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# Larvicidal effect of compounds isolated from *Maerua siamensis* (Capparidaceae) against *Aedes aegypti* (Diptera: Culicidae) larvae

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**Abstract**

**Background:** Dengue is a major problem for humanity. Most people use insecticides to eliminate larvae of *Aedes aegypti*, which requires heavy chemicals use that affects the environment and human health. Therefore, in this research, the focus was on the larvicidal efficacy of pure compounds from the leaves and twigs of *Maerua siamensis* against the larvae of *A. aegypti*.

**Results:** Larval mortality was observed after a 24-h exposure. The 1H-indole-3 acetonitrile glycosides capparilo-side A and capparilloside B and the triterpene lupeol showed strong larvicidal effects (24-h $LC_{50}$ = 71.14, 99.79 and 133.03 ppm). After 48 h, capparilloside B caused the most potential mortality with an $LC_{50}$ of 1.56 ppm and lupeol had the highest lethal concentration at $LC_{50}$ = 158.71 ppm. Additionally, consistency was observed between the toxicity tests and detoxification enzyme activity. Most compounds, except for lupeol and vanillin, reduce the activity of glutathione-*s*-transferase, whereas no significant differences were between control and treated groups for carboxylesterase.

**Conclusions:** Capparilloside A and capparilloside B are good potential larvicide agents. They showed larvicidal activity against *Ae. aegypti* larvae with $LC_{50}$ = 71.14 and 99.79 ppm at 24 and 48 h, respectively.

**Keywords:** *Maerua siamensis*, Larvicidal agent, *Aedes aegypti*, Toxicity, Detoxification enzyme

## Background

The yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae) is a primary vector that causes dengue and dengue haemorrhagic fever [1]. In the 20th century, more than 1.8 billion people (more than 70%) in 110 countries were at risk of dengue infection. In the past decade, because of the public health problems [2], most people use insecticides to eliminate the mosquitoes. However, the current use of insecticides has caused inhibition of cholinesterases and chromosomal aberrations in human peripheral leukocytes [3, 4]. Additionally, these insecticides also contaminate air, water, and soil in surrounding areas and therefore cause mortality to animals [5, 6]. Thus, other insecticidal substances with lower toxicity to the environment than the ones in current use are urgently required [7].

Some plant compounds can eliminate larvae. The crude ethyl acetate extract of leaves of *Acalypha fruticosa* shows significant larvicidal activity with lethal concentration values $LC_{50}$ and $LC_{90}$ of 253.08 and 455.40 ppm, respectively [8]. The methanol extract from the leaves of *Ocimum sanctum* against fourth instar larvae of *Ae. aegypti* has an $LC_{50}$ value of 429.54 ppm [9]. Deguelin and tephrosin, rotenoid types isolated from the seeds of *Millettia dura* show potent larvicidal activity with $LC_{50}$ = 1.6 and 1.4 µg/ml at 24 h, respectively [10]. The methanol extract of the leaves of *Atalantia monophylla* (Rutaceae) shows larvicidal activity against second stages *Ae. aegypti* with a lethal concentration = 0.002 mg/l [11].

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Plants of Capparidaceae are found in tropical and subtropical regions of the world. Most plants of this family are found in Africa with 17 genera and 450 species [12]. Four genera are found in Thailand [13]: *Capparis*, *Cleome*, *Crateva,* and *Maerua*. All 90 species in the genus *Maerua* are found in tropical Asia, including the African continent. The crude extracts of plants in this genus have biological activities and ethnomedical applications. The aqueous root extract of *M. oblongifolia* is an anti-diabetic in rats [14]. The crude methanol extract of leaves of *M. angolensis* DC is active against *Streptococcus pyogenes, Escherichia coli,* and *Neisseria gonorrhoeae* [15]. The ethyl acetate fraction of the tuber parts of *M. pseudopetalosa* shows cytotoxic activity in brine shrimp larvae [16].

In this study, we focused on the isolation of larvicidal agents from *Maerua siamensis* (Kurt) Pax, the only species of the genus found in Thailand. The isolation of natural compounds from the leaves and twigs of this plant led to the separation of eight known compounds with promising insecticidal bioactivity.

