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

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Abstract. The German cockroach, *Blattella germanica*, is considered an important medical and economic pest in Thailand. The insecticidal activities of hexane, acetone and ethanol extracts derived from six Thai botanicals, namely, *Piper retrofractum* Vahl, *Stemona tuberosa* Lour, *Derris elliptica* (Wall.) Benth., *Rhinacanthus nasutus* (L.), *Butea superba* Roxb., and *Foeniculum vulgare* Mill. were used to evaluate their toxicities against a variety of developmental stages of *B. germanica* including nymphs, adults and gravid females. All botanical extracts showed high insecticidal activity (up to 100%) after a 24-hour exposure at 200 μg/insect, using 2 μl/insect 1% Tween-20 in distilled water as negative control. Extracts from *P. retrofractum* gave the highest mortality to all *B. germanica* developmental stages, with the acetone extract showing maximal insecticidal activity against both gravid (LD_{50} = 39 μg/insect; LD_{90} = 52 μg/insect) and adult (LD_{50} = 53 μg/insect; LD_{90} = 104 μg/insect) stages after a 6-hour exposure. Isolation and purification of the main components of *P. retrofractum* acetone extract by column chromatography led to a fraction with highest insecticidal activity against nymphs. Subsequent purification by thin layer chromatography led to identification of piperine as the major bioactive compound. This is the first report of insecticidal activity from *P. retrofractum* extracts against *B. germanica* and holds promise as a potential biopesticide.

Keywords: *Blattella germanica*, botanical extract, German cockroach, insecticidal activity

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INTRODUCTION

Cockroach is considered an important pest of concern to medical and public health. Cockroaches are commonly found in homes, hospitals, hotels and restaurants (Yeom *et al*, 2012). The insect is used as a key indicator of insanitary conditions as they are able to carry various human pathogens, including bacteria, viruses and protozoa, moreover, they contribute to the cause of allergies and asthma (Slater *et al*, 2007; Mindykowski *et al*, 2010).

In Thailand, more than ten species of cockroaches have been described, including the American cockroach *(Periplaneta americana),* Australian cockroach (*P. australasiae*) and German cockroach (*Blattella germanica*) (Tawatsin *et al*, 2001; Sriwichai *et al*, 2002; Chompoosri *et al*, 2004). However, *B. germanica* is considered as an important medical and economic pest because it has a shorter generation time and higher fecundity than the other cockroaches, which makes it difficult to control. Conventional synthetic insecticides, such as pyrethroids, carbamates and organophosphates are very popularly used compounds for controlling cockroaches (Lee *et al*, 1996; Syed *et al*, 2014). However, these insecticides have several adverse effects including acute or chronic toxicity to humans and animals. They can also have an adverse impact on the food chain of the ecosystem (Govindarajan *et al*, 2011; Amerasan *et al*, 2012; Muthukumaran *et al*, 2015). Furthermore, cockroaches can develop resistance to these insecticides (Pai *et al*, 2005). Due to these problems, an alternative approach is to search for effective botanical extracts, which are safe to humans and the environment.

Thailand has an abundance of plants, extracts of which are used in many applications in place of chemicals as they are more environmental friendly and less toxic (Kamaraj *et al*, 2009; Koodalingam *et al*, 2009; Elango *et al*, 2010). Moreover, several Thai botanicals have long been employed as insecticides or repellents for pest and insect control (Promsiri *et al*, 2006; Tawatsin *et al*, 2006; Phasomkusolsil and Soonwera, 2010). For example, Chang and Ahn (2002) reported that methanol extract of fruit from *Illicium verum* has insecticidal activity against adults of *B. germanica* when used as a fumigation agent. Thavara *et al* (2007) demonstrated that essential oils of *Citrus hystrix* (Kaffir lime) exhibit complete repellency against *P. americana* and *B. germanica* and also show a high repellency (87.5%) against *Neostylopyga rhombifolia* under laboratory conditions. Ling *et al* (2009) stated that essential oils of *Piper aduncum* (spike pepper) can have insecticidal activity against adults and nymphs stage of *P. americana*. Tine *et al* (2011) revealed that azadirachtin from kernel of neem tree (*Azadirachta indica*) has an insecticidal effect against newly emerged adults of *Blatta orientalis* under laboratory conditions. According to Sittichok *et al* (2013) essential oils derived from Thai plants *Cymbopogon citatus* (lemon grass), *C. nardus* (citronella grass) and *Syzygium aromaticum* (clove) had highly repellent effects on adult *P. americana*.

