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# Toxicity of isolated phenolic compounds from *Acorus calamus* L. to control *Spodoptera litura* (Lepidoptera: Noctuidae) under laboratory conditions

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## Abstract

**Background:** *Spodoptera litura* (Lepidoptera: Noctuidae), the tobacco cutworm, is an prominent agricultural pest. To reduce the use of chemical pesticides that cause health problems and that leave residue in the environment, some botanical pesticides have been developed. Our research aimed to evaluate the insecticidal efficacy of *Acorus calamus* L. extract for the control of *S. litura* under laboratory conditions.

**Results:** After 24 h of topical application, the *A. calamus* methanolic crude extract showed toxicity against secondary instar *S. litura* larvae ($LD_{50}$ ~ 7.438 µg/larva). Chrysin (5,7-dihydroxy flavone), one of the isolated phenolic compounds, showed optimal control efficiency ($LD_{50}$ ~ 2.752 µg/larva) and showed a reduction in carboxylesterase activities, which have detoxification reduction roles in larvae.

**Conclusion:** Chrysin in the crude extract of *A. calamus* may be an active compound to control this pest, and it may be applied as an alternative to minimize the usage of chemical insecticides.

**Keywords:** *Spodoptera litura*, Noctuidae, *Acorus calamus*, Chrysin

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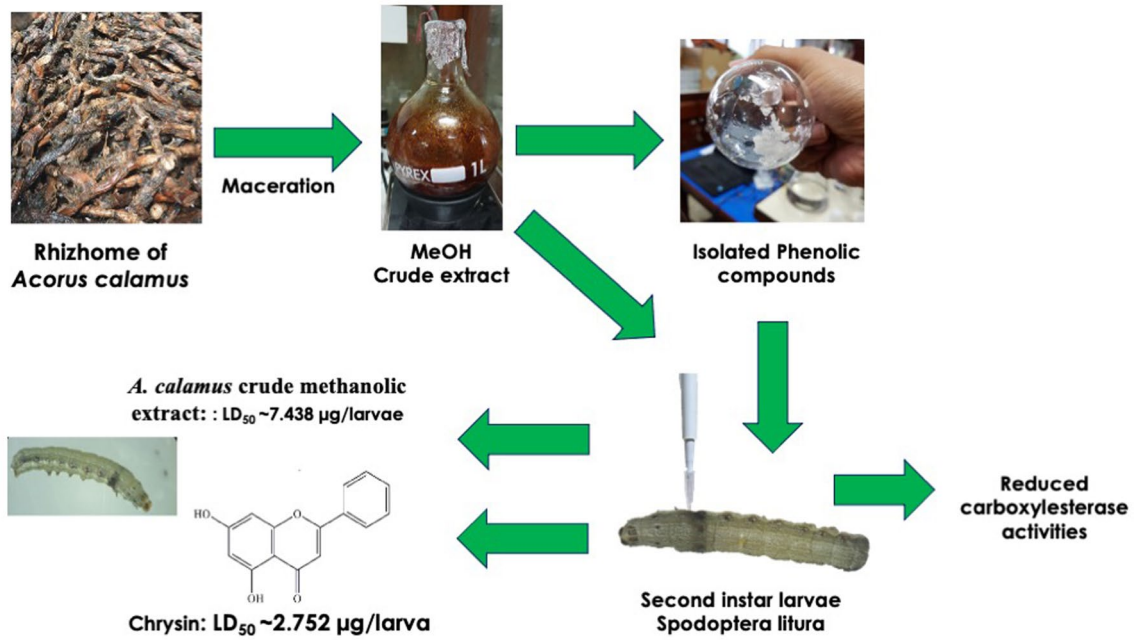
**Graphical Abstract**