## Methods

### General experimental procedure

Column chromatography (CC) used silica gel 60 (70–230 mesh, Merck Millipore, Darmstadt, Germany). Preparative thin-layer chromatography (Prep-TLC) used Kieselgel 60 PF254 (0.5 mm Merck Millipore, Darmstadt Germany). Benzenesulfonyl fluoride hydrochloride (AEBSF), EDTA, 1-chloro-2,4-dinitrobenzene (CDNB), glutathione-*s*-transferase, potassium phosphate buffer (pH 7.2), carboxylesterase and 4-nitrophenyl acetate (pNPA) were purchased from Sigma-Aldrich. The melting point was recorded on an electrothermal instrument. Optical rotations were determined on a JASCO DIP-370 digital polarimeter using a 50 mm microcell (1 ml). UV spectra were measured in EtOH or MeOH with a JASCO 530 spectrometer, and IR spectra were recorded on a Perkin Elmer 2000. The $^{1}$H-NMR, $^{13}$C-NMR, DEPT, and 2-D NMR spectra were recorded on a Bruker Ascend™ 400 MHz or Bruker AVANCE 500 MHz. in $CDCl_3$ using TMS as an internal standard, unless otherwise stated. Finally, HR-TOF-MS results were recorded on a Micromass model VQ-TOF2.

The leaves and twigs of *M. siamensis* were collected in Nakhonsawan Province, Thailand, in August 2015. A voucher specimen (BKF No. 180668) was deposited in and identified by the Forest Herbarium, Royal Forest Department in Bangkok.

Dried and finely powdered leaves and twigs (1.70 kg) of *M. siamensis* were percolated 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 crude MeOH: EtOAc (1:1) soluble fraction (105.00 g) was obtained. The active MeOH: EtOAc (1:1) soluble fraction was separated by Si-gel CC ($SiO_2$, 1.8 kg, $CH_2Cl_2$–hexane and MeOH–$CH_2Cl_2$ gradients) to give fractions $A_1$–$A_5$. Fraction $A_1$ (2.57 g) provided glochidone (**3**) (125.20 mg) after two consecutive Si-gel CCs ($CH_2Cl_2$–hexane gradients), followed by recrystallisation from MeOH–$CH_2Cl_2$. Fraction $A_3$ (13.20 g) afforded fractions $B_1$–$B_7$ after Si-gel CC (acetone–hexane, and MeOH–acetone gradients). Fraction $B_2$ (5.20 g) gave fractions $C_1$–$C_5$ after Si-gel CC (EtOAc–hexane, and MeOH–EtOAc gradients). Fraction $C_1$ (1.52 g) gave lupeol (**4**) (121.20 mg) as white needles after recrystallisation from MeOH–$CH_2Cl_2$. Fraction $C_2$ (1.07 g) yielded chrysoeriol (**5**) (80.70 mg) after two consecutive CCs (1st CC: Si-gel, EtOAc–hexane gradient; 2nd CC: Sephadex LH-20, MeOH), followed by recrystallisation from ethanol. Fraction $C_3$ (1.11 g) yielded cappariloside A (**1**) (51.70 mg) after separation by CC ($CH_2Cl_2$–hexane gradients), followed by recrystallisation from MeOH. Fraction $B_3$ (5.05 g) provided cappariloside B (**2**) (14.80 mg) after two consecutive Si-gel CCs (1st CC: EtOAc–hexane gradient; 2nd CC: acetone–hexane gradient), followed by prep-TLC (2% MeOH–$CH_2Cl_2$ as the eluent) and recrystallisation from EtOAc–hexane. Fraction $B_5$ (2.15 g) after two consecutive Si-gel CCs (1st CC: $CH_2Cl_2$–hexane and $CH_2Cl_2$–MeOH gradients; 2nd CC: EtOAc–hexane, and MeOH–EtOAc gradients) gave fractions $D_1$–$D_7$. Fraction $D_6$ (180.80 mg) provided cinnamic acid (**6**) (17.30 mg) after recrystallisation from MeOH–$CH_2Cl_2$. The residue (98.20 mg) yielded 3,4-dimethoxybenzoic acid (**7**) (21.20 mg) after recrystallisation from $CH_2Cl_2$–hexane. Fraction $B_6$ (3.42 g) was further purified by Si-gel CC (acetone–hexane gradient), followed by prep-TLC (2% MeOH–$CH_2Cl_2$) to provide fractions $E_1$–$E_4$. Fraction $E_3$ (174.00 mg) gave vanillin (**8**) (32.10 mg) following recrystallization from EtOAc. The structures of all pure compounds are shown in Fig. 1.

