insecticidal activity of crude extracts from six Thai botanicals, such as *Piper retrofratum* (Java long pepper), *Stemona tuberosa* (Wild asparagus), *Derris elliptica* (Derris), *Rhinacanthus nasutus* (White crane flower), *Butea superba* (Red kwao krua) and *Foeniculum vulgare* (Fennel) against various stages of *B. germanica* under laboratory conditions. Moreover, Thai botanical extract which shows the highest insecticidal activity against various stage of *B. germanica* were investigate the main component by column chromatography and thin layer chromatography (TLC).

The benefits of the study were to obtain some Thai botanical extracts that may be used as natural insecticides for the controlling of *B. germanica* which are inexpensive and safe for humans and the environment.

Key words: German cockroach, *Blattella germanica*, Thai botanical extracts, Botanical insecticides

1.2 Research questions

Major Research question

1.2.1 Do the six Thai botanical extracts (*P. retrofractum*, *S. tuberosa*, *D. elliptica*, *R. nasutus*, *B. superba* and *F. vulgare*) have insecticidal effect against *B. germanica*?

Minor Research questions

1.2.2 Do the various developmental stages of *B. germanica* have different susceptibility to Thai botanical extracts?

1.2.3 Do the different solvents used for extraction have effect on the efficacy of extraction of Thai botanical extracts?

1.2.4 What is the main component of Thai botanicals which has highest insecticidal effects?

1.3 Objectives

1.3.1 To evaluate and compare efficacy of the six Thai botanical extracts (*P. retrofractum*, *S. tuberosa*, *D. elliptica*, *R. nasutus*, *B. superba* and *F. vulgare*) for toxicity against *B. germanica* by using a topical application bioassay under laboratory conditions.

1.3.2 To compare susceptibility of various stages of *B. germanica* to Thai botanical extracts.

1.3.3 To compare efficacy of different solvents on the extraction of Thai botanical extracts.

1.3.4 To investigate the main component of Thai botanical extracts which shows the highest insecticidal activity against various stages of *B. germanica* among six Thai botanicals.

1.4 Hypothesis

1.4.1 Thai botanical extracts have an insecticidal efficacy effect on *B. germanica*.

1.4.2 Thai botanical extracts have different effects on various developmental stages of *B.germanica*.

1.4.3 Different solvents used for extraction have different effects on the efficacy of extraction of Thai botanical extracts.

1.4.4 Thai botanical extracts contain a main component which has insecticidal activity.

1.5 Definitions of key terms

***B. germanica*:** One of the most important cockroaches in medical science and a public health problem because they are major causes of allergy, irritation, food spoilage and can carry many pathogens of infectious diseases, such as food poisoning, typhoid and pneumonia. Moreover, they are also an intermediate host of parasites, such as tapeworms.

Thai Botanical extracts: Thai botanicals are used in this study, including *P. retrofractum* (Java long pepper), *S. tuberosa* (Wild asparagus), *D. elliptica* (Derris), *R. nasutus* (White crane flower), *B. superba* (Red kwao krua) and *F. vulgare* (Fennel) were extracted by the maceration method.

Insecticidal activity: The contact toxicity of Thai botanical extracts against *B. germanica* were determined by using topical application bioassay.

Topical application bioassay: The method used to evaluate contact toxicity of Thai botanical extracts is via application of the botanical extract to the first abdominal segment of each cockroach by a microapplicator [8].

Death of *B. germanica*: Death of *B. germanica* were determined by observing the following criteria:

1. The inability to move when prodded with forceps.

2. The cockroaches lying on their back when touched on their abdomen with forceps and the insects not able to right themselves to normal posture within two minutes [8].

Lethal doses LD_{50} and LD_{90} values: The dose causing mortality effects in 50% and 90% of the population of treated insects, respectively**.**

Lethal time LT_{50} and LT_{90} values: The time required for 50% and 90% of the population of insects to die after treated to botanical insecticides, respectively.

1.6 Limitation of the study

The efficacy of crude extracts from six Thai botanicals, such as *P. retrofractum*, *S. tuberosa*, *D. elliptica*, *R. nasutus*, *B. superba* and *F. vulgare* was evaluated against nymph, adult and gravid stages of *B. germanica* by using topical application method under laboratory conditions. Moreover, Thai botanical extract which shows the highest insecticidal activity against various stage of *B. germanica* were investigate the main component by colum chromatography and thin layer chromatography (TLC).

1.7 Benefits and application of this study

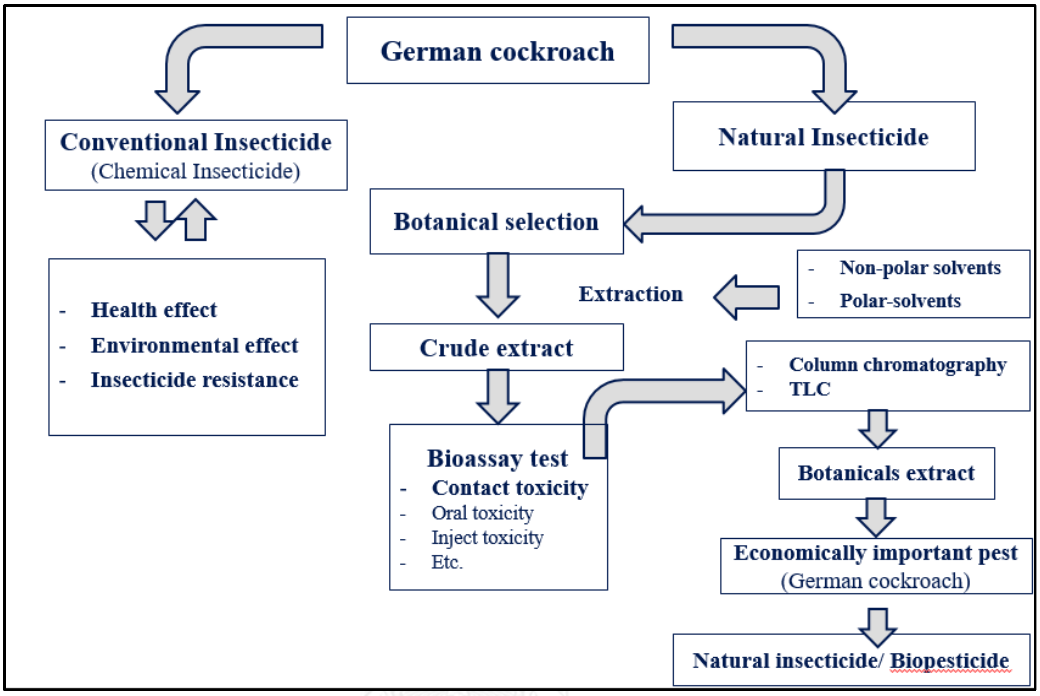
1.7.1 Advantages of using Thai botanicals as an alternative strategy for controlling *B. germinica* are that Thai botanicals are safe for the human and are also environmental friendly. Furthermore, there have been no reports of developed resistance by cockroaches against these botanicals.

1.7.2 Thai botanical extracts could be used for controlling *B. germinica* instead of chemical insecticides in areas where chemical insecticides are not appropriate.

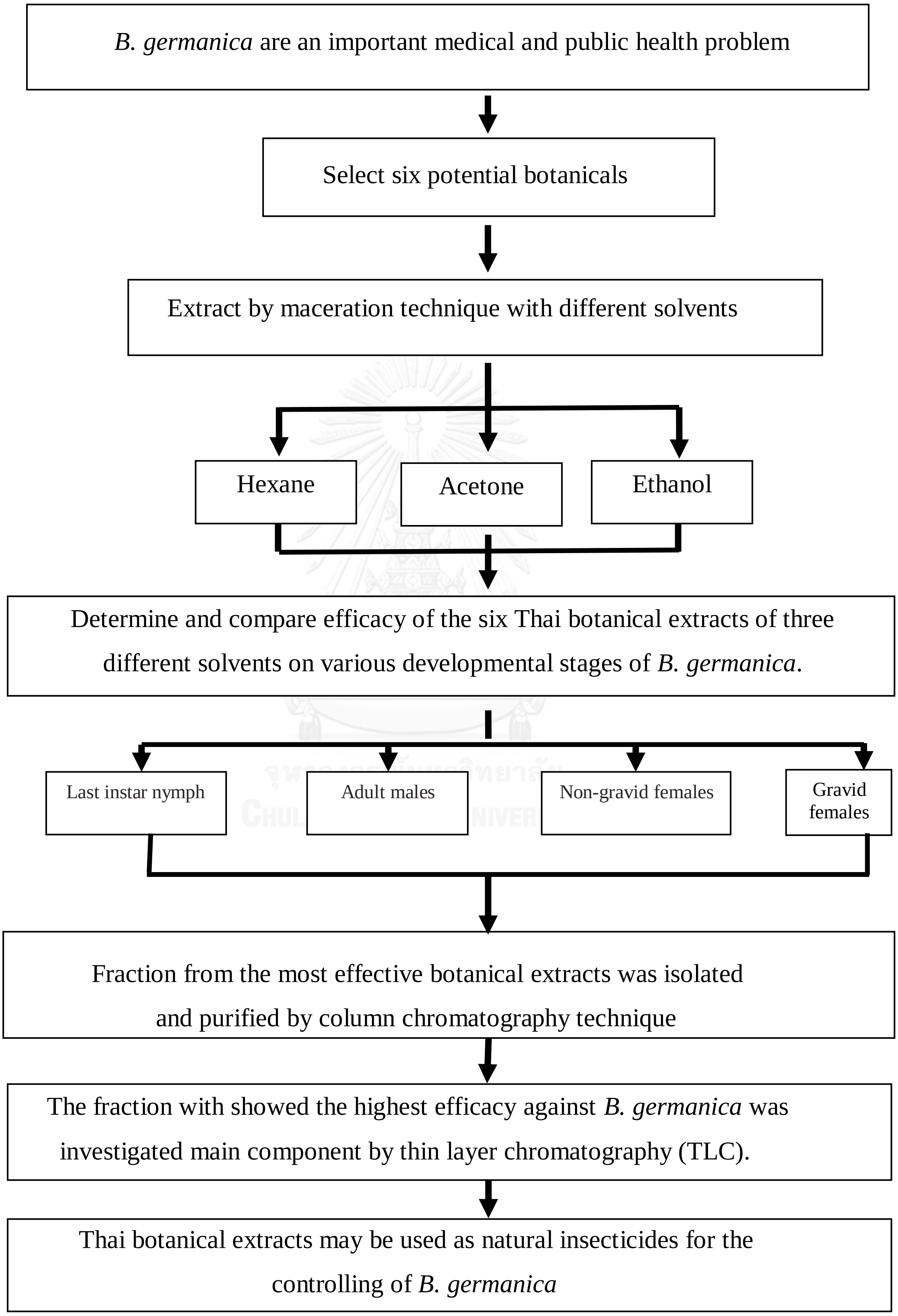
1.7.3 Thai botanical extracts could be used in integrated pest management programs (IPM) against *B. germinica*.

1.7.4 The extracts obtained from the plants that use in this study could be developed as commercial product that will be acceptable to a worldwide market.

1.8 Conceptual framework



1.9 Experimental study



CHAPTER II
LITERATURE REVIEW

2.1 German cockroach, *B. germanica*

B. germanica is the most common cockroach found in homes, hospitals, hotels, restaurants, supermarkets and other buildings. They are considered an important pest in medical and public health because they carry many pathogenic microorganisms of disease, such as dysentery, typhoid, pneumonia and they are also intermediate hosts of several kinds of parasites, such as tapeworms. Moreover, cast skins and feces of cockroaches are associated with allergies and asthma in humans which is the second most importance arthropod allergen after the house dust mites [28-30].

B. germanica is also widely distributed in urban areas. The survey report of Sriwichai *et al.* (2002) observed that the *B. germanica* was abundance in all types of buildings in Bangkok, Thailand, especially in grocery establishments [4].

Scientific classification

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Orthoptera

Family: Blattellidae

Genus: *Blattella*

Species: *germanica*

Common Name: German cockroach

External morphology

Egg stage

Egg case or ootheca is tiny, brown in color and about 1/4 inches in length. Each egg case contains 30-40 eggs (Fig. 1).

Figure 1 Egg cases (oothecae) of *B. germanica*

Nymph stage

Nymph stages are smaller than adult stages and have undeveloped wings and reproductive organs. They are also dark brown to black in color with a single light stripe running down the length of the pronotum. The number of molts to adult stage varies, but the most frequently reported number of molts is six and the stage between each molting is called an instar (Fig. 2).

Figure 2 Newly molted adult *B. germanica*

Adult stage

Adult stages are a flattened, oval shape and have spiny legs, long filamentous antennae and are brown to dark brown in color with two light stripes running through the length of the pronotum. Adult sizes are about 1/2- 5/8 inches in length and they have wings but rarely fly. In addition, male and female adult *B. germanica* can be separate as follows: the adult male has a thin body and a slender, posterior abdomen which is tapered, terminal segments of abdomen are visible, are not covered by tegmina and are light brown in color (Fig. 3). Adult females have a thick body, the posterior of the abdomen is round, the entire abdomen is covered by tegmina and they are also slightly darker than the adult male [27, 31, 32] (Fig. 4).

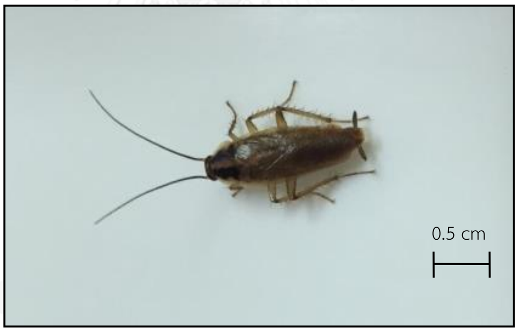
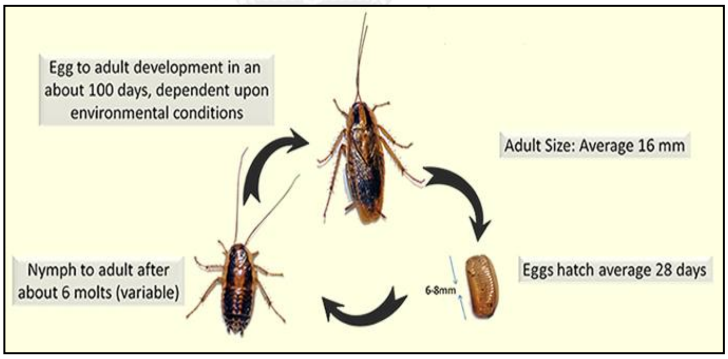


Figure 3 Adult male *B. germanica*

Figure 4 Adult female *B. germanica* with ootheca

Life cycle

B. germanica has three developmental stages called incomplete metamorphosis which consists of egg, nymph and adult. The duration of the life cycle is approximately three months. Female adult *B. germanica* are different from other cockroaches because they carry egg cases or oothecas which overhang from their abdomen until the eggs are ready to hatch. Eggs hatch within 1 month and the nymph develops to adult within 1-3 months depending on food, temperature and humidity. Adult female cockroaches live approximately 6-12 months and may produce four to eight egg cases during their lives, while males live approximately 6-9 months. *B. germanica* has a shorter generation time than other cockroaches as they produce more than 2 generations per year, therefore it is difficult to control them [33-36] (Fig. 5).



Source From: http://www.acvcsd.org/services/vectorid/cockroaches.htm

Figure 5 Life cycle of *B. germanica*

2.2 Method of cockroach control

Method of cockroach control can be categorized as follows:

2.2.1 Physical control

Physical control is the management of the environment to interfere with the cockroach population. For example, food can be stored in closed containers or sealed in plastic bags. Food garbage and water can be removed overnight and cabinets, sinks and refrigerators can be kept clean [37, 38].

2.2.2 Biological control

Biological control is the use of natural enemies to control the cockroach. The natural enemies of cockroaches include [37, 38] :

Predators

- Vertebrate predators, such as frogs, turtles and mice.
- Invertebrate predators, such as mites, roundworms and centipedes.

Parasites

- Parasitic wasps in genera *Evania*, *Hyptia* and *Tetrastichus* that lay their eggs in the egg case of some cockroaches, including the American cockroach (*P. americana*), Oriental cockroach (*B. orientalis*) and Brown-banded cockroach (*Supella longipalpa*).

Microbes

- Abamectin is a natural toxin produced by a soil-inhabiting fungus namely *Streptomyces avermitilis*. Abamectin acts as both a stomach poison when ingestion or a contact toxicity when attach to the cockroach body.

2.2.3 Chemical control

Chemical control can be categorized by source of product, such as synthetic chemicals and natural products.

2.2.3.1 Synthetic chemicals

Synthetic chemicals that are widely used in Thailand can be divided into four major chemical groups based on their chemical structures, such as chlorinated hydrocarbons or organochlorine, organophosphates, carbamates, syntheticpyrethroids and a special class, Insect Growth Regulators (IGRs).

Chlorinated hydrocarbons or Organochlorine groups

Chlorinated hydrocarbons or Organochlorine groups insecticides**,** such as dichlorodiphenyltrichloroethane (DDT). The mode of action for chemicals in this group is to interfere with the nervous system of the insect. Chemicals in this class are considered to be hazardous due to their long lasting effects. High levels of these insecticides can also cause harm to humans as they affect the central nervous system and are also carcinogen therefore they should not be used for cockroach control [38-41].

Organophosphate groups

Organophosphates include chlorpyrifos, diazinon, and acephate. The mode of action of organophosphates is to inhibit acetylcholynesterase (AChE) which is an important enzyme in the nervous system of insects. However, chemicals in this group can also affect the nervous system of the human body in the long term, thus these insecticides should not be used for cockroach control [38, 39, 42].

Carbamate groups

Carbamate groups are insecticides such as propoxur and carbaryl. These insecticides have a broad spectrum of activity and cause a rapid knockdown of insects. Moreover, the mode of action of chemicals in this group is to inhibit acetylcholynesterase (AChE) which is an important enzyme in the nervous system of insects. Furthermore, carbamate groups have similar effects on human as organophosphate groups, which is to affect the nervous system [38, 39, 42].

Synthetic pyrethroid groups

Synthetic pyrethroids emerged from natural pyrethrins which include permethrin, tetramethrin, deltamethrin, cypermethrin and cyfluthrin. Synthetic pyrethroids have fast knock-down activity especially against flying insects. The mode of action of synthetic pyrethroidsis to induce a toxic effect by acting on sodium channels in the nerves of insects. In addition, chemicals in this group can induce increased movement in insects that then increases exposure to the chemical and is sometimes referred to as the flushing effect. However, chemicals in the synthetic pyrethroid groups cause irritations to the human body, such as the eyes, skin, respiratory tract and are also highly toxic to fish and aquatic organisms [38, 41, 43-45].

Insect Growth Regulators or IGRs

Insect Growth Regulators (IGRs) are insecticides. The mode of action of IGRs is to interfere with regulatory pathways or inhibit specific biochemical pathways. They also affect processes essential for insect growth and development. The advantages of IGRs are that they are less toxic to humans and other non-target organisms. However, IGRs are often mixed with an insecticide because they cannot directly kill cockroaches on their own. The effects of IGRs have been observed on growth and development of nymphs and also have some effect to the fertility of adults [37, 38, 46].

2.2.3.2 Natural products

Natural products are chemicals from botanicals or herbs that can be extracted by using several methods. Examples of chemical extract from plants include pyrethrin from *C. cinerariaefolium* (Pyrethrum) and azadirachtin from *A. indica* (Neem) [24, 28, 47]. The advantages of using botanical products is that they are non-toxic to the users and are environmental friendly, thus botanicals are safer to use than synthetic insecticides.

2.3 Botanicals

Botanicals or herbs is defined as any part of a plant or plant product that can be used to prevent or treat a disease and also protect from insects [48, 49].

Botanicals for insect control

Botanicals are natural insecticides that are made from plant extracts. Todate, more than 2,000 types of plants in the world are found to have an effect for controlling insects in various functions, such as killing adult insects (adulticides), killing the larva (larvicides), repelling insects (repellent) and attracting insects (attractant). Moreover, botanicals can be divided according to the mode that they harm the insects, such as contact poisons or stomach poisons. In addition, botanicals can also be classified according to the type of effect on the insects, such as nervous poisons, respiratory poisons or antifeedants [50-52].

Chemical constituents of herbs

Several groups of phytochemicals are found in different parts and types of botanicals. Therefore, before use of a botanicals, It is necessary to know the active chemical constituents. The chemical constituents commonly found in botanicals are fat, proteins, carbohydrates, amino acids, enzymes and vitamins. Furthermore, other groups of chemical constituents commonly found in botanicals are shown in Table 1 [53-55];

Azadirachtin

Azadirachtin is a triterpenoid obtained from plants in the Meliaceae family, especially from *A. indica* (Neem). Azadirachtin shows multiple effects in the pest and insect including several species of cockroaches through ingestion or contact. Azadirachtin can cause antifeedant activity, growth regulation, oviposition repellency or attractancy and changes in biological fitness [3, 56-62].

Pyrethrin

Pyrethrin is an extract from *C. cinerariaefolium* (Pyrethrum). It is found to have an effect in controlling insects both on the contact toxicity and nervous system. They are considered to be an effective insecticide and pyrethrin is used in almost all insecticide products. In 1980, synthetic chemicals with properties similar to pyrethrin were synthesized and named as synthetic pyrethroids [43, 44, 63, 64].

Piperine

Piperine is an alkaloid substance which is obtained from some plants in the Piperaceae family, especially from *P. nigrum* (Black pepper) and *P. retrofratum* (Java long pepper). They are found to have an effect for controlling insects including insecticide and repellent activity. The mode action of piperine are affects the central nervous system of insect by inhibit acetylcholynesterase (AChE) which is an important enzyme in the nervous system of insects. [65, 66].

Rotenone or Rotenoids

Rotenone or Rotenoids is an alkaloid substance which is obtained from several plants, such as *D. elliptica* (Derris) and *Tephrosia purpurea* (Wild indigo). The mode of action involves the inhibition of electron transport in the mitochondria and blocking phosphorylation of ADP to ATP, thus inhibiting insect metabolism. This causes harm to the insects either by direct contact or ingestion. However, rotenone should be used carefully because it is highly toxic to fish and aquatic organisms [67, 68].

Stemofoline

Stemofoline is an alkaloid substance which is obtained from some plants particularly *S. tuberose* (Wild Asparagus). The mode of action of stemofoline is to inhibit acetylcholynesterase (AChE) which is an important enzyme in the nervous system of insects. Moreover, they can cause antifeedant activity in insects. [69-71].

Flavonoid

Flavonoid is an extract found in several plants, such as *B. superba* (Red kwao krua) and *T. purpuria* (Wild indigo). Acetylcholinesterase (AChE) is the main site of action of the flavonoids and they show insecticidal activity [72-74].

Saponin

Saponin is an extract found in several plants, such as *Surcuma longa* (Turmeric), *Croton tiglium* (Purging croton) and *Corchorus olitorius* (Tossa jute). The mechanisms of saponin are affects the neurotoxicity and can cause retardation in development and decreased reproduction of insect. They are found to have an effect in controlling insects via insecticide and repellent activity [75-77].

Cyanogenic glycosides

Cyanogenic glycosides can be found in several plants, such as *Manihot esculenta* (Cassava) and *Lantana camara* (Lantana). They have been discovered to have an effect in controlling insects via contact toxicity and antifeedants [78, 79].

Tannin

Tannin is an extract from some plants, such as *Annona squamosal* (Sugar apple) and *Acorus calamu*s (Mytle grass).They are found to have an effect in controlling insects via contact toxicity and antifeedants [80, 81].

Table 1 Chemical constituent of botanicals and their activity.

Chemical constituent	Activity	Main source	Report of the effect against insects and protozoa	Ref.
Azadirachin	An antifeedant, insect growth regulatory and cause reproductive effects	-*A. indica*	- *B. orientalis* - *Ae. aegypti* - *Cx.quinquefasciatus* - *An. stephensi* - *Musca domestica* - *Chilo auricilius*	[24] [82] [83] [84]
Pyrethrin	A contact toxicity which affects the nervous system	-*C.cinerariaefolium*	- *Tribolium castanum*	[85]
Piperine	An insecticide with repellent activity and against promastigotes of intracellular parasitic protozoa in vitro	- *P. retrofratum*	- *L. donovani* - *Ae. aegypti* - *Cx.quinquefasciatus*	[86] [87]
		-*P. nigrum*	- *Ae. aegypti* - *Ae. togoi* - *Cx. pipien pallens*	[88]
Retenone	Inhibits insect metabolism	- *D. elliptica*	- *Plutella xylostella* - *Ae. aegypti* - *Cx.quinquefasciatus* - *An. dirus* - *Mn. uniformis*	[89] [90]
Stemofoline	Antifeedant activity	- *S. tuberosa*	- *Ae. aegypti* - *Cx. quinquefasciatus* - *An. dirus* - *Mn. uniformis* - *Spodoptera exigua*	[90] [69]
Flavonoid	Insecticide activity	- *B. superba*	- *Ae. aegypti* - *Cx. quinquefasciatus*	[91]

Table 1 Chemical constituent of botanicals and their activity (Cont.)