## Background

Noctuidae, which contains some of the most prominent insect pests in agriculture, is the largest family in the order Lepidoptera. Our research interest lies in the tobacco cutworm [*Spodoptera litur*a (Lepidoptera: Noctuidae)], which is an important crop pest in regions worldwide [1]. This species feeds on many varieties of plants, such as corn, rice, peanuts, sorghum, Bermuda grass, and cotton [2, 3]. This species parasitizes the plants through vigorous larval eating patterns, often leaving the leaves destroyed. Additionally, moths are responsible for decreasing yield in agricultural crops [4]. Currently, the management of this pest mainly relies on synthetic chemicals, including organophosphates, carbamates, and pyrethroids. However, *S. litura* has developed substantial resistance to various classes of chemicals following the long-term utilization of synthetic pesticides [5–7]. Several farmers use high doses of synthetic insecticides. But inappropriate use of synthetic insecticides can result in greater environmental problems and the loss of on-farm biodiversity, as well as damage to human health [8].

Botanical insecticides have been used to control many pests over the last two decades, as they show minimal toxicity to mammals. Several isolated compounds from plant extracts or essential oils have been evaluated for their insecticidal activity [9]. For example, neem has had a substantial global impact as a botanical insecticide [10–13]. There are many publications that show that plant extracts such as neem, rotenone, toosendanin [14], *Itoa orientalis* Hemsl [12], piperine, β-asarone [15] and *Alpinia galanga* [16] efficiently control *S. litura*.

This research aimed to evaluate the efficiency of *Acorus calamus* L. for controlling *S. litura*. Humans use the extract of *Acorus calamus* L. to prevent stress and memory deficits, because it inhibits inflammation and controls oxidative stress [17]. In addition, *A. calamus* is a botanical extract for pest management and a supplement in primary health care worldwide [18]. Numerous documents have recorded that *A. calamus* has toxic effects against many insects [19, 20]. Its essential rhizome oil showed a sterilizing effect against the eggs of *Sitophilus granaries* L., *Sitophilus oryzae* L., and *Callosobruchus chinensis* L. [21], and it showed larvicidal activity against *Culex quinquefasciatus* L. [19]. This plant originated in Asia, but has been cultivated in South Africa since early colonial times for use in treating indigestion, flatulence and diarrhoea. It is distributed throughout the countryside and has become naturalized [22]. The compound β-asarone, isolated from the *A. calamus* rhizome, is a potent growth inhibitor and antifeedant to the variegated cutworm *Peridroma saucia* L. [20].

In this research, we also investigated phenolic compounds isolated from *A. calamus*. Many studies have described that *A. calamus* has various phenolic compounds [23–25]. Phenolic compounds were recorded as toxic to some insects, such as red imported fire ants

(*Solenopsis invicta*) [26], termites [27], and fall armyworms [28].

It has proven especially difficult to characterize phenonic activity in insect herbivores. Appel and Schultz (1992) [29] described that variation among insect species in midgut conditions, mainly redox potential, and pH, yields variable impacts of phenolics on insects. Redox conditions and pH are influenced by intrinsic physiological characteristics of the insect and by foliar oxidative enzymes, nonenzymatic oxidants, and reductants. All may affect detoxification enzymes and may be related to toxicity by pesticides. In many cases, oxidative activation may be necessary before biological impacts can be observed.

In this study, we produced an effective crude extract from the *A. calamus* rhizome with a methanolic solvent and isolated the phenolic compounds to control *S. litura*. There is little research on the efficacy of phenolics isolated from *A. calamus* on *S. litura*. The aim of this work was to determine the toxic effects of *A. calamus* on *S. litura* and of the detoxification enzyme activities to estimate insect resistance.

## Materials and methods

### Insect rearing

*S. litura* larvae were obtained from the Animal Toxicology and Physiology Research Unit Laboratory of Kasetsart University, Bangkok, Thailand. *S. litura* larvae were raised in $23 \times 13 \times 7$ $cm^3$ plastic boxes during the first- to third-instar larval stage and in $26 \times 40 \times 20$ $cm^3$ plastic boxes for the fourth-instar larval stage. The temperature was set to $25 \pm 2$ °C with 75% relative humidity and a photoperiod of 16:8 (L:D). *S. litura* larvae were fed an artificial diet (a mixture of 240 g green bean, 25 g agar, 40 ml mixed vitamin solution, 5 g ascorbic acid, 40 ml amoxil solution, 3 g sorbic acid, 5 g methylparaben, 20 g yeast, 4 ml of 40% formalin and 1.41 L water). The artificial diet ingredients were the same as those described by Yooboon et al. [30]. To allow oviposition, the moths were moved to cages with filter papers and 10% v/v sugar solution in water after emergence. The second instars were used in topical application assays as described below. All experimental procedures were performed with approval of the Animal Ethics Committee of Kasetsart University.

