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Research article

Insecticidal activity of *Piper retrofractum* fruit extracts and isolated compounds against *Spodoptera litura*

Atcharee Ratwatthananon[a], Thitaree Yooboon[b], Vasakorn Bullangpoti[b], Wanchai Pluempanupat[a,*]

[a] *Department of Chemistry and Center of Excellence for Innovation in Chemistry, Special Research Unit for Advanced Magnetic Resonance, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.*

[b] *Animal Toxicology and Physiology Speciality Research Unit, Department of Zoology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.*

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Abstract

Secondary metabolites found in plants have been recognized as potent botanical insecticides that are safer than synthetic insecticides. The potential of *Piper retrofractum* Vahl. fruit extracts and their isolated compounds was investigated against the second instar of *Spodoptera litura* Fab. using topical application. The results showed that the hexane extract was the most potent with a median lethal dose (LD_{50}) value of 0.87 µg/larva at 24 hr and 48 hr posttreatment. Among seven isolated compounds from the hexane extract, methyl piperate had the highest toxicity to *S. litura* with LD_{50} values of 2.00 and 1.60 µg/larva at 24 hr and 48 hr posttreatment, respectively, followed by pipernonaline, piperanine and retrofractamide D, respectively. The fruits of *P. retrofractum* could have potential for the development of novel insecticides to control *S. litura* for use in an integrated pest management program.

Introduction

Spodoptera litura Fab. (Lepidoptera: Noctuidae) is a polyphagous insect pest that is dispersed throughout Asia and is having a high impact on reduced agricultural yields where its larvae can be a severe pest of vegetables such as cabbages, lettuces, kale, soybeans, mung beans and corn (Su et al., 2012). Feeding by the larvae damages leaves, followers, stems, and head sections throughout the year in Thailand and synthetic insecticides such as pyrethroids, organophosphates and carbamates, have been used to control this insect (Ruttanaphan et al., 2019). Unfortunately, these chemicals take a long time to decompose so that residues can affect the agricultural products, consumers and the environment (Gavrilescu, 2005).

An alternative approach for the control of *S. litura* is to use botanical insecticides that are safer and less harmful to the environment than synthetic insecticides. Many naturally occurring compounds from plants have been used to control this insect such as *Alpinia galanga* (L.) Willd. (Pengsook et al., 2020), *Acorus calamus* L. (Yooboon et al., 2019), *Brucea Javanica* L. (Mao et al., 2019), *Piper nigrum* L. (Fan et al., 2011) and *Piper longum* L. (Park et al., 2002). However, the botanical insecticides may not be useful in the long term due to their rapid rate of decomposition and the development of insecticide resistance in insect pests (Ahmad et al., 2009). Therefore, alternative plants are needed as a source of novel insecticides to control this insect species. Additionally, the identification of active ingredients is needed, since the chemical structures of active compounds have also suggested structural modification as a means of exploring novel insecticides (Tharamak et al., 2020), which are more potent and can be used for the control of resistant insects (Hüter, 2011).

* Corresponding author.
E-mail address: fsciwcp@ku.ac.th (W. Pluempanupat)

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Piper retrofractum Vahl. belongs to the family Piperaceae. This plant is used as a traditional medicine (Kim et al., 2011) and displays a wide variety of biological activities: antioxidant (Jadid et al., 2017), anti-obesity activity (Muharini et al., 2015), antitussive (Kubo et al., 2013), antifungal (Luyen et al., 2014), antidiabetic (Kim et al., 2011) and antibacterial (Tewtrakul et al., 2011). Interestingly, this plant has shown insecticidal activity against various insects such as *Spodoptera exigua* Hübner (Ratwatthananon et al., 2017), *Plutella xylostella* L. (Kraikrathok et al., 2013), *Blattella germanica* L. (Saenmanot et al., 2018) and *Culex quinquefasciatus* Say (Wiwattanawanichakun et al., 2018). Furthermore, previous studies found that the ethanolic *P. retrofractum* fruit extract and its major components had high toxicity to *S. litura* (Yooboon et al., 2019). The present study has extended the identification of other active ingredients from the most active extract, which was produced using sequential extraction. The aim of the present study was to investigate the contact toxicity of seven isolated compounds from the hexane extract of the fruits of *P. retrofractum* against *S. litura*.