Chemical constituent	Activity	Main source	Report of the effect against insects and protozoa	Ref.
Saponin	Insecticide and repellent activity	- *Croton tiglium*	- *Ae. aegypti* - *Cx. quinquefasciatus* - *An. dirus* - *Mn. Uniformis*	[90]
Cyanogenic glycosides	Contact toxicity and antifeedants	- *Manihot esculenta* - *Lantana camara*	- *Ae. aegypti*	[92, 93]
Tannin	Contact toxicity and antifeedants	- *A. squamosa*	- *An. stephensi* - *P. xylostella*	[94] [95]

2.4 Botanicals used in this study

Thailand has various types of botanicals, so this study has criteria to select botanical which induce insecticidal activity against *B. germanica*. The selection criteria of botanicals insecticides that used in this study are as follows;[96]

- The botanicals are commonly available in Thailand.
- The botanicals are widely distributed in Thailand.
- The botanicals could have additional uses (e.g. medicinal use)
- The botanicals have to contain chemical constituents that are assumed to have the effect against insects.
- Insecticidal activity has never been investigated with relation to *B. germanica*.

Thai botanicals that were used in this study consist of *P. retrofractum*, *S. tuberosa*, *D. elliptica*, *R. nasutus*, *B. superba* and *F. vulgare* (Table 2).

Table 2 List of Thai botanicals used in this study.

Scientific name	**Common name**	**Thai name**	**Family**	**Part used**	**Main components**
Piper retrofractum Vahl.	Java long pepper	Di-pli	Piperaceae	Fruit	Piperine
Stemona tuberosa Lour.	Wild Asparagus, Stemona	Non-tai-yak	Stemonaceae	Root	Stemofoline/ Rotenone
Derris elliptica Benth.	Derris, Tuba Root	Hang-lai-deang	Fabaceae	Root	Rotenone
Rhinacanthus nasutus (L.) Kurz..	White crane flower	Thong-phan-chang	Acanthaceae	Root	Flavonoid
Butea superba Roxb.	Red kwao krua	Kwao-khrua-deang	Papilionaceae	Root	Flavonoid
Foeniculum vulgare Mill.	Fennel	Tian-kao-pleuak	Umbelliferae	Seed	Flavonoid

2.4.1 *Piper retrofractum* Vahl.

Scientific name: *Piper retrofractum* Vahl.

Synonyms: *Piper chaba* Hunter.

Common name: Java long pepper

Thai name: Di-pli

Family: Piperaceae

Part used: Fruit (Fig. 6)

Botanical description:

The plant is a climber with flexuous branches, stem cylindrical, glabrous and prominent nodes. Fruits are broadly rounded, connate and agnate to stalk of bract with 1 seed. The fruits turn from green to red when they are ripe [49].

Used in traditional medical:

The fruit of *P. retrofractum* has been used in Thai traditional medicine and has been reported to be useful for treatment of bronchial asthma, bronchitis and muscle pain. The stem has also been reported to alleviate alley post-delivery pain in mothers and also to be useful against rheumatic pains and diarrhea. The root has been reported to be useful for asthma, bronchitis and tuberculosis [65, 97].

Chemical constituents:

The principle active constituents of the fruit are piperine, pipernonaline, dehydropipernonaline, piperlonguminine, piperundecalidine, while the main constituents of the essential oil of the fruits are β-caryophyllene, β-bisabolene, α-curcumene, pentagecane, caryophyllene oxide, heptadec-8-ene and heptadecane. The stem has been reported to contain piperine, β-sitosterol and piplartine. The stem bark has been reported to contain lignan and alkaloids, such as piper amine, 2, 4-

decadienoic acid piperidine, kusunokinin and pellitorine. Roots have been reported to contain alkamides, such as piperine, sylvatine, piplartine, piperlonguminine and β-sitosterol. Moreover, amino acids and monosaccharides have also been identified [86, 97-100]

Toxicity of *P. retrofractum* for controlling insects:

According to Chansang *et al.* (2005) who described that the aqueous extracts of *P. retrofractum* fruit showed larvicidal activity against *Ae. Aegypti* and *Cx. quinquefasciatus* mosquito larvae [87]. In addition, the hexane, ethyl acetate, methanol, and acetone extracts of *P. retrofractum* have anti-leishmania effects against promastigotes of *L. donovani* in vitro [86].

Figure 6 Fruit of *P. retrofractum*

2.4.2 *Stemona tuberosa* Lour.

Scientific name: *Stemona tuberosa* Lour.

Synonyms: *Stemona collinsae* Craib.

Common name: Wild Asparagus**,** Stemona

Thai name: Non-tai-yak

Family: Stemonaceae

Part used: Root (Fig. 7)

Botanical description:

The plant stem is woody around the base and its roots and tubers are cream in color. Leaves are usually broadly ovate or ovate to lanceolate and seeds usually have 5-8 appendages on the funicle or arillate.

Used in traditional medical:

The root of *S. tuberosa* has been used in Thai traditional medicine and has been reported to be useful against respiratory diseases, such as bronchitis and tuberculosis. Furthermore, the roots have been shown to prevent agriculture pests, domestic insects and also have insecticide activity. Thick roots can be used for treatment of malaria fever, chronic cough and cold. The leaves can be useful against night blindness [101-104].

Chemical constituents:

The principle active constituents of the root are stemofoline, 16, 17-didehydro-16(E)-stemofoline, rotenone, stemonine, stemonidine, stemonacetal, stemonal, stemonone, isostemonidine and tuberostemonine [69-71, 105].

Toxicity of *S. tuberose* for controlling insects:

Komalamisra *et al*. (2005) investigated the effect of ethanolic extracts from *S. tuberose* root on mosquito larvae. The result indicated larvicidal activity against 4 mosquito vector species, *Ae. aegypti, Cx. quinquefasciatus, An. dirus* and *Mn. uniformis* in laboratory conditions. Moreover, Sawangjit (2000) showed the efficacy of roots extracted of *S. tuberosa* against the 2nd instar beet armyworm *Spodoptera exigua* Hubn [69, 89].

Figure 7 Root of *S. tuberosa*

2.4.3 *Derris elliptica* Benth.

Scientific name: *Derris elliptica* Benth.

Synonyms: *Deguelia elliptica* (Roxb.) Taub., *Galedupa elliptica* Roxb., *Pongamia elliptica* Wall.

Common name: Derris, Tuba root

Thai name: Hang-lai-daeng

Family: Fabaceae

Part used: Root (Fig. 8)

Botanical description:

The plant is a branch densely brown, leaflets in 4-6 pairs and brown-silky in color, petals are bright red in color and fruit have a narrow-winged along one margin [67].

Used in traditional medical:

The root of *D. elliptica* have been used in Thai traditional medicine are reported to be useful to treat scabies and has been reported to have insecticidal properties in various insects such as fleas, ticks and lice. However, it is toxic to fish and aquatic organisms [106].

Chemical constituents:

The principle active constituents of the root are rotenone, resin, tannin, and saponin which are used to kill some insect pests especially those in the order Homoptera. Leaves have been reported to contain pipecolic acid, tubaic and β-tubaic acids, imino alcohol, 2, 5-dihydroxymethyl-3, 4 dihydroxypyrrolidine [67].

Toxicity of *D. elliptica* for controlling insects:

Visetson and Milne (2001) have investigated roots extracted from *D. elliptica* on the Diamondback Moth, *P. xylostella* larvae. The results showed that the rotenone from *D. elliptica* root extract has shown insecticidal activity against *P. xylostella* larvae (92). Furthermore, the ethanolic extracts from *D. elliptica* root showed larvicidal activity against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. dirus* and *Mn. uniformis* mosquito larvae [90].

Figure 8 Root of *D. elliptica*

2.4.4 *Rhinacanthus nasutus* (L.) Kurz.

Scientific name: *Rhinacanthus nasuta* (L.) Kurz.

Synonyms: *Rhinacanthus communis* Nees.

Common name: White crane flower

Thai name: Thong-phan-chang

Family: Acanthaceae

Part used: Root (Fig. 9)

Botanical description:

The plant is an undershrub, leaves are elliptic-lanceolate, acute, entire, lanceolate and glabrous. The flowers consist of the upper lip and the lower lip which are white in color. The fruit is club-shaped and contains 4 seeds [107].

Used in traditional medical:

The various parts of *R. nasutus* have been used in Thai traditional medicine are reported to be useful to treat many diseases, such as diabetes, hypertension, pulmonary tuberculosis, hepatitis and several skin diseases, such as eczema, tinea versicolor, ringworm, and herpes. Furthermore the leaves, roots and seeds are used as an antidote for snake bites [107-109].

Chemical constituents:

The principle active constituents of *R. nasutus*are the flavonoids, steroids, terpenoids, anthraquinones, lignans, naphthoquinone and analogues. Roots and leaves have been reported to contain a red resinous substance, rhinacanthin and frangulic acids. Flowers have been reported to contain flavone derivatives, flavanol-3-glucoside and rutin [107, 110].

Toxicity of *R. nasutus* for controlling insects:

Muthukrishnan and Pushpalatha (2001) discovered that the *R. nasutus* leaf extract are effective at decreasing the fecundity of the three mosquitoes, *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* and the hatchability of their eggs [111]. This is similar to Komalamisra *et al*. (2005) who reported that the ethanolic extracts from *R. nasuta*s showed larvicidal activity against *Ae. aegypti, Cx. quinquefasciatus, An. dirus* and *Mn. Uniformis* [90].

Source from: http://ran4u.com/

Figure 9 Root of *R. nasutus*

2.4.5 *Butea superba* Roxb.

Scientific name: *Butea superba* Roxb.

Common name: Red kwao krua

Thai name: Kwao-khrua-deang

Family: Papilionaceae

Part used: Root (Fig. 10)

Botanical description:

The branch of the tree has three leaves, flowers are yellow and orange in color and the long tuber root of the plant is buried under the ground. This plant reproduces through seed dispersal and tuber root [72].

Used in traditional medical:

The tuber and stem of the plant are used in medicines as it is believed to enhance human health and increase male sexual performance [72].

Chemical constituents:

The principle active constituents of the root are the flavonoid (3, 7, 3'-Trihydroxy-4'-methoxyflavone) and flavonoid glycoside (3 ,3'-dihydroxy-4'-methoxyflavone-7-O-β-D-glucopyranoside) [72].

Toxicity of *B. superba* for controlling insects:

According to Lapcharoen *et al.* (2005), *B. superba* extract exhibited larvicidal activity and insect growth regulators (IGRs) on late 3rd and early 4th instar larvae of *Ae. Aegypti* and *Cx. quinquefasciatus* [91].

Figure 10 Root of *B. superba*

2.4.6 *Foeniculum vulgare* Mill.

Scientific name: *Foeniculum vulgare* Mill.

Synonyms: *Foeniculum dulce (Miller.), Foeniculum officinale, Foeniculum capillaceum Gilib* and *Anethum foeniculum* L.

Common name: Fenel

Thai name: Tian-kao-pleuak

Family: Umbelliferae

Part used: Seed (Fig. 11)

Botanical description:

The plant is tall and glabrous, leaves are either tri or tetrapinnate with very narrow, linear or subulate segments. Flowers are small and yellow in color, fruits are about 6 mm. long and oblong-ellipsoid [112].

Used in traditional medical:

The seed of *F. vulgare* has been used in Thai traditional medicine and has been found to be useful for gastrointestinal disorders, such as indigestion, flatulence and colic pain. The leaves can be used as a diuretic and the roots can be used as a purgative. Moreover, the essential oils of these plants have been reported to have antimicrobial activity [112].

Chemical constituents:

The principle active constituent of the fruits and leaves is the flavonoid. The essential oils have been reported to contain estragole, fenchone, anethone, coumarin, xanthotoxin, bergapten, psoralen, scoparone and vanillin [112, 113].

Toxicity of *F. vulgare* for controlling insects:

The fruit of *F. vulgare* has showed insecticidal activities against adults of three coleopteran stored-product insects, including *Sitophilus oryzae*, *Callosobruchus chinensis* and *Lasioderma serricorne* by using direct contact application and fumigation methods [114].

Figure 11 Seed of *F. vulgare*

CHAPTER III
MATERIALS AND METHODS

3.1 Experimental place

This research was carried out at the laboratory of the Parasitology Department, Faculty of Medicine, Chulalongkorn University, Department of Materials Science, Faculty of Science, Chulalongkorn University and the Department of Plant Pest Management Technology, Faculty of Agricultural technology, King Mongkut's Institute of Technology Ladkrabang.

3.2 Materials and methods

3.2.1 Botanicals

Source of botanicals

The thrive roots of *S. tuberosa*, *D. elliptica*, *R. nasutus* and *B. superba*, fruits of *P. retrofractum* and seeds of *F. vulgare* were purchased from a traditional herbal medicine store, Chao-Krom-Poe, Chinatown, Bangkok, Thailand. The botanicals then were identified at Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

Preparation of extracts

The botanical materials were washed throughly with distilled water, cut into pieces (size<2 cm), dried in the shade and ground into fine powder using an electric blender. The fine powders (1kg) were extracted with 1,000 ml of three different solvents with increasing polarity: hexane, acetone and ethanol by maceration at room temperature (30±2ºC) for 3 days with frequent agitation. The resultant liquid was filtered by Whatman filter paper no.1. The solvents were removed by evaporation using a rotary evaporator under the following conditions (Table 3) [115, 116]:

Table 3 Solvents and their condition.

Solvents	Vacuum pressure	Water bath temperature
Hexane	360 mbar	55°C
Acetone	556 mbar	55°C
Ethanol	175 mbar	55°C

The crude extracts were weighed and kept at room temperature (30±2ºC) for drying and were stored in a brown bottle, protected from light and kept in a refrigerator at 4 ºC until use.

Preparing the stock solution and concentrations

Standard stock solutions were prepared at 25% (w/v) by dissolving 10 g of extracts in 10 ml of 2% Tween-20 in 10 ml of distilled water. From the stock solution, different concentrations, such as 2, 4, 6, 8 and 10% were prepared by dissolving in 2% Tween-20 in distill water (1:1) and these solutions were used for the topical application bioassay test [117].

3.2.2 Cockroaches

Source of Cockroaches

B. germanica were obtained from Biology and Ecology Section, Medical Entomology Group, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand.

Rearing of *B. germanica*

B. germanica were reared according to the standard protocols of the Biology and Ecology Section, National Institute of Health, Thailand. Cockroaches were reared in plastic containers (30×30×30 cm) under an ambient temperature (27-30 ºC) , humidity (70-90% RH) and photoperiod of 12:12 h light: dark cycle. The upper inside surface of the rearing container were smeared with petroleum jelly to prevent the cockroaches from escaping [8, 20, 118] (Fig. 12).

The cockroaches were fed with dry dog food and water provided *ad libitum* according to Habes *et al*. (2006) [119] (Fig. 12). Last instar nymph (3-4 weeks), adult males, non-gravid females (age 6-8 weeks) and gravid females (female with egg case) of *B. germanica* were employed for toxicity testing under laboratory conditions.

Figure 12 Rearing of German cockroaches

3.2.3 Topical application bioassay

Mortality bioassays were undertaken according to the standard method described by McDonald *et al.* (1996) with modification by topical application [120]. A group of 10 unsexed nymphs, gravid females and adult cockroaches (five males and five non-gravid females) were used for each concentration according to Lee *et al*. (1996), Liu *et al.* (2011), Pai *et al.* (2005) and Tunaz *et al*. (2009) [8, 10, 22, 26]. Cockroaches were anesthetized shortly (<5 min) with carbon dioxide to facilitate handling. Each Thai botanical extracts at a different dose (2, 4, 6, 8 and 10%) (w/v) were used for the toxicity test and 2 µl of each concentration were topically applied on the first abdominal segment of each cockroach with a hand microapplicator [3, 8, 121] (Fig. 13-14).

Cockroaches in the control group were tested with water that is mixed with 2% Tween-20 (1:1) (2 µl per insect) (124). All cockroaches were kept under the same conditions and mortality was assessed at 1, 2, 4, 6, 24 and 48 hours after treatment. The death of the German cockroach was determined by observing the following criteria: 1.The inability to move when prodded with forceps. 2. The cockroaches lying on their back when touched on their abdomen with forceps and the insects not able to right themselves to normal posture within two minutes [8, 29]. Each experiment was repeated four times.

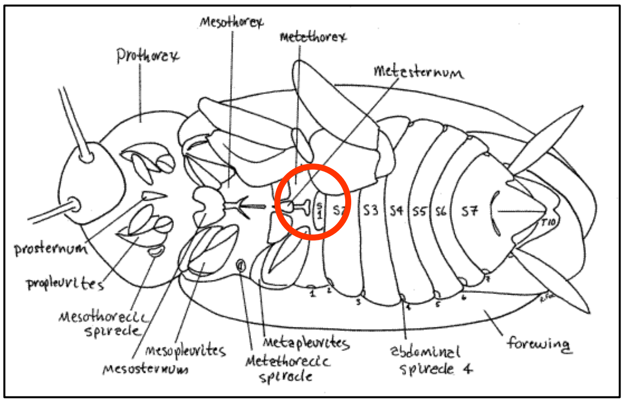


Figure 13 First abdominal segment of cockroach

Figure 14 Hand microapplicator

3.2.4 Main component of botanicals extracts

This study, Thai botanical extract which shows the highest insecticidal activity against various stage of *B. germanica* among six Thai botanicals is acetone extract of *P. retrofractum*. Moreover, among three stage including nymph, adult and gravid female, the nymph stage were the most tolerant. Therefore, the acetone extract of *P. retrofractum* was isolated and purified the component by column chromatography over silica gel with toluene per ethyl acetate as 70 per 30, volume by volume were used as a mobile phase and 10 milliliter of each fractions was collected [71, 122-125]. After that, each fraction was analyzed by Thin Layer Chromatography (TLC).

The fractions with a same TLC pattern were combined and each fraction was tested against nymph stage of *B. germanica* which showed the most tolerant stage in this study under laboratory condition. The fraction with showed the highest efficacy against nymph stage was investigated main component by two dimension development Thin Layer Chromatography (TLC) [126, 127].

3.3 Data analysis

Yield (%)

The percentage yield of each botanical crude extract with different solvents was calculated as follow:

$$\%\ \text{yield (w/w)} = \frac{\text{Weight of crude extract (g)}}{\text{Weight of raw materials (g)}} \times 100$$

Mortality rate (%)

The percentage mortality was calculated as follows:

$$\text{Mortality rate (\%)} = \frac{\text{Total dead cockroaches}}{\text{Total cockroaches in the experiment}} \times 100$$

If the % mortality rate of the control groups occurs within 5% and 20%, the percentage mortality should be adjusted by using Abbott’s formula as follows:

$$\text{Mortality rate (\%)} = \frac{\text{Death rate of testing group} - \text{Death rate of controlled group} \times 100}{100 - \text{Death rate of controlled group}}$$

If the control mortality rate is over 20%, the test was discarded and repeated.

Dose mortality

The data obtained was statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). All differences were considered significant at $p \leq 0.05$. The LD_{50} and LD_{90} which is the dose causing mortality effect in 50% and 90% of the treated cockroaches in the topical application tests were calculated by the Probit analysis program. Moreover, the LT_{50} and LT_{90} which is the time required for 50% and 90% of the population of insects to die after treated to botanical insecticides, respectively were also calculated by the Probit analysis program [31].

3.4 Ethical consideration

The present study was approved by the animal research ethics committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (IRB. 26/2558).

CHAPTER IV
RESULTS

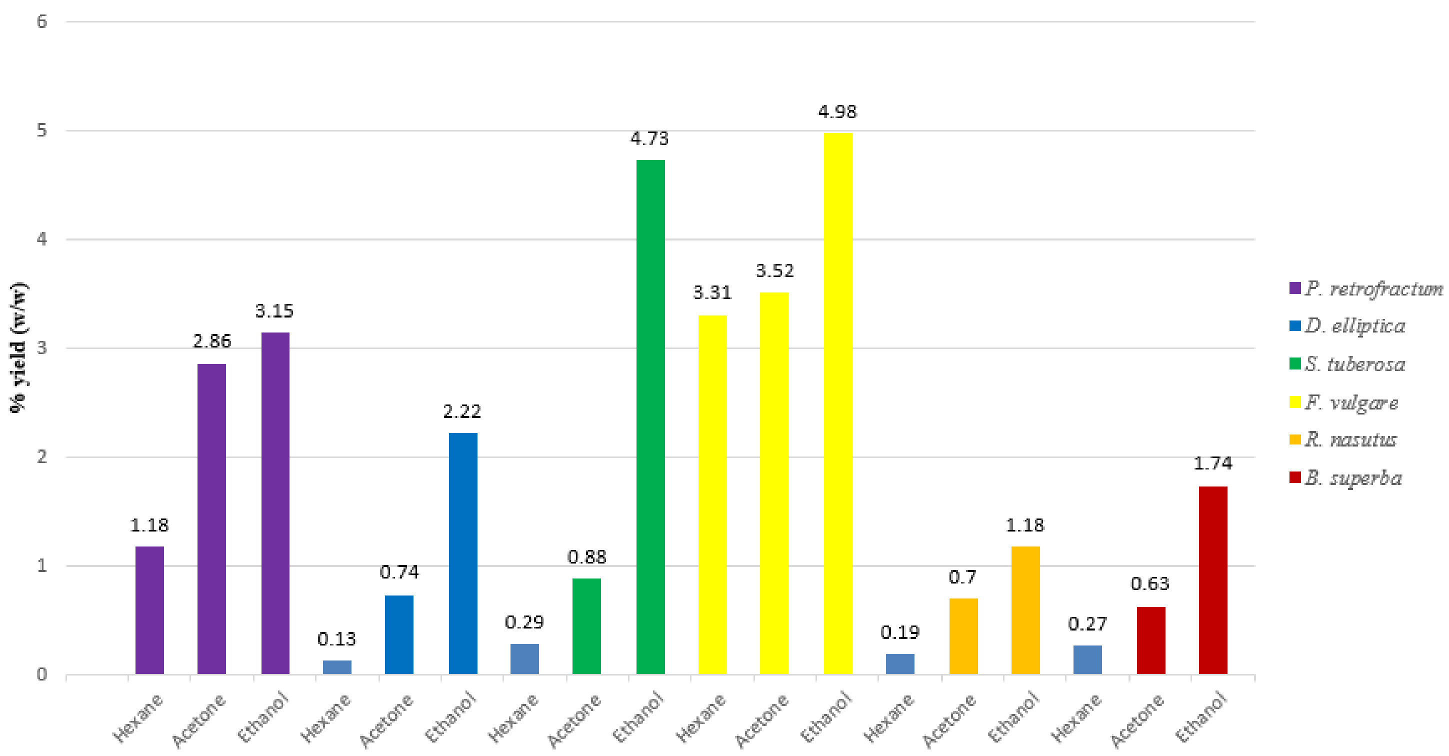
4.1 Effect of solvent types on the yield of Thai botanical

One kilogram of each botanical was used for this study and the yield (%) when using different type of solvent with increasing polarity: hexane, acetone and ethanol by maceration technique were calculated and physical characteristic were showed in Table 4.

Table 4 Percent yield of each Thai botanical with various solvents used.

Type of Botanical	Solvent	% yield (w/w)	Physical characteristic
P. retrofractum	Hexane	1.18	Red-brown and liquid
	Acetone	2.86	Red-brown and crystal
	Ethanol	3.15	Red-brown and sticky
D. elliptica	Hexane	0.13	Green-brown and sticky
	Acetone	0.74	Dark-brown and sticky
	Ethanol	2.22	Dark-brown and sticky
S. tuberosa	Hexane	0.29	Yellow-green and liquid
	Acetone	0.88	Dark-brown and sticky
	Ethanol	4.73	Dark-brown and sticky
F. vulgare	Hexane	3.31	Dark-green and liquid
	Acetone	3.52	Dark-green and liquid
	Ethanol	4.98	Dark-green and liquid
R. nasutus	Hexane	0.19	Dark-cork and sticky
	Acetone	0.70	Dark-cork and sticky
	Ethanol	1.18	Dark-cork and sticky
B. superba	Hexane	0.27	Yellow-green and crystal
	Acetone	0.63	Light-brown and crystal

Figure 15 Histogram showing the percent yield (w/w) of each Thai botanical with various solvents used.