### Plant material

*A. calamus* was collected from Chiangmai Province, Thailand, and was registered at the Princess Sirindhorn Plant Herbarium of the Plant Varieties Protection Division, Department of Agriculture, Thailand, with voucher No. BKF 071393. Rhizomes were cleaned and dried at room temperature, powdered with a grinding machine (WF-10) and stored in Zip-lock bags.

### Extraction method

The rhizomes of *A. calamus* were extracted with methanol (MeOH) to produce toxic crude methanol, and its bioactive compounds were isolated with the modified method of Nobsathian et al. [31].

### Identification

The air-dried and finely ground powder of the *A. calamus* L. rhizome was extracted with methanol. The crude extract was filtered and dried using a rotary evaporator (IKA®RV10 basic, Thailand) and stored at 4 °C until further use in the experiments. The isolated compound was obtained by using chromatography techniques.

The dried and finely ground powdered rhizome (1.70 kg) of *A. calamus* was soaked with MeOH at room temperature to produce a crude MeOH extract (215.00 g). After dissolution in MeOH:EtOAc (1:1) and solvent removal, a soluble fraction (105.000 g) of crude MeOH:EtOAc (1:1) was obtained. After removing solvents, the obtained active MeOH:EtOAc (1:1) fraction (816.0 g) was divided into three portions. Each portion (116.6 g) was subjected to VLC over Si-gel (200 g) on a sintered glass funnel (i.d. 13.0 cm × 4.3 cm) and eluted with EtOAc–hexanes and MeOH–EtOAc gradients. Fractions (1000 mL each) were collected and combined based on their thin layer chromatography behaviours to produce fractions. $A_1$–$A_6$. Fraction $A_3$ (2.57 g) provided 4-hydroxyacetophenone (4) (125.20 mg) after two consecutive Si-gel CCs (CH2Cl2–hexane gradients), followed by recrystallization from MeOH–$CH_2Cl_2$. Fraction $A_3$ (13.20 g) produced fractions $B_1$–$B_4$ after Si-gel CC (ethyl acetate–hexanes and MeOH–ethyl acetate gradients). Fraction B3 (5.20 g) produced fractions $C_1$–$C_6$ after Si-gel CC (acetone–hexanes and MeOH–acetone gradients). Fraction C1 (1.52 g) produced pachypophyllin (1) (121.2 mg) as a white needle after recrystallization from MeOH–ethyl acetate. Fraction $B_3$ (780.1 mg) provided galangin (2) (101.1 mg) after recrystallization from MeOH–CH2Cl2. The residue of fraction B3 (211.4 mg) yielded chrysin (3) (21.20 mg) after recrystallization from CH2Cl2–hexanes.

All pure compounds were verified by their physical properties and spectroscopic data, as found in the literature [32–35].

### Toxicity assay

The toxicities of the crude extract and isolated compounds were determined following the method of Yooboon et al. [9]. The extract and compounds were individually applied directly to the thoracic region of the second instars (42.31 mg/larva), as this stage of larvae were widespread on other plants, with a microapplicator at a

dose of 0–30 µg/larva, using acetone as a control because it was used as the dissolving solvent (five replicates, five treatments, $n=150$ per dose). After application, each larva was placed in a Petri dish with filter paper and then moved to a new sealed plastic tray ($30\times20\times10$ $cm^3$), provided with an artificial diet, covered with a black cover, and placed under controlled conditions in a rearing room with moistened filter paper. Mortality was counted after 24 h of exposure. The median lethal dose was calculated by probit analysis (StatPlus Program for Mac 2017, AnalystSoft, Walnut, CA, USA).