Thus Thai botanicals contain insecticidal or repellent substances and, importantly, there has been no report, to the best of our knowledge, of resistance by pests and insects against Thai botanicals, highlighting the attractiveness of Thai botanicals as alternative substances for controlling German cockroaches. Hence, the present study determined the insecticidal activity of organic solvent extracts derived from six Thai botanicals, namely, *Butea superba* Roxb. (red '*kwao krua*'), *Derris elliptica* (Wall.) Benth. (Derris), *Foeniculum vulgare*

Mill. (Fennel), *Piper retrofractum* Vahl (Java long pepper), *Rhinacanthus nasutus* (L.) (white crane flower), and *Stemona tuberosa* Lour. (wild asparagus), against various developmental stages of *B. germanica* including nymph, adult and gravid stages under laboratory conditions. In addition, the main component of Thai botanical extracts with highest insecticidal effect against *B. germanica* was identified.

MATERIALS AND METHODS

Botanical material

Thriving roots of *B. superba*, *D. elliptica, R. nasutus* and *S. tuberosa*, fruits of *P. retrofractum* and seeds of *F. vulgare* (1 kg each) were purchased from a traditional herbal medicine store, Bangkok, Thailand (Table 1). The plants were identified at the Plant Production Technology Section, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Lat Krabang, Bangkok, Thailand.

Preparation of botanical extract

The botanical materials were washed thoroughly with distilled water and cut into pieces (<2 cm in size), then left to dry in the shade and ground into fine powder using an electric blender. The fine powders were extracted using a sequential extraction procedure with 1,000 ml aliquots of three different solvents with increasing polarity, namely, hexane, acetone and ethanol, by maceration at room temperature (30 ± 2ºC) for three days with frequent agitation. The solvents were removed by evaporation (AhbiRami *et al*, 2014; Govindarajan and Sivakumar, 2015). The extracts were weighed, dried at room temperature (30 ± 2ºC) and stored in brown bottles at 4ºC until used. Extracts (40, 80, 120, 160 and 200 μg/μl 1% Tween-20 in distilled water/insect) were prepared for a topical application bioassay test (Rahman and Akter, 2006).

Cockroach

B. germanica was obtained from the Biology and Ecology Section, Medical Entomology Group, National Institute of the Health, Department of Medical Science, Ministry of Public Health, Thailand and reared according to standard protocol (Thavara *et al*, 2007). Cockroaches were maintained in the insectary of the Laboratory Animal Center, Faculty of Medicine, Chulalongkorn University, Bangkok under ambient temperature (27-30ºC) and humidity (70-90%) and photoperiod of 12:12 hour light:dark cycle. Cockroaches were fed dry dog food and water *ad libitum*. The upper inside surfaces of the rearing container were smeared with petroleum jelly to prevent cockroaches from escaping (Habes *et al*, 2006). *B. germanica* final instar nymphs (3-4 weeks old), adult males, non-gravid females (6-8 weeks old) and gravid females (with egg case) were employed for toxicity testing under laboratory conditions.

Topical application bioassay

A group of 10 each of unsexed nymph, gravid female and adult cockroaches (five males and five non-gravid females) were used in each experiment. Cockroaches were anesthetized briefly (<5 minutes) with carbon dioxide to facilitate handling (Tunaz *et al*, 2009; Liu *et al*, 2011). Each botanical extract of different concentrations (2 μl/insect) were topically applied with a micro applicator (Burkard Manufacturing, Rickmansworth, United Kingdom) onto the first abdominal segment of each cockroach (Ferrero *et al*, 2007). Cockroaches in the negative control group were treated with vehicle (2 μl/insect). All cockroaches were kept under the same conditions and mortality was assessed at 1, 2, 4, 6, 24 and

Table 1
Thai botanicals used in the study.

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

48 hours post-treatment. Each experiment was carried out in quadruplicate. Death was determined based on the following criteria: (i) inability to move when prodded with forceps, and (ii) inability to right to a normal posture within two minutes (Tungtrongchitr *et al*, 2012).