As shown in the table and graph above, the study exhibited that the percent yield and appearances of the extract was found to be different dependent on the type of solvent used. One kilogram of each botanical was used for this study to obtain the yield as follows: *P. retrofractum*, the ethanol extract was the highest yield (3.15%), followed by the acetone extract and hexane extract (2.86% and 1.18%), respectively. The ethanol extract of *D. elliptica* was highest yield (2.22%), followed by the acetone extract and hexane extract (0.74% and 0.13%), respectively. Similar to the ethanol extract of *S. tuberosa*, was highest yield (4.73%), followed by the acetone extract and hexane extract (0.88% and 0.29%), respectively (Table 4 and Fig. 15)

Moreover, *F. vulgare*, the ethanol extract was the highest yield (4.98%), followed by the acetone extract and hexane extract (3.52% and 3.31%), respectively. The ethanol extract of *R. nasutus* was the highest yield (1.18%), followed by the acetone extract and hexane extract (0.70% and 0.19%), respectively. In addition, *B. superba*, the ethanol extract was the highest yield (1.74%), followed by the acetone extract and hexane extract (0.63% and 0.27%), respectively.

Therefore, when considering the effect of solvent types on the yield of Thai botanical, it could be seen that all of six Thai botanical, the ethanol extract was highest yield, followed by the acetone extract and hexane extract respectively. Moreover, the ethanol and acetone extracts were also found higher yield than hexane extract in all of six Thai botanical. Nevertheless, the yield of hexane extract from *D. elliptica* (0.13%), *S. tuberose* (0.29%), *R. nasutus* (0.19%) and *B. superba* (0.27%) showed very low of yield. Therefore, in this study these four Thai botanical from the hexane extract were not selected to insecticidal activity test on *B. germanica* cockroach.

4.2 Insecticidal activity of Thai botanical extract to the *B. germanica*

Thai botanical extract including *P. retrofractum, D. elliptica, S. tuberosa, F. vulgare*, *R. nasutus* and *B. superba* under various solvent (hexane, acetone and ethanol) and different dose (2, 4, 6, 8 and 10%) (w/v) were used for the insecticidal activity test on various stages of *B. germanica* (nymph, adult and gravid female) by topical application test under laboratory condition. In addition, the percentage mortality of *B. germanica* was daily observed and recorded at 1^{st}, 2^{nd}, 4^{th}, 6^{th} 24^{th} and 48^{th} hours post experiment. The results of the study were as follows:

4.2.1 Insecticidal activity of Thai botanical extract to the nymph stage of *B. germanica*

All of six Thai botanical extracts could be used to induce mortality of nymph stage of *B. germanica* under laboratory condition. The *P. retrofractum* extract at the concentration 10% w/v exhibited the highest efficacy against nymph stage of *B. germanica*, caused 92-97 % mortality at 6 hours post experiment, followed by *S. tuberose* and *D. elliptica* caused 47% and 45% mortality, respectively. While 12-25 %, 15-20% and 5-10% mortality induced by *B. superba*, *F. vulgare* and *R. nasutus*, respectively, were showed at the same concentration. Moreover, the *P. retrofractum* extract lead to significantly higher mortality rates ($P<0.05$) when compared to those induced by other extract. However, all of six Thai botanical extracts cannot induce complete mortality against nymph stage of *B. germanica* in this study (Table 5, Fig. 16).

Furthermore, *P. retrofractum* extract exhibited highest toxicity against nymph stage with LD_{50} value ranged from 3.46 % w/v to 4.50 w/v and LD_{90} value ranged from 6.53 % w/v to 8.94 % w/v, followed by *S. tuberose* extract with LD_{50} value ranged from 9.26 % w/v to 9.81 % w/v and LD_{90} value ranged from 15.69 % w/v to 7.30 % w/v, and *D. elliptica* extract with LD_{50} value ranged from 10.04 % w/v to 10.79 % w/v and LD_{90} value ranged from 19.51 % w/v to 22.09 % w/v, respectively; whereas *F. vulgare, B. superba* and *R. nasutus* were the less toxic botanical extract with LD_{50} value ranged from 13.80 % w/v to 17.60 % w/v, 14.90 % w/v to 18.50 % w/v and 19.54 % w/v to 23.12 % w/v and LD_{90} value ranged from 21.11 % w/v to 27.17 % w/v, 23.15 % w/v to 29.34 % w/v and 29.39 % w/v to 38.64 % w/v, respectively (Table 5, Fig. 16).

In addition, when considering the solvent extract of the six Thai botanical, our results showed that the acetone extract of *P. retrofractum, S. tuberosa* and *F. vulgare* bright about the higher efficacy against nymph stage of *B. germanica* when compared to those solvent extract by the ethanol and hexane extract. On the other hand, the ethanol extract of *D. elliptica, R. nasutus* and *B. superba* caused the highest efficacy against nymph stage of *B. germanica,* followed by the acetone extract (Table 5, Fig. 16).

4.2.2 Insecticidal activity of Thai botanical extract to the adult stage of *B. germanica*

The efficacy of the six Thai botanical extracts against adult stage of *B. germanica.* This result found that all of six Thai botanical extracts could be used to induce mortality of adult stage of *B. germanica* under laboratory condition. The highest mortality rate (92-100%) of adult stage of *B. germanica* occurred when concentration 10 % w/v of *P. retrofractum* extract were used, followed by *D. elliptica* and *S. tuberose* caused 52-60 % and 52-57% mortality at 6 hours post experiment, respectively; whereas *R. nasutus*, *B. superba* and *F. vulgare* caused 11%, 12-13% and 13-16% mortality, respectively. Moreover, the *P. retrofractum* extract caused significantly higher mortality rates ($P<0.05$) when compared to those induced by other extract (Table 6, Fig. 17).

According to the Table 6, *P. retrofractum* extract exhibited highest toxicity against adult stage with LD_{50} value ranged from 2.61 % w/v to 4.00 % w/v and LD_{90} value ranged from 5.01 % w/v to 7.98 % w/v, followed by *D. elliptica* extract with LD_{50} value ranged from 8.52 % w/v to 8.86 % w/v and LD_{90} value ranged from 14.85 % w/v to 15.53 % w/v, and *S. tuberose* extract with LD_{50} value ranged from 8.64 % w/v to 9.11 % w/v and LD_{90} value ranged from 14.87 % w/v to 14.94 % w/v, respectively; while *R. nasutus*, *B. superba* and *F. vulgare* were the less toxic botanical extract with LD_{50} value ranged from 11.61 % w/v to 11.87 % w/v, 12.09 % w/v to 13.03 % w/v and 13.02 % w/v to 16.96 % w/v and LD_{90} value ranged from 14.99 % w/v to 15.59 % w/v, 16.24 % w/v to 17.73 % w/v and 17.53 % w/v to 25.51 % w/v, respectively

Furthermore, when considering the solvent extract of the six Thai botanical, this results found that the acetone extract of *P. retrofractum*, *F. vulgare* and *B. superba* induce the higher efficacy against adult stage of *B. germanica* when compared to those solvent extract by the ethanol and hexane extract; while the ethanol extract of *D. elliptica*, *S. tuberose* and *R. nasutus* showed the higher efficacy against adult stage of *B. germanica* when compared to the acetone extract. However, only acetone extract of *P. retrofractum* at concentration 10% (w/v) exhibited complete insecticidal activity (100%) against adult stage of *B. germanica* at 6 hours post experiment (Table 6, Fig. 17).

4.2.3 Insecticidal activity of Thai botanical extract to the gravid stage of *B. germanica*

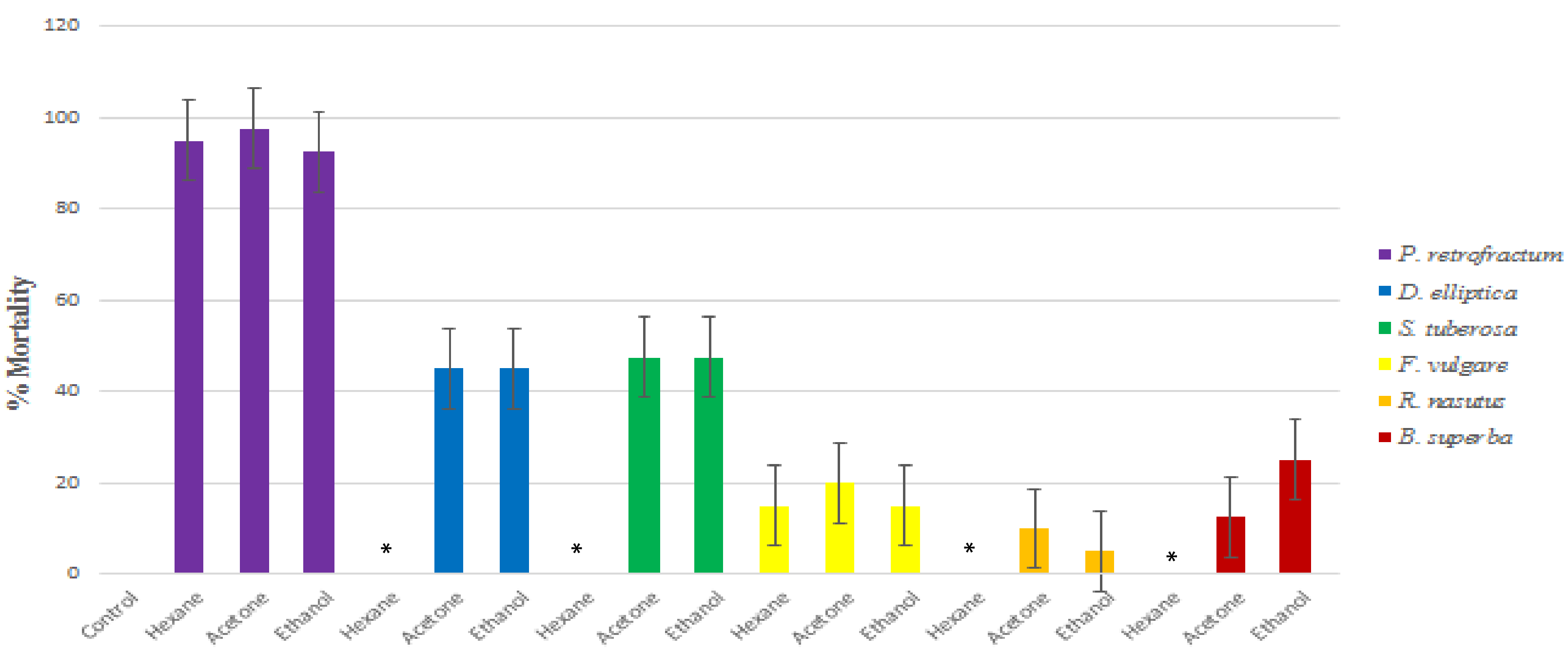
The extracts from six Thai botanical that used in this study exhibited insecticidal efficacy against gravid stage of *B. germanica*. Especially, the *P. retrofractum* extract induced complete mortality (100%) of gravid stage of *B. germanica* at 6 hours post experiment when concentration 4%, 6%, 8% and 10% w/v were used, followed by the concentration 10% w/v of *D. elliptica* and *S. tuberose* resulted in 75-77% and 65-70% mortality, respectively; whereas the extract of *B. superba*, *F. vulgare* and *R. nasutus* resulted in 42-47%, 30-47% and 30-35% mortality, respectively, showed at the same concentration. Moreover, the *P. retrofractum* extract caused significantly higher mortality rates ($P<0.05$) when compared to those induced by other extract. (Table 7, Fig. 18)

When looking at the table 7, it is evident that, *P. retrofractum* extract exhibited highest toxicity against gravid stage with LD_{50} value ranged from 1.97 % w/v to 2.14 % w/v and LD_{90} value ranged from 2.65 % w/v to 2.83 % w/v, followed by *D. elliptica* extract with LD_{50} value ranged from 6.17 % w/v to 6.32 % w/v and LD_{90} value ranged from 12.79 % w/v to 13.84 % w/v, and *S. tuberose* extract with LD_{50} value ranged from 6.38 % w/v to 7.19 % w/v and LD_{90} value ranged from 13.54 % w/v to 14.47 % w/v, respectively; whereas *B. superba*, *F. vulgare* and *R. nasutus* were less toxic botanical extract against gravid stage with LD_{50} value ranged from 9.83 % w/v to 9.87 % w/v, 9.35 % w/v to 14.90 % w/v and 10.40 % w/v to 11.61 %

w/v and LD_{90} value ranged from 14.65 % w/v to 15.83 % w/v, 14.63 % w/v to 25.63 % w/v and 15.52 % w/v to 18.57 % w/v, respectively.

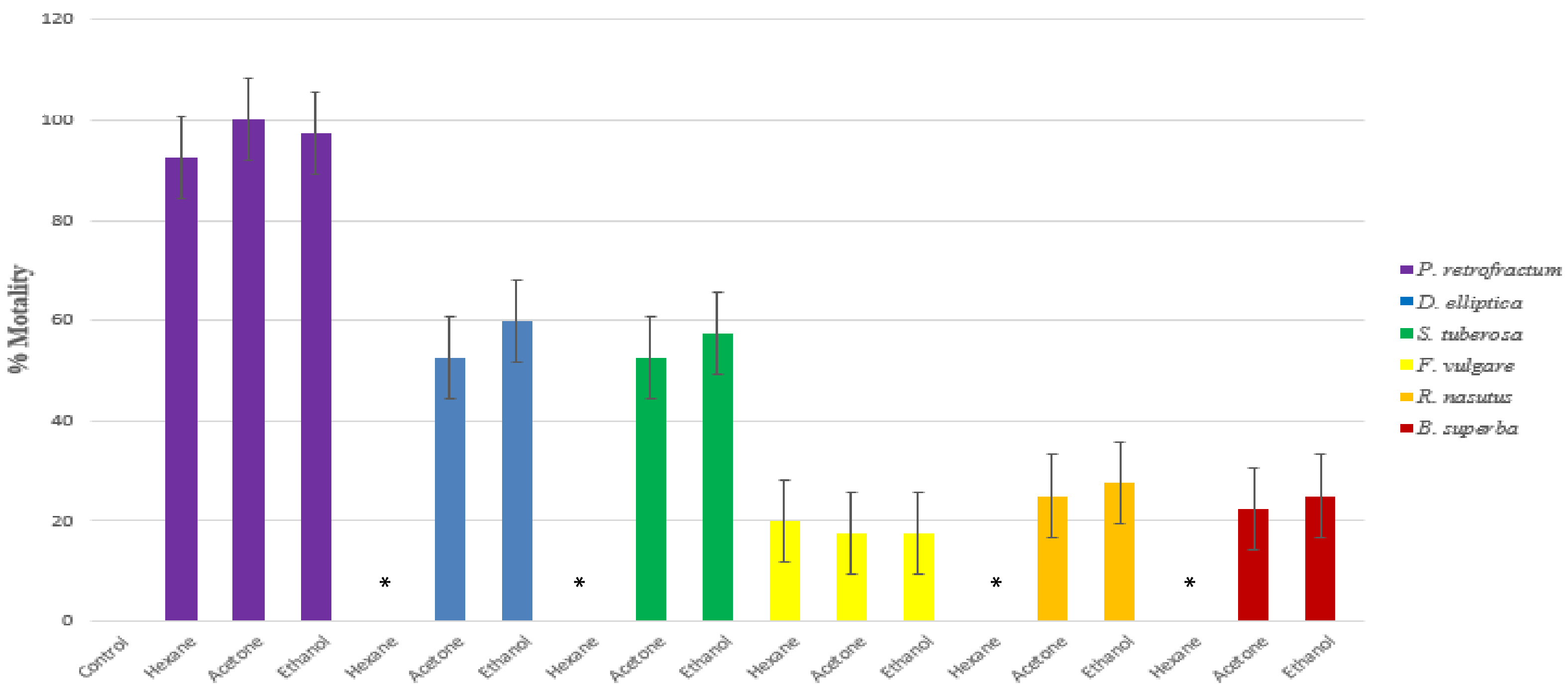
In addition, when considering the solvent extract of the six Thai botanical, this results found that the acetone extract of *P. retrofractum* and *F. vulgare* caused higher efficacy against gravid stage of *B. germanica* when compared to those solvent extract by the ethanol and hexane extract. However, the ethanol extract of *D. elliptica*, *S. tuberose*, *R. nasutus* and *B. superba* lead to the highest efficacy against gravid stage of *B. germanica*, followed by the ethanol extract. Furthermore, all solvent extract of *P. retrofractum* induced complete mortality of gravid stage of *B. germanica* at 6 hours post experiment. (Table 7, Fig. 18).

Figure 16 Histogram showing the percentage mortality of Thai botanical extract at concentration 10% (w/v) on nymph stage of *B. germanica* at 6 hour post experiment.



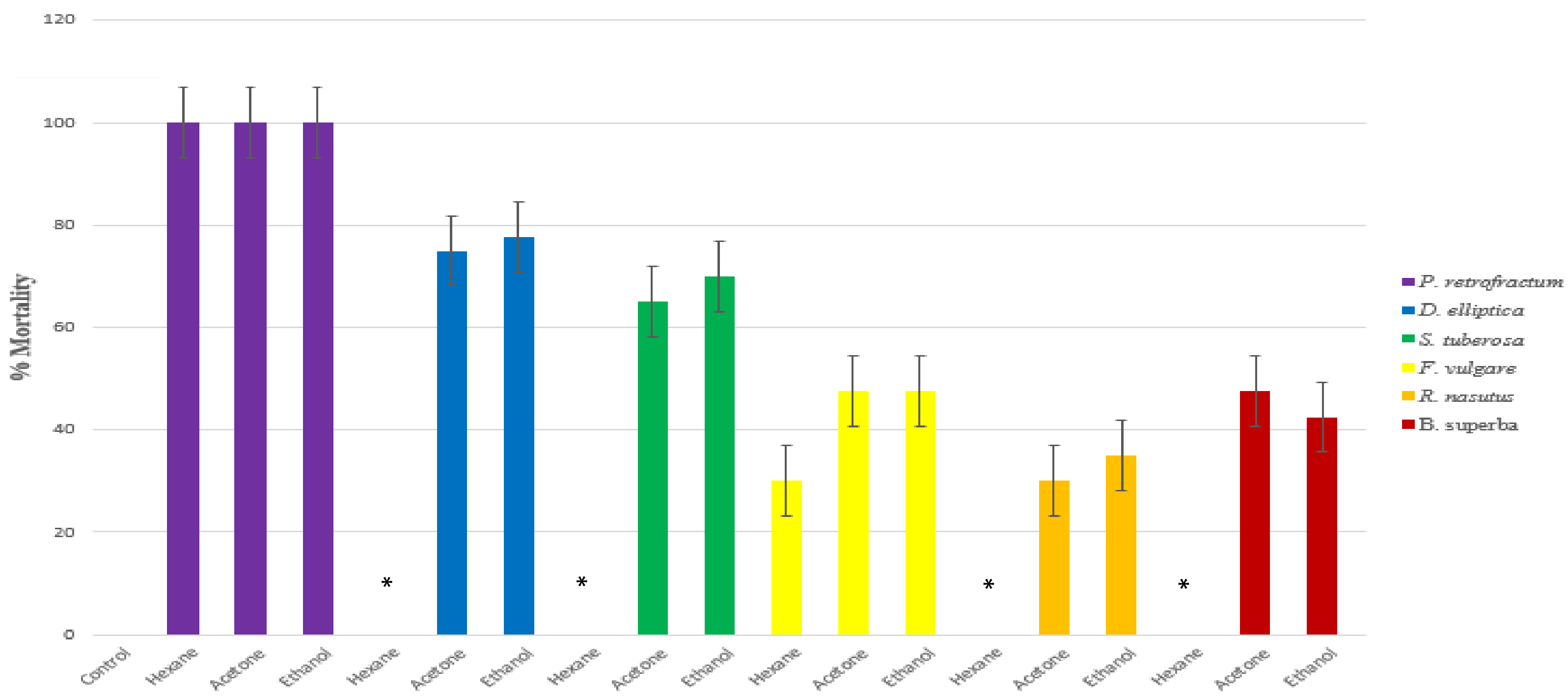
*Percentage yield (w/w) of hexane extract had very low.

Figure 17 Histogram showing the percentage mortality of Thai botanical extract at concentration 10% (w/v) on adult stage of B. germanica at 6 hour post experiment.



*Percentage yield (w/w) of hexane extract had very low.

Figure 18 Histogram showing the percentage mortality of Thai botanical extract at concentration 10% (w/v) on gravid stage of B. germanica at 6 hour post experiment.



*Percentage yield (w/w) of hexane extract had very low.

Table 5 Comparison of percentage mortality of nymph stage in different type of botanical at different solvent and levels of concentration**,** 6 hours post experiment.

Types of herbs	Solvent	% Mortality (6 hours post experiment) Concentration (% w/v)						LD_{50} (% w/v)	LD_{90} (% w/v)
		Control	2%	4%	6%	8%	10%		
P. retrofractum	Hexene	0.00 ± 0.00^{c}	47.50 ± 0.96^{Ab}	42.50 ± 0.96^{Ab}	42.50 ± 0.50^{Ab}	95.00 ± 1.00^{Aa}	95.00 ± 0.58^{Aa}	4.50	8.94
	Acetone	0.00 ± 0.00^{c}	45.00 ± 0.58^{Ab}	42.50 ± 0.96^{Ab}	95.00 ± 0.58^{Ab}	97.50 ± 0.50^{Aa}	97.50 ± 0.50^{Aa}	3.46	6.53
	Ethanol	0.00 ± 0.00^{c}	47.50 ± 0.50^{Ab}	47.50 ± 0.96^{Ab}	48.00 ± 0.58^{Ab}	95.00 ± 0.58^{Aa}	92.50 ± 0.96^{Aa}	4.34	8.93
D. elliptica	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00 ± 0.00^{c}	15.00 ± 0.00^{Bb}	22.50 ± 0.50^{Bb}	20.00 ± 0.00^{Bb}	32.50 ± 0.50^{Ba}	45.00 ± 1.29^{Ba}	10.79	19.51
	Ethanol	0.00 ± 0.00^{c}	37.50 ± 0.96^{Bb}	30.00 ± 0.82^{Bb}	27.50 ± 1.26^{Bb}	45.00 ± 0.58^{Ba}	45.00 ± 1.29^{Ba}	10.04	22.09
S. tuberosa	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00 ± 0.00^{c}	10.00 ± 0.82^{Bb}	17.50 ± 0.96^{Bb}	20.00 ± 0.82^{Bb}	52.50 ± 0.50^{Ba}	47.50 ± 0.96^{Ba}	9.26	15.69
	Ethanol	0.00 ± 0.00^{c}	12.50 ± 0.50^{Bb}	27.50 ± 0.96^{Bb}	10.00 ± 0.50^{Bb}	47.50 ± 0.96^{Ba}	47.50 ± 0.50^{Ba}	9.81	17.30
F. vulgare	Hexene	0.00 ± 0.00^{c}	2.50 ± 0.50^{Cb}	2.50 ± 0.50^{Cb}	10.00 ± 0.82^{Cb}	7.50 ± 1.50^{Ca}	15.00 ± 0.58^{Ca}	17.60	27.17
	Acetone	0.00 ± 0.00^{c}	0.00 ± 0.00^{Cc}	2.50 ± 0.50^{Cb}	5.00 ± 0.58^{Cb}	10.00 ± 0.00^{Ca}	20.00 ± 0.82^{Ca}	13.80	19.67
	Ethanol	0.00 ± 0.00^{c}	0.00 ± 0.00^{Cb}	2.50 ± 0.50^{Cb}	5.00 ± 0.58^{Cb}	15.00 ± 1.00^{Ca}	15.00 ± 0.58^{Ca}	14.50	21.11
R. nasutus	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00 ± 0.00^{c}	7.50 ± 0.96^{Cb}	5.00 ± 0.58^{Cb}	7.50 ± 0.96^{Cb}	15.00 ± 0.58^{Ca}	10.00 ± 0.82^{Ca}	23.12	38.64
	Ethanol	0.00 ± 0.00^{c}	0.00 ± 0.00^{Cb}	2.50 ± 0.50^{Cb}	5.00 ± 0.58^{Cb}	12.50 ± 0.96^{Ca}	5.00 ± 0.58^{Ca}	19.54	29.39
B. superba	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00 ± 0.00^{c}	5.00 ± 0.58^{Cb}	2.50 ± 0.50^{Cb}	2.50 ± 0.50^{Cb}	15.00 ± 0.58^{Ca}	12.50 ± 0.50^{Ca}	18.58	29.34
	Ethanol	0.00 ± 0.00^{c}	5.00 ± 0.58^{Cb}	5.00 ± 0.58^{Cb}	5.00 ± 0.58^{Cb}	12.50 ± 1.26^{Ca}	25.00 ± 1.29^{Ca}	14.90	23.15
	p-value					<0.05			

Note: Different alphabets above the average number represent different pairs.
*Percentage yield (w/w) of hexane extract had very low

Table 6 Comparison of percentage mortality of adult stage in different type of botanical at different solvent and levels of concentration**,** 6 hours post experiment.