### Detoxification enzyme assays

This research determined some of the major detoxification enzymes in insects, carboxylesterase (CE) and glutathione-*S*-transferase (GST) activity, using a microplate reader technique. Second instars of *S. litura* were treated with *A. calamus* crude extract and its isolated compounds at the median lethal dose ($LD_{50}$) that was determined from the topical application.

Five replicates of the surviving larvae 24 h after treatment were homogenized in 1% Triton-X-100 and 0.5 mL of 100 mM potassium phosphate buffer, pH 7.2. After centrifugation at 12,000 rpm for 15 min at 4 °C, the supernatant was used as an enzyme source.

CE activity was determined with a microplate reader by a modified method from Bullangpoti et al. [36], which uses *p*-nitrophenyl acetate (pNPA) as the substrate. Enzyme activity was measured in kinetic mode at 410 nm and 37 °C for 90 s. The activity was determined by the extinction coefficient of 176.4705 for pNPA.

To determine GST activity with a microplate reader, the method of Oppenoorth et al. [37], which used 150 mM 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate, was modified. The optical density was recorded in kinetic mode at 340 nm and 37 °C for 30 s. The extinction coefficient of GST activity was 0.0096.

The protein content of each fraction that was used as an enzyme source was determined by the method of Bradford (1976) [38] using a Bradford kit (Bio–Rad Laboratories, Hercules, CA, USA) before measuring enzyme activities. Three biological replicates per enzyme fraction were estimated.

All statistical analyses were performed in the StatPlus Program (StatPlus Program for Mac 2017, AnalystSoft, Walnut, CA, USA).

## Results

### Crude and isolation compound

The percent yields of crude methanol, the 1:1 MeOH:EtOAc soluble fraction and all pure compounds are shown in Tables 1 and 2.

**Table 1** %yield of crude methanol, 1:1 MeOH:EtOAc soluble fraction

| Sample | Dry weight (g) | Crude extract (g) | % Yield |
|---|---|---|---|
| Powder of rhizome | 1700.00 | 215.00 | 12.65 |
| Soluble fraction | 215.00 | 105.00 | 48.84 |

**Table 2** %yield of all pure compounds

| Sample | Weight of soluble fraction (g) | Weight of pure compounds (mg) | % Yield |
|---|---|---|---|
| Pachypophyllin | 105.00 | 121.20 | 0.1152 |
| Galangin | 105.00 | 101.10 | 0.0963 |
| Chrysin | 105.00 | 21.20 | 0.0202 |
| 4-Hydroxyacetophenone | 105.00 | 125.20 | 0.1192 |

After purification, four pure compounds (Fig.1) were based on melting points and spectral analysis data as described below:

**Pachypophyllin** (1): colourless needles from recrystallization with ethyl acetate–hexane, mp 158–159.2 °C; $[\alpha]^{25}$ 0 (c. 0.8;$CH_2Cl_2$), UV $\lambda_{max}$... nm (logs): 227 (3.24) and 289 (4.11); FTIR $\upsilon_{max}$ $cm^{-1}$: 2972, 2926, 2868, 1711, 1461, 1389, 1299, 1189, 1073, 1002, 982, and 924; $^1$H NMR (400 MHz, CDCl3): δ 6.98 (s, 2H, H-6/6′), 6.48 (*s*, 2H, H-3 /3'), 3.87 (*m*, 2H, H-7/7′), 3.86 (*s*, 6H, 5/5′-$OCH_3$), 3.85 (*s*, 6H, 4/4′-$OCH_3$), 3.75 (*s*, 6H, 2/2′-$OCH_3$), 2.31 (*dt*, 5,1, 2,1 Hz, 2H, H-8α), 1.94 (*dt*, 5,1, 2,1 Hz, 2H, H-8β), $^{13}$C NMR (100 MHz, CDCl3): δ 151.0 (C-4/4′), 147.5 (C-2/2′), 143.1 (C-5/5′),124.6 (C-1/1′), 111.8 (C-6/6′), 97.8 (C-3/3′), 40.5 (C-7/7′), 56.6 (5/5′-OCH3), 56.5 (2/2′-OCH3), 56.2 (5/5′-OCH3), 27.0 (C-8/8′). HR MS (ESI-TOF): *m/z* found: (m/z 411.1780 $[M+Na]^+$, (calcd. for $C_{22}H_{28}O_6Na$, 411.1784).