Isolation and purification of bioactive component

Extract showing highest insecticidal effect against *B. germanica* was subjected to purification by column chromatography and 10 ml aliquot of each fraction was analyzed by thin layer chromatography (TLC) (Dua *et al*, 2009; Deng *et al*, 2012; Kongkiatpaiboon and Gritsanapan, 2012). Fractions with the same TLC profile were combined and tested against *B. germanica.* The fraction with the highest efficacy against *B. germanica* nymphs was analyzed further by two-dimensional TLC using hexane per ethyl acetate (40: 60 v/v) solvent system and Dragendorff's reagent (Kolhe *et al*, 2011) to identify the main bioactive compound.

Statistical analysis

Percent mortality data were subjected to probit analysis for calculating LD_{50}, LD_{90}, and other statistics at 95% confidence limit of upper (UCL) and lower (LCL) confidence limits using SPSS version 23 software (IBM, Armonk, NY). Results with p-value <0.05 are considered statistically significant.

RESULTS

Yield of botanical extracts

One kg of each botanical produced the following yields: acetone, ethanol and hexane extract of *B. superba* of 0.63%, 1.74% and 0.27%, respectively; *D. elliptica* of 0.74%, 2.22% and 0.13%, respectively; *F. vulgare* of 3.52%, 4.98% and 3.31%, respectively; *P. retrofractum* of 2.86%, 3.15% and 1.18%, respectively; *R. nasutus* of 0.70%, 1.18% and 0.19%, respectively; and *S. tuberosa* of 0.88%, 4.73% and 0.29%, respectively. Due to their low yields, the hexane extracts, except those of *F. vulgare* and *P. retrofractum*, were not selected for insecticidal activity tests on *B. germanica*.

Insecticidal activity of botanical extracts against *B. germanica*

Among the extracts of the six botanicals, *P. retrofractum* extracts showed the highest mortality when compared to other extracts of the other five botanicals at every experimental time points for gravid (Fig 1A), adult (Fig 1B) and nymph (Fig

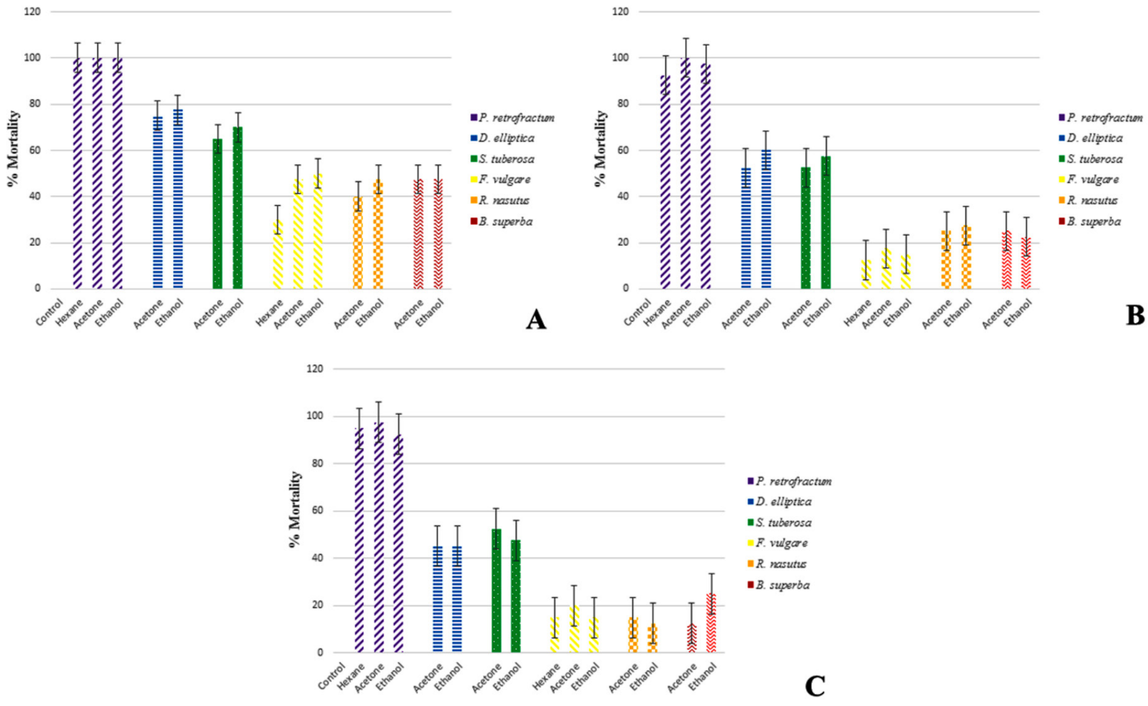


Fig 1-Histogram showing the percentage mortality of Thai botanical extract at concentration 200 μg/insect on gravid **(A)**, adult **(B)** and nymph **(C)** stage of *B. germanica* after 6 hours post-exposure.