Types of herbs	Solvent	% Mortality (6 hours post experiment) Concentration (% w/v) Control	2%	4%	6%	8%	10%	LD_{50} (% w/v)	LD_{90} (% w/v)
P. retrofractum	Hexene	0.00±0.00^{c}	40.00±0.82^{Ab}	45.00±1.00^{Ab}	80.00±0.82^{Ab}	92.50±0.50^{Aa}	92.50±0.50^{Aa}	4.00	7.98
	Acetone	0.00±0.00^{c}	50.00±0.82^{Ab}	77.50±0.50^{Ab}	97.50±0.50^{Ab}	97.50±0.50^{Ab}	100.00±0.00^{Aa}	2.61	5.01
	Ethanol	0.00±0.00^{c}	45.00±1.00^{Ab}	75.00±1.29^{Ab}	82.50±0.96^{Ab}	95.00±0.58^{Aa}	97.50±0.50^{Aa}	3.12	6.59
D. elliptica	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00^{c}	5.00±0.58^{Bb}	22.50±0.96^{Bb}	27.50±0.50^{Bb}	47.50±0.96^{Ba}	52.50±1.50^{Ba}	8.86	14.85
	Ethanol	0.00±0.00^{c}	15.00±1.29^{Bb}	32.50±0.50^{Bb}	25.00±1.29^{Bb}	45.00±0.58^{Ba}	60.00±1.15^{Ba}	8.52	15.53
S. tuberosa	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00^{c}	2.50±0.50^{Bb}	22.50±0.50^{Bb}	25.00±0.58^{Bb}	42.50±0.96^{Ba}	52.50±0.50^{Ba}	9.11	14.94
	Ethanol	0.00±0.00^{c}	7.50±0.50^{Bb}	27.50±0.50^{Bb}	27.50±0.50^{Bb}	45.00±0.58^{Ba}	57.50±0.50^{Ba}	8.64	14.87
F. vulgare	Hexene	0.00±0.00^{c}	3.30±0.58^{Cb}	3.30±0.58^{Cb}	3.30±0.58^{Cb}	3.30±0.58^{Ca}	20.00±1.00^{Ca}	16.96	25.51
	Acetone	0.00±0.00^{c}	0.00±0.00^{Cc}	0.00±0.00^{Cc}	2.50±0.50^{Cb}	10.00±0.00^{Ca}	17.50±0.50^{Ca}	13.02	17.53
	Ethanol	0.00±0.00^{c}	0.00±0.00^{Cc}	2.50±0.50^{Cb}	5.00±0.58^{Cb}	12.50±0.50^{Ca}	17.50±0.96^{Ca}	14.14	20.38
R. nasutus	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00^{c}	0.00±0.00^{Cc}	0.00±0.00^{Cc}	2.50±0.50^{Cb}	10.00±0.82^{Ca}	25.00±0.58^{Ca}	11.87	15.59
	Ethanol	0.00±0.00^{c}	0.00±0.00^{Cc}	0.00±0.00^{Cc}	2.50±0.50^{Cb}	7.50±0.96^{Ca}	27.50±0.50^{Ca}	11.61	14.99
B. superba	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00^{c}	0.00±0.00^{Cc}	0.00±0.00^{Cc}	2.50±0.50^{Cb}	15.00±1.00^{Ca}	22.50±0.96^{Ca}	12.09	16.24
	Ethanol	0.00±0.00^{c}	0.00±0.00^{Cc}	2.50±0.50^{Cb}	2.50±0.50^{Cb}	2.50±0.50^{Ca}	25.00±0.58^{Ca}	13.03	17.73
	p-value				<0.05				

Note: Different alphabets above the average number represent different pairs.
*Percentage yield (w/w) of hexane extract had very low.

Table 7 Comparison of percentage mortality of gravid stage in different type of botanical at different solvent and levels of concentration**,** 6 hours post experiment.

Types of herbs	Solvent	% Mortality (6 hours post experiment) Concentration (% w/v)						LD_{50} (% w/v)	LD_{90} (% w/v)
		Control	**2%**	**4%**	**6%**	**8%**	**10%**		
P. retrofractum	Hexene	0.00±0.00^{c}	40.00±0.82^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	2.14	2.83
	Acetone	0.00±0.00^{c}	52.50±0.96^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	1.97	2.65
	Ethanol	0.00±0.00^{c}	45.00±0.58^{Ab}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	100.00±0.00^{Aa}	2.07	2.76
D. elliptica	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00^{d}	27.50±0.50^{Bc}	47.50±0.96^{Bb}	50.00±0.82^{Bb}	52.50±0.96^{Bb}	75.00±1.29^{Ba}	6.32	13.84
	Ethanol	0.00±0.00^{d}	27.50±0.50^{Bc}	47.50±0.96^{Bb}	55.00±0.58^{Bb}	50.00±0.82^{Bb}	77.50±0.50^{Ba}	6.17	12.79
S. tuberosa	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00^{d}	20.00±0.82^{Bc}	45.00±0.58^{Bb}	47.50±0.50^{Bb}	47.50±0.50^{Bb}	65.00±1.29^{Ba}	7.19	14.47
	Ethanol	0.00±0.00^{d}	27.50±0.96^{Bc}	50.00±0.82^{Bb}	50.00±0.82^{Bb}	55.00±0.58^{Bb}	70.00±0.82^{Ba}	6.38	13.54
F. vulgare	Hexene	0.00±0.00^{c}	12.50±0.50^{Cb}	10.00±0.82^{Cb}	12.50±0.50^{Cb}	17.50±0.96^{Cb}	30.00±0.82^{Ca}	14.90	25.63
	Acetone	0.00±0.00^{d}	5.00±0.58^{Cc}	15.00±0.58^{Cb}	22.50±0.96^{Cb}	47.50±1.26^{Ca}	47.50±0.50^{Ca}	9.35	15.21
	Ethanol	0.00±0.00^{c}	5.00±0.58^{Cb}	7.50±0.96^{Cb}	7.50±0.96^{Cb}	50.00±0.82^{Ca}	47.50±0.96^{Ca}	9.60	14.63
R. nasutus	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00^{c}	7.50±0.96^{Cb}	5.00±0.58^{Cb}	10.00±0.82^{Cb}	40.00±0.82^{Ca}	30.00±0.82^{Ca}	11.61	18.57
	Ethanol	0.00±0.00^{c}	2.50±0.50^{Cb}	5.00±0.58^{Cb}	5.00±0.58^{Cb}	47.50±0.50^{Ca}	35.00±0.58^{Ca}	10.40	15.52
B. superba	*Hexene	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00^{c}	10.00±0.82^{Cb}	7.50±0.50^{Cb}	12.50±0.96^{Cb}	45.00±0.58^{Ca}	47.50±0.50^{Ca}	9.87	15.83
	Ethanol	0.00±0.00^{c}	2.50±0.50^{Cb}	5.00±0.58^{Cb}	10.00±0.82^{Cb}	47.50±0.50^{Ca}	42.50±0.96^{Ca}	9.83	14.65
	p-value				<0.05				

Note: Different alphabets above the average number represent different pairs.
*Percentage yield (w/w) of hexane extract had very low.

4.2.4 Lethal time (LT_{50}, LT_{90})

Six Thai botanical extract including *P. retrofractum, D. elliptica, S. tuberosa, F. vulgare, R. nasutus* and *B. superba* at concentration 2, 4, 6, 8 and 10% (w/v) under various solvent such as hexane, acetone and ethanol were used for the insecticidal activity test on nymph, adult and gravid stage of *B. germanica* and the percentage mortality was daily observed and recorded at 1^{st}, 2^{nd}, 4^{th}, 6^{th} 24^{th} and 48^{th} hours post experiment, moreover LT_{50} and LT_{90} values which is the time required for 50% and 90% of the population of cockroaches to die after treated to botanical insecticides, respectively were analyzed.

4.2.4.1 Lethal time of Thai botanical extract to the nymph stage of *B. germanica*

As can be seen in the table 8 – 12 and Figure 19, it was found that time increased the percentage mortality of nymph stage of *B. germanica* increased in all concentrations of the six botanical extract. Moreover, the LT_{50} values of *P. retrofractum* extract (at 2, 4, 6, 8 and 10% concentration) on nymph stage were the lowest with LT_{50} values ranged from 4.46 hours to 18.00 hours, followed by *D. elliptica* extract with LT_{50} values ranged from 21.75 hours to 36.38 hours and *S. tuberosa* extract with LT_{50} values ranged from 21.89 hours to 32.81 hours, respectively; while *F. vulgare, R. nasutus* and *B. superba* extract gave LT_{50} values ranged from 36.65 to 55.33, 35.31 to 4.75 and 36.30 to 50.77 hours, respectively.

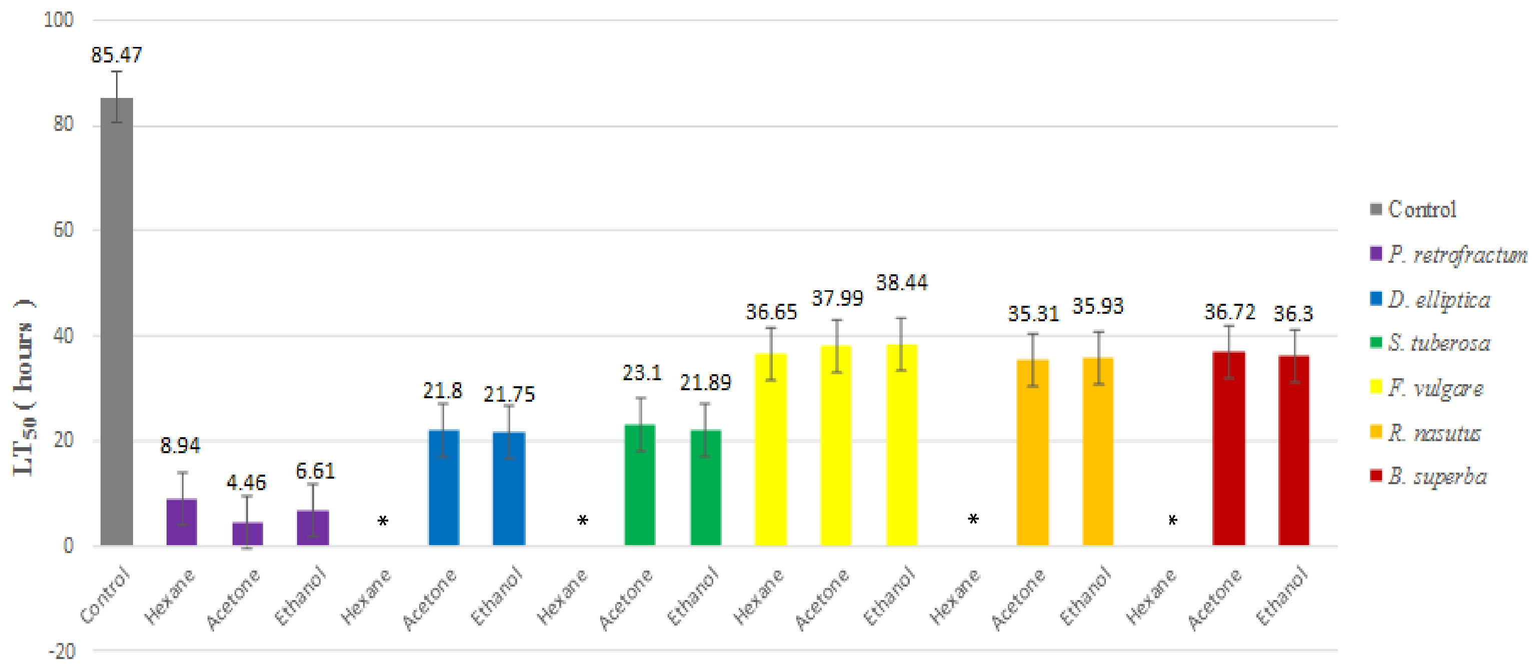
4.2.1.2 Lethal time of Thai botanical extract to the adult stage of *B. germanica*

As can be seen in the table 13 – 17 and Figure 20, it was found that time increased the percentage mortality of adult stage of *B. germanica* also increased in all concentrations of the six botanical extract. Furthermore, The LT_{50} values of *P. retrofractum* extract (at 2, 4, 6, 8 and 10% concentration) were the lowest with LT_{50} values ranged from 2.42 hours to 17.75 hours, followed by *D. elliptica* extract with LT_{50} values ranged from 18.70 hours to 38.03 hours and *S. tuberosa* extract with LT_{50} values ranged from 18.38 hours to 39.21 hours, respectively; while *F. vulgare*, *R. nasutus* and *B. superba* extract gave LT_{50} values ranged from 34.58 to 5.79, 35.71 to 48.76 and 27.79 to 51.07 hours, respectively.

4.2.1.3 Lethal time of Thai botanical extract to the gravid stage of *B. germanica*

The lethal time (LT_{50}, LT_{90}) of gravid state of *B. germanica*, it was found that as the time passed, percentage mortality of *B. germanica* also increased in all concentrations of the six botanical extract. The LT_{50} values of *P. Retrofractum* extract (at 2, 4, 6, 8 and 10% concentration) were the lowest with LT_{50} values ranged from 1.73 hours to 6.38 hours, followed by *D. elliptica* extract with LT_{50} values ranged from 11.51 hours to 31.16 hours and *S. tuberosa* extract with LT_{50} values ranged from 13.78 hours to 34.64 hours, respectively; while *F. vulgare*, *R. nasutus* and *B. superba* extract gave LT_{50} values ranged from 27.41 to 41.62, 30.53 to 45.11 and 29.38 to 38.75 hours, respectively (Table 18 - 22, Figure 21).

Figure 19 Histogram showing the medium lethal time (LT50) of Thai botanical extract at concentration 10% (% w/v) on nymph stage of *B. germanica.*



*Percentage yield (w/w) of hexane extract had very low.

Table 8 Percentage mortality of nymph stage of *B. germanica* died with concentration 2% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	5.00±0.58	12.50±1.26	35.00±0.58	47.5±0.96	87.50±0.50	87.50±0.50	16.44	38.24
	Acetone	0.00±0.00	5.00±0.58	10.00±1.41	22.50±1.50	45.0±0.50	92.50±0.50	92.50±0.50	15.25	32.25
	Ethanol	0.00±0.00	2.50±0.50	5.00±0.58	25.00±0.58	47.5±0.58	92.50±0.50	92.50±0.50	15.33	31.95
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	7.50±0.50	15.00±0.00	42.50±0.50	62.50±6.50	36.38	63.87
	Ethanol	0.00±0.00	2.50±0.50	10.00±0.82	25.00±0.58	37.50±0.96	52.50±0.50	72.50±0.50	27.99	59.27
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	10.00±0.82	47.50±0.50	72.50±0.50	32.75	55.49
	Ethanol	0.00±0.00	2.50±0.50	2.50±0.50	5.00±0.58	12.50±0.50	50.00±0.82	70.00±0.82	32.81	57.19
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	20.00±0.82	37.50±0.50	51.65	79.49
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.50±0.50	37.50±0.50	32.30	76.48
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±0.82	32.50±0.96	55.33	80.29
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	7.50±0.96	32.50±0.96	47.50±0.50	44.75	74.36
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	32.50±0.50	47.50±0.50	44.47	68.79
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	27.50±0.96	37.50±0.96	50.77	82.19
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	40.00±0.00	50.00±0.82	42.12	68.05
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 9 Percentage mortality of nymph stage of *B. germanica* died with concentration 4% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality 0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	2.50±0.50	2.50±0.58	37.50±0.96	42.50±0.96	87.50±0.50	87.50±0.50	17.45	38.06
	Acetone	0.00±0.00	2.50±0.50	7.50±0.96	32.50±1.26	42.50±0.96	95.00±0.58	95.00±0.58	13.58	28.25
	Ethanol	0.00±0.00	5.00±0.58	17.50±0.96	27.50±1.26	47.50±0.96	90.00±0.82	90.00±0.82	15.54	35.45
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	5.00±0.58	12.50±0.96	17.50±0.50	22.50±0.50	50.00±0.82	75.00±0.58	29.00	57.48
	Ethanol	0.00±0.00	2.50±0.50	10.00±0.82	22.50±0.50	30.00±0.82	47.50±0.50	75.00±0.58	28.77	58.22
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	17.50±0.96	52.50±0.96	70.00±0.82	32.15	56.59
	Ethanol	0.00±0.00	2.50±0.50	5.00±0.58	12.50±0.96	27.50±0.96	50.00±1.41	72.50±0.50	30.28	57.72
Foeniculum	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	20.00±0.00	42.50±0.50	49.10	75.20
vulgare	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	7.50±0.50	15.00±0.58	40.00±0.82	53.13	86.04
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	15.00±1.29	35.00±1.29	54.11	82.20
	*Hexane	-	-	-	-	-	-	-	-	-
Rhinacanthus nasutus	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	40.00±0.82	50.00±0.82	42.12	68.05
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	37.50±0.96	52.50±0.50	41.72	65.97
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	32.50±0.50	42.50±0.96	46.63	73.70
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	40.00±0.82	47.50±0.96	43.14	70.83
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 10 Percentage mortality of nymph stage of *B. germanica* died with concentration 6% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	2.50±0.50	7.50±0.96	25.00±1.73	42.50±0.50	87.50±1.50	87.50±1.50	18.00	38.23
	Acetone	0.00±0.00	15.00±0.58	25.00±1.29	45.00±0.58	95.00±0.58	95.00±0.58	95.00±0.58	5.30	26.13
	Ethanol	0.00±0.00	5.00±0.58	7.50±0.96	27.50±0.50	48.00±0.58	92.50±0.96	92.50±0.96	14.86	32.00
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	5.00±0.58	10.00±0.82	15.00±0.58	20.00±0.00	45.00±0.58	72.50±0.50	31.20	60.02
	Ethanol	0.00±0.00	2.50±0.50	7.50±0.96	15.00±1.00	27.50±1.26	50.00±0.82	72.50±0.50	30.00	58.31
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	7.50±0.50	12.50±0.50	20.00±0.82	50.00±0.82	70.00±0.82	31.55	59.43
	Ethanol	0.00±0.00	2.50±0.50	5.00±0.58	10.00±0.50	17.50±0.50	45.00±0.58	72.50±0.96	32.03	58.09
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	10.00±0.82	22.50±0.96	47.50±0.96	46.89	76.46
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	12.50±0.50	32.50±0.96	50.00±0.82	50.00±0.82	38.88	75.90
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	15.00±0.58	45.00±1.29	49.54	77.50
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	7.50±0.96	40.00±0.82	55.00±0.58	40.16	66.25
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	45.00±0.58	57.50±0.50	38.51	63.13
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	35.00±0.58	50.00±0.82	43.08	67.95
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	40.00±0.82	50.00±0.82	42.12	68.05
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 11 Percentage mortality of nymph stage of *B. germanica* died with concentration 8% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality 0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	10.00±0.82	22.50±1.71	45.00±1.00	95.00±0.58	95.00±0.58	95.00±0.58	8.23	25.00
	Acetone	0.00±0.00	10.00±0.82	27.50±1.26	50.00±0.82	97.50±0.50	97.50±0.50	97.50±0.50	6.34	18.87
	Ethanol	0.00±0.00	7.50±0.96	22.50±0.96	47.50±0.96	95.00±1.00	95.00±1.00	95.00±1.00	8.22	24.88
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	7.50±0.96	12.50±0.96	27.50±0.50	32.50±0.50	55.00±1.29	75.00±0.58	26.53	57.50
	Ethanol	0.00±0.00	5.00±0.58	7.50±0.50	17.50±0.50	45.00±0.58	70.00±0.82	77.50±0.50	23.50	50.25
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	10.00±1.41	17.50±1.50	32.50±1.26	52.50±0.50	62.50±0.96	77.50±1.26	21.91	54.42
	Ethanol	0.00±0.00	2.50±0.50	5.00±0.58	20.00±0.82	47.50±0.96	70.00±0.82	77.50±0.50	23.48	49.95
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	5.00±1.00	7.50±0.96	7.50±0.96	37.50±0.96	50.00±0.82	42.78	74.19
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	32.50±1.26	40.00±0.00	42.50±0.58	55.00±1.29	37.20	78.69
	Ethanol	0.00±0.00	2.50±0.50	2.50±0.50	2.50±0.50	15.00±1.00	40.00±0.82	52.50±0.50	40.95	71.73
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	7.50±0.96	15.00±0.58	52.50±0.50	60.00±0.82	35.54	62.67
	Ethanol	0.00±0.00	0.00±0.00	2.50±0.50	2.50±0.50	12.50±0.96	52.50±0.50	57.50±0.96	36.71	63.67
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	15.00±0.58	45.00±0.58	55.00±0.58	38.90	66.80
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	12.50±1.26	47.50±0.50	55.00±0.58	38.55	65.76
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	2.50±0.50	120.11	171.02

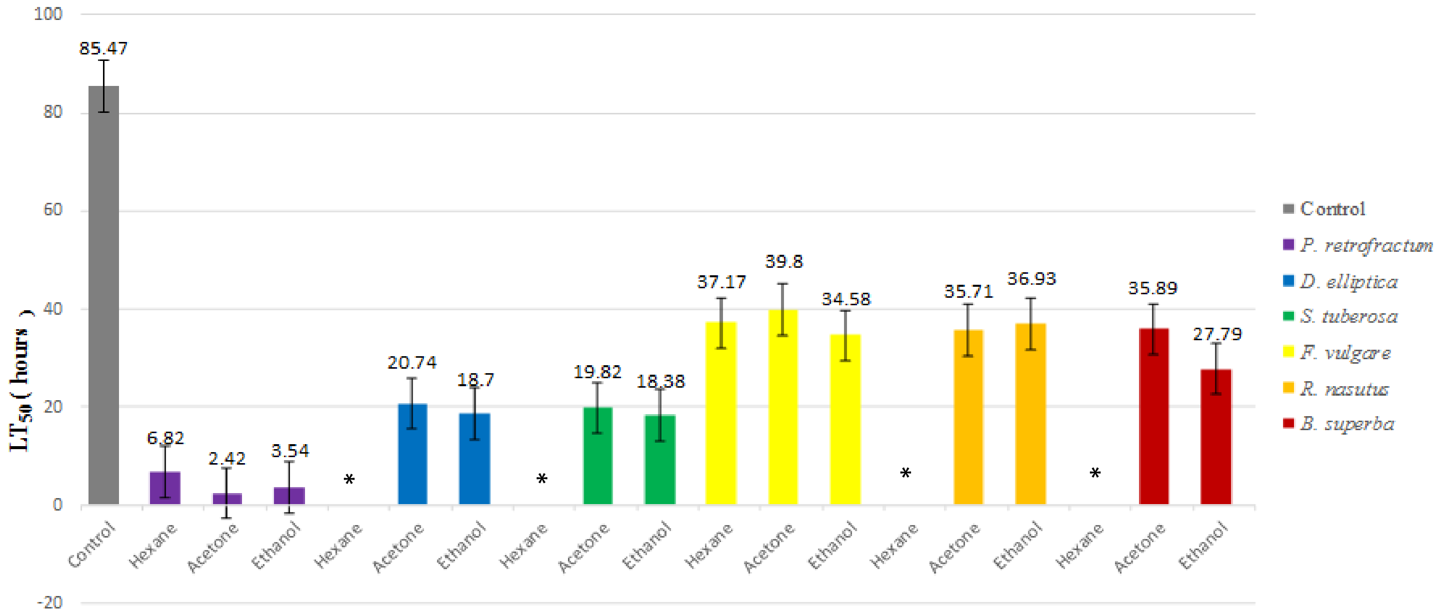
*Percentage yield (w/w) of hexane extract had very low.