Materials and Methods

General

The solvents used were purified using standard protocols. A rotary evaporator (IKA®RV10 basic, Thailand) was used at the Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz AVANCE III HD spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). The chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane (TMS). Splitting patterns were designated as: s, singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; ddd, doublet of doublet of doublets; t, triplet; m, multiplet; q, quintet and br, broad. Coupling constants (*J*) were reported in hertz (Hz). High-resolution mass spectra (HRMS) were recorded on a MAXIS (Bruker).

Plant materials

The dried fruits of *P. retrofractum* (2.2 kg) were collected in September 2018 from Village No. 9, Ban Hong district, Ban Hong sub-district, Lamphun province, Thailand. A voucher specimen (BK 68866) was preserved in the Bangkok Herbarium, Plant Varieties Protection Office, Department of Agriculture, Bangkok, Thailand.

Extraction

The dried fruits of *P. retrofractum* were ground into a powder and extracted using maceration and sequential extraction over 7 d using hexane, dichloromethane, ethyl acetate, and methanol. All crude extracts were filtered using vacuum filtration with a Buchner funnel and the solvent was removed using a rotary evaporator and stored at 4°C in a refrigerator.

Isolation

The most active hexane extract (3 g) was fractionated using column chromatography with 100% hexane to 50% hexane in ethyl acetate to provide seven fractions. Fraction 3 was purified using recrystallization with ethanol to produce methyl piperate (**1**, 0.55%). Fraction 4 was subjected to silica gel column chromatography with 65% hexane in ethyl acetate to give four subfractions. Subfraction 4–2 was further purified using preparative thin layer chromatography (PTLC) with 75% hexane in ethyl acetate to obtain (2*E*,4*E*,14*Z*)-*N*-isobutylicosa-2,4,14-trienamide (**2**, 0.55%). The purification of fraction 5 using silica gel column chromatography and eluting with 70% hexane in ethyl acetate produced four subfractions. Subfraction 5–1 was further purified using PTLC with 80% hexane in ethyl acetate to produce guineensine (**3**, 0.88%). Fraction 6 was fractionated using silica gel column chromatography and eluting with 70% hexane in ethyl acetate to provide four subfractions. Retrofractamide D (**4**, 0.32%) was isolated from subfraction 6–4 using PTLC with 70% hexane in ethyl acetate. Next, fraction 7 was purified using silica gel column chromatography with 80% to 65% hexane in ethyl acetate to produce two subfractions. Subfraction 7–1 was purified using silica gel column chromatography and eluting with 75% hexane in ethyl acetate to give pipernonaline (**5**, 0.55%). Subfraction 7–2 was fractionated using silica gel column chromatography and eluting with 70% hexane in ethyl acetate to obtain two subfractions. Subfraction 7–2–1 was purified using PTLC with 70% hexane in ethyl acetate to produce piperine (**6**, 0.22%), whereas subfraction 7–2–2 was purified using PTLC using 70% hexane in ethyl acetate to produce piperanine (**7**, 0.32%).

Details of the fraction purifications are provided below:

Methyl piperate (**1**): Colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 15.3, 10.8 Hz, 1H), 7.02 (d, *J* = 1.6 Hz, 1H), 6.94 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.86 (s, 1H), 6.82 (d, *J* = 1.7 Hz, 1H), 6.80 (s, 1H), 6.75 (d, *J* = 10.8 Hz, 1H), 6.01 (s, 2H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.75, 148.73, 148.44, 145.14, 140.43, 130.69, 124.64, 123.11, 120.08, 108.69, 106.03, 101.54, 51.68. HRMS (ESI) Calculated for $C_{13}H_{12}NNaO_4$ 255.0633 ($[M+Na]^+$), Found 255.0636 (Venkatasamy et al., 2004).