1C) stages, or at the highest concentration (200 μg/insect) at 6 hours post-exposure with extracts from *B. superba*, *F. vulgare* and *R. nasutus* being the least effective (Tables 2-4). The acetone, ethanol and hexane extract of *P. retrofractum* showed 100% lethality against gravid stage at 6 hours post-exposure (LD_{50} = 39.4, 41.2 and 42.5 μg/insect; LD_{90} = 51.9, 53.9 and 55.1 μg/insect, respectively). With adult stage, only *P. retrofractum* acetone extract showed 100% lethality at 6 hours post-exposure (LD_{50} = 53.2 μg/insect; LD_{90} = 103.7 μg/insect), followed by ethanol and hexane extract (LD_{50} = 62.4 and 80.1 μg/insect; LD_{90} = 131.9 and 159.7 μg/insect, respectively). However, all extracts of the six botanicals were unable to produce 100% killing of *B. germanica* nymph stage (Table 5) and among the six botanicals, *P. retrofractum* acetone extract exhibited highest efficacy against the nymph stage followed by ethanol and hexane extract, (LD_{50} = 69.3, 89.1 and 91.4 μg/insect; LD_{90} = 129.7, 181.3 and 174.3 μg/insect, respectively) (Fig 2).

Identification of the major bioactive component

As the acetone extract from *P. retrofractum* had the highest efficacy against all various developmental stages of *B. germanica*, this extract was subjected to column chromatography and each fraction subsequently was tested against the nymph stage of *B. germanica*. The results found that, fractionation of extract of *P. retrofractum* by using silica gel column

Table 2
Insecticidal activity of organic solvent extracts from Thai botanicals against gravid stage of *Blattella germanica* at 6 hours post-exposure.

Thai botanical	Extraction solvent	Vehicle[a]	Percent mortality ± SD Concentration (μg/insect)				
			40	80	120	160	200
Piper retrofractum	Hexane	0	40.0 ± 0.8	100	100	100	100
	Acetone	0	52.0 ± 1.0	100	100	100	100
	Ethanol	0	45.0 ± 0.6	100	100	100	100
Derris elliptica	Acetone	0	27.5 ± 0.5	47.0 ± 1.0	50.0 ± 0.8	52.0 ± 1.0	75.0 ± 1.0
	Ethanol	0	27.5 ± 0.5	47.0 ± 1.0	50.0 ± 0.8	55.0 ± 0.6	77.5 ± 0.5
Stemona tuberosa	Acetone	0	20.0 ± 0.8	45.0 ± 0.6	47.5 ± 0.5	50.0 ± 0.8	65.0 ± 1.0
	Ethanol	0	27.0 ± 1.0	47.5 ± 0.5	50.0 ± 0.8	55.0 ± 0.6	70.0 ± 0.8
Foeniculum vulgare	Hexane	0	12.5 ± 0.5	10.0 ± 0.8	12.5 ± 0.5	17.0 ± 1.0	30.0 ± 0.8
	Acetone	0	5.0 ± 0.6	15.0 ± 0.6	22.0 ± 1.0	47.0 ± 1.0	47.5 ± 0.5
	Ethanol	0	5.0 ± 0.6	7.0 ± 1.0	27.0 ± 1.0	47.0 ± 1.0	50.0 ± 0.8
Rhinacanthus nasutus	Acetone	0	5.0 ± 0.6	7.0 ± 1.0	10.0 ± 0.8	30.0 ± 0.8	40.0 ± 0.8
	Ethanol	0	2.5 ± 0.5	5.0 ± 0.6	12.5 ± 0.5	35.0 ± 0.6	47.5 ± 0.5
Butea superba	Acetone	0	7.5 ± 0.5	10.0 ± 0.8	12.0 ± 1.0	45.0 ± 0.6	47.5 ± 0.5
	Ethanol	0	2.5 ± 0.5	5.0 ± 0.6	10.0 ± 0.8	42.0 ± 1.0	47.5 ± 0.5

[a]1% Tween-20 in distilled water.