**Galangin** (2): yellow powder from recrystallization with ethyl acetate–hexane, mp. 215–215 °C; UV $\lambda_{max}$... nm (logs): 267 (3.44) and 370 (3.55); FTIR $\upsilon_{max}$ $cm^{-1}$: 3607–3084, 1659, 1600, 1550 and 1260; $^1$H NMR (400 MHz, DMSO-$d_6$): δ 12.36 (1H, *br.s*, 5-OH), 8.14 (2H, *m,* H-4′), 7.53 (3H, *m*, H-3′, 4′, 5′), 6.45 (1H, *br.s,* H-8′), and 6.21 (1H, *br.s*, H-6′), $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ: 176.4 (C-4), 164.3 (C-7), 160.8 (C-5), 155.9 (C-9), 145.5 (C-2), 137.0 (C-3), 130.8 (C-1′), 139.92 (C-4′), 128.6 (C-2′, 6′), 127.6 (C-3′, 5′), 103.3 (C-10), 98.4 (C-6), and 93.6 (C-8); HR MS (ESI-TOF): *m/z* found 316.0331 $[M+Na]^+$, (calcd. for $C_{15}H_{10}O_5Na$, 316.0324).

**Chrysin** (3): yellow powder from recrystallization with MeOH, m.p. 270.3–274.0 °C; UV $\lambda_{max}$... nm (logs): 212 (4.66), 268 (4.56), 313 (4.17); FTIR $\upsilon_{max}$ $cm^{-1}$: 3434 (O–H stretching of phenol), 3012 (aromatic C-H stretching),

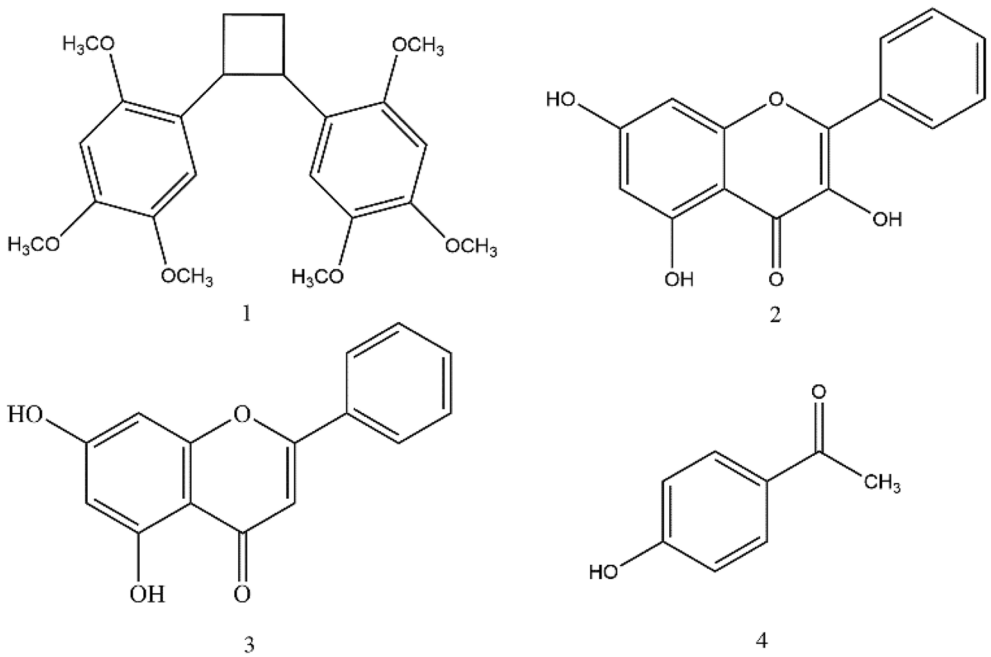



**Fig. 1** Phenolic compounds isolated from *A. calamus* were (1) pachypophyllin (2) galangin (3) chrysin and (4) 4-hydroxyacetophenone

