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Evaluation of *Alpinia galanga* (Zingiberaceae) extracts and isolated *trans*-cinnamic acid on some mosquitoes larvae

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Abstract

Background: Mosquitoes are vectors for diseases damaging human health and thus, there is an urgent need for insecticidal compounds to control their population. The objective of this study was to determined the efficiency from *trans*-cinnamic acid isolated from *Alpinia galanga* (Zingiberales: Zingiberaceae) for control of *Aedes aegypti* (Dipetera: Culicidae), *Anopheles dirus* B (Dipetera: Culicidae) and *Culex quinquefasciatus* (Dipetera: Culicidae).

Methods: *Alpinia galanga* (Zingiberales:Zingiberaceae) was extracted by soaking in a sequence of solvents (hexane, dichloromethane, ethyl acetate and methanol), and the isolated *trans*-cinnamic was separated by preparative thin layer chromatography. All crude extracts and isolated *trans*-cinnamic were evaluated for their control and affect on detoxification enzyme activities of the third-instar larvae of each mosquito species in laboratory conditions.

Results: Our results showed that the hexane crude extract had the best control efficiency in all species, particularly in *Cx. quinquefasciatus*. The *trans*-cinnamic acid, isolated compound from hexane crude extract showed as active ingredient against third-instar larvae of each mosquito species. Mortality in this case may result from the inhibition of carboxylesterase.

Conclusion: These results indicated that *A. galanga* which had *trans*-cinnamic acid as active ingredient compound could represent a promising naturally occurring control agent for all three mosquito species. However, this research consider as an initial prospective study, the other side effect on nontarget species need to concerned before used as commercial product.

Keywords: *Alpinia galanga*, *Aedes aegypti*, *Anopheles dirus* B, *Culex quinquefasciatus*, Detoxification enzymes, Acetylcholinesterase

Background

Mosquitoes are carriers of diseases causing serious health problems in tropical regions. A variety of mosquito species transmit many diseases to humans and animals in a species-dependent manner; for example, *Aedes aegypti* is the vector for dengue fever, Zika fever and Chikungunya, *Anopheles dirus* B is a vector for malaria in Asian forested zones and *Culex quinquefasciatus* is a vector for zoonotic diseases that affect humans and wild and domestic animals. Almost all these diseases are severe problems in many countries, which makes it necessary to control mosquito populations. Although there are many mosquito pesticides available on the market currently, mosquitoes can develop resistance to pesticides, especially synthetic pesticides, and thus, it becomes necessary to increase the dosage used to control mosquitoes, introducing additional risks to human health and to the environment.

The previous studies found that one of the important mechanisms for insecticide resistance is by the increasing

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of detoxification enzymes activities, including carboxylesterase (CarE) and glutathione-*S*-transferase (GST) [13, 18, 40]. Many reports showed mosquitoes using detoxification enzymes and become resistant to many synthetic pesticides [21, 24, 25]. Alternative ways are required to counteract insecticide resistance development.

Acetylcholinesterase (AChE) plays a crucial act for every function of living being including insects. AChE catalyses the hydrolysis of acetylcholine, a neurotransmitter for cholinergic neurotransmission in insects. Most of current insecticides such as organophosphorus is based on inhibiting AChE by hydrolyse the neurotransmitter acetylcholine to terminate neuronal excitement at the postsynaptic membrane [11]. In mosquitoes, resistance is usually caused by less inhibition of the target enzyme in the resistant strains [2, 28, 41]. The understanding of the pesticide action on enzymes in insect pests should enable to produce selective and effective insecticides with low mammalian toxicity (Arshia et al. 2016).