Table 3
Insecticidal activity of organic solvent extracts from Thai botanicals against adult stage of *Blattella germanica* at 6 hours post-exposure.

Thai botanical	Extraction solvent	Vehicle[a]	Percent mortality ± SD Concentration (μg/insect)				
			40	80	120	160	200
Piper retrofractum	Hexane	0	40.0±0.8	45.0±1.0	80.0±0.8	92.5±0.5	92.5±0.5
	Acetone	0	50.0±0.8	77.5±0.5	95.0±0.6	97.5±0.5	100
	Ethanol	0	45.0±1.0	75.0±1.0	83.0±1.0	95.0±0.6	97.5±0.5
Derris elliptica	Acetone	0	5.0±0.6	22.0±1.0	27.5±0.5	48.0±1.0	53.0±1.0
	Ethanol	0	15.0±1.0	25.0±1.0	32.5±0.5	45.0±0.6	60.0±1.0
Stemona tuberosa	Acetone	0	2.5±0.5	22.5±0.5	25.0±0.6	43.0±1.0	52.5±0.5
	Ethanol	0	7.5±0.5	25.0±0.6	27.5±0.5	45.0±0.6	57.5±0.5
Foeniculum vulgare	Hexane	0	0	0	2.5±0.5	3.3±0.6	12.5±0.5
	Acetone	0	0	0	2.5±0.5	12.5±0.5	17.5±0.5
	Ethanol	0	0	0	5.0±0.6	10.0±0.0	15.0±1.0
Rhinacanthus nasutus	Acetone	0	0	0	2.5±0.5	10.0±0.8	25.0±0.6
	Ethanol	0	0	0	2.5±0.5	8.0±1.0	27.5±0.5
Butea superba	Acetone	0	0	0	2.5±0.5	15.0±1.0	25.0±0.6
	Ethanol	0	0	0	2.5±0.5	8.0±1.0	23.0±1.0

[a]1% Tween-20 in distilled water.

Table 4
Insecticidal activity of organic solvent extracts from Thai botanicals against nymph stage of *Blattella germanica* at 6 hours post-exposure.

Thai botanical	Extraction solvent	Vehicle[a]	Percent mortality ± SD Concentration (µg/insect)				
			40	80	120	160	200
Piper retrofractum	Hexane	0	40.0±0.8	43.0±1.0	48.0±1.0	95.0±1.0	95.0±0.6
	Acetone	0	43.0±1.0	45.0±0.6	95.0±0.6	97.5±0.5	97.5±0.5
	Ethanol	0	45.0±0.6	48.0±1.0	48.0±0.6	93.0±1.0	93.0±1.0
Derris elliptica	Acetone	0	15.0±0.0	20.0±0.0	22.5±0.5	32.5±0.5	45.0±1.0
	Ethanol	0	28.0±1.0	30.0±0.8	38.0±1.0	45.0±0.6	45.0±1.0
Stemona tuberosa	Acetone	0	10.0±0.8	18.0±1.0	20.0±0.8	48.0±1.0	52.5±0.5
	Ethanol	0	10.0±0.5	12.5±0.5	28.0±1.0	48.0±1.0	47.5±0.5
Foeniculum vulgare	Hexane	0	2.5±0.5	5.0±0.6	8.0±1.0	10.0±0.8	15.0±0.6
	Acetone	0	0	2.5±0.5	5.0±0.6	10.0±0.0	20.0±0.8
	Ethanol	0	0	2.5±0.5	5.0±0.6	15.0±1.0	15.0±0.6
Rhinacanthus nasutus	Acetone	0	2.5±0.5	5.0±0.6	8.0±1.0	10.0±0.8	15.0±0.6
	Ethanol	0	0	2.5±0.5	5.0±0.6	10.0±0.8	13.0±1.0
Butea superba	Acetone	0	0	2.5±0.5	5.0±0.6	15.0±0.6	12.5±0.5
	Ethanol	0	0	5.0±0.6	8.0±1.0	13.0±1.0	25.0±1.0

[a]1% Tween-20 in distilled water.

chromatography led to separation of 14 fractions (F1-F14), with F5 having the highest lethality against the nymph stage, with 100% mortality achieved at 200 µg/insect at 4 hours post-exposure (Table 6). F5 was further purified by one-dimensional followed by two-dimensional TLC, which resulted in the identification of piperine, a major alkaloid in the fruit of *P. retrofractum*, as the main bioactive compound (Fig 3).