1654 (C=O stretching of ketone), 1612 (C=C stretching), 1577 (aromatic C=C stretching), 1169 (C–O stretching of phenol), 1120, 1101, 1077, 1033, 999, 908, 842, 807, 782, 747, 733, 711, 693, 674, 642, 612, 572, 559, 511, 502, 461, 430, 405 $cm^{-1}$; $^{1}H$ (400 MHz, DMSO-$d_6$) δ 12.79 (1 H, *s*, OH-5) 10.89 (1H, *s*, OH-7) 8.00 (2H, *d*, J = 8.0 Hz, H-2′, 6′) 7.51–7.56 (3H, *m*, H-3′, 4′, 5′), 6.89 (1H, *s*, H-3), 6.47 (1H, *s*, H-8), 6.18 (1H, *s*, H-6); $^{13}C$ (100 MHz, DMSO-$d_6$) δ 163.46 (s,C-2), 105.27 (s,C-3), 182.27 (s,C-4), 157.82 (C-9), 161.96 (s, C-5), 99.35 (C- 6), 164.69 (C-7), 94.95 (C-8), 104.41 (C-10), 131.43 (s, C-1'), 126.23 (C-2′,6′), 129.6 (C-3′,5′); HR MS (ESI-TOF): *m/z* found 277.0481 $[M+Na]^+$, (calcd. for $C_{15}H_{10}O_4Na$, 277.0477).

**4-hydroxyacetophenone** (4): white powder from recrystallization with MeOH, m.p. 106.3–107.7 °C; UV $\lambda_{max...}$ nm (logs): 219 (4.09) and 275 (4.23); FTIR $\upsilon_{max}$ $cm^{-1}$: 3310 (O–H stretching of phenol), 1664 (C=O stretching of ketone), 1604, 1578, 1512 (aromatic C=C stretching), 1108 and 1074 (C–O stretching of phenol), 1022, 962, 849, 817, 668, 590, 568, 498, 489, 414 $cm^{-1}$; $^{1}H$ (400 MHz, $CDCl_3$) (δ ppm) 7.91 (2H, *d*, J = 8.5 Hz, H-2,6), 7.03 (2H, *d*, J = 8.5 Hz, H-3,5), 2.56 (*s*, 3H) ppm. $^{13}C$ NMR (100 MHz, $CDCl_3$): 129.0 (C-1), 132.0 (C-2,6), 115.5 (C-3,5), 198.4 (C-7), 161.3 (C-4), 26.3 ($CH_3$); HR MS (ESI-TOF): *m/z* found 159.0426 $[M+Na]^+$, (calcd. for $C_8H_8O_2Na$, 159.0422).

**Table 3** $LD_{50}$ values (μg/larvae)[1] of the crude methanolic extract and isolated phenolic compounds from *A. calamus* against second instar *S. litura* larvae under laboratory conditions

| Extract | Number of insects/dose (*n*) | $LD_{50}$[1] | UCL | LCL | Chi-square | *P*-level |
|---|---|---|---|---|---|---|
| *A. calamus* crude methanolic extract | 150 | 7.438 | 7.507 | 7.369 | 3.21 | 0.043 |
| Pachypophyllin | 150 | 6.512 | 6.623 | 6.398 | 1.56 | 0.031 |
| Galangin | 150 | 4.718 | 4.717 | 4.729 | 1.29 | 0.063 |
| Chrysin | 150 | 2.752 | 2.821 | 2.683 | 0.77 | 0.089 |
| 4-Hydroxyacetophenone | 150 | 3.217 | 3.293 | 3.179 | 6.94 | 0.065 |
| Control[2] | 150 | – | – | – | – | – |