From the past, the discovery and development of pesticides with mosquito larval toxicity derived from plant extracts have become a topic of interest to many researchers. At present, plant extract products are attractive choices for use in integrated pest management programmes to reduce the residues in the environment as it has biodegradable efficiency. Many studies have used plant extracts to control mosquitoes, such as the essential rhizome oil of *Blumea mollis* or *Piper retrofractum* against *Cx. quinquefasciatus* larvae [5, 37], *Curcuma aromatica* or *Dalbergia oliveri* to control *Ae. aegypti* [7, 33]. In addition, *Cymbopogon proximus*, *Lippia multiflora* and *Ocimum canum* have been used against *Ae. aegypti* and *A. gambiae* [3]; *Juniperus macropoda* and *Pimpinella anisum* against *A. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* [34]; *Citrus bergamia*, *Cuminum myrrha* and *Pimenta racemosa* against *Ae. aegypti* and *Cx. pipiens pallens* [23] and *Chloroxylon swietenia* against *Ae. aegypti* and *A. stephensi* [20]. In addition, essential oils of *Cyperus rotundus*, *Alpinia galanga* and *Cinnamomum verum*, were found to synergistic activity with permethrin or repellent against *Ae. aegypti* [6, 27].

This present study evaluated the use of rhizome *Alpinia galanga*, a herb in the Zingiberaceae family that is commonly used in cooking, especially in Thai cuisines, to control three species of mosquito larvae. Previous research has shown that plants belonging to the Zingiberaceae family, including *Curcuma xanthorrhiza*, *Curcuma zedoaria*, *Kaempferia galanga* and *Kaempferia pandurata*, showed toxicity to Spodoptera littoralis [31]. This present study was focused on analysing and comparing the rhizome of *A. galanga* toxicity in *Ae. aegypti*, *A. dirus* B and *Cx. quinquefasciatus* larvae under laboratory conditions. We chose the rhizome of *A. galanga* for this research because it is easily found and grown in Thailand or other countries in SE Asia; thus, it could probably be used easily throughout the year and provide a low-cost form of mosquito control. The development and use of locally available plants is an alternative strategy for the control of mosquito would cause the decreasing use of conventional synthetic pesticides. Additionally, the activities of detoxification enzymes (CarE and GST) and AChE activities were. Knowing the mechanism of action will help to control the resistance in insects' pests, which is an increasing problem in insect control.

Materials and methods

Mosquito culture

Egg clusters of *Ae. aegypti*, *Anopheles dirus* B and *Cx. quinquefasciatus* Thailand laboratory strains were received from the Ministry of Public Health, Nonthaburi province, Thailand. The larvae were reared in plastic boxes (size 50 × 30 cm) with net covers. The larvae were reared in an insect room with artificial fish diet and a controlled rearing environment of 28 °C, 70% relative humidity (RH) and 14:10 of dark:light (D:L) photoperiod without xenobiotic exposure at the Department of Zoology, Faculty of Science, Kasetsart University. Third-instar larvae were used for larvicidal efficiency testing.

Plant materials

The rhizomes of *A. galanga* were collected from Nonthaburi province, Thailand, in May 2017, and subsequently, rhizomes were removed and sliced. The sliced rhizomes were shade-dried by a hot air oven at 60 °C for 3 days. The dried plant material was powdered in a blender at the Faculty of Forestry, Kasetsart University, Bangkhean Campus, Thailand. The powder was stored in zip-lock bags in a refrigerator at 4 °C to prevent sample contamination.

Plant extraction

The dry *A. galanga* rhizome powder (1 kg) was extracted sequentially with 4 l of hexane, dichloromethane, ethyl acetate and methanol by soaking the plant material at room temperature for 7 days. Each crude extract was filtered through filter paper (Whatman No. 1) using a vacuum pump and dried using a rotary evaporator (IKA model RV 10 basic, Thailand) and then stored at 4 °C before use in the experiments.

The most active hexane extract (200 mg) was subjected to preparative thin layer chromatography ($L \times W$ 20 cm × 20 cm, Silica gel 60 PF254 for preparative thin layer chromatography, Merck) and eluted with 20% EtOAc in hexane to obtain three fractions. Due to their polarity, fraction 3 was first chosen to purify by

preparative thin layer chromatography using 50% EtOAc in hexane. One isolated compound was identified as *trans*-cinnamic acid white solid (0.5 mg, 0.25%) by spectral analyses. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz AVANCE III HD spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C).

trans-Cinnamic acid white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (d, $J=16.0$ Hz, 1H), 7.60–7.58 (m, 2H), 7.46–7.43 (m, 3H) and 6.52 (d, $J=16.0$ Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.4, 147.1, 134.1, 130.8, 128.9, 128.4 and 117.3. The NMR data were consistent with those from Kalinowska et al. [17].