Table 12 Percentage mortality of nymph stage of *B. germanica* died with concentration 10% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality 0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	7.50±0.50	12.50±1.26	45.00±1.29	95.00±0.58	95.00±0.58	95.00±0.58	8.94	24.99
	Acetone	0.00±0.00	20.00±0.82	50.00±0.82	55.00±0.58	97.50±0.50	97.50±0.50	97.50±0.50	4.46	18.41
	Ethanol	0.00±0.00	10.00±1.15	47.50±1.26	57.50±0.50	92.50±0.96	92.50±0.96	92.50±0.96	6.61	29.09
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	10.00±0.82	12.50±0.96	17.50±0.50	45.00±1.29	72.50±0.96	80.00±0.82	21.80	48.46
	Ethanol	0.00±0.00	5.00±0.58	12.50±0.96	22.50±0.96	45.00±1.29	72.50±0.50	80.00±0.82	21.75	48.21
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	10.00±1.41	17.50±0.96	22.50±1.26	47.50±0.96	67.50±0.96	75.00±0.58	23.10	54.46
	Ethanol	0.00±0.00	7.50±0.96	10.00±0.82	27.50±0.96	47.50±0.50	72.50±0.50	77.50±0.50	21.89	50.32
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	15.00±0.58	47.50±1.26	60.00±0.82	36.65	62.83
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	20.00±0.82	22.50±1.26	45.00±0.58	55.00±1.29	37.99	72.87
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	15.00±0.58	42.50±0.50	57.50±0.96	38.44	65.60
Rhinacanthus nasutua	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	10.0±00.82	52.50±0.50	62.50±0.50	35.31	59.54
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	5.00±0.58	50.00±0.82	62.50±0.50	35.93	60.88
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	7.50±0.00	10.00±0.58	12.50±0.50	50.00±0.82	57.50±0.96	36.72	67.73
	Ethanol	0.00±0.00	2.50±0.50	2.50±0.50	5.00±1.00	25.00±1.29	50.00±0.82	57.50±0.96	36.30	67.22
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	7.50±0.50	85.47	120.16

*Percentage yield (w/w) of hexane extract had very low.

Figure 20 Histogram showing the medium lethal time (LT50) of Thai botanical extract at concentration 10% (% w/v) on adult stage of *B. germanica*



*Percentage yield (w/w) of hexane extract had very low.

Table 13 Percentage mortality of adult stage of *B. germanica* died with concentration 2% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	40.00±0.82	92.50±0.50	92.50±0.50	17.75	32.29
	Acetone	0.00±0.00	0.00±0.00	5.00±0.58	30.00±1.15	50.00±0.82	95.00±0.58	95.00±0.58	13.47	27.83
	Ethanol	0.00±0.00	0.00±0.00	2.50±0.50	17.50±0.96	45.00±1.00	92.50±0.50	92.50±0.50	16.26	32.03
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	2.50±0.50	2.50±0.50	5.00±0.58	42.50±0.50	60.00±1.15	38.03	63.68
	Ethanol	0.00±0.00	2.50±0.50	2.50±0.50	7.50±0.96	15.00±1.29	45.00±0.58	65.00±0.58	34.99	62.17
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	37.50±0.50	60.00±0.82	39.21	61.51
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.50±0.50	40.00±0.00	62.50±0.50	37.69	60.87
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.30±0.58	13.30±0.58	33.30±0.58	55.79	85.00
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15.00±0.58	40.00±0.82	50.72	74.81
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	30.00±0.82	42.50±0.50	46.96	72.56
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.00±0.58	47.50±0.50	45.80	69.43
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	20.00±0.82	42.50±0.96	48.76	73.12
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.50±0.50	40.00±0.82	51.07	74.48
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	17.50±0.50	40.00±0.82	50.33	74.99
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 14 Percentage mortality of adult stage of *B. germanica* died with concentration 4% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	0.00±0.00	2.50±0.50	17.50±0.96	45.00±1.00	92.50±0.50	92.50±0.50	16.26	32.03
	Acetone	0.00±0.00	0.00±0.00	12.50±0.50	27.50±0.50	77.50±0.50	95.00±1.00	95.00±1.00	11.46	26.45
	Ethanol	0.00±0.00	0.00±0.00	2.50±0.50	27.50±0.96	75.00±1.29	95.00±0.58	95.00±0.58	12.13	26.54
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	22.50±0.96	62.50±0.96	72.50±0.96	29.44	53.21
	Ethanol	0.00±0.00	0.00±0.00	2.50±0.50	15.00±1.00	32.50±0.50	57.50±0.50	70.00±0.82	29.56	57.35
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	7.50±0.50	22.50±0.50	50.00±0.82	65.00±0.58	33.78	60.70
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	27.50±0.50	55.00±1.29	75.00±0.58	29.59	53.20
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.30±0.58	13.30±0.58	40.00±1.00	51.78	78.39
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	17.50±0.50	42.50±0.50	49.17	73.08
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	27.50±.096	47.50±0.96	45.46	70.66
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	17.50±0.96	45.00±0.58	48.09	71.29
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	22.50±0.96	47.50±0.50	46.24	69.56
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	22.50±0.50	42.50±0.50	48.32	73.07
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	22.50±0.50	47.50±0.50	46.40	71.21
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 15 Percentage mortality of adult stage of *B. germanica* died with concentration 6% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	0.00±0.00	12.50±1.26	37.50±0.96	80.00±0.82	90.00±0.82	90.00±0.82	13.09	33.69
	Acetone	0.00±0.00	2.50±0.50	17.50±0.96	57.50±0.50	97.50±0.50	97.50±0.50	97.50±0.50	6.75	18.59
	Ethanol	0.00±0.00	2.50±0.50	10.00±1.15	30.00±0.82	82.50±0.96	92.50±0.50	92.50±0.50	12.31	30.26
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	7.50±0.50	27.50±0.50	57.50±1.50	70.00±0.82	30.42	56.39
	Ethanol	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	25.00±1.29	62.50±0.96	77.50±0.96	27.76	50.22
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	25.00±0.58	50.00±0.00	67.50±0.96	32.80	59.36
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	5.00±1.00	27.50±0.50	57.50±0.50	72.50±0.96	29.96	54.22
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.30±0.58	20.00±0.00	46.70±0.58	47.26	72.53
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	2.50±0.50	17.50±0.50	52.50±0.50	45.48	69.93
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	17.50±0.96	45.00±0.58	48.09	71.29
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	27.50±0.50	42.50±0.96	47.62	74.39
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	32.50±0.96	52.50±0.50	42.61	66.68
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	27.50±0.50	50.00±0.00	44.46	68.94
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	22.50±0.50	47.50±0.50	46.40	71.21
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 16 Percentage mortality of adult stage of *B. germanica* died with concentration 8% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	5.00±1.00	10.00±0.82	57.50±1.50	92.50±0.50	92.50±0.50	92.50±0.50	9.66	29.06
	Acetone	0.00±0.00	10.00±1.41	37.50±0.96	87.50±0.50	97.50±0.50	97.50±0.50	97.50±0.50	3.93	16.90
	Ethanol	0.00±0.00	12.50±0.96	35.00±0.58	67.50±1.50	95.00±0.58	95.00±0.58	95.00±0.58	5.86	24.08
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	15.00±1.29	47.50±0.96	67.50±1.71	82.50±1.50	23.05	46.46
	Ethanol	0.00±0.00	0.00±0.00	2.50±0.50	10.00±0.82	45.00±0.58	70.00±0.82	80.00±0.82	23.99	47.44
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	5.00±0.58	20.00±0.00	42.50±0.96	55.00±1.29	72.50±0.96	27.79	57.70
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	15.00±0.58	45.00±0.58	67.50±0.96	80.00±1.41	24.14	48.04
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	3.30±0.58	36.70±1.15	50.00±1.00	42.76	68.00
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±0.00	40.00±0.82	55.00±0.58	40.11	66.09
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.50±0.50	47.50±0.50	55.00±0.58	38.63	65.13
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±0.82	37.50±0.96	60.00±0.82	38.80	63.34
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.50±0.96	45.00±0.58	60.00±0.82	37.65	61.48
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15.00±1.00	40.00±0.82	55.00±0.58	39.91	67.25
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	47.50±0.50	55.00±0.58	39.12	62.91
Control		0.00±0.00	0.00±0.00C	0.00±0.00C	0.00±0.00C	0.00±0.00	2.50±0.50	2.50±0.50	120.11	171.02

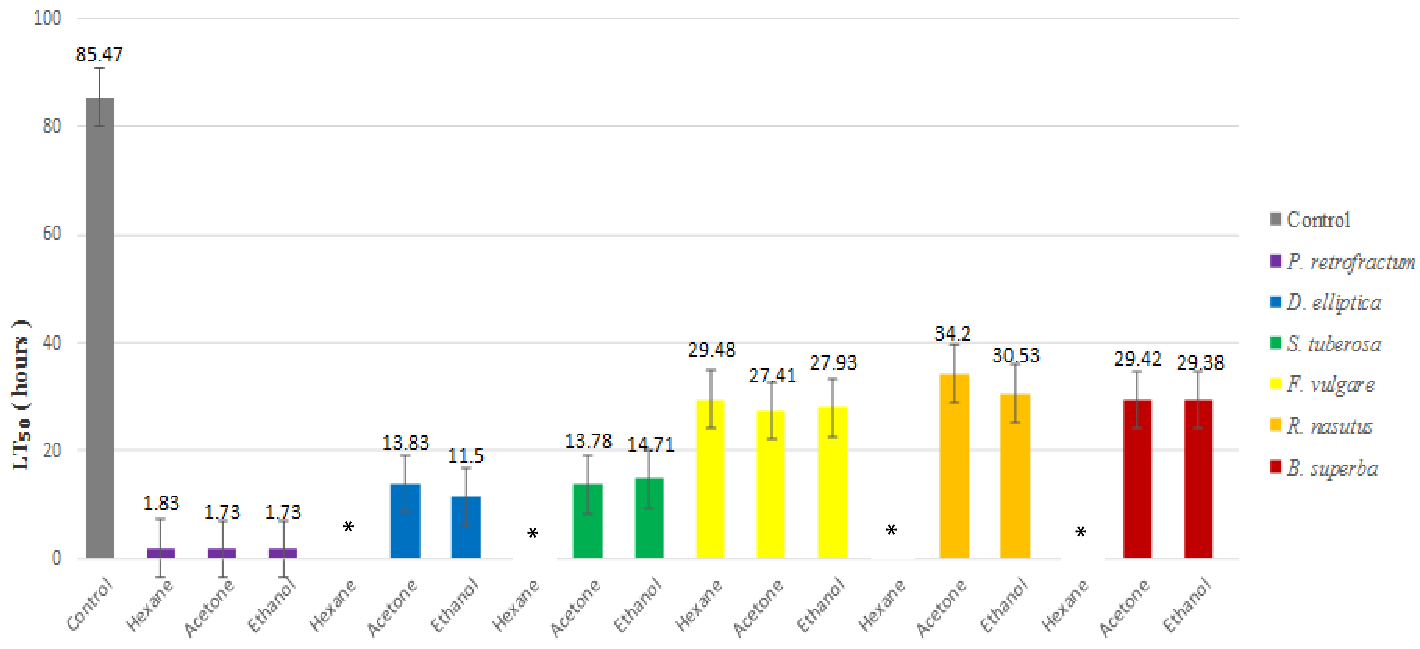
*Percentage yield (w/w) of hexane extract had very low.

Table 17 Percentage mortality of adult stage of *B. germanica* died with concentration 10% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality 0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	7.50±0.50	32.50±0.50	72.50±0.96	92.50±0.50	92.50±0.50	92.50±0.50	6.82	28.59
	Acetone	0.00±0.00	12.50±0.50	42.50±1.26	87.50±1.26	100.00±0.00	100.00±0.00	100.00±0.00	2.42	3.96
	Ethanol	0.00±0.00	10.00±1.15	45.00±0.58	87.50±1.26	97.50±0.50	97.50±0.50	97.50±0.50	3.54	16.77
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	7.50±0.50	25.00±0.58	52.50±1.50	72.50±1.71	82.50±1.71	20.74	45.84
	Ethanol	0.00±0.00	0.00±0.00	5.00±0.58	30.00±1.15	60.00±1.15	77.50±0.96	85.00±1.29	18.70	42.23
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	25.00±1.00	30.00±0.82	52.50±0.50	72.50±1.26	80.00±0.82	19.82	48.89
	Ethanol	0.00±0.00	2.50±0.50	12.50±0.96	32.50±0.50	57.50±0.50	75.00±1.00	85.00±0.58	18.38	43.42
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	20.00±1.00	50.00±1.00	56.70±0.58	37.17	64.75
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	17.50±0.50	40.00±0.82	55.00±0.58	39.80	67.79
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	17.50±0.96	45.00±0.58	67.50±0.50	34.58	58.51
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.00±0.58	50.00±0.00	60.00±0.82	35.71	63.19
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	27.50±0.50	42.50±0.50	60.00±0.82	36.93	65.33
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	22.50±0.96	45.00±0.58	62.50±0.50	35.89	62.31
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.00±0.58	40.00±0.82	60.00±0.00	27.79	57.70
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	7.50±0.50	85.47	120.16

*Percentage yield (w/w) of hexane extract had very low.

Figure 21 Histogram showing the medium lethal time (LT50) of Thai botanical extract at concentration 10% (% w/v) on gravid stage of *B. germanica.*



*Percentage yield (w/w) of hexane extract had very low.

Table 18 Percentage mortality of gravid stage of *B. germanica* died with concentration 2% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	5.00±1.00	15.00±1.73	30.00±0.82	40.00±0.82	100.00±0.00	100.00±0.00	6.38	10.82
	Acetone	0.00±0.00	5.00±1.00	15.00±1.00	35.00±0.58	52.50±0.96	100.00±0.00	100.00±0.00	5.48	9.12
	Ethanol	0.00±0.00	5.00±0.58	25.00±0.58	37.50±0.96	45.00±0.58[Ae]	100.00±0.00	100.00±0.00	5.74	10.21
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	7.00±0.50	17.50±0.50	27.50±0.50	47.50±0.50	70.00±0.82	31.16	61.06
	Ethanol	0.00±0.00	2.50±0.50	2.50±0.50	17.50±0.50	27.50±0.50	52.50±0.96	77.50±0.50	28.07	53.71
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	2.50±0.50	7.50±0.50	20.00±0.82	50.00±0.82	62.50±0.96	34.64	63.26
	Ethanol	0.00±0.00	2.50±0.50	5.00±0.58	17.50±0.96	27.50±0.96	47.50±0.50	70.00±0.82	31.25	60.75
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.50±0.50	52.50±0.50	62.50±1.26	35.27	59.42
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	45.00±1.00	52.50±0.96	40.30	65.52
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	40.00±0.82	52.50±0.50	41.20	66.39
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.50±0.96	37.50±0.50	50.00±0.82	42.55	69.31
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	35.00±0.58	52.50±0.50	42.16	66.33
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.00±0.82	47.50±0.50	55.00±0.58	38.75	64.62
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	45.00±0.58	60.00±0.82	37.95	60.42
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 19 Percentage mortality of gravidt stage of *B. germanica* died with concentration 4% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality 0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	5.00±0.58	25.00±1.29	52.50±0.50	100.00±0.00	100.00±0.00	100.00±0.00	3.42	5.31
	Acetone	0.00±0.00	12.50±0.96	40.00±0.82	60.00±0.82	100.00±0.00	100.00±0.00	100.00±0.00	2.98	5.09
	Ethanol	0.00±0.00	10.00±0.82	17.50±0.96	50.00±0.82	100.00±0.00	100.00±0.00	100.00±0.00	3.50	4.48
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	7.00±0.50	22.50±0.50	47.50±0.96	72.50±0.96	77.50±0.50	22.79	49.52
	Ethanol	0.00±0.00	0.00±0.00	7.50±0.50	27.50±0.50	47.50±0.96	75.00±1.29	75.00±1.29	22.90	50.71
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	5.00±0.58	17.50±0.50	45.00±0.58	70.00±0.82	75.00±0.58	24.60	51.65
	Ethanol	0.00±0.00	5.00±0.58	7.50±0.50	22.50±0.50	50.00±0.82	70.00±0.82	75.00±0.58	23.50	52.37
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	10.00±0.82	55.00±0.58	70.00±0.82	32.47	55.06
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	15.00±0.58	52.50±0.50	65.00±0.58	34.17	58.64
	Ethanol	0.00±0.00	0.00±0.00	2.50±0.50	5.00±1.00	7.50±0.96	37.50±0.96	52.50±0.96	41.62	70.32
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58	35.00±0.58	45.00±0.58	45.11	72.54
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	42.50±0.50	52.50±0.96	40.71	66.81
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	2.50±0.50	2.50±0.50	2.50±0.50	7.50±0.50	45.00±1.29	60.00±0.82	37.44	63.59
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	47.50±0.50	62.50±0.50	36.47	59.71
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 20 Percentage mortality of gravid stage of *B. germanica* died with concentration 6% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	17.50±0.50	25.00±1.29	42.50±0.50	100.00±0.00	100.00±0.00	100.00±0.00	3.44	5.79
	Acetone	0.00±0.00	17.50±0.96	37.50±0.05	57.50±0.50	100.00±0.00	100.00±0.00	100.00±0.00	2.99	5.23
	Ethanol	0.00±0.00	10.00±0.82	32.50±0.96	55.00±1.29	100.00±0.00	100.00±0.00	100.00±0.00	3.20	5.27
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	5.00±0.58	10.00±0.82	22.50±0.50	50.00±0.82	75.00±1.29	80.00±0.82	21.15	47.35
	Ethanol	0.00±0.00	5.00±0.58	15.00±0.58	25.00±0.58	55.00±0.58	75.00±0.58	77.50±0.96	20.90	49.62
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	5.00±0.58	10.00±0.82	22.50±0.50	47.50±0.50	67.50±1.26	80.00±0.82	22.44	49.40
	Ethanol	0.00±0.00	2.50±0.50	10.00±0.82	25.00±0.58	50.00±0.82	70.00±0.82	80.00±0.82	21.84	48.58
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	2.50±0.50	10.00±0.00	12.50±0.50	50.00±0.82	60.00±0.00	35.95	63.96
	Acetone	0.00±0.00	2.50±0.50	2.50±0.50	7.50±0.50	22.50±0.96	52.50±0.96	62.50±1.26	34.02	62.88
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	7.50±0.96	52.50±0.96	65.00±0.58	34.72	57.75
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	10.00±0.82	37.50±0.50	50.00±0.82	42.53	71.02
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	42.50±0.50	57.50±0.96	38.95	63.58
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	12.50±0.96	42.50±1.26	65.00±0.58	35.79	61.39
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	10.00±0.82	52.50±0.50	67.50±0.96	33.78	56.62
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	-	-

*Percentage yield (w/w) of hexane extract had very low.

Table 21 Percentage mortality of gravid stage of *B. germanica* died with concentration 8% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality 0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	10.00±0.82	25.00±0.58	50.00±0.82	100.00±0.00	100.00±0.00	100.00±0.00	3.40	5.46
	Acetone	0.00±0.00	22.50±0.96	50.00±0.82	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	1.87	2.98
	Ethanol	0.00±0.00	15.00±1.00	50.00±1.41	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	1.93	2.93
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	5.00±0.58	10.00±0.82	25.00±0.58	52.50±0.96	80.00±0.82	82.50±1.26	19.37	44.10
	Ethanol	0.00±0.00	7.50±0.50	12.50±0.50	25.00±0.58	50.00±0.82	82.50±0.96	85.00±1.29	18.19	41.74
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	7.50±0.50	15.00±0.58	25.00±0.58	47.50±0.50	75.00±0.58	82.50±0.50	22.50	45.93
	Ethanol	0.00±0.00	2.50±0.50	7.50±0.50	25.00±0.58	55.00±0.58	67.50±0.96	85.00±1.29	20.52	45.22
Foeniculum vulgare	Hexane	0.00±0.00	2.50±0.50	2.50±0.50	7.50±0.96	17.50±0.96	57.50±0.96	62.50±1.26	33.48	61.16
	Acetone	0.00±0.00	2.50±0.50	5.00±0.58	10.00±0.82	47.50±1.26	57.50±0.96	67.50±0.50	29.31	60.26
	Ethanol	0.00±0.00	2.50±0.50	2.50±0.50	12.50±0.50	50.00±0.82	60.00±0.82	62.50±0.50	30.38	63.66
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	2.50±0.50	5.00±0.58	40.00±0.82	52.50±0.50	65.00±0.58	32.15	61.71
	Ethanol	0.00±0.00	0.00±0.00	0.00±0.00	10.00±0.82	47.50±0.50	62.50±0.50	67.50±0.50	28.85	57.81
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	10.00±0.82	45.00±0.58	60.00±0.82	67.50±0.96	29.46	58.34
	Ethanol	0.00±0.00	0.00±0.00	2.50±0.50	7.50±0.50	40.75±0.50	60.00±0.82	67.50±0.96	29.81	58.23
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	2.50±0.50	120.11	171.02

*Percentage yield (w/w) of hexane extract had very low.

Table 22 Percentage mortality of gravid stage of *B. germanica* died with concentration 10% (% w/v) at different hours post experiment

Types of herbs	Solvent	% Mortality								
		0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	48 hrs.	LT_{50}	LT_{90}
Piper retrofractum	Hexane	0.00±0.00	30.00±0.82	47.50±0.96	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	1.83	3.05
	Acetone	0.00±0.00	22.50±0.50	60.00±0.82	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	1.73	2.74
	Ethanol	0.00±0.00	22.50±0.96	60.00±0.82	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	1.73	2.74
Derris elliptica	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	7.50±0.96	25.00±1.29	50.00±0.82	75.00±1.29	77.50±1.26	85.00±1.73	13.83	42.96
	Ethanol	0.00±0.00	12.50±0.96	25.00±1.00	50.00±0.82	77.50±0.50	82.50±0.50	90.00±0.82	11.51	36.56
Stemona tuberosa	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	10.00±0.82	27.50±0.50	52.50±0.50	65.00±1.29	80.00±1.41	85.00±1.00	13.78	42.67
	Ethanol	0.00±0.00	7.50±0.50	22.50±0.50	55.00±0.58	70.00±0.82	72.50±0.50	85.00±1.29	14.71	44.73
Foeniculum vulgare	Hexane	0.00±0.00	0.00±0.00	7.50±0.50	15.00±0.58	30.00±0.82	57.50±0.50	70.00±0.82	29.48	57.88
	Acetone	0.00±0.00	0.00±0.00	7.50±0.50	37.50±0.50	47.50±0.50	57.50±1.26	67.50±0.50	27.41	62.87
	Ethanol	0.00±0.00	0.00±0.00	0.50±0.58	17.50±1.26	47.50±0.96	60.00±0.82	70.00±0.82	27.93	57.31
Rhinacanthus nasutus	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	0.00±0.00	15.00±0.58	30.00±0.82	52.50±1.26	60.00±0.82	34.20	65.61
	Ethanol	0.00±0.00	0.00±0.00	5.00±0.58	7.50±0.50	35.00±0.58	57.50±0.50	67.50±0.50	30.53	58.90
Butea superba	*Hexane	-	-	-	-	-	-	-	-	-
	Acetone	0.00±0.00	0.00±0.00	5.00±0.58	10.00±0.82	47.50±0.50	57.50±0.50	67.50±0.96	29.42	59.85
	Ethanol	0.00±0.00	0.00±0.00	5.00±0.58	10.00±0.82	42.50±0.96	60.00±0.82	67.50±0.50	29.38	58.90
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	7.50±0.50	85.47	120.16

*Percentage yield (w/w) of hexane extract had very low.

4.3 Susceptibility of various stages of the *B. germanica* to Thai botanical extracts.

The efficacy of the six Thai botanical extracts against German cockroach, *B. germanica* were studied and susceptibility of various stages of the *B. germanica* including, nymph, adult and gravid were compared at different solvent of each Thai botanical extracts.

4.3.1 Susceptibility of three stages of *B. germanica* to *P. retrofractum* extracts.

As can be seen from the table 23 and figure 22, the toxicity of three difference solvent including, hexane, acetone and ethanol extract of *P. retrofractum* on nymph, adult and gravid stage of *B. germanica* were investigated. This study found that among three stage, gravid stage was the most susceptible ($P<0.05$) with LD_{50} value ranged from 1.97 to 2.14 % w/v and LD_{90} value ranged from 2.65 to 2.83 % w/v, followed by adult stage with LD_{50} value ranged from 2.61 to 4.00 %w/v and LD_{90} value ranged from 5.01 to 7.98 % w/v, and nymph stage with LD_{50} value ranged from 3.46 to 4.5 % w/v and LD_{90} value ranged from 6.53 to 8.94 % w/v, respectively, at 6 hour post experiment.

Moreover, when compare the efficacy among three different solvent of *P. retrofractum* on *B. germanica*, the result showed that the acetone extract of *P. retrofractum* caused the highest mortality with LD50 of 1.97 % w/v for gravid stage, LD_{50} of 2.61 % w/v for adult stage and LD50 of 3.46 % w/v for nymph stage, respectively, followed by ethanol extract of *P. retrofractum* with LD_{50} of 2.07 % w/v for gravid stage, LD_{50} of 3.12 % w/v for adult stage and LD_{50} of 4.34 % w/v for nymph stage, respectively, and hexane extract of *P. retrofractum* was the least efficacy against *B. germanica* with LD_{50} of 2.14 % w/v for gravid stage, LD_{50} of 4.00 % w/v for adult stage and LD_{50} of 4.50 % w/v for nymph stage , respectively (Table 23, Fig. 22).