(2*E*,4*E*,14*Z*)-*N*-Isobutylicosa-2,4,14-trienamide (**2**): Colorless solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (dd, *J* = 15.0, 9.9 Hz, 1H), 6.16–5.99 (m, 2H), 5.77 (d, *J* = 15.1 Hz, 1H), 5.72 (s, 1H), 5.36–5.31 (m, 2H), 3.14 (dd, *J* = 12.3, 5.8 Hz, 2H), 2.12 (dd, *J* = 13.7, 7.1 Hz, 2H), 2.05–1.97 (m, 4H), 1.85–1.73 (m, 1H), 1.62 (dt, *J* = 15.2, 7.8 Hz, 1H), 1.43–1.37 (m, 2H), 1.29 (ddd, *J* = 19.8, 9.3, 4.0 Hz, 16H), 0.93–0.84 (m, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.72, 143.43 141.55, 129.98, 128.32, 121.76, 47.10, 32.08, 31.89, 29.90, 29.19, 28.93, 28.72, 27.30, 27.02, 22.45, 20.24, 14.11. HRMS (ESI) Calculated for $C_{24}H_{45}NNaO$ 384.3242 ($[M+Na]^+$), Found 384.3235 (Wu et al., 2004).

Guineensine (**3**): Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (dd, *J* = 15.1, 9.9 Hz, 1H), 6.89 (d, *J* = 1.5 Hz, 1H), 6.80–6.69 (m, 2H), 6.32– 6.20 (m, 1H), 6.18–5.96 (m, 3H), 5.93 (s, 2H), 5.74 (d, *J* = 14.9 Hz, 1H), 5.50 (s, 1H), 3.16 (t, *J* = 6.3 Hz, 2H), 2.15 (q, *J* = 7.0 Hz, 4H), 1.38 (dd, *J* = 42.4, 6.1 Hz,

9H), 0.92 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.32, 141.53, 129.51, 128.39, 121.82, 120.34, 108.36, 105.53, 101.05, 47.08, 33.01, 29.47, 29.11, 28.78, 20.26. HRMS (ESI) Calculated for $C_{24}H_{33}NNaO_3$ 406.2358 ([M+Na]$^+$), Found 406.2360 (Wu et al., 2004).

Retrofractamide D (**4**): Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (dd, *J* = 12.0, 4.0 Hz, 1H), 6.98 (br s, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 6.81–6.73 (m, 3H), 6.67 (d, *J* = 8.0 Hz, 1H), 5.97 (s, 2 H), 5.93 (d, *J* = 8.0 Hz, 2H), 5.56 (br s, 1H), 3.19 (t, *J* = 6.4 Hz, 2H), 1.82 (m, 1H), 1.70 (br s, 6H), 0.94 (d, *J* = 8.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.45, 147.11, 143.61, 141.51, 130.99, 129.73, 129.02, 124.77, 122.99, 122.79, 128.65, 105.87, 101.45, 47.25, 28.77, 20.28. HRMS (ESI) Calculated for $C_{21}H_{27}NNaO_3$ 364.1889 ([M+Na]$^+$), Found 364.1883 (Banerji et al., 1985).

Pipernonaline (**5**): Yellow oil; ^{1}H NMR (400 MHz, C_3D_6O) δ 7.42 (d, *J* = 1.7 Hz, 1H), 7.26 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.21 (d, *J* = 7.9 Hz, 1H), 7.16 (t, *J* = 7.0 Hz, 1H), 6.88 (dt, *J* = 14.9, 1.5 Hz, 1H), 6.80 (dt, *J* = 15.8, 1.5 Hz, 1H), 6.60 (dt, *J* = 15.8, 6.9 Hz, 1H), 6.41 (s, 2H), 4.01–3.94 (m, 4H), 2.73–2.60 (m, 4H), 2.14–2.04 (m, 2H), 1.99–1.90 (m, 9H).^{13}C NMR (100 MHz, C_3D_6O) δ 155.14, 143.71, 140.22, 139.20, 131.59, 130.85, 118.57, 115.70, 111.59, 43.00, 42.40, 38.49, 35.06. HRMS (ESI) Calculated for $C_{21}H_{28}NO_3$ 342.2069 ([M+H]$^+$), Found 342.2075 (Wu et al., 2004).

Piperine (**6**): Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (ddd, *J* = 14.7, 8.4, 1.8 Hz, 1H), 6.96 (d, *J* = 1.6 Hz, 1H), 6.87 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.78–6.70 (m, 3H), 6.42 (d, *J* = 14.7 Hz, 1H), 5.94 (d, *J* = 4.7 Hz, 2H), 3.56 (s, 4H), 1.74–1.46 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.59, 148.34, 148.26, 142.64, 138.38, 131.12, 125.51, 122.65, 120.20, 108.64, 105.82, 101.42, 47.07, 43.38, 26.87, 25.80, 24.81. HRMS (ESI) Calculated for $C_{17}H_{19}NNaO_3$ 308.1263 ([M+Na]$^+$), Found 308.1278 (Wu et al., 2004).