Larvicidal bioassay

The assays were performed with a slight modification of the standard protocol recommended by the World Health Organization [42] under laboratory conditions. Each plant extract was dissolved in dimethyl sulphoxide (DMSO). To prepare a series of extracts with different concentrations, 0.5 ml of DMSO solution containing the extract compound was wholly mixed with 249 ml of distilled water in a bowl 10 cm in diameter to give a range of concentrations (0–2000 ppm). Four batches of 25 early third-instar larvae of each mosquito were maintained in 250 ml of aqueous solution, with a final total number of 100 larvae for each concentration. This experiment was conducted under laboratory conditions at 28 °C, 70% RH and 14:10 D:L photoperiod. Temephos were used for positive control in each case. During the experiment, the mosquitoes were not fed. Whereas, positive control used is Temephos. The mosquito larval mortality was recorded as the LC_{50} values after 24 and 48 h by Probit analysis using StatPlus version on a Mac (AnalystSoft company, Canada).

Mode of action study

Third-instar larvae of each mosquito were treated with crude extracts and *trans*-cinnamic acid compound of *A. galanga* at the LC_{50} value concentration, and the control group was treated with DMSO (25 larvae/replication). After 24 h of treatment, the surviving mosquitoes were used for enzyme extraction.

The extraction method was modified from Phankaen et al. [32]. Surviving third-instar larvae of each mosquito were placed in a microtube and kept on ice. After that, thirty larvae was ground with homogenized buffer [0.1 M potassium phosphate buffer mix with 1 mM ethylenediaminetetraacetic acid (EDTA) at pH 8.0], then centrifuged (Hettich model Universal 16R, Germany) at 4 °C and 10,000×*g* for 10 min and the supernatant was transferred to a new tube. The supernatant was centrifuged further at 100,000*g* and 4 °C for 60 min. The supernatant was used to analyse carboxylesterase (CE), glutathione-*S*-transferase (GST) and acetylcholinesterase (AChE).

The CE activity was analysed by a para-nitrophenyl acetate (pNPA) assay modified from Phankaen et al. [32]. 50 mM phosphate buffer was mixed with the supernatant, then 10 mM pNPA was added and the solution was measured by kinetic mode at $\lambda_{max}=410$ nm at 37 °C using a microplate reader (Biotek PowerWave XS microplate spectrophotometer, USA). The CE activity was determined using an extinction coefficient of 176.4705 for pNPA. Three biological replicates were tested per treatment.

The method for determining GST activity was that of Oppenoorth et al. [30]. The reaction solution contained 100 µl of enzyme solution, 200 µl of 50 mM potassium phosphate buffer (pH 7.3) and 10 µl of 150 mM 1-chloro-2,4-dinitrobenzene (CDNB). Optical density was recorded at intervals of 30 s for 3 min at 37 °C and 340 nm with a microplate reader (Biotek PowerWave XS microplate spectrophotometer, US). The GST activity was determined using an extinction coefficient of 0.0096 for CDNB. Three biological replicates were tested per treatment.

The AChE activity analysis method was modified from Ellman et al. [14]. A 100 mM potassium phosphate buffer pH 8.0 was mixed with 50 µl supernatant and incubated at 30 °C for 30 min, then substrates [10 mM of Ellman's reagent (DTNB), 0.1 mM EDTA, 100 mM of acetylthiocholine iodide (ASCh) and 100 mM phosphate buffer (pH 7.2)] were added and the solution was measured at $\lambda_{max}=412$ nm by kinetic mode. The activity was shown by a yellow colour generated by the reaction of DTNB (5, 5′-dithiobis (2-nitrobenzoic acid)) using a microplate reader (Biotek PowerWave XS microplate spectrophotometer, USA), and the AChE activity was converted to nM of acetylthiocholine hydrolysed per min ($\varepsilon 412$ nm $=1.36\times10^{4}$ M^{-1} cm^{-1}). Three biological replicates were tested per treatment.