DISCUSSION

This present study indicates that organic solvent extracts of six Thai botanicals had toxic effects on various developmental stages of *B. germanica*. Among the six Thai botanicals, extracts of *P. retrofractum* proved to be the most insecticidal activity against *B. germanica*. Several reports have demonstrated that *P. retrofractum* have been used bioinsecticides against various insects. For example, Chansang *et al* (2005) reported larvicidal activity of nine Thai botanical aqueous extracts including those from *Allium sativum, Cleome viscosa, Curcuma longa, Gynura pseudochina, Spilanthes paniculata, P. retrofractum, Rhinacanthus nasutus, Syzpgium aromaticum,* and *Zingiber montanum*. They also found that *P. retrofractum* extract has the highest larvicidal activity against 3rd and 4th instar larvae of *Culex quinquefasciatus* and *Aedes aegypti* with LD_{50} value of 135 mg/l and LD_{90} value of 79 mg/l. Kraikrathok *et al* (2013), evaluating extracts of *P. interruptum*, *P. nigrum*, *P. retrofractum*, and *P. sarmentosum* against diamondback moth, *Putella xylostella* third instars, reported extract of *P. retrofractum* having the highest larvicidal activity with an LD_{50} value of 237 ppm. In the study of

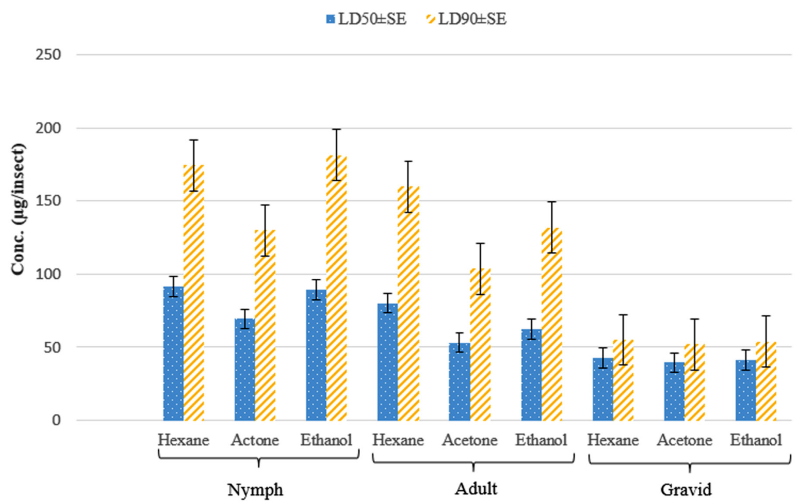
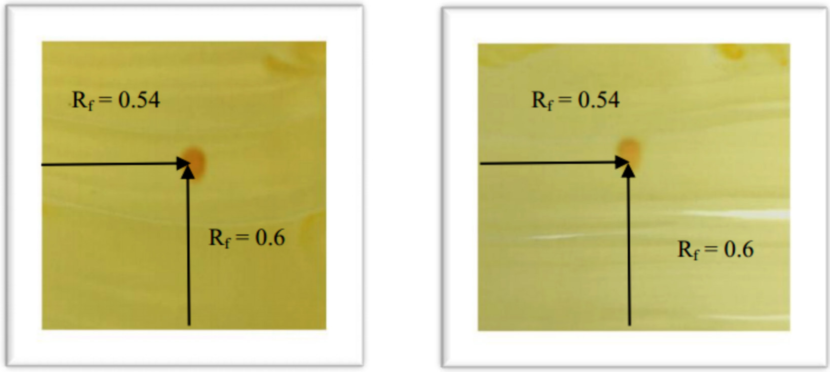


Fig 2-Graph showing the LD_{50} and LD_{90} value of *P. retrofractum* extract on various developmental stage of *B. germanica* after 6 hours post exposure.



a. b.

Fig 3-Two dimension development thin layer chromatography (TLC) of (a.) piperine analytical standard and (b.) fraction 5 from acetone extract of *P. retrofractum*.