Cappariloside A (**1**) [17]: amorphous solid; m.p. 228.0–229.0 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 267 (4.79), 278 (4.61), 289 (4.14) nm.; IR (KBr disc) $\nu_{max}$ 3525, 3495, 3400, 3359, 1625, 1590, 1508, 1170, 1084; HR-TOF-MS (ESI positive) m/z 357.1069 $[M + Na]^+$ (calcd. for $C_{16}H_{18}N_2O_6Na$, 357.1063).

Cappariloside B (**2**) [17]: amorphous solid; m.p. 230.1–231.3 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 272 (4.00), 279 (3.88), 289 (4.12) nm.; IR (KBr disc) $\nu_{max}$ 3390, 2855, 2255, 1625, 1540, 1510, 1120 $cm^{-1}$; HR-TOF-MS (ESI positive) m/z 519.1589 $[M + Na]^+$ (calcd. for $C_{22}H_{28}N_2O_{11}Na$, 519.1591).

Glochidone (**3**) [18]: colorless needles; m.p. 166.0–166.6 °C; UV (EtOH) $\lambda_{max}$ (log $\varepsilon$) 228 (3.96), 333 (1.73).; IR (KBr disc) $\nu_{max}$ 2945, 2873, 1662 (C=O stretching of conjugated ketone), 1456, 1382, 1284, 1229, 1161, 1103,

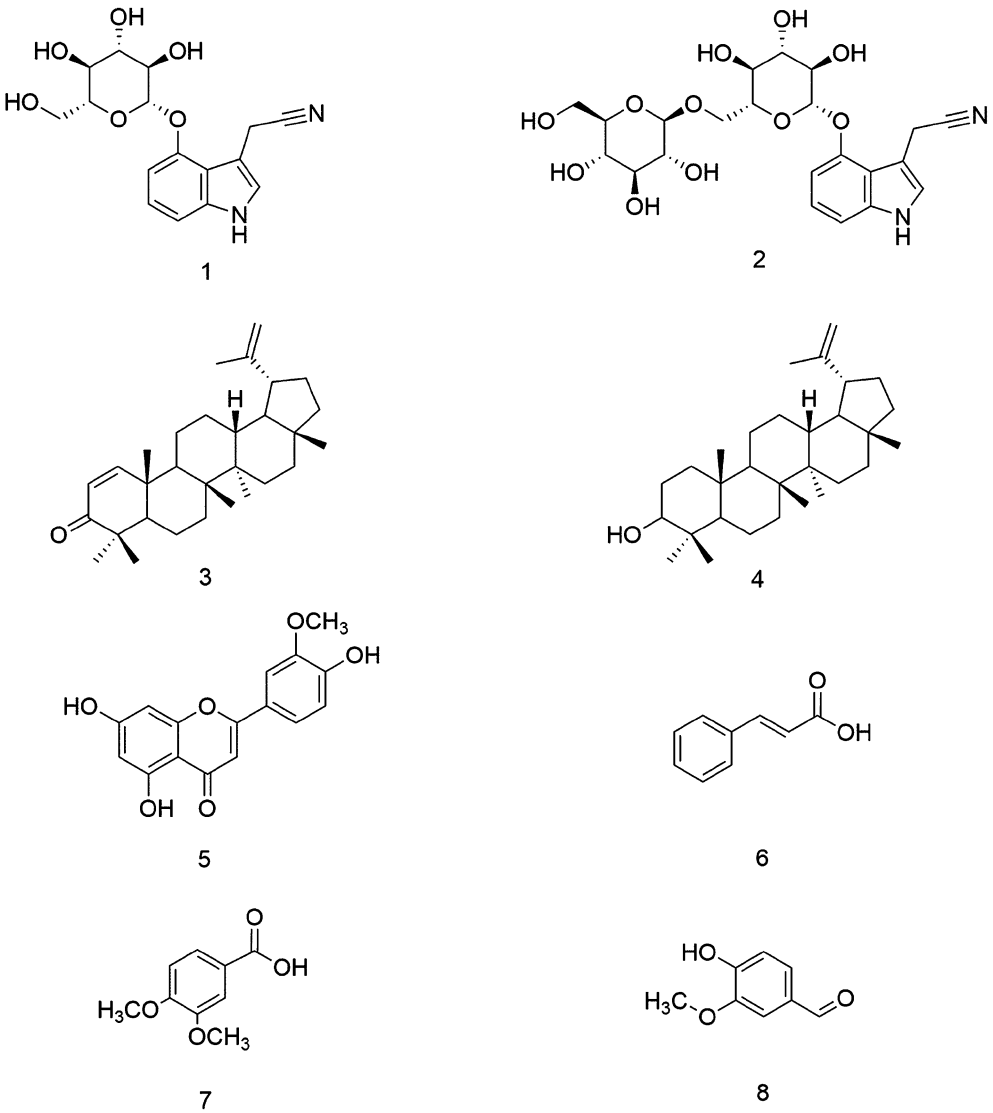


**Fig. 1** Compounds isolated from *Maerua siamensis*. All compounds were purified and their structures were elucidated based on spectroscopic data as described in the text