A statistical comparison of all enzyme activity was performed using Tukey's test in the StatPlus for Mac programme (AnalystSoft Inc., Canada). The protein content of each fraction used as an enzyme source was determined by the Bradford method [4] before measuring the enzyme activity for the total analysed protein.

Results

The amount of the extracts obtained from the various solvent extractions are given in Table 1. The methanol crude extract provided the highest amount of extract, whereas dichloromethane gave the lowest amount (Table 1). The acute toxicity of various concentrations of the rhizome of *A. galanga* extracts on the mortality of all

mosquito larvae showed that the crude hexane extract had the highest toxicity at both 24 and 48 h post-treatment (Table 2). Mortality occurred in a dose-dependent manner, and no mortality was observed in the controls.

The toxicity of *trans*-cinnamic acid was higher than that of the hexane crude extract (Table 2). Mortality occurred in a dose or time-dependent manner, and no mortality was observed in the controls. Similar to that of the crude extract, the toxicity of *trans*-cinnamic acid in *Cx. quinquefasciatus* larvae was higher than that in other species.

The effects of each crude extract and the active ingredient *trans*-cinnamic acid on *Ae. aegypti* showed that the CE was induced in hexane crude extract and *trans*-cinnamic acid. GST on *Ae. aegypti* was induced significantly by all crude extracts and *trans*-cinnamic acid (Table 3). AchE on *Ae. aegypti* was induced significantly by all crude extracts; whereas *trans*-cinnamic acid were reduced (Table 3). The effect of each crude extract and *trans*-cinnamic acid on *A. dirus* B showed that the CE was reduced. However, GST were induced on *A. dirus* B (Table 3). AchE in *A. dirus* B almost induced except *A. dirus* B larvae tested on *trans*-cinnamic acid (Table 3). The enzyme activities in *Cx. quinquefasciatus* showed significant reductions for CE and GST, whereas AChE activity was induced in all crude extracts expet larvae tested on *trans*-cinnamic acid (Table 3).

Discussion

All three mosquitoes chosen for the present experiments are essential vectors that transmit many important diseases to humans and domestic pests and have been reported to develop faster resistance to many pesticides, causing unsuccessful control. Thus, it is necessary to determine other control methods. This research using *A. galanga* to control all three mosquito species, as this plant species is commonly used and easily grown in Thailand, which can reduce the economic cost of production.

Alpinia galanga is a plant belonging to the Zingberaceae family. We chose the rhizome of *A. galanga* for this research because it is easily found and grown in Thailand or other countries in SE Asia; thus, it could probably be used easily throughout the year and provide a low-cost form of mosquito control. This small-scale laboratory experiment aimed to evaluate the effectivity of *A. galanga* extract used as pesticide. Further research focusing on the application in the environment is indispensable to estimate the economic feasibility.

This plant family has been shown by many reports to have insecticidal activity towards many pests. In the control of mosquitoes, for example, *A. galanga* essential oils could synergy in the adulticidal efficacy by improvement of permethrin toxicity [6] or repellency effect on *Ae. aegypti* adult [29]. Abdullah et al. [1] reported antifeedant, repellent and toxic effects of *A. galanga* rhizomes on *Coptotermes gestroi* and *Coptotermes curvignathus*. Rathy et al. [36] also reported 100% mortality of third-instar larvae of *Aedes* sp. after 72 h exposure to *A. galanga* leaf water distillation extract. Misni et al. [27] described the effectiveness of microencapsulated essential oils of *A. galanga* in lotion formulations against *Cx. quinquefasciatus*. Moreover, toxicity of *A. galanga* can be found in larvae stage of other insects such as *Spodoptera litura* [35] or in *Plutella xylostella* [9]. Although *A. galanga* has been shown by many previous reports to have insecticidal activity towards many pests, however, there are no reports yet describing control the isolated compound like trans-cinnamic acid from *A. galanga* on *Ae. aegypti*, *A. dirus* B and *Cx. quinquefasciatus* larvae yet.