[1] $LD_{50}$ represents the median lethal dose

[2] Control is treatment with acetone only

### Toxicity results

The toxicity in terms of the median lethal dose ($LD_{50}$) values of *A. calamus* methanolic crude extract, pachypophyllin, galangin, chrysin and 4-hydroxyacetophenone were 7.438, 6.512, 4.718, 2.752 and 3.217 µg/larva, respectively (Table 3). There are no mortality occurs in control treatment. From our results, the *A. calamus* methanolic extract showed toxicity on second instar *S. litura* larvae under laboratory conditions, and chrysin could be an active ingredient, as it showed the highest toxicity (Table 1, LD50 = 2.752 µg/larva). No mortality occurred in the control group. Although other isolated phenolic compounds, such as pachypophyllin, galangin and 4-hydroxyacetophenone, showed lower relative toxicities, they still all showed toxicity to the larvae. In addition, the toxicity to *S. litura* second instar larvae was dose-dependent.

### Detoxification enzyme activities

Detoxification enzyme analysis was carried out with the methanolic crude extract and all isolated phenolic compounds to determine the detoxification ability of the treated larvae. Our results showed that CE activity was reduced after treatment with all the isolated extracts (Table 4) compared with that of the control (the treatment in which larvae were not exposed to any plant compounds), except for after treatment with the crude methanolic extract of *A. calamus*. Galangin showed the highest reduction in activity, with an approximately 1.4-fold reduction. GST activity was significantly induced after all treatments compared to that of the control group (Table 4). The crude methanolic extract showed the highest induction (1.65- times). Among the phenolic compounds, galangin showed the highest induction (1.5 times) (Table 4).

## Discussion

This is the first study that showed that crude *A. calamus* methanolic extract had an $LD_{50}$ value of 7.438 ± 69.8 µg/larva against secondary instars of *S. litura*. The toxicity of the bioeffective *A. calamus* methanolic extract on *S. litura* was also confirmed (Table 3).

Yooboon et al. [15] reported that this plant's crude ethanolic extract exhibited contact toxicity and antifeedant activity against second instar *S. litura*. Moreover, the highest concentration of *A. calamus* caused an approximately 90% mortality of *Callosobruchus analis* at 13.2625 µg/cm$^2$ [39]. Several reports have published that *A. calamus* showed some insecticidal efficiency; for example, the leaf-dipping toxicity of *A. calamus* ethanolic extracts caused mortality against third instar larvae of *P. xylostella* [40].

Melani et al. [41] showed that *A. calamus* essential oil caused the highest toxicity on *S. litura* larvae ($LD_{50}$ values ~ 5.86 µg/larvae). Compared with other plants using the same solvent extract, the *A. galangal* methanolic extract showed toxicity and growth inhibition against *S. litura* larvae [42].

According to Imam and Tajuddeen [43], *A. calamus* phytochemical studies have reported glycosides, saponins, flavonoids, tannins, mucilage, polyphenolic compounds, and some volatile oils in this plant. There have been some reports that have shown that beta-asarone is a compound contained within *A. calamus* that demonstrated insecticidal efficiency against the controls of *Prostephanus truncates* (Horn) [44] and *S. litura* [45]. Several reported flavonoid compounds were also found in the *A. calamus* extract, such as uvangoletin, galangin, chrysin (5,7-dihydroxy flavone), 5-hydroxy-4', 7-dimethoxy-flavone and pinostrobin [46]. Moreover, there have been a few reports on the efficiency of other flavonoid compounds against *S. litura*. In addition, this

**Table 4** Detoxification enzyme activities[1] of second instar *S. litura* larvae after treatment with the crude extract and the isolated phenolic compounds from *A. calamus* at the median lethal dose

| | Carboxylesterase[c] | Effect | Glutathione-*S*-transferase[3] | Effect |
|---|---|---|---|---|
| Control[4] | 776.60 ± 25.40a | – | 4.98 ± 0.001e | – |
| *A. calamus* crude extract | 780.97 ± 31.51a | No effect | 8.20 ± 0.003a | Induction |
| Pachypophyllin | 739.63 ± 66.32b | Reduction | 6.06 ± 0.002d | Induction |
| Galangin | 553.21 ± 15.80d | Reduction | 7.46 ± 0.005b | Induction |
| Chrysin | 631.85 ± 19.42c | Reduction | 6.30 ± 0.001c | Induction |
| 4-Hydroxyacetophenone | 657.85 ± 94.16c | Reduction | 7.06 ± 0.002d | Induction |