947, 888, 825 $cm^{-1}$; HR-TOF-MS (ESI positive) m/z 427.2044 $[M + Na]^+$ (calcd. for $C_{30}H_{28}ONa$, 427.2038); Optical rotation: $[\alpha]_{589}^{30}$ +68.6° (*c* 0.1, $CHCl_3$).

Lupeol (**4**) [19]: white powder; m.p. 212.4–213.0 °C; IR ($CHCl_3$) $\nu_{max}$ 3486, 2933, 1640, 1473, 1384, 1037, 870 $cm^{-1}$; HR-TOF-MS (ESI positive) m/z 449.3750 $[M + Na]^+$ (calcd. for $C_{30}H_{50}ONa$, 449.3759).

Chrysoeriol (**5**) [20]: yellow powder; m.p. 325.1–326.2 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 269 nm (3.44), 340 nm (4.52) nm.; IR (KBr disc) 3350, 3088, 2925, 1777, 1719, 1655, 1607, 1561, 1506, 1256 $cm^{-1}$; HR-TOF-MS (ESI positive) m/z 323.0540 $[M + Na]^+$ (calcd. for $C_{16}H_{12}O_6Na$, 323.0532).

Cinnamic acid (**6**) [21]: white powder; m.p. 193.9–194.3 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 272 (2.85) nm.; IR (KBr disc) $\nu_{max}$ 3448, 1711, 1638, 1577, 1551, 1450 $cm^{-1}$; HR-TOF-MS (ESI positive) showed $[M + Na]^+$ 171.0411 [M + Na] + (calcd. for $C_9H_8O_2Na$, 171.0422).

3,4-Dihydroxybenzoic acid (**7**) [22]: white powder; m.p. 130.9–131.0 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 212 (4.66), 268 (4.56) 313 (4.17) nm.; IR (KBr disc) $\nu_{max}$ 3200, 2839, 1674, 1603 $cm^{-1}$; HR-TOF-MS (ESI positive) m/z 177.0170 $[M + Na]^+$ (calcd. for $C_7H_6O_4Na$, 177.0164).

Vanillin (**8**) [23]: white powder; m.p. 81.0–83.2 °C; UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 221 (3.66), 271 (3.56) nm.; IR (KBr disc) $\nu_{max}$ 3475, 3444, 3184, 2733, 2669 $cm^{-1}$;

HR-TOF-MS (ESI positive) showed $[M + Na]^+$ 175.0380 $[M + Na] +$ (calcd. for $C_7H_6O_4Na$, 175.0371).

#### Mosquito strain

Eggs of *Ae. aegypti* (Thailand laboratory strain) were received from the Ministry of Public Health, Thailand. Larvae were reared in 500-ml glass beakers containing water, fed a fish diet and maintained in our culture room at 28 °C and 70% RH with a 14:10 DL photoperiod. The same conditions were used for the pupae. After emergence, adults were maintained in cages, in the same locale.

#### Larvicidal toxicity assay

The larvicidal bioassay against third instars of *Ae. aegypti* was modified from the WHO (1981) method under laboratory conditions at 28 ± 1 °C and, 70% RH 14:10 DL photoperiod. Twenty-five larvae were placed in a small cup filled with 50 ml of distilled water, to which 0.5 ml of each extract dissolved in 0.5% acetone was added. The final concentration range for each treatment was from 125 to 1000 ppm dissolved in acetone. For all concentrations, 15 replicates were used per concentration. In controls, 0.5 ml of 0.5% acetone was used in each case. During the aqueous dispersion test, mosquito larvae were not provided with food. After 24 and 48 h, mortality of the larvae in each treatment was recorded.

#### Mode of action studied

The larvae that survived the $LC_{50}$ value treatment at 24 h of exposure were homogenised in 0.5 ml of homogenised buffer [100 mM phosphate buffer (pH 7.2) and 1% Triton X-100]. The homogenate was centrifuged at 10,000×*g* for 15 min at 4 °C, and the supernatant was used as the enzyme source.

To measure in vivo enzymes activities, 24 h $LC_{50}$ value-treated larvae were homogenised in buffer A [100 mM phosphate buffer (pH 7.2) containing 1 M DTT, 100 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) and 0.5 M EDTA]. The homogenate was centrifuged at 10,000×*g* for 5 min at 4 °C, and the resultant supernatant was used for carboxylesterase and glutathione-*s*-transferase activity analyses.

Carboxylesterase (CE) activity was determined by the modified method of Kumrungsee [24]. Enzyme solution 50 µl, was mixed with 4-nitrophenyl acetate (pNPA) (10 mM in DMSO) and phosphate buffer (50 mM, pH 7.4). Enzyme activity was measured at 410 nm and 37 °C for 90 s with a microplate reader in the kinetic mode. The activity of CE was determined using the extinction coefficient of 176.4705 for pNPA.

The method for determining glutathione-*s*-transferase (GST) activity was according to Oppenoorth [25]. The reaction solution contained 100 µl of enzyme solution, 50 mM potassium phosphate buffer (pH 7.3) and 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. The GST activity was determined from the extinction coefficient of 0.0096 for CDNB.

Three biological replicates per treatment were analysed. The protein content of each fraction used as an enzyme source was determined by the Bradford method before measuring enzyme activities.

#### Statistical analyses

Probit analysis was used to calculate $LC_{50}$ and $LC_{90}$ values were determined using the STATPLUS program (version 2017). The range of detoxification enzyme activity from each treatment was compared using one-way analysis of variance (ANOVA).

## Results and discussion

All pure compounds isolated from *M. siamensis* were identified by the comparison of their physical properties and spectroscopic data with those reported in the literature [17–23].

The larvicidal activities in the different periods of exposure to *Ae. aegypti* larvae are presented in Tables 1 and 2. Cappariloside A had the lowest lethal concentration with an $LC_{50}$ of 71.14 ppm, and vanillin had the highest $LC_{50}$ value at = 2846 ppm at 24 h of exposure. After 48 h, cappariloside B caused maximum mortality with an $LC_{50}$ 1.56 ppm and lupeol had the highest lethal concentration $LC_{50}$ = 158.71 ppm.

The effect of pure compounds on detoxification enzyme activity is shown in Table 3. Consistency was found between toxicity tests and detoxification enzyme activity. Most compounds except for lupeol and vanillin, reduce glutathione-*s*-transferase activity whereas, differences between the control and treated groups for carboxylesterase were not significant.

For triterpenes, glochidone and lupeol; lupeol ($LC_{50}$ = 133.03 ppm) had better activity than betulinic acid [26] ($LC_{50}$ = 142 ppm), but the activity of betulinic acid was better than that of glochidone ($LC_{50}$ = 382.34 ppm) after 24 h. No reports on the larvicidal activity of indole alkaloids are available. Cappariloside A and B were actively toxic ($LC_{50}$ = 71.14 and 99.79 ppm, respectively). Finally, cinnamic acid, 3,4-dimethoxybenzoic and vanillin were weakly toxic. However, after 48 h, cappariloside B, cinnamic acid, lupeol, cappariloside A, 3,4-dimethoxybenzoic acid and chrysoeriol were actively toxic ($LC_{50}$ = 1.56, 22.76, 58.87, 71.14, 72.54 and 77.55 ppm, respectively). Vanillin and

**Table 1 The $LC_{50}$ and $LC_{90}$ values (ppm) of compounds isolated from *Maerua siamensis* against 3rd instars of *Aedes aegypti* after 24 h of exposure**

| Compounds | Slope | $LC_{50} \pm SE$ (ppm) | $LC_{90} \pm SE$ (ppm) | $\chi^2$ |
|---|---|---|---|---|
| Capparilosides A | 0.89 ± 0.37 | 71.14 ± 5.41a | 1980.51 ± 182.71a | 1.48 |
| Capparilosides B | 1.14 ± 0.21 | 99.79 ± 2.85a | 1329.68 ± 46.53a | 0.6 |
| Glochidone | 1.68 ± 0.20 | 382.34 ± 35.95a | 2210.21 ± 56.45a | 0.95 |
| Lupeol | 1.28 ± 0.21 | 133.03 ± 26.98a | 1340.41 ± 40.19a | 0.39 |
| Chrysoeriol | 1.12 ± 0.46 