4.3.2 Susceptibility of three stages of *B. germanica* to *D. elliptica* extracts.

The insecticidal activity of *D. elliptica* extracts against nymph, adult and gravid stage of *B. germanica* were explored and the result showed that gravid stage was the most susceptible ($P<0.05$) with LD_{50} value ranged from 6.17 to 6.32 % w/v and LD_{90} value ranged from 12.79 to 13.84 % w/v, followed by adult stage with LD_{50} value ranged from 8.52 to 8.86 % w/v and LD_{90} value ranged from 14.85 to 15.53 % w/v, and nymph stage with LD_{50} value ranged from 10.04 to 10.71 % w/v and LD_{90} value ranged from 19.51 to 22.09 % w/v, respectively, at 6 hour post experiment (Table 24, Fig. 23).

Furthermore, the efficacy between two different solvent including, acetone and ethanol extract of *D. elliptica* against *B. germanica*, the results showed that the ethanol extract of *D. elliptica* caused higher efficacy on mortality when compared the acetone extract. The ethanol extract of *D. elliptica* gave LD_{50} of 6.17 % w/v for gravid stage, LD_{50} of 8.52 % w/v for adult stage and LD_{50} of 10.04 % w/v for nymph stage, respectively; while acetone extract of *D. elliptica* gave LD_{50} of 6.32 % w/v for gravid stage, LD_{50} of 8.86 % w/v for adult stage and LD50 of 10.79 % w/v for nymph stage, respectively, (Table 24, Fig. 23).

4.3.3 Susceptibility of three stages of *B. germanica* to *S. tuberosa* extracts.

According to the table 25 and figure 24, the gravid stage was the most susceptible on *S. tuberosa* extracts (P<0.05) with LD_{50} value ranged from 6.38 to 7.19 % w/v and LD_{90} value ranged from 13.54 to 14.47 % w/v, followed by adult stage with LD_{50} value ranged from 8.64 to 9.11 % w/v and LD_{90} value ranged from 14.87 to 14.94 % w/v, and nymph stage with LD_{50} value ranged from 9.26 to 9.81 % w/v and LD_{90} value ranged from 15.69 to 17.30 % w/v, respectively, at 6 hour post experiment.

Moreover, the efficacy between acetone and ethanol extract of *S. tuberosa* against B. *germanica*. The results discovered that on adult and gravid stages, the ethanol extract of *S. tuberosa* lead to higher efficacy on mortality when compared the acetone extract. The ethanol extract of *S. tuberosa* gave LD_{50} of 6.38 % w/v for gravid stage and LD_{50} of 8.64 % w/v for adult stage; whereas on nymph stage, acetone extract of *S. tuberosa* (LD_{50} of 9.26 % w/v) gave higher efficacy than ethanol extract (LD_{50} of 9.81 % w/v), (Table 25, Fig. 24).

4.3.4 Susceptibility of three stages of *B. germanica* to *F. vulgare* extracts.

The toxicity of three difference solvent of *F. vulgare* extracts on various stage of *B. germanica* were studied and result showed that gravid stage was the most susceptible (P<0.05) with LD_{50} value ranged from 9.35 to 14.90 % w/v and LD_{90} value ranged from 14.63 to 25.63 % w/v, followed by adult stage with LD_{50} value ranged from 13.02 to 16.96 % w/v and LD_{90} value ranged from 17.53 to 25.51 % w/v, and nymph stage with LD_{50} value ranged from 13.80 to 17.60 % w/v and LD_{90} value ranged from 19.67 to 27.17 % w/v, respectively, at 6 hour post experiment (Table 26, Fig. 25).

In addition, the efficacy among three different solvent of *F. vulgare* against *B. germanicat*, This results showed that the acetone extract of *F. vulgare* caused highest efficacy on mortality with LD_{50} of 9.35 % w/v for gravid stage, LD_{50} of 13.02 % w/v for adult stage and LD_{50} of 13.80 % w/v for nymph stage, respectively, followed by ethanol extract of *F. vulgare* with LD_{50} of 9.60 % w/v for gravid stage, LD50 of 14.14 % w/v for adult stage and LD_{50} of 14.50 % w/v for nymph stage, respectively, and hexane extract of *F. vulgare* was the least efficacy against *B. germanica* with LD_{50} of 14.90 % w/v for gravid stage, LD_{50} of 16.96 % w/v for adult stage and LD_{50} of 17.60 % w/v for nymph stage , respectively (Table 26, Fig. 25).

4.3.5 Susceptibility of three stages of *B. germanica* to *R. nasutus* extracts.

The insecticidal activity of *R. nasutus* extracts against nymph, adult and gravid stage of *B. germanica* were explored and this study found that gravid stage was the most susceptible stage ($P<0.05$) with LD_{50} value ranged from 10.40 to 11.61 % w/v and LD_{90} value ranged from 15.52 to 18.57 % w/v, followed by adult stage with LD_{50} value ranged from 11.61 to 11.87 % w/v and LD_{90} value ranged from 14.99 to 15.59 % w/v, and nymph stage with LD_{50} value ranged from 19.54 to 23.12 % w/v and LD_{90} value ranged from 29.39 to 38.64 % w/v, respectively, at 6 hour post experiment (Table 27, Fig. 26).

Moreover, this result reveals that the ethanol extract of *R. nasutus* caused higher efficacy on mortality when compared the acetone extract. The ethanol extract of *R. nasutus* gave LD_{50} of 10.40 % w/v for gravid stage, LD50 of 11.61 % w/v for adult stage and LD_{50} of 19.54 % w/v for nymph stage, respectively; while acetone extract of *R. nasutus* gave LD_{50} of 11.61 % w/v for gravid stage, LD_{50} of 11.87 % w/v for adult stage and LD_{50} of 23.12 % w/v for nymph stage, respectively (Table 27, Fig. 26).

4.3.6 Susceptibility of three stages of *B. germanica* to *B. superba* extracts.

When considering the table 28 and figure 27, it is evident that among three stages, gravid stage was the most susceptible on *B. superba* extracts ($P<0.05$) with LD_{50} value ranged from 9.83 to 9.87 % w/v and LD_{90} value ranged from 14.65 to 15.83 % w/v, followed by adult stage with LD50 value ranged from 12.09 to 13.03 % w/v and LD_{90} value ranged from 16.24 to 17.73 % w/v, and nymph stage with LD_{50} value ranged from 14.90 to 18.58 % w/v and LD_{90} value ranged from 23.15 to 29.34 % w/v, respectively, at 6 hour post experiment.

Furthermore, the efficacy between acetone and ethanol extract of *B. superba* against *B. germanica*. This study showed that on nymph and gravid stage, the ethanol extract of *B. superba* induced higher mortality when compared the acetone extract. The ethanol extract of *B. superba* gave LD_{50} of 9.83 % w/v for gravid stage and LD_{50} of 14.90 % w/v for nymph stage; whereas on adult stage, the acetone extract of *B. superba* (LD_{50} of 12.09 % w/v) gave higher efficacy than ethanol extract (LD_{50} of 13.03 % w/v) (Table 28, Fig. 27).

Based on the aforementioned results, the efficacy of the six Thai botanical extracts against German cockroach, *B. germanica* were investigated and susceptibility of various stages of the *B. germanica* including, nymph, adult and gravid were compared at different solvent of each Thai botanical extracts. The results found that among three stages; gravid stage was the most susceptible, followed by adult and nymph stage in all of six Thai botanical. Furthermore, at the 6^{th} hour post experiment, all solvents extracts including hexane, acetone and ethanol from *P. retrofractum* at highest concentration (10% w/v) showed complete mortality (100%) against gravid stage of *B. germanica*; whereas only acetone extract of *P. retrofractum* at the same concentration showed complete mortality (100%) against adult stage of *B. germanica*. Nevertheless, all solvents extracts of *P. retrofractum* cannot induce complete mortality against nymph stage of *B. germanica*.

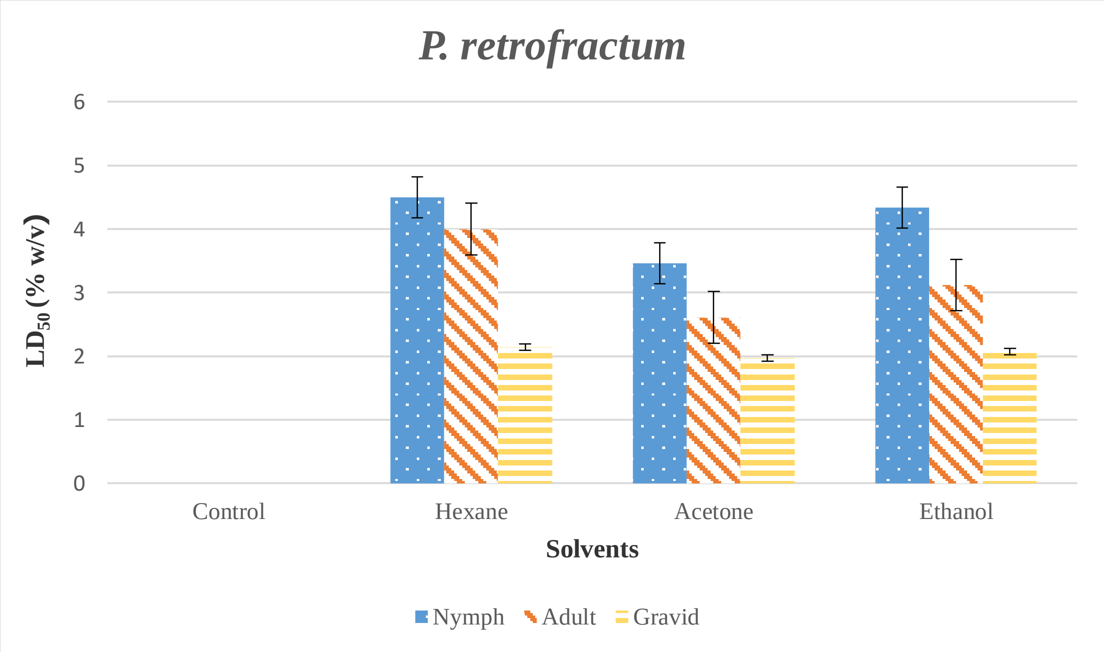


Figure 22 Histogram showing the medium lethal dose (LD_{50}) of *P. retrofractum* extract on various stage of *B. germanica* at 6 hour post experiment.
*Percentage yield (w/w) of hexane extract had very low.

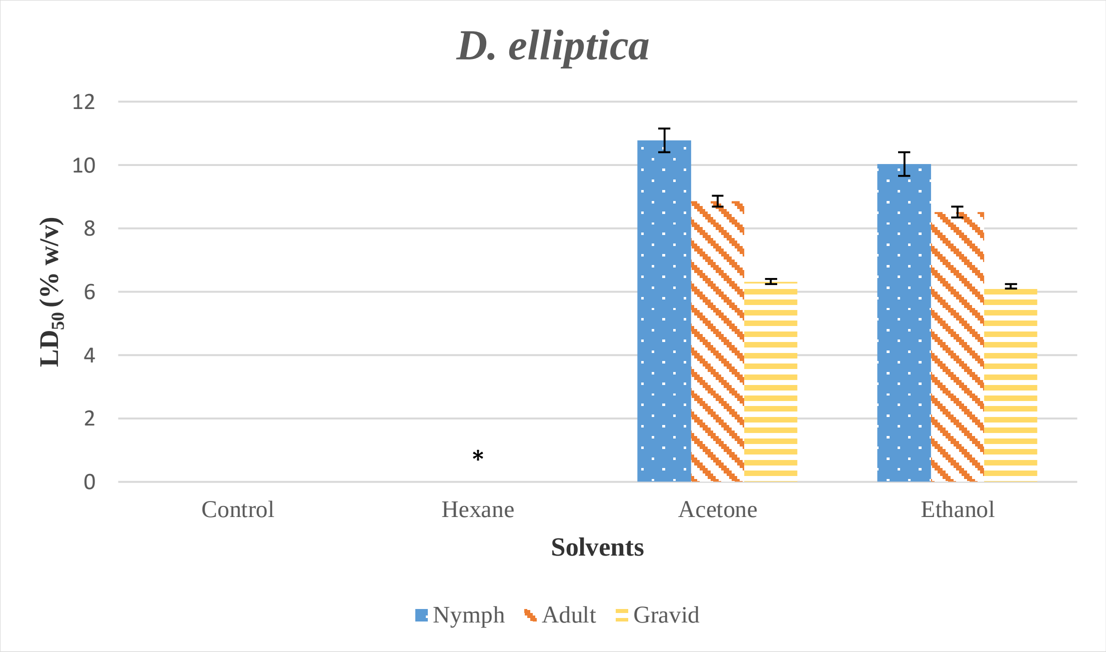


Figure 23 Histogram showing the medium lethal dose (LD_{50}) of *D. elliptica* extract on various stage of *B. germanica* at 6 hour post experiment.
*Percentage yield (w/w) of hexane extract had very low.

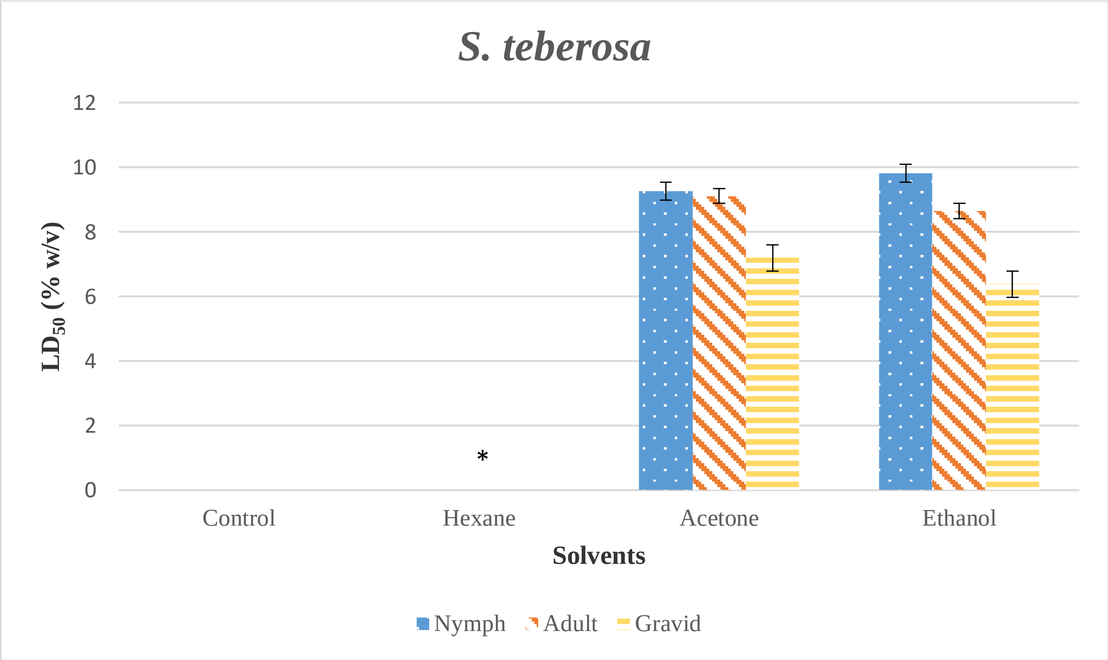


Figure 24 Histogram showing the medium lethal dose (LD_{50}) of *S. teberosa* extract on various stage of *B. germanica* at 6 hour post experiment.
*Percentage yield (w/w) of hexane extract had very low.

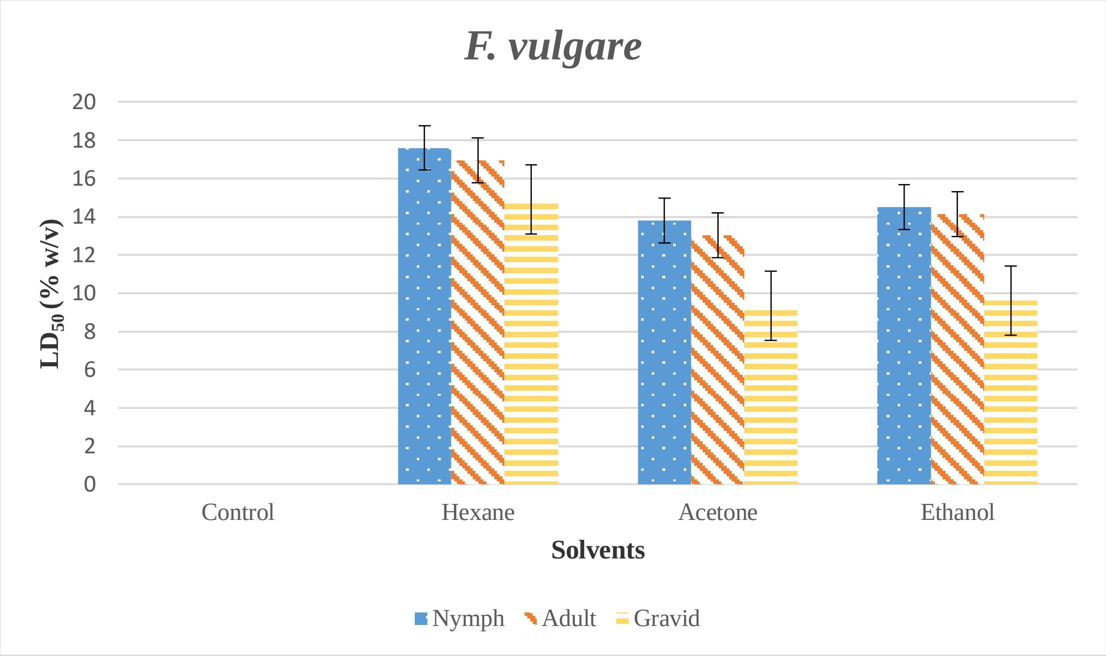


Figure 25 Histogram showing the medium lethal dose (LD_{50}) of *F. vulgare* extract on various stage of *B. germanica* at 6 hour post experiment.
*Percentage yield (w/w) of hexane extract had very low.

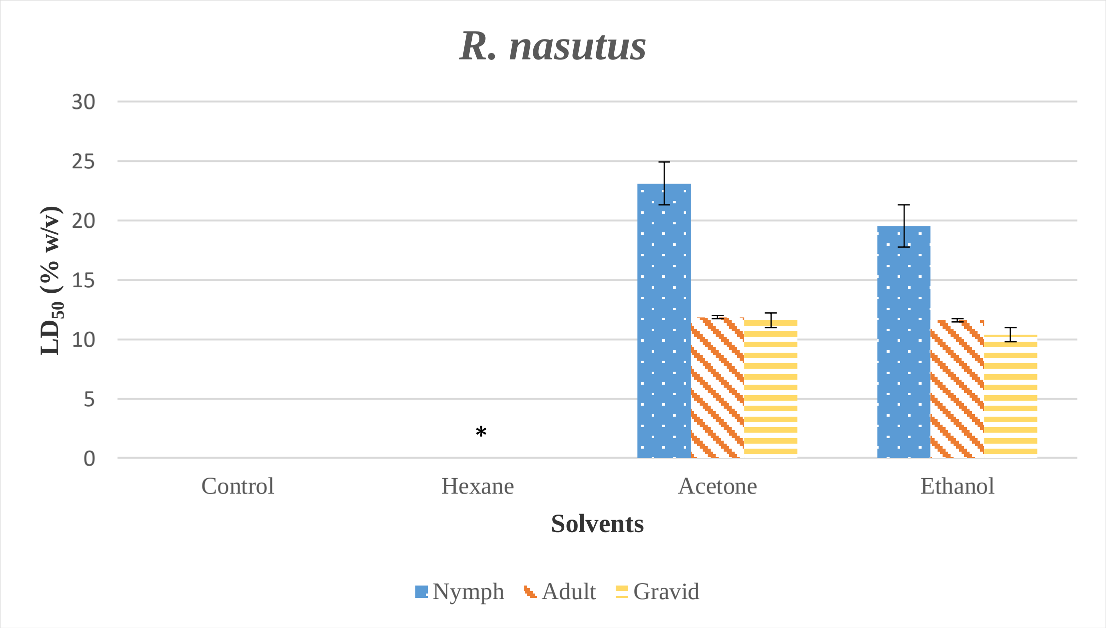


Figure 26 Histogram showing the medium lethal dose (LD_{50}) of *R. nasutus* extract on various stage of *B. germanica* at 6 hour post experiment.
*Percentage yield (w/w) of hexane extract had very low.

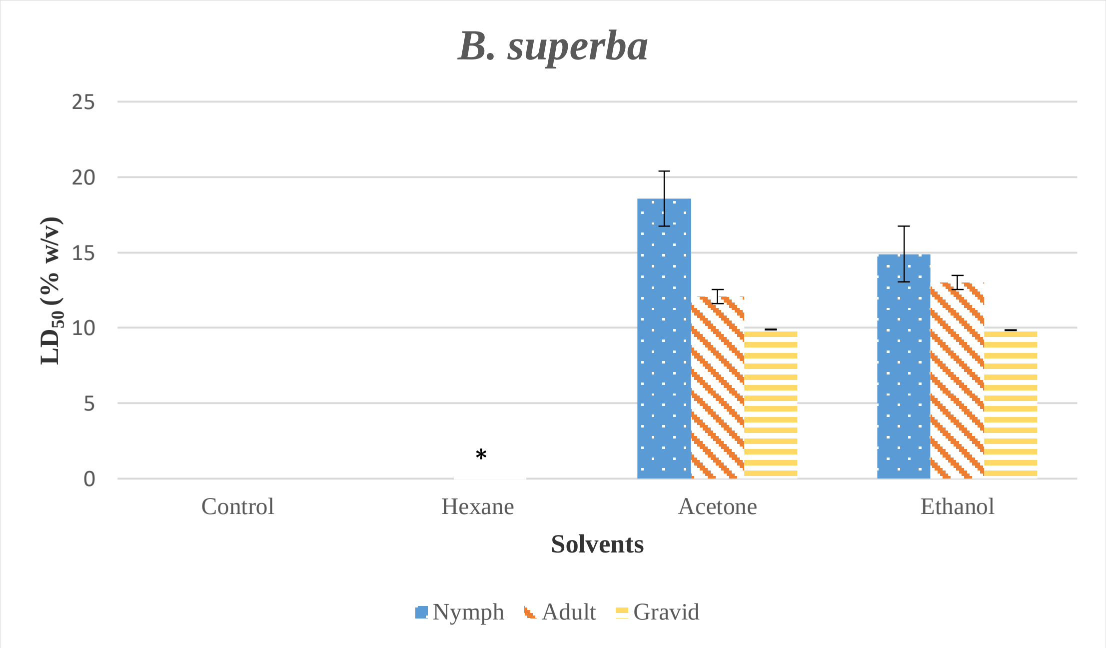


Figure 27 Histogram showing the medium lethal dose (LD_{50}) of *B. superba* extract on various stage of *B. germanica* at 6 hour post experiment.
*Percentage yield (w/w) of hexane extract had very low.

Table 23 The percentage mortality of *P. retrofractum* extract on the three developmental stages of *B. germanica* at 6 hour post experiment.

Types of herbs	**Solvent**	**Concentration**	**Control**	**% Mortality**		
				Nymph	**Adult**	**Gravid female**
Piper retrofractum	Hexane	2%	0.00±0.00	47.50±0.96	40.00±0.82	40.00±0.82
		4%	0.00±0.00	42.50±0.96	45.00±1.00	100.00±0.00
		6%	0.00±0.00	42.50±0.50	80.00±0.82	100.00±0.00
		8%	0.00±0.00	95.00±1.00	92.50±0.50	100.00±0.00
		10%	0.00±0.00	95.00±0.58	92.50±0.50	100.00±0.00
		LD_{50}	-	4.50	4.00	2.14
		LD_{90}	-	8.94	7.98	2.83
	Acetone	2%	0.00±0.00	45.00±0.58	50.00±0.82	52.50±0.96
		4%	0.00±0.00	42.50±0.96	77.50±0.50	100.00±0.00
		6%	0.00±0.00	95.00±0.58	97.50±0.50	100.00±0.00
		8%	0.00±0.00	97.50±0.50	97.50±0.50	100.00±0.00
		10%	0.00±0.00	97.50±0.50	100.00±0.0	100.00±0.00
		LD_{50}	-	3.46	2.61	1.97
		LD_{90}	-	6.53	5.01	2.65
	Ethanol	2%	0.00±0.00	47.50±0.50	45.00±1.00	45.00±0.58
		4%	0.00±0.00	47.50±0.96	75.00±1.29	100.00±0.00
		6%	0.00±0.00	48.00±0.58	82.50±0.96	100.00±0.00
		8%	0.00±0.00	95.00±0.58	95.00±0.58	100.00±0.00
		10%	0.00±0.00	92.50±0.96	97.50±0.50	100.00±0.00
		LD_{50}	-	4.34	3.12	2.07
		LD_{90}	-	8.93	6.59	2.76

*Percentage yield (w/w) of hexane extract had very low.