[1] In all experiments, the mean values followed by the same letter within the same column are not significantly different using Tukey's HSD test ($P > 0.05$). In each experiment, 30 larvae/treatment in five replicates were used ($n = 150$ per treatment)

[2] Carboxylesterase activity ± SE (nM p-nitrophenol/min/mg protein)

[3] Glutathione-*S*-transferase activity ± SE ($\times 10^{-3}$ CDNB conjugated product/mg protein/min)

[4] Control is treatment with acetone only

research found three flavonoid compounds (galangin, chrysin, and pachypophyllin) that showed toxicity to *S. litura* larvae, with $LD_{50}$ values of 6.512 ± 114.2 µg/larva, 4.718 ± 114 µg/larva, and 2.752 ± 69.2 µg/larva for pachypophyllin, galangin, and chrysin, respectively (Table 1). Chrysin seemed to be an active ingredient (Table 3).

Chrysin is a phenolic compound in several plants. In a previous report, chrysin contributed to antioxidant activity as an immune enhancer in fish [47]. Avila et al. [48] calculated an $LC_{50}$ of 28.79 ppm for this compound against *S. frugiperda*. Chrysin also had antifeedant activity on *S. litura* larvae [49].

Herbivorous animals normally use detoxification enzymes to metabolize xenobiotic compounds [50]. The mechanism of *S. litura* resistance towards insecticides must be understood to increase control efficiency by studying the functions of the detoxification enzyme activities in *S. litura*. Several reports found that resistance in *S. litura* was dependent on certain detoxification enzyme activities, such as GST, CE, and cytochrome P450. The enzyme activities of CE and GST were observed to be higher in *Spodoptera spp.* that are resistant to pesticides [51, 52].

Our results showed that CE had significantly reduced activities in larvae that survived for 24 h after exposure to all isolated phenolic compounds from *A. calamus* compared to the control group and the *A. calamus* crude extract group.

Generally, at the biochemical level, CE plays a role in the tolerance to allelochemical intake [53]. Our results showed that CE reduction affected some specific functions in insects, such as the neurotransmitter acetylcholine or juvenile hormones [54, 55]. Pesticides that induced these enzymes always showed resistance mechanisms [53, 56].

However, as seen in Table 4, GST activity was significantly induced; they were catalysed enzymes that detoxified the endogenous compounds involved in *Spodoptera* spp. resistance mechanisms [57]. Lepidoptera always showed an increase in GST activity during pesticide resistance [58]. Our results indicate that GST could be an important detoxification enzyme of *S. litura* to fight against the activity of the *A. calamus* extract. However, this process must be evaluated in further studies.

## Conclusions

The crude methanolic extract of *A. calamus* and its isolated compound, chrysin, can cause toxicity to *S. litura* larvae (Table 1, $LD_{50}$ = 2.752 µg/larva). There was a significant reduction in detoxification enzymes as CE activity among the isolated compounds (Table 4). Chrysin could be a main active ingredient to control *S. litura* and may be used as an alternative to minimize the application of chemical insecticides.

### Abbreviations

$LD_{50}$: Median lethal dose; L:D: Light:dark; CE: Carboxylesterase; GST: Glutathione-*S*-transferase; pNPA: *p*-Nitrophenylacetate; CDNB: 1-Chloro-2,4-dinitrobenzene; MeOH: Methanol (MeOH).

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### Authors' contributions

VB and SN designed the experiment. PW, VB and TY performed the experiments. VB, SN, PW and NK wrote and reviewed the paper. All author checked all the details, read and approved the final manuscript.

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### Availability of data and materials

All data are presented in Tables 1, 2, 3, 4 and Fig. 1.

## Declarations

### 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 have no conflicts of interest.

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## Publisher's Note