Piperanine (**7**): Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (ddd, *J* = 14.7, 8.7, 1.5 Hz, 1H), 7.02–6.92 (m, 1H), 6.88 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.85–6.57 (m, 1H), 6.28–6.10 (m, 1H), 5.90 (s, 2H), 3.62 (dt, *J* = 37.9, 14.1 Hz, 4H), 2.68 (t, *J* = 7.6 Hz, 2H), 2.52–2.36 (m, 2H), 1.71–1.44 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.65, 147.70, 144.22, 142.67, 138.40, 135.13, 125.48, 121.43, 108.98, 108.62, 108.28, 105.80, 101.40, 100.91, 34.68, 34.60, 24.74. HRMS (ESI) Calculated for $C_{17}H_{21}NNaO_3$ 310.1419 ([M+Na]$^+$), Found 310.1414 (Wu et al., 2004).

Insect rearing

All experiments were performed with the approval of the Animal Ethics Committee of Kasetsart University (No. OACKU01059). *S. litura* larvae were reared on an artificial diet (Tharamak et al., 2020) and before testing for contact toxicity were kept at 27°C, 65 ± 5% relative humidity and an L16:D8 photoperiod in the insect-rearing room of the Department of Zoology, Faculty of Science, Kasetsart University, Bangkok, Thailand.

Contact toxicity bioassay

The median lethal doses (LD_{50}) were determined of the extracts and isolated compounds against second instars of *S. litura* using a topical application. Sample concentrations in the range 0.25–40 µg/larva were prepared in acetone. Each sample (2 µL) was slowly applied to the thorax region of *S. litura* using a micro applicator in five replicates each consisting of a population of 10 larvae (*n* = 50 per treatment) and acetone alone was used as the control. The treated larvae were transferred into Petri dishes (diameter 100 mm) under controlled conditions with an artificial feeding diet and then mortality was recorded at 24 hr and 48 hr posttreatment. Larvae that did not move after prodding with a fine brush were considered dead. The LD_{50} values were determined using Probit analysis in the StatPlus Program (2018 version, Analyst Company, Canada).

Results

The sequential extraction of the fruits of *P. retrofractum* revealed that the highest yield was produced by the dichloromethane solvent (4.28%) and the lowest yield by the ethyl acetate solvent (1.27%), as shown in Table 1. A comparison of the contact toxicity among the four extracts for control of *S. litura* showed that the hexane extract had the greatest efficacy with an LD_{50} value of 0.87 µg/larva at 24 hr posttreatment, while LD_{50} values were obtained above 9 µg/larva for the other extracts (Table 2). All tested extracts produced the same mortality at 24 hr and 48 hr posttreatment.

The chemical constituents of the hexane extract were isolated using appropriate chromatography techniques. Seven known compounds were identified: methyl piperate (**1**), (2*E*,4*E*,14*Z*)-*N*-isobutylicosa-2,4,14-trienamide (**2**), guineensine (**3**), retrofractamide D (**4**), pipernonaline (**5**), piperine (**6**) and piperanine (**7**), as shown in Fig. 1.

Table 1 Characteristics and amounts of different crude extracts of fruits of *P. retrofractum* from different solvent extractions

Extract	Weight (g)	Yield[1] (% wt/wt)	Characteristic
Hexane	57.06	2.59	Orange oil
Dichloromethane	94.22	4.28	Dark brown gum
Ethyl acetate	28.04	1.27	Dark brown gum
Methanol	54.18	2.46	Dark brown gum

[1] (Weight of crude extract/weight of dried plant) × 100

Table 2 Contact toxicity of *P. retrofractum* fruit extracts against *S. litura*

Extract	Time (hr)	LD_{50}	LCL	UCL	Slope ± SE	*p*-value	χ^2
Hexane	24	0.87	0.50	1.04	1.45 ± 0.15	0.05	18.14
	48	0.87	0.50	1.04	1.45 ± 0.15	0.05	18.14
Dichloromethane	24	12.93	9.20	19.02	1.08 ± 0.13	0.21	9.68
	48	12.93	9.20	19.02	1.08 ± 0.13	0.21	9.68
Ethyl acetate	24	9.36	6.50	14.14	1.74 ± 0.26	0.01	18.64
	48	9.36	6.50	14.14	1.74 ± 0.26	0.01	18.64
Methanol	24	25.40	15.33	56.88	1.11 ± 0.20	0.002	22.48
	48	25.40	15.33	56.88	1.11 ± 0.20	0.002	22.48