In addition, the extraction method used in previous reports was water distillation method or Soxhlet extraction. The extraction method used in this present work is by using soaking method which have main goal to prevent any destruction of compounds inside *A. galanga* no form of external thermal energy. Based on that, a soaking extraction at room temperature was chosen since it is not known which exact compounds inside *A. galanga* are active ones regarding the influence on the metabolism of the targeted mosquito larvae.

Larval stages of mosquitoes were chosen to study in this present work as there are easy targets for pesticides because they breed in water and can be dealt with in this habitat conveniently. The results showed that the control efficiency varied among the *A. galanga* extracts on mosquito larvae species, with the crude hexane extract having the most active larvicidal effect on all species (24 h LC_{50} ~ 24–53 ppm, Table 2), and *Cx. quinquefasciatus* was the most susceptible to the extracts compared to the other species. Thus, *A. galanga* extract, which has *trans*-cinnamic acid as an active ingredient, can control the larvae of all three essential mosquito species. Our results indicated that the crude *A. galanga* hexane extract had higher toxicity than the other crude extracts from the *A. galanga*. However, the toxicity is less than synthetic pesticide like Temephos.

Table 1 Information about the rhizome of *A. galanga* extracts which was extracted sequentially with hexane, dichloromethane, ethyl acetate and methanol by soaking

Solvent	Weight (g)	Yield[a] (% wt:wt)	Characteristics
Hexane	24.8	1.21	Yellow oil
Dichloromethane	16.00	0.80	Yellow oil
Ethyl acetate	18.94	0.95	Dark brown gum
Methanol	76.85	3.84	Black gum

[a] Weight of crude extract/weight of dried rhizome × 100

Table 2 LC_{50} ± SE values (PPM)[a] of *A. galanga* extract on *Aedes aegypti*, *Anopheles dirus* B and *Culex quinquefasciatus* larvae

Extract[b]	*Aedes aegypti*		*Anopheles dirus* B		*Culex quinquefasciatus*	
	24 h	48 h	24 h	48 h	24 h	48 h
H	53.39 ± 2.27ab	41.02 ± 3.56a	36.11 ± 4.46b	17.11 ± 1.39b	24.35 ± 3.78b	18.39 ± 1.297b
D	63.49 ± 5.06a	42.69 ± 2.32a	63.40 ± 2.50c	23.66 ± 2.49c	41.21 ± 5.46c	24.46 ± 4.12c
E	123.29 ± 5.91c	111.49 ± 8.76b	79.35 ± 5.15d	52.79 ± 4.62d	46.59 ± 5.11c	26.45 ± 4.10c
M	1717.05 ± 18.49d	1611.76 ± 10.49c	423.06 ± 9.16e	242.21 ± 3.85e	127.86 ± 2.14d	56.34 ± 1.45d
Cin	45.54 ± 4.48b	37.51 ± 3.76a	24.15 ± 3.78a	14.96 ± 1.12a	6.74 ± 0.68a	7.29 ± 6.22a
T	0.0177 ± 0.003	0.0200 ± 0.003	0.003 ± 0.0001	0.001 ± 0.0003	0.06 ± 0.007e	0.01 ± 0.007

[a] For all experiments, results followed by the same letters within the same column are not significantly different by Tukey's test ($P < 0.05$)

[b] Extracts: H, hexane crude extract; D, dichloromethane crude extract; E, ethyl acetate crude extract; M, methanol crude extract; Cin, *trans*-cinnamic acid; T, positive control (Temephos)

Table 3 Enzyme activity of surviving mosquito larvae after 24 h exposure to *A. galanga* extracts or *trans*-cinnamic acid