Table 24 The percentage mortality of *D. elliptica* extract on the three developmental stages of *B. germanica* at 6 hour post experiment.

Types of herbs	Solvent	Concentration	Control	% Mortality Nymph	Adult	Gravid female
Derris elliptica	Acetone	2%	0.00±0.00	15.00±0.00	5.00±0.58	27.50±0.50
		4%	0.00±0.00	22.50±0.50	22.50±0.96	47.50±0.96
		6%	0.00±0.00	20.00±0.00	27.50±0.50	50.00±0.82
		8%	0.00±0.00	32.50±0.50	47.50±0.96	52.50±0.96
		10%	0.00±0.00	45.00±1.29	52.50±1.50	75.00±1.29
		LD_{50}	-	10.79	8.86	6.32
		LD_{90}	-	19.51	14.85	13.84
	Ethanol	2%	0.00±0.00	37.50±0.96	15.00±1.29	27.50±0.50
		4%	0.00±0.00	30.00±0.82	32.50±0.50	47.50±0.96
		6%	0.00±0.00	27.50±1.26	25.00±1.29	55.00±0.58
		8%	0.00±0.00	45.00±0.58	45.00±0.58	50.00±0.82
		10%	0.00±0.00	45.00±1.29	60.00±1.15	77.50±0.50
		LD_{50}	-	10.04	8.52	6.17
		LD_{90}	-	22.09	15.53	12.79

*Percentage yield (w/w) of hexane extract had very low.

Table 25 The percentage mortality of *S. tuberosa* extract on the three developmental stages of *B. germanica* at 6 hour post experiment.

Types of herbs	Solvent	Concentration	% Mortality			
			Control	Nymph	Adult	Gravid female
Stemona tuberosa	Acetone	2%	0.00±0.00	10.00±0.82	2.50±0.50	20.00±0.82
		4%	0.00±0.00	17.50±0.96	22.50±0.50	45.00±0.58
		6%	0.00±0.00	20.00±0.82	25.00±0.58	47.50±0.50
		8%	0.00±0.00	52.50±0.50	42.50±0.96	47.50±0.50
		10%	0.00±0.00	47.50±0.96	52.50±0.50	65.00±1.29
		LD_{50}	-	9.26	9.11	7.19
		LD_{90}	-	15.69	14.94	14.47
	Ethanol	2%	0.00±0.00	12.50±0.50	7.50±0.50	27.50±0.96
		4%	0.00±0.00	27.50±0.96	27.50±0.50	50.00±0.82
		6%	0.00±0.00	10.00±0.50	27.50±0.50	50.00±0.82
		8%	0.00±0.00	47.50±0.96	45.00±0.58	55.00±0.58
		10%	0.00±0.00	47.50±0.50	57.50±0.50	70.00±0.82
		LD_{50}	-	9.81	8.64	6.38
		LD_{90}	-	17.30	14.87	13.54

*Percentage yield (w/w) of hexane extract had very low.

Table 26 The percentage mortality of *F. vulgare* extract on the three developmental stages of *B. germanica* at 6 hour post experiment.

Types of herbs	Solvent	Concentration	Control	% Mortality Nymph	Adult	Gravid female
Foeniculum vulgare	Hexene	2%	0.00±0.00	2.50±0.50	3.30±0.58	12.50±0.50
		4%	0.00±0.00	2.50±0.50	3.30±0.58	10.00±0.82
		6%	0.00±0.00	10..00±0.82	3.30±0.58	12.50±0.50
		8%	0.00±0.00	7.50±1.50	3.30±0.58	17.50±0.96
		10%	0.00±0.00	15.00±0.58	20.00±1.00	30.00±0.82
		LD_{50}	-	17.60	16.96	14.90
		LD_{90}	-	27.17	25.51	25.63
	Acetone	2%	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58
		4%	0.00±0.00	2.50±0.50	0.00±0.00	15.00±0.58
		6%	0.00±0.00	5.00±0.58	2.50±0.50	22.50±0.96
		8%	0.00±0.00	10.00±0.00	10.00±0.00	47.50±1.26
		10%	0.00±0.00	20.00±0.82	17.50±0.50	47.50±0.50
		LD_{50}	-	13.80	13.02	9.35
		LD_{90}	-	19.67	17.53	15.21
	Ethanol	2%	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.58
		4%	0.00±0.00	2.50±0.50	2.50±0.50	7.50±0.96
		6%	0.00±0.00	5.00±0.58	5.00±0.58	7.50±0.96
		8%	0.00±0.00	15.00±1.00	12.50±0.50	50.00±0.82
		10%	0.00±0.00	15.00±0.58	17.50±0.96	47.50±0.96
		LD_{50}	-	14.50	14.14	9.60
		LD_{90}	-	21.11	20.38	14.63

*Percentage yield (w/w) of hexane extract had very low.

Table 27 The percentage mortality of *R. nasutus* extract on the three developmental stages of *B. germanica* at 6 hour post experiment.

			% Mortality			
Types of herbs	**Solvent**	**Concentration**	**Control**	**Nymph**	**Adult**	**Gravid female**
Rhinacanthus nasutus	Acetone	2%	0.00±0.00	7.50±0.96	0.00±0.00	7.50±0.96
		4%	0.00±0.00	5.00±0.58	0.00±0.00	5.00±0.58
		6%	0.00±0.00	7.50±0.96	2.50±0.50	10.00±0.82
		8%	0.00±0.00	15.00±0.58	10.00±0.82	40.00±0.82
		10%	0.00±0.00	10.00±0.82	25.00±0.58	30.00±0.82
		LD_{50}	-	23.12	11.87	11.61
		LD_{90}	-	38.64	15.59	18.57
	Ethanol	2%	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50
		4%	0.00±0.00	2.50±0.50	0.00±0.00	5.00±0.58
		6%	0.00±0.00	5.00±0.58	2.50±0.50	5.00±0.58
		8%	0.00±0.00	12.50±0.96	7.50±0.96	47.50±0.50
		10%	0.00±0.00	5.00±0.58	27.50±0.50	35.00±0.58
		LD_{50}	-	19.54	11.61	10.40
		LD_{90}	-	29.39	14.99	15.52

*Percentage yield (w/w) of hexane extract had very low.

Table 28 The percentage mortality of *B. superba* extract on the three developmental stages of *B. germanica* at 6 hour post experiment.

			% Mortality			
Types of herbs	**Solvent**	**Concentration**	**Control**	**Nymph**	**Adult**	**Gravid female**
Butea superba	Acetone	2%	0.00±0.00	5.00±0.58	0.00±0.00	10.00±0.82
		4%	0.00±0.00	2.50±0.50	0.00±0.00	7.50±0.50
		6%	0.00±0.00	2.50±0.50	2.50±0.50	12.50±0.96
		8%	0.00±0.00	15.00±0.58	15.00±1.00	45.00±0.58
		10%	0.00±0.00	12.50±0.50	22.50±0.96	47.50±0.50
		LD_{50}	-	18.58	12.09	9.87
		LD_{90}	-	29.34	16.24	15.83
	Ethanol	2%	0.00±0.00	5.00±0.58	0.00±0.00	2.50±0.50
		4%	0.00±0.00	5.00±0.58	2.50±0.50	5.00±0.58
		6%	0.00±0.00	5.00±0.58	2.50±0.50	10.00±0.82
		8%	0.00±0.00	12.50±1.26	2.50±0.50	47.50±0.50
		10%	0.00±0.00	25.00±1.29	25.00±0.58	42.50±0.96
		LD_{50}	-	14.90	13.03	9.83
		LD_{90}	-	23.15	17.73	14.65

*Percentage yield (w/w) of hexane extract had very low.

On the whole, among the six Thai botanical extract, *P. retrofractum* was the most efficacy against all stage of *B. germanica* including nymph, adult and gravid stage at every experimental time intervals, followed by *D. elliptica* and *S. tuberosa,* while *F. vulgare, R. nasutus* and *B. superba* were the least effective. Moreover, in all stage, gravid female was the most susceptible, followed by adult stage and nymph stage in all of six Thai botanical. In considering the solvent, the ethanol and acetone exhibited more efficacy of extraction than hexane.

Furthermore, at all solvent and levels of concentration of the six Thai botanical extracts, it was found that as the time passed, percentage mortality of *B. germanica* increased, however, the 24 and 48 hours post experiment of all of six Thai botanical extract more toxicity on three developmental stage than 1, 2, 4 and 6 hours post experiment. In addition, the acetone extract of *P. retrofractum* at highest concentration (10% w/v) exhibited complete insecticidal activity (100%) against both adult and gravid stage of *B. germanica* after 6th hours post experiment. However, all solvents and all concentrations extracts from *P. retrofractum* cannot induce complete insecticidal activity against nymph stage of *B. germanica.*

4.4 Main component of Thai botanical extracts

This study indicated that among the six Thai botanical extract, the acetone extract from *P. retrofractum* was the most efficacy against all various stage of *B. germanica* by topical application method. Moreover, among three stage including nymph, adult and gravid, the nymph stage were the most tolerant.

Therefore, the acetone extract from *P. retrofractum* was isolated and purified the main component by column chromatography and each fraction was tested against nymph stage of *B. germanica* in laboratory condition. The results found that, fractionation of acetone extract of *P. retrofractum* by using silica gel column chromatography led to separation of 14 fractions (F1-F14) (Fig. 28 - 29). After that, each fraction at different concentrations (2% and 10% w/v) was used for the toxicity test against nymph stage by topical application method. The percentage mortality of nymph stage against each fractions of acetone extract of *P. retrofractum* showed in Table 29.

Figure 28 The acetone extract from *P. retrofractum* was isolate and purified the main component by column chromatography**.**

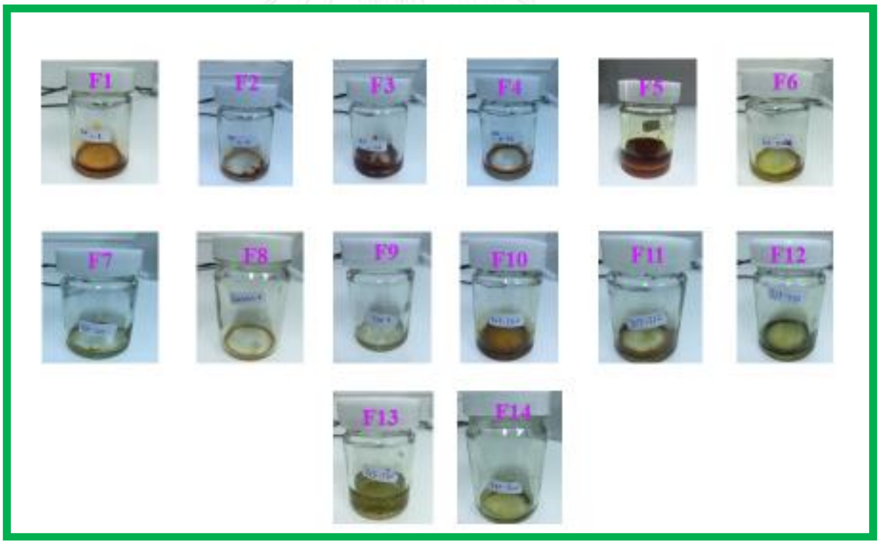


Figure 29 Fractionation of acetone extract of *P. retrofractum* led to separation of 14 fractions (F1-F14).

Table 29 The percentage mortality of nymph stage against different concentrations of each fractions of acetone extract of *P. retrofractum*

Fractions	% yield	% Mortality											
	hours	1 hour		2 hours		4 hours		6 hours		24 hours		48 hours	
	Conc.	2%	10%	2%	10%	2%	10%	2%	10%	2%	10%	2%	10%
F1	0.028	0.00±0.00	40.00±0.82	0.00±0.00	55.00±0.58	0.00±0.00	62.50±0.50	0.50±0.58	72.50±0.96	1.25±0.50	72.50±0.96	2.00±0.82	72.50±0.96
F2	0.027	0.00±0.00	40.00±0.82	0.50±0.58	55.00±0.58	1.25±0.96	67.50±0.96	1.75±0.50	72.50±0.96	3.25±0.96	85.00±0.58	4.00±0.82	92.50±0.50
F3	0.158	0.25±0.50	40.00±0.82	0.50±0.58	55.00±0.58	0.50±0.58	67.50±0.96	0.75±0.96	72.50±0.96	2.25±0.50	80.00±0.82	2.50±0.58	85.00±0.58
F4	0.077	0.00±0.00	40.00±0.82	0.75±0.96	47.50±1.26	1.00±0.82	62.50±0.50	1.50±0.58	72.50±0.96	1.75±0.50	87.50±1.50	2.25±0.50	90.00±1.15
F5	1.088	0.75±0.96	87.50±0.96	2.00±0.82	97.50±0.00	2.50±1.29	100.00±0.00	3.50±0.00	100.00±0.00	5.00±0.00	100.00±00.00	5.50±0.00	100.00±0.00
F6	0.040	0.50±0.58	5.00±0.58	1.25±0.96	20.00±0.82	1.25±0.96	25.00±0.58	1.75±0.96	42.50±0.96	1.75±0.50	62.50±0.96	2.00±0.82	75.00±0.58
F7	0.005	0.00±0.00	10.00±0.82	0.25±0.50	15.00±0.58	0.25±0.50	25.00±0.58	0.25±0.50	35.00±1.00	0.50±0.58	45.00±0.58	1.25±0.50	55.00±0.58
F8	0.076	0.00±0.50	10.00±0.50	0.25±0.50	15.00±0.50	0.25±0.50	25.00±0.50	0.25±0.58	35.00±0.58	0.50±0.58	45.00±0.58	1.25±0.58	55.00±0.58
F9	0.023	0.00±0.50	10.00±0.50	0.25±0.50	15.00±0.50	0.25±0.58	25.00±0.58	0.25±0.50	35.00±0.50	0.50±0.50	45.00±0.50	1.25±0.50	55.00±0.50
F10	0.011	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50
F11	1.011	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
F12	0.877	0.25±0.50	2.50±0.50	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50	0.25±0.50	5.00±0.58
F13	0.100	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50	0.00±0.00	2.50±0.50
F14	0.002	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.25±0.50	0.00±0.00
Control		0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.50	2.50±0.50

When considering the table above, it could be seen that fractions 5 gave the highest efficacy against nymph stage of *B. germanica* when comparing with the other fractions. Furthermore, at concentration 10% (w/v) showed completely mortality (100%) after 4 hours post experiment. Therefore, fraction 5 may assumed that it have to contain the main component. Consequently, the fraction 5 was investigated main component by thin layer chromatography (TLC) and detected piperine which a major alkaloid in the fruit of *P. retrofractum* by Dragendorff's reagent with two dimension development TLC. The solvents system used hexane per ethyl acetate (40:60 v/v) and dichloromethane per methanol (95:5 v/v). The result found that the retention factor value (R_f) of piperine analytical standard and the fraction 5 from acetone extract of *P. retrofractum* were the same (Fig. 30).

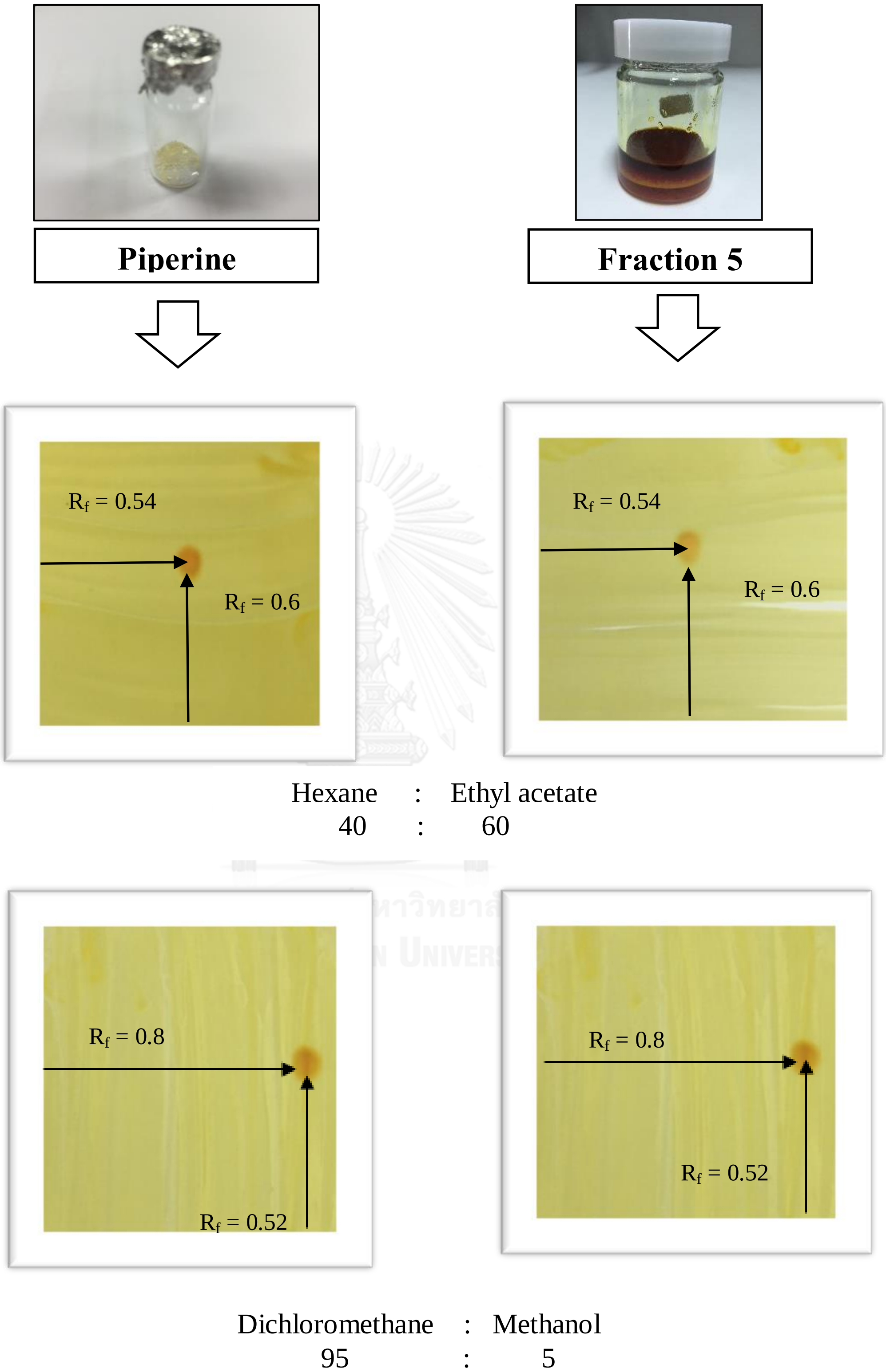


Figure 30 Two dimension development Thin Layer Chromatography (TLC) of piperine analytical standard and fraction 5 from acetone extract of *P. retrofractum* by used two solvents system.

CHAPTER V
DISCUSSIONS

5.1 Effect of solvent types on the yield of Thai botanical extract

In considering the solvent, this study used three different solvent with increasing polarity such as hexane, acetone and ethanol. This study found that, the ethanol gave the highest % yield (w/w) in all the six Thai botanical, followed by acetone and hexane, respectively.

The results of our study were similar to the previous study, Kraikrathok *et al.* (2013) who evaluated the extracts of *P. retrofractum* against diamondback moth, *Plutella xylostella* third instars larvae under laboratory conditions. These plants were prepared by using sequential extraction with four different solvents including hexane, dichloromethane, ethyl acetate and ethanol and the result showed that the percent yields of the ethanol extracts were higher (10.91 w/w) when compare with hexane extract (8.80 w/w) [128]. A similar finding was reported by Tikum *et al.* (2008), who investigated percentage yield of *S. tuberosa* when extract with different solvent with increasing polarity such as hexane, dichloromethane and methanol and this study found that hexane (non-polar solvents) had lower percentage yield of crude extract (0.21 % yield w/w) than methanol extract (polar solvents) (7.14 % yield w/w) [129]. Moreover, Kongkiatpaiboon and Gritsanapan (2013) evaluated the yield of didehydrostemofoline alkaloid which a major active component of *S. collinsiae* root, the study found that the yield of didehydrostemofoline alkaloid of *S. collinsiae* root increased with the increasing polarity (acetone < acetonitrile < 70% ethanol < methanol). They suggested that the methanol and 70% ethanol were the efficient solvents for extracting didehydrostemofoline from the roots of *S. collinsiae* with nearly the same yields of 0.393 ± 0.007 and 0.388 ± 0.001% w/w, respectively. However, 70% ethanol was the recommended solvent for extracting S. collinsiae roots because it was less hazardous than methanol [130].

In 2003, Oktay *et al*. investigated yield of *F. vulgare* seed extracts when extract with water and ethanol and the result found that water extract had higher yield of crude extract with cause 16.20 (g/100g) than ethanol extract with cause 10.95 (g/100g) [131]. Chukeatirote and Saisavoey (2009) examined crude extracts of *B. superba* which were prepared by using sequential extraction with three different solvents including, hexane, ethyl acetate and methanol. The study showed that the percent yield of the extract was found to be highly dependent on the type of solvent used. The percent yields of the methanol extracts of *B. superba* were the highest with cause 15.34 w/w, followed by ethyl acetate extract with cause 0.36 w/w and hexane extract with cause 0.13 w/w, respectively. Furthermore, they also examined crude extracts of *Pueraria mirifica* with the percent yields of the methanol extracts were the highest (11.37 w/w), followed by ethyl acetate extract (0.58 w/w) and hexane extract (0.21 w/w), respectively. And *Mucuna macrocarpa*, the percent yields of the methanol extracts were the highest (11.46 w/w), followed by ethyl acetate extract (0.34 w/w) and hexane extract (0.29 w/w), respectively [132]. Another similarity is Suklampoo *et al.* (2010) who study the extraction of pomelo peel of Khao-yai and Thong-dee varieties with hexane, ethylacetate and 95% ethanol and this study found that ethylacetage had more percentage yield of crude extract than hexane and the highest yield of crude extract was obtained from the extraction with ethanol [133].

As a result of this study indicate that, the ethanol exhibited efficient solvents for extraction than acetone and hexane in all of the six Thai botanicals. However, the hexane extract from *D. elliptica, S. tuberosa, F. vulgare, R. nasutus* and *B. superba* showed very low of % yield. Thus, in this study these four botanical from the hexane extract were not selected to insecticidal activity test. One factor that may explain effect of solvent types on the yield of botanical extract is properties of the component in the botanicals that different solubility depend on types of solvent used.

On the other hand, the results of efficacy of solvent type on the yield of *D. elliptica* extract of our study were in contrast to the previous study by Sae-Yuna *et al.* (2006) who study the effects of experimental variables, such as solvent, temperature and pressure, on pressurized liquid extraction (PLE) techniques for the efficient extraction of rotenone from the stem and root of *D. elliptica* and *D. malaccensis*. They showed that chloroform (non-polar solvents) was determined to be a good extraction solvent with lead to rotenone content 40.6%, w/w compared to commonly used solvent, 95% ethanol (polar solvents) with lead to rotenone content 15.0%, w/w [134]. However, the efficacy on the yield in plant may depend on several factors including plant population, plant parts, developmental stage of the plant, types of solvents used, extraction techniques, and types of equipment used and the solubility of the phytochemicals in the plant.

5.2 Insecticidal activity of Thai botanical extract to the *B. germanica*

This present study indicated that crude extract of six Thai botanical including *P. retrofractum, D. elliptica, S. tuberosa, F. vulgare, R. nasutus* and *B. superba* had a highly toxic effect against nymph, adult and gravid stage of *B. germanica*. This result similar to previous study that found the extracts of unripe and ripe fruits of P. retrofractum from Thailand showed significantly high activity against 3rd and 4th instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* [87]. And the extracts of P. retrofractum also showed against Diamondback moth, *Plutello xylostella* third instars larvae under laboratory conditions [128]. Moreover, *P. retrofractum* has been reported to antileishmanial activity, the n-hexane, ethyl acetate, methanol, and acetone extracts of *P. retrofractum* exhibited significant in vitro activity at 100 mg /ml against promastigotes of *L. donovani* [86]. Furthermore, two botanical insecticide formulations, mixtures of *P. retrofractum* and *A. squamosa* (RS) extracts and *Aglaia odorata* and *A. squamosa* (OS) extracts showed efficacy decreased the population of *Crocidolomia pavonana* and *P. xylostella* [135].