LD_{50} = lethal dosage that kills 50% of exposed larvae, expressed in micrograms per larva;
LCL = lower confidence limit; UCL = Upper confidence limit

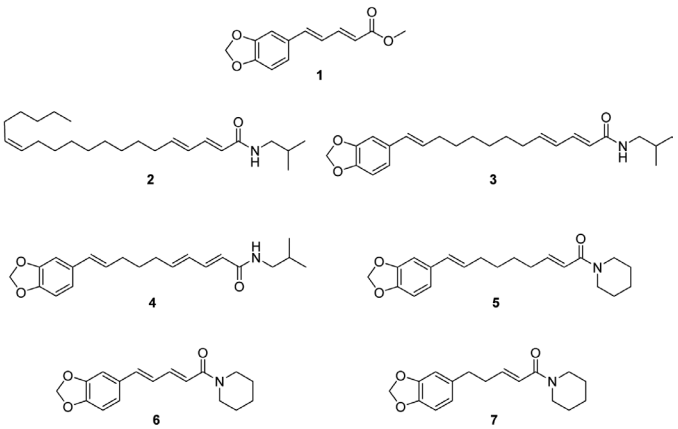

Fig. 1 Chemical structures of seven isolated compounds from hexane extract

All the isolated compounds were examined for their contact toxicity against *S. litura* to determine the active ingredient (Table 3). The results showed that methyl piperate (**1**) had the greatest toxicity with LD_{50} values of 2.00 and 1.60 µg/larva at 24 hr and 48 hr posttreatment, respectively, followed by pipernonaline (**5**), piperanine (**7**) and retrofractamide D (**4**) with LD_{50} values in the ranges 2.27–2.93 and 1.22–2.25 µg/larva at 24 hr and 48 hr posttreatment, respectively. On the other hand, (2*E*,4*E*,14*Z*)-*N*-isobutylicosa-2,4,14-trienamide (**2**), guineensine (**3**) and piperine (**6**) were less toxic.

Discussion

Botanical insecticides have low levels of hazardous properties and result in no harmful residues in the environment and consequently, they offer an alternative approach to reduce the use of synthetic insecticides (Aktar et al., 2009). *P. retrofractum* in the genus *Piper* is widely distributed in tropical regions of the world. *P. retrofractum* fruits have been used as a spice in food and traditional medicine (Muharini et al., 2015; Sholikhah, 2016). This plant has also been used as an insecticide to control various insect pests (Isman, 2014; Saenmanot et al., 2018; Subsuebwong et al., 2016). Regarding *S. litura*, the ethanolic extract of *P. retrofractum* fruits has been reported to be more effective than other plants (*A. calamus*, *A. galanga*, *Curcuma longa* L. and *Sphagneticola trilobata* L.) according to Yooboon et al. (2019); in addition, a mixture of *P. retrofractum* and *A. calamus* ethanolic extracts had a synergistic effect that enhanced

Table 3 Contact toxicity of seven isolated compounds against *S. litura.*

Compound	Time (hr)	LD_{50}	LCL	UCL	Slope ± SE	*p*-Value	χ^2	Potency ratio
Methyl piperate (**1**)	24	2.00	1.62	2.60	1.40 ± 0.20	0.46	1.54	1
	48	1.60	1.29	2.00	1.39 ± 0.20	0.50	1.40	-
(2*E*,4*E*,14*Z*)-*N*-isobutylicosa-2,	24	14.38	10.16	24.22	0.91 ± 0.13	0.43	2.76	7.19
4, 14-trienamide (**2**)	48	10.73	7.92	16.30	0.95 ± 0.13	0.93	0.14	-
Guineensine (**3**)	24	10.97	8.60	15.00	1.01 ± 0.11	0.38	5.33	5.48
	48	8.54	5.42	16.99	0.99 ± 0.17	0.31	5.96	-
Retrofractamide D (**4**)	24	2.93	2.34	3.67	1.36 ± 0.20	0.97	0.06	1.46
	48	1.41	1.12	1.79	1.34 ± 0.20	0.98	0.04	-
Pipernonaline (**5**)	24	2.27	1.87	2.88	1.63 ± 0.21	0.47	1.52	1.14
	48	2.25	1.84	2.92	1.52 ± 0.20	0.28	2.54	-
Piperine (**6**)	24	>40	-	-	-	-	-	>20
	48	4.97	3.55	8.91	1.34 ± 0.22	0.79	0.47	-
Piperanine (**7**)	24	2.60	2.14	3.14	1.62 ± 0.20	0.51	1.35	1.30
	48	1.22	1.00	1.47	1.62 ± 0.20	0.39	1.89	-