Compound[c]	Acetylcholinesterase[a,b]			Carboxylesterase[a,b]			Glutathione-*S*-transferase[a,b]		
	Ae. aegypti	*A. dirus* B	*Cx. quinquefasciatus*	*Ae. aegypti*	*A. dirus* B	*Cx. quinquefasciatus*	*Ae. aegypti*	*A. dirus* B	*Cx. quinquefasciatus*
C	13.80 ± 0.01a	12.72 ± 0.01b	13.94 ± 0.03c	110.96 ± 0.12b	125.10 ± 0.07e	107.70 ± 0.06e	2.99 ± 0.27a	4.35 ± 0.39a	5.60 ± 0.06e
H	14.62 ± 0.06c	13.46 ± 0.04c	15.71 ± 0.03e	124.46 ± 0.07e	104.47 ± 0.12d	85.55 ± 0.08d	4.44 ± 0.40b	6.21 ± 0.07b	5.04 ± 0.03e
D	15.91 ± 0.01d	14.08 ± 0.02e	13.67 ± 0.02b	112.73 ± 0.08c	101.56 ± 0.05c	76.02 ± 0.08b	5.42 ± 0.04e	6.96 ± 0.68b	5.13 ± 0.01b
E	17.00 ± 0.01e	13.53 ± 0.01d	15.23 ± 0.01d	117.44 ± 0.10d	95.74 ± 0.08a	72.88 ± 0.03a	5.32 ± 0.04d	6.21 ± 0.10b	5.40 ± 0.04d
M	22.85 ± 0.08f	14.76 ± 0.01f	16.52 ± 0.01f	108.05 ± 0.07a	100.85 ± 0.03b	73.82 ± 0.01a	6.13 ± 0.08f	5.66 ± 0.11c	4.91 ± 0.05a
Cin	13.73 ± 0.02b	12.52 ± 0.02a	12.99 ± 0.04a	127.91 ± 0.09f	104.35 ± 0.08d	79.64 ± 0.06c	4.93 ± 0.04c	12.65 ± 0.17d	5.53 ± 0.04c

[a] For all experiments, results followed by the same letters within the same column are not significantly different by Tukey's test ($P < 0.05$)

[b] Units of enzymes: Acetylcholinesterase = μM acetylcholinesterase/min/mg of protein; carboxylesterase, nM *p*-nitrophenol/min/mg protein; glutathione-*S*-transferase, mM CDNB conjugated product/mg protein/min

[c] Compounds: C, Comtrol, H, hexane crude extract; D, dichloromethane crude extract; E, ethyl acetate crude extract; M, methanol crude extract; Cin, *trans*-cinnamic acid

Many investigators have studied the chemistry of galangal rhizome. Scheffer et al. [38] analysed a rhizome sample from Indonesia and reported 1,8-cineole (47.3%), α-pinene (11.5%), β-pinene (7.1%), α-thujene (6.2%), terpinen-4-ol (6.0%), α-terpineol, limonene (4.3% each) and many other compounds in lesser concentrations. De Pooter et al. [12] analysed a sample from Malaysia and reported (E)-farnesene (18.2%), β-bisabolene (16.2%), α-bergamontene (10.7%) and α-pinene (10.2%) as the important components. Janssen and Scheffer [15] determined that the oil of *A. galanga* contained 1′-acetoxychavicol acetate, 1′-acetoxyeugenol acetate and 1′-hydroxychavicol acetate as trace constituents.

Our study indicated that the *trans*-cinnamic acid isolated from *A. galanga* hexane extract could control third-instar mosquito larvae. *trans*-Cinnamic acid seems to be one of the active ingredients; in this case, this compound is well known and has been reported to be an insecticide to other species. However, this report is the first report on the potential use of *trans*-cinnamic acid for mosquito control. The present study suggests that *A. galanga* plays a significant role in mosquito larval control, with *trans*-cinnamic acid as the active compounds.

Cinnamic acid esters and their derivatives are widely distributed in plants, including cereals, legumes, oilseeds, fruits, vegetables and tea or coffee beverages [8]. Due to their frequent occurrence in plants and low toxicity to humans, animals and the environment [16, 22], cinnamic acid derivatives have attracted many toxicologists. In previous research, cinnamic acid derivatives have been described with actions such as antimicrobial [39], anti-inflammatory [10] and pesticidal on *Spodoptera littoralis* [31].