P. retrofractum toxicity on mammals by Jaijoy *et al* (2010), aqueous extract of the fruits do not produce acute or subchronic toxicity in either female or male rats at dose of 5,000 mg/kg body weight for acute toxicity and 300-1,200 mg/kg body weight for subchronic toxicity.

The presence of piperrine in *P. retrofractum* has been reported by others. Parmar *et al* (1997) and Taufiq-Ur-Rahman *et al* (2005) reported the principle active constituent of the fruit of *P. retrofractum* is piperine. Bao *et al* (2014) explored piperidine alkaloids in family Piperaceae collected in different areas and reported piperine as the major component of dried fruits of *P. longum*, *P. nigrum* and *P. retrofractum*. Fabarani (2012) reported crude methanol extract of *P. retrofractum* fruit contains methyl piperate, N-isobutyl-2E, 4E, 14Z-elcosatrienamide piperine, and oleic acid.

Piperine is dark yellow in color, aromatic and pungent in odor, and has a biting taste. It is soluble in chloroform, ethanol and methanol, but insoluble in water. Its R_f value is 0.33-0.55 demonstrating good separation by TLC (Banerji *et al*, 2002; Raman and Galkar, 2002; Kolhe *et al*, 2011). Previous studies have suggested piperine as a potential bioinsecticide. For example, Samuel *et al* (2016) demonstrated that *P. nigrum* and piperine cause mortality to third and fourth instars of *An. arabiensis*, *An. coluzzii*, *An. funestus*, *An. gambiae*, and *An. quadriannulatus*; in addition, a mixture of *P. nigrum* and piperine causes

Table 5
Fifty (LD_{50}) and 90 (LD_{90}) percent lethal dose of *Piper retrofractum* organic solvent extracts against nymph, adult and gravid stages of *Blattella germanica* at 6 hours post-exposure.

Stage	Extraction solvent	LD_{50} (LCL-UCL) (μg/insect)	LD_{90} (LCL-UCL) (μg/insect)
Nymph	Hexane	91.4 (30.3-146.2)	174.3 (127.5-388.9)
	Acetone	69.3 (16.3-111.5)	129.7 (93.8-272.5)
	Ethanol	89.1 (12.6-150.3)	181.3 (129.6-468.1)
Adult	Hexane	80.1 (37.7-114.5)	159.7 (123.1-259.1)
	Acetone	53.2 (20.0-79.4)	103.7 (77.9-175.5)
	Ethanol	62.4 (18.2-93.9)	131.9 (99.2-221.3)
Gravida	Hexane	42.5	55.1
	Acetone	39.4	51.9
	Ethanol	41.2	53.9

[a]Due to high frequency of complete mortality in gravid stage, LD_{50} and LD_{90} values are estimation by probit analysis. LCL, lower confidence limit; UCL upper confidence limit.

high mortality of *An. gambiae*. Freire-de-Lima *et al* (2008) reported piperine caused morphological alteration of *Trypanosoma cruzi* epimastigotes. Piperine has insecticidal activity to eggs of *Spodoptera frugiperda* and *Diatraea saccharalis* (Tavares *et al*, 2011), and to *Musca domestica* and promastigotes of *Leishmania donovani* both *in vitro* and *in vivo* (Kapil, 1993; Raay *et al*, 1999; Veerareddy *et al*, 2004; Bodiwala *et al*, 2007). Three *Piper* amines isolated from *P. nigrum*, namely, pellitorine, pipercid and piperine, show toxicity to male *Callosobruchus chinensis* ranging from 0.15 to 20 μg/insect (Dev and Koul, 1999).

Piper amines including dihydropiperine, dihydropiperlonguminine, pipercide, piperine, piperlonguminine of family Piperaceae are known to act as neurotoxins in insects. The mode of action in insect is as a contact poison acting on the central nervous system (de Paula *et al*, 2000). Therefore, *Piper* amines of family Piperaceae could replace contact insecticides, specifically chemical neurotoxic compounds such as carbamates, organophosphates and pyrethroids to reduce insect resistance to these chemical insecticide, and to decrease environmental exposure to humans and animals (Jensen *et al*, 2006; Scott *et al*, 2008). Based on the aforementioned, piperine from fruit of *P. retrofractum* might be the main toxic agent against the various developmental stages of *B. germanica*.