In 2005, Komalamisra *et al.* investigated the effect of ethanolic extracts from S. tuberose root on mosquito larvae, and the result indicated that roots ethanolic extracts of *S. tuberose* showed larvicidal activity against four mosquito vector species including *Ae. aegypti*, *Cx. quinquefasciatus*, *An. dirus* and *Mn. uniformis* in laboratory conditions. Sawangjit (2000) also reported the efficacy of roots extracted of *S. tuberosa* against the 2nd instar beet armyworm *Spodoptera exigua* Hubn [69, 89].

Moreover, Visetson and Milne (2001) evaluated roots extracted from *D. elliptica* on the Diamondback moth, *P. xylostella* larvae, and the results showed that the rotenone from *D. elliptica* root extract has shown insecticidal activity against *P. xylostella* larvae (92). As well as, the ethanolic extracts from *D. elliptica* root showed larvicidal activity against *Ae. aegypti*, *Cx.quinquefasciatus*, *An. dirus* and *Mn. uniformis* mosquito larvae [90]. Furthermore, Muthukrishnan and Pushpalatha (2001) discovered the *R. nasutus* leaf extract are effective at decreasing the fecundity of the three mosquitoes including *Cx. quinquefasciatus, An. stephensi* and *Ae. aegypti* on the hatchability of their eggs [111]. This is similar to Komalamisra *et al*. (2005) who reported the ethanolic extracts from *R. nasutas* showed larvicidal activity against *Ae. aegypti, Cx. quinquefasciatus, An. dirus* and *Mn. uniformis* [90].

Furthermore, Lapcharoen *et al.* (2005) stated that *B. superba* extract exhibited larvicidal activity and insect growth regulators (IGRs) on late 3rd and early 4th instar larvae of *Ae. Aegypti* and *Cx. quinquefasciatus* [91]. Moreover, the fruit of *F. vulgare* has shown insecticidal activities against adults of three coleopteran stored-product insects, including *Sitophilus oryzae*, *Callosobruchus chinensis* and *Lasioderma serricorne* by using direct contact application and fumigation methods [114]. Pitasawat *et al*. (2007) also reported essential oil of F. vulgare showed larvicidal activity on *Ae. agypti* and *An. dirus* [136].

Nevertheless, this study indicated that among the six Thai botanical extracts, *P. retrofractum* was proved to be the most insecticidal activity against all various stage of *B. germanica* including nymph, adult and gravid stage. Similar results were found by Chansang *et al.* (2005) demonstrated the larvicidal activity of ten Thai botanical aqueous extract including P. *retrofractum, Curcuma longa, Syzpgium aromaticum, Allium sativum, Spilanthes paniculata, Gynura pseudochina, Zingiber* *montanum, Rhinacanthus nasutus* and *Cleome viscosa*. The study found that *P. retrofractum* has showed the highest larvicidal activity against 3rd and 4th instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti* in the laboratory with LD_{50} value of 135 mg/l and LD_{90} value of 79 mg/l, respectively [87]. Similar to Kraikrathok *et al.* (2013) who evaluated the extracts of *P. retrofractum, P. sarmentosum*, *P. interruptum* and *P. nigrum* against diamondback moth, *P. xylostella* third instars under laboratory conditions. The results also reported that the extract of *P. retrofractum* was the most active with LD_{50} value of 237 ppm [128].

P. retrofractum is commonly known as Java long pepper and belong to family piperaceae. In Thai commonly called "Di-pli", which is common grown throughout Thailand and other countries in Southeast Asia such as Indonesia, Malaysia, Philippines and Vietnam. It has various applications in traditional medicine in Southeast Asia. In Thailand, the fruit of *P. retrofractum* used as a spice for curries, preserves and pickles and has been frequently used in traditional medicine for treatment of food digestion, blood circulation, asthma, influenza and hypertension. The stem has been reported to alleviate alley post-delivery pain in mothers and also to be useful against rheumatic pains and diarrhea. The root has been reported to be useful for asthma, bronchitis and tuberculosis [97, 98]. Therefore, *P. retrofractum* listed as a source of spices and herbal drugs in Thai Herbal Pharmacopoeia (THP 2000) [87, 137- 138].

Several reports indicate that *P. retrofractum* and many other species belonging to the family Piperaceae have been used as natural insecticides for various insects and protozoa (Table 30). For example, *P. retrofractum* extract showed larvicide efficacy against *Ae. aegypti, Cx. quinquefasciatus* and *P. xylostella* [87, 128]. It also showed against promastigotes of *L. donovani* in vitro[86]. Ethanol extract of *P. nigrum* exhibited adulticide efficacy against *M. domestica*, and it essential oil can repellent and oviposition deterrent on various mosquitoes, for instance *Ae. albopictus, Ae. aegypti, Ae. togoi, An. dirus, Cx. quinquefasciatus* and *Cx. pipien pallens* [12, 88, 139]. Moreover, *P. longum* expressed both larvicide and adulticide against *Ae. aegypti* [140-142]. The ethanol extract of *P. ribesoides* and *P. sarmentosum* expressed larvicide and adulticide activity against *Ae. aegypti* [140, 141]. Furthermore, the essential oil of *P. aduncum* proved repellent activity against *Ae. albopictus* [143]. And the essential oil of *P. betle* exhibited repellent activity against *Ae. albopictus, Ae. aegypti, An. dirus* and *Cx. quinquefasciatus* [12].

Table 30 The efficacy of family Piperaceae extracts against insects and protozoa.

Plant species	**Extracts used**	**Report of the effect against insects**	**Activity**	**Reference**
P. retrofractum	Water extract	*Ae. aegypti*, *Cx.quinquefasciatus*	Larvicide	[87]
	Hexane, Dichloromethane, Ethyl acetate and Ethanol extract	*P. xylostella*	Larvicide	[128]
	Hexane, Ethyl acetate, Acetone and Methanol extract	*L. donovani*	Promastigote - killing	[86]
P. nigrum	Ethanol extract	*M. domestica*	Adulticide	[139]
	Essential oil	*Ae. albopictus* *Ae. aegypti*, *An. dirus*, *Cx. quinquefasciatus*	Repellent, oviposition deterrent	[12]
P. longum	Ethanol extract	*Ae. aegypti*	Larvicide	[140]
	Essential oil	*Ae. aegypti*	Adulticide	[142]
P. ribesoides	Ethanol extract	*Ae. aegypti*	Larvicide	[140]
	Ethanol extract	*Ae. aegypti*	Adulticide	[141]
P.sarmentosum	Ethanol extract	*Ae. aegypti*	Larvicide	[140]
	Ethanol extract	*Ae. aegypti*	Adulticide	[141]
P. aduncum	Essential oil	*Ae. albopictus*	Repellent	[143]
P. betle	Essential oil	*Ae. albopictus* *Ae. aegypti*, *An. dirus*, *Cx. quinquefasciatus*	Repellent, oviposition deterrent	[12]

In addition, the toxicity of *P. retrofractum* on mammalian was reported by Jaijoy *et al.* (2010) who evaluated the acute and subchronic toxicity of aqueous extract from the fruits of *P. retrofractum* in both male and female rats, and the results showed that the aqueous extract of *P. retrofractum* fruits did not produce acute or subchronic toxicity in either female or male rats at doses of 5,000 mg/kg body weight for acute toxicity and 300–1,200 for subchronic toxicity. This study proved that *P. retrofractum* extract does not produce acute or subchronic toxicity in either female or male rats and neither gross abnormalities nor histopathological changes were observed [138].

The advantage of all of the previously mentioned attributes including the fact that botanical are safe for the human and are also environmental safety, as well as it less toxic when compared to chemical products. Moreover, botanical also prevent the insect develop resistance to chemical insecticides, and low or no mammalian toxicity. Furthermore, based on the study finding, *P. retrofractum* crude extract could be used as an alternative strategy for controlling various stage of *B. germanica* instead of chemical insecticides in areas where chemical insecticides are not appropriate and/or used in integrated pest management programs (IPM) against *B. germanica* [144, 145].

5.3 Susceptibility of various developmental stages of the *B. germanica* to Thai botanical extracts.

When compare susceptibility of three developmental stages of German cockroach, *B. germanica* to the six Thai botanical extracts. This study found that among three stages, gravid female was the most susceptible, followed by adult stage and nymph stage in all of six Thai botanical. Our results agree with those of Phillips *et al*. (2010) who determined the fumigant toxicity of 12 essential oil components including carvacrol, 1,8-cineole, trans-cinnamaldehyde, citronellic acid, eugenol, geraniol, S-(-)-limonene, (-)-linalool, (-)-menthone, (+)-α-pinene, (-)-β-pinene, and thymol on B. germanica and found that 1, 8-cineole was the most toxic essential oil component and result indicated that the LC50 values for gravid females (5.3 mg/liter air) were the lowest followed by adult males (6.8 mg/liter air), adult females (8.4 mg/liter air) and large nymphs (11.4 mg/liter air), respectively [31].

Similar results were found by Abd-Elghafar *et al*. (1990) demonstrated the toxicity of ten insecticide including bendiocarb, chlorpyrifos, cyfluthrin, cypermethrin, fenvalerate, hydramethylnon, malathion, propetamphos, propoxur and pyrethrins against males, gravid and non-gravid female of *B. germanica*. The study found that males and gravid females were generally more susceptible than non-gravid females to the ten insecticides that they tested, and the non-gravid female were the most tolerant to all insecticide tested among the three adult stage/sexes tested [146]. Moreover, Koehler *et al*. (1993) who studied the differential susceptibility of *B. germanica* cockroach sexes and nymphal age to insecticides such as bendiocarb, chlorpyrifos, and cypermethrin, and the results also proves that male cockroaches have the highest susceptibility and late instar nymphs were the most tolerant in all insecticide because late instar nymphs had higher LC_{50} (µg per insect) and LD_{50} (µg/mg) than adult males and female cockroach, and females had significantly higher LC_{50} (µg per insect) than males [147].

Furthermore, Qian *et al*. (2010) investigated differential susceptibility of age group of nymphs staged of *B. germanica* with consisted of 2 week-old nymphs are primarily 3rd instars, 4 week-old nymphs are primarily 4th instars, 5 week-old nymphs are primarily 5th instars and 6 week-old nymphs are primarily 6th instars on insecticides such as dichlorvos and propoxur by using a topical application bioassay. The results showed that the 6 week-old nymphs were more tolerant to dichlorvos and propoxur than the other ages tested with LD_{50} values of dichlorvos and propoxur were 2.003 µg per insect and 5.296 µg per insect, respectively. The tolerance ratios of 18.55 fold and 4.98 fold for LD_{50} were obtained from 6-weekold nymphs compared to 4 week-old nymphs [148]. In addition, Alejandra *et al*. (2014) evaluated differential susceptibility of nymphs and adults stage of *B. germanica* (Blattodea: Blattellidae) and *P. fuliginosa* (Blattodea: Blattidae) to native isolates of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*. The results showed differences in susceptibility between the two species of cockroaches and between nymphs and adults stage of the same species. Moreover, adult stage is more susceptible than nymph stage in both species [149].

From the overall reports, it was proved that gravid female and adult stage was more susceptible than nymph stage, and gravid female was more susceptible than adult stage. This may be due to 1). Lipid content: the weight differences affect insecticide susceptibility. Therefore, female cockroaches having higher weight and higher mass than the males due to differential lipid content and thus affecting insecticide toxicity [8, 150, 151]. 2). Metabolic rate: differences in metabolic rate found in the adult male and female and the nymphs, thus the higher metabolism of nymph stage may lead to faster excretion of the toxic substance from the body [21, 147]. Therefore, stage and sex differences should be considered when determining insecticide toxicity for *B. germanica* cockroaches.

5.4 Main component of Thai botanical extracts

This study indicated that among the six Thai botanical extract, the acetone extract from *P. retrofractum* was the most efficacy against all various stage of *B. germanica* including nymph, adult and gravid stage by topical application method. Therefore, the acetone extract from *P. retrofractum* was isolated and purified the main component by column chromatography. After that, each fraction was analyzed by Thin Layer Chromatography (TLC). The fractions with a same TLC pattern were combined and the fraction with showed the highest efficacy against *B. germanica* was investigated main component by two dimension development Thin Layer Chromatography (TLC). Our study proved that the fraction 5 from acetone extract of *P. retrofractum* have to contain the main component which is piperine. The results obtained were similar to those observed for chemical constituents of *P. retrofractum* in previous study. For instance, Parmar *et al.* (1997) and Rahman *et al*. (2005), reported the principle active constituents of the fruit of *P. retrofractum* is piperine [97, 98]. Moreover, Bao *et al*. (2014) explored piperidine alkaloids in family Piperaceae that collected in different areas. The result exhibited that piperine was showed to be a major component of dried fruits of *P. retrofractum, P. nigrum and P. longum,* however the content of piperine in *P. longum* was lower than in *P. retrofractum* and *P. nigrum* [152]. Furthermore, Fabarani (2012) investigated methanol crude extract of the fruits of *P. retrofractum* and found that the fruits of P. retrofractum crude extract contain four compounds such as piperine, oleic acid, N-isobutyl-2E,4E,14Z-eicosatrienamide and methyl piperate. The crude extracts and piperine were found to show the strongest inhibition against both gram positive bacteria such as *Bascillus subtilis* and *Staphylococcus aureus* with MIC and MBC values of 225 µg/mL [153]. In addition, Scott et al. 2005 stated that the compounds found in family Piperaceae such as *P. nigrum* seed and *P. tuberculatum* leaf extract were piperamides which including piperine, piperlonguminine, dihydropiperlonguminine, dihydropiperine and pipercide. The modes actions of piperamides are several such as contact toxicity, synergism, repellent and antifeedant properties in insect [154].

Piperine is known as a major alkaloid in the fruit of *P. retrofractum* and many other species belonging to the family Piperaceae such as *P. retrofractum, P. longum* and *P. nigrum*. The characteristics of piperine are black and yellow in color, aromatic and pungent in odour, and biting taste. It was found to be soluble in various solvents such as methanol, ethanol and chloroform but insoluble in water. The melting point of piperine was found to be 129 0C (standard range (129-130 0C). Amount of total ash content was found to be 1% w/w and acid insoluble ash was found to be 0.5%w/w. The Rf value was found to 0.55 and 0.33 which showed good separation of piperine for Thin Layer Chromatography (TLC) method, however different samples have different Rf value. For Ultra Violet Spectrophotometer analysis (UV) method the λmax was found to be 360nm and the absorbance observed was 2.65. Moreover, Piperine in *P. nigrum* was separated from other compounds by HPLC and compared with the standard Piperine peak, the λmax was found to be 343nm [155-157].

Moreover, many previous studies suggested piperine as potential botanical insecticides. The study of toxicity of piperine for insecticidal activity against insects and protozoa, for example, Samuel et al. (2016) explored the larvicidal effects of *P. nigrum* and piperine against third and fourth instar Anopheles larvae and found that the *P. nigrum* and piperine lead to mortality in *An. gambiae, An. arabiensis, An.*

coluzzii, An. quadriannulatus and *An. funestus*. Moreover, *P. nigrum* and piperine mixtures caused high mortality in the *An. gambiae*. However, *P. nigrum* proving significantly more toxic than piperine and they concluded that *P. nigrum* shows potential as a larvicide for the control of certain malaria vector species [158]. Moreover, Freire-de-Lima *et al*. (2008) examined the toxic effects of piperine against *Trypanosoma cruzi* and the result exhibited that the kinetics of the morphological alterations induced by piperine on *T. cruzi* epimastigote [159]. Furthermore, insecticide activity of piperine to eggs of *Spodoptera frugiperda* and *Diatraea saccharalis* was reported by Tavares *et al*. (2011) , and it was reported to show against *M. domestica* and promastigotes of *L. donovani* both in vitro and in vivo [86, 160-164]. In addition, three of piperaminds isolate from *P. nigrum* such as piperine, pellitorine and pipercid showed against male of been weevil, *Callosobruchus chinensis* with ranged in toxicity from 20, 2 and 0.15µg/insect, respectively [165].

Nevertheless, the efficacy of the plant extracts in *P. retrofractum* and many other species belonging to the family Piperaceae against insect has been correlated with the concentration of piperamides in the extract [166]. Furthermore, the toxicity and chemical component of the plant are varies greatly according to the geographical origin of the plant, plant parts, developmental stage of the plant, methods of extraction and solvent used [167, 168] The appearance of main component in family Piperaceae that collected in different areas was study by Bao *et al*. (2014) who analyzed quantitative of piperine and piperlonguminine which main component of family Piperaceae in 11 samples collected in different geographical and ecological regions. The result showed that the average content of piperine in *P. longum* (18.26 mg/g, range 12.05–33.23 mg/g) was lower than *P. nigrum* (40.09 mg/g, range 29.57–54.23

mg/g) and *P. retrofractum* (31.08 mg/g), Moreover, a sample of *P. longum* from Vietnam and a sample of *P. retrofractum* from Ishigaki and Japan, showed high contents of piperine (31.08 mg/g) and piperlonguminine (1.93 mg/g), respectively. In contrast, a sample of *P. betle* from Taiwan showed very low content of piperine (0.06 mg/g), and piperlonguminine was not detected. The study suggested that *P. betle* may be chemotaxonomically different from *P. retrofractum, P. nigrum* and *P. longum* although they all belong to the genus Piper. Moreover, the quantitative content of piperine and piperlonguminine in the same species but collected in different areas are dissimilar [152]. In 1988, Semler and Gross investigated the distribution of piperine in vegetative parts of *P. nigrum*, and the study found that the most recognized compound from *P. nigrum* seed is piperine. It is present in the highest concentration of all secondary compounds in the seed of the plant; however, 2 to 10 fold differences can occur between samples [169]. According to Singh et al. 2004 who revealed the most abundant compounds in the *P. nigrum* essential oil acetone extract were piperine (33.5%) and piperolein B (13.7%) as well as unidentified alkaloid constituents (5.5 and 6.3%) and other identified piperamides (<3.4%) [170].

Furthermore, the environmental persistence of piperamides of family Piperaceae was investigated by Scott et al. (2003) who revealed that piperamides are degraded quickly under full sunlight and under ultraviolet (UV) lamp exposure, Moreover, pure piperine degraded quickly with a half-life of approximately 40 minute. Similar to half-life of piperine in *P. guineense* seed extract after exposure to full sunlight was approximately 49 minute. Furthermore, *P. nigrum* extracts were applied to the soil and the half-life for piperamides was one to several days depending on the time of year. As a result, the benefit of short residual activity of family Piperaceae seed extracts are more acceptable for organic certification [166, 171].

In addition, Piperamides of family Piperaceae are known to act as neurotoxins in the insect. The mode of entry into insect of piperine are a contact poison and the mode of action affects in central nervous system (CNS) of insect [66, 98]. Moreover, Scott et al. (2008) stated that Piperamides of *P. nigrum* extract are highly effective when applied as a synergist with other botanical insecticide such as pyrethrum. Therefore, Piperamides of family Piperaceae might still replace contact insecticides, specifically chemical neurotoxic compounds such as carbamates, organophosphates and pyrethroids for reduce the insect resistance to chemical insecticide, and it decrease environmental exposure to conventional synthetic insecticide [166, 172].

Therefore, this study used topical application method; hence, the route of entry into the *B. germanica* is a contact poison and the mode of action may affects in central nervous system (CNS). The target site may entering through the cuticle, fat body and other tissues of *B. germanica* [173]. Base on the aformentioned, piperine in the fruit of *P. retrofractum* in this study may cause toxic effect against various stage of *B. germanica* and our results indicate that acetone extract of *P. retrofractum* may useful insecticidal activity against *B. germanica*.

CHAPTER VI
CONCLUSION

German cockroaches, *B. germanica* is considered an important medical and economic pest because they have a short generation time and high fecundity that makes it difficult to control. Conventional synthetic insecticides, such as pyrethroids, carbamates, and organophosphates are common methods used for controlling cockroaches. But, these insecticides have several adverse effects including acute or chronic toxicity to humans and animals. They can also have an impact on food chain in the ecosystem. Moreover, the cockroach can develop resistance to these insecticides. Due to the problems described previously, an alternative approach is to search for effective botanical extracts which are safe to humans and the environment.

The present study is aimed to determine the insecticidal activity of crude extracts from six Thai botanicals, such as *P. retrofractum*, *S. tuberosa*, *D. elliptica*, *R. nasutus*, *B. superba* and *F. vulgare* against various stages of *B. germanica* including nymph, adult and gravid under laboratory conditions. This study indicated that these six Thai botanical extract exhibited potential to be used as natural insecticide against *B. germanica*. Among the six Thai botanical extract, *P. retrofractum* was the most efficacy, followed by *D. elliptica* and *S. tuberosa*, while *F. vulgare*, *R. nasutus* and *B. superba* were the least effective. Furthermore, in all stage, gravid female was the most susceptible, followed by adult stage and nymph stage in all of six Thai botanical. In considering the solvent, the ethanol and acetone exhibited more efficacy of extraction than hexane. In addition, at every various solvent and levels of concentration of each Thai botanical extracts, it was found that time increased the percentage mortality of *B.*

germanica also increased, however, the 24 and 48 hours post experiment of all of six Thai botanical extract more toxicity on three developmental stages than 1, 2, 4 and 6 hours post experiment. In addition, the acetone extract of *P. retrofractum* at concentration 10% (w/v) exhibited complete insecticidal activity (100%) against both adult and gravid stage of *B. germanica* at 6th hours post experiment. However, all the six Thai botanical cannot induce complete insecticidal activity against nymph stage of *B. germanica*.

The advantages of using botanical extracts include the fact that botanical are safe for the human and are also environmental safety, as well as it less toxic when compared to chemical products. Furthermore, based on the study finding, *P. retrofractum* crude extract could be used as an alternative strategy for controlling various stage of *B. germanica* instead of chemical insecticides in areas where chemical insecticides are not appropriate and used in integrated pest management programs (IPM) against *B. germanica*. However, insecticidal activity of the Thai botanical extracts against *B. germanica* in field environment and appropriate formulations of this extracts should be determined.

CONSIDERATIONS OF THIS STUDY

1. Botanicals cultivated in different areas may show different results from this study due to dissimilar climate conditions, lights, temperature, humidity and minerals in the soil which can affect growth and chemicals in botanicals.
2. In this study, the preparation for botanical extracts was conducted by maceration technique which may give different results than by using other extraction methods.
3. Botanical extracts should be kept in a refrigerator to prevent spoilage. Moreover, they may lose their activity when coming into contact with water for too long, thus the botanicals used in this study were stored in a crude extract form.
4. Insecticidal activity of the Thai botanical extracts against *B. germanica* in field environment and appropriate formulations of this extracts should be determined.

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APPENDIX

APPENDIX A

Research equipment

1. Rotary evaporator (Dragonlab, China)
2. Hand micro-applicator (Burkard Manufacturing Company Ltd.)
3. Column Chromatography
4. Mortar and pestle
5. Water bath: Memmert WB 45 (Memmert, German)
6. Hot oven incubator (Memmert, Germany)
7. Spin down centrifuge: Force mini (Liolab international co., ltd, Thailand)
8. Votex mixture (Scientific industry, USA)
9. Flask size 500 ml and 250 ml (Pyrex, USA)
10. Beaker size 1,000 ml and 500 ml (Pyrex, Germany)
11. Autopipette (Eppendorf, USA)
12. Pipet tip size 200 µl and 10 µl (Eppendorf, Germany)
13. Refrigerator 4 oC (Sanyo, Japan)
14. Whatman filter paper no.1.
15. Electric blender (Philips)
16. Plastic containers (30×30×30 cm)
17. Plastic containers (15×15×15 cm)
18. Cotton bun
19. Plastic cup
20. Science glassware size 1ml and 10 ml (Pyrex, Germany)

APPENDIX B

Chemicals and reagents preparations

Chemicals and reagents

1. Hexane
2. Acetone
3. Ethanol
4. Ethyl acetate
5. Toluene
6. Sulfuric acid
7. Anisaldehyde
8. 2% tween 20
9. Piperine (Sigma-Aldrich, USA)
10. Silica Gel (70-230 mesh)
11. Silica Gel (230-400 mesh)
12. TLC Silica gel
13. Iodine
14. Bismuth subnitrate
15. Potassium iodine
16. Acetic acid
17. Carbon dioxide
18. Petroleum jelly
19. Dry dog food

Reagents preparations

1. Dragendorff's reagent preparation

1. bismuth subnitrate 0.85 g
2. potassium iodine 8 g
3. acetic acid
4. water

Solution A: 0.85 g of bismuth subnitrate in 10 ml of acetic acid and 40 ml of water.

Solution B: 8 g of potassium iodine in 20 ml of water.

Mix reagents together as follows: 5 ml A + 5 ml B + 20 ml acetic acid + 100 ml water.

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