From Table 3, it seems that carboxylesterase is one of the reasons causing mortality in mosquitoes, especially *A. dirus* B and *Cx. quinquefasciatus* larvae. It is evident that the toxicity in *Ae. aegypti* is less than that in the other two species, as their detoxification enzymes seem to be significantly induced by some extracts, especially hexane crude extract and cinnamic acid. There is a large amount of research showing that detoxification enzymes play an essential role to chemical pesticide resistance

and that their levels increase to eliminate pesticides or other xenobiotics from an animal's body. For example, Kaur et al. [19] reported that the induction of detoxification enzyme activities depended on both the duration of treatment and the concentration; prolonged treatment and high dose showed a higher increase in enzyme activities. Zhou et al. [44] showed that extracts from *Illicium verum* fruit induced EST activity in *Myzus persicae* and *Lymantria dispar* after they were fed on a diet of aspen leaves supplemented with phenolic glycosides. Yonggyun et al. [40] and Karuppaiah et al. [18] described insecticide resistance in *Spodoptera litura* by overexpression of detoxification enzymes. Similarly, in other insect species, *Oedaleus asiaticus* [13], *Amsacta albistriga* [26] and *Musca domestica* [43], cytochrome P450, CE and GST were reported to play roles in the development of chemical pesticides resistance.

Insects including mosquitoes are known for their ability to develop resistances to insecticides. Presently there are insects resistant to every synthetic chemical insecticide used. There are many factors to developing resistance. An insecticide is detoxified by one or more enzymes before it can reach its site of action. Mixed-function oxidases or other enzymes are involved. Most resistance may develop to only a single synthetic insecticide such as organophosphates, carbamates, pyrethroids, neonicotinoids, etc. But, there are so many ingredients in galangal crude extract, plant extract based pesticides. It is not easy to develop themselves to resistance so many compound at the same time.

As pesticides generally act on neural enzymes, the AChE activity analysis showed that the AChE activities in all three species were increased. Observation of the behaviour of the treated larvae clearly showed that they were motionless. Thus, the effect of *A. galanga* or *trans*-cinnamic acid may have another mode of action on neuronal Na^+/K^+ icon channels or other proteins, which will require further study.

Conclusion

This is the report to show an effect of *A. galanga* solvent extracts and *trans*-cinnamic acid as pesticides on *Ae. aegypti, A. dirus* B and *Cx. quinquefasciatus* larvae. Overall, the relationships among detoxification and the acute toxicity of *A. galanga* suggest that the *A. galanga* extracts which has *trans*-cinnamic acid as active ingredient compound affect the mortality of *Ae. aegypti*, *A. dirus* B and *Cx. quinquefasciatus* larvae. Our data will also be useful for generating a database for plant natural pesticidal studies and will be used in the future larger scale for vector control in developing countries, especially in Southeast Asia.

Abbreviations

RH: relative humidity; CarE: carboxylesterase; GST: glutathione-*S*-transferase; AChE: acetylcholinesterase; *A. galanga*: *Alpinia galanga*; D:L: dark:light; NMR: nuclear magnetic resonance; DMSO: dimethyl sulphoxide; LC_{50}: median lethal concentrations; EDTA: ethylenediaminetetraacetic acid; pNPA: para-nitrophenyl acetate; CDNB: 1-chloro-2,4-dinitrobenzene; DTNB: Ellman's reagent or 5, 5′-dithiobis (2-nitrobenzoic acid); ASCh: acetylthiocholine iodide.

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Authors' contributions

VB and WP designed the experiment. AP, TY and WPo performed the experiments. VB and WP wrote and reviewed the paper. All author checked all the details. All authors read and approved the final manuscript.

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Availability of data and materials

All data are presented in Tables 1, 2, 3

Ethics approval and consent to participate

All experimental procedures in this research were performed with the approval of an appropriate animal Ethics Committee of Kasetsart University, Thailand.

Consent for publication

This research has been confirmed for publication in the journal.

Competing interests

The authors declare that they have no competing interests.

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