LD_{50} = lethal dosage that kills 50% of the exposed larvae, expressed in micrograms per larva; LCL = lower confidence limit; UCL = upper confidence limit; Potency ratio = LD_{50} value of compound/LD_{50} value of methyl piperate (**1**) at 24 hr posttreatment.

toxicity against *S. litura*. However, in the current study, a comparison of the LD_{50} values against *S. litura* at 24 hr posttreatment between the hexane extract (LD_{50} = 0.87 µg/larva) and the ethanolic extract in the previous work (LD_{50} = 5.58 µg/larva) showed that the hexane extract was approximately 6.41-fold more potent than the ethanolic extract. Similarly, the hexane extract was comparatively more potent than the most active ethyl acetate extracts from *A. galanga* rhizomes (Pengsook et al., 2020) and *Phaseolus lathyroides* L. seeds (Pipattanaporn et al., 2015) against this insect pest. These results suggested that extraction with hexane could produce beneficial ingredients present in the fruits of *P. retrofractum* for the development of commercial insecticides.

The chemical components of *P. retrofractum* fruits have been reported in several studies as alkaloids, terpenoids, lignans, flavones, propenylphenols, kawapyrones and dihydrochalcones (Kubo et al., 2013). The results of the present study found that almost all the isolated compounds from the hexane extract were alkaloids (Compounds **2–7**) except for methyl piperate (**1**). The insecticidal activities of these compounds have also been reported. For example, piperine (**6**), isolated from *Piper ribesioides* Wall. had high toxicity toward *Aedes aegypti* L. (Kumrungsee et al., 2018). Pipernonaline (**5**), isolated from *P. longum*, had toxicity activity against *S. litura* (Park et al., 2002). However, there have been no reports identifying methyl piperate (**1**) as a potent insecticide. This compound has been first reported in the current study as having insecticidal activity against *S. litura*. Regarding contact toxicity, methyl piperate (**1**) was the most active compound with LD_{50} values of 2.00 and 1.60 µg/larva at 24 hr and 48 hr posttreatment, respectively, whereas pipernonaline (**5**), retrofractamide D (**4**) and piperanine (**7**) were slightly less potent than methyl piperate (**1**). However, piperine (**6**) did not appear to have any potential effect on the mortality of *S. litura*. A similar result was obtained when piperine was used to treat *S. exigua* (Ratwatthananon et al., 2017).

Based on the LD_{50} values, methyl piperate (**1**) was less potent than the hexane crude extract. However, the toxicity of pure compounds may have a potentially synergistic effect in mixtures that may lead to the development of more potent botanical insecticides and reduce the potential of resistance developing toward the pure compound (Koul and Walia, 2009). Methyl piperate (**1**) is an alternative approach for use as a botanical insecticide and it has potential for the modification of its chemical structure for the development of novel insecticides that are less toxic than synthetic insecticides. Furthermore, this compound may provide a significant reduction in antioxidant ability (Nakatani et al., 1986), which may avoid losing insecticidal activity due to its degradation by temperature and light (Turek and Stintzing, 2013).

The *P. retrofractum* fruit extracts and the active composition were first examined for their insecticidal activity against *S. litura* using topical application. The hexane extract and methyl piperate (**1**) could be further developed for use as an alternative botanical insecticide. In addition, the effect on detoxification enzymes and biochemical interactions should be a further focus for understanding the trends of resistance and the possible mode of action of *S. litura* after treatment using the hexane extract and methyl piperate (**1**).

Conflict of Interest

The authors declare that there are no conflicts of interest.

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