In conclusion, this study clearly shows that organic solvent extracts of *P. retrofractum* and their crude fraction have highest insecticidal activity against *B. germanica* compared to extracts from the other five Thai botanicals. The active compound was identified as piperine from the fruit. Thus, extracts of *P. retrofractum* have the potential to be used as bioinsecticides that are inexpensive and safe to humans and the environment for the controlling of *B. germanica* instead of current chemical insecticides in areas where the latter insecticides are not appropriate, and should be integrated into pest manage-

Table 6
Percent mortality of *Blattella germanica* nymph stage following exposure to *Piper retrofractum* acetone extract fractions from column chromatography.

Fraction	Percent mortality ± SD											
	1 hour		2 hours		4 hours		6 hours		24 hours		48 hours	
Concentration (µg/insect)	40	200	40	200	40	200	40	200	40	200	40	200
F1	0	40.0±0.8	0	55.0±0.6	0	62.5±0.5	0.5±0.6	73.0±1.0	1.2±0.5	73.0±1.0	2.0±0.8	73.0±1.0
F2	0	40.0±0.8	0.5±0.6	55.0±0.6	1.3±1.0	68.0±1.0	1.7±0.5	73.0±1.0	3.3±1.0	85.0±0.6	4.0±0.8	92.5±0.5
F3	0.2±0.5	40.0±0.8	0.5±0.6	55.0±0.6	0.5±0.6	68.0±1.0	0.7±1.0	73.0±1.0	2.2±0.5	80.0±0.8	2.5±0.6	85.0±0.6
F4	0	40.0±0.8	0.8±1.0	48.0±1.0	1.0±0.8	62.5±0.5	1.5±0.6	73.0±1.0	1.7±0.5	88.0±1.0	2.2±0.5	90.0±1.0
F5	0.8±1.0	88.0±1.0	2.0±0.8	97.5±0.0	3.0±1.0	100	3.5±0.0	100	5.0±0.0	100	5.5±0.0	100
F6	0.5±0.6	5.0±0.6	1.3±1.0	20.0±0.8	1.3±1.0	25.0±0.6	1.7±1.0	43.0±1.0	1.7±0.5	63.0±1.0	2.0±0.8	75.0±0.6
F7	0.0±0.5	10.0±0.8	0.2±0.5	15.0±0.6	0.2±0.5	25.0±0.6	0.2±0.5	35.0±1.0	0.5±0.6	45.0±0.6	1.2±0.5	55.0±0.6
F8	0.0±0.5	10.0±0.5	0.2±0.5	15.0±0.5	0.2±0.5	25.0±0.5	0.2±0.6	35.0±0.6	0.5±0.6	45.0±0.6	1.2±0.5	55.0±0.6
F9	0.0±0.5	10.0±0.5	0.2±0.5	15.0±0.5	0.2±0.6	25.0±0.6	0.2±0.5	35.0±0.5	0.5±0.5	45.0±0.5	1.2±0.5	55.0±0.5
F10	0	0	0	0	0	0	0	2.5±0.5	0	2.5±0.5	0	2.5±0.5
F11	0	0	0	0	0	0	0	0	0	0	0	0
F12	0	2.5±0.5	0	2.5±0.5	0	2.5±0.5	0	2.5±0.5	0	2.5±0.5	0.2±0.5	5.0±0.6
F13	0	2.5±0.5	0	2.5±0.5	0	2.5±0.5	0	2.5±0.5	0	2.5±0.5	0	2.5±0.5
F14	0	0	0	0	0	0	0	0	0	0	0.2±0.5	0
Vehicle[a]	0	0	0	0	0	0	0	0	0	0	0	0

[a]1% Tween-20 in distilled water.

ment programs against *B. germinica*. This is the first report of insecticidal activity of *P. retrofractum* extracts against German cockroach, *B. germanica*. However, insecticidal activity of the extracts against *B. germanica* in field environment and appropriate formulations of this extracts need to be determined.

ACKNOWLEDGEMENTS

The study was supported by the Ratchadapiseksompotch Fund (RA59/050), the Faculty of Medicine, Chulalongkorn University, the National Science and Technology Development Agency (Thailand) Research Chair Grant, and the Integrated Innovation Academic Center (IIAC) Chulalongkorn University Centenary Academic Development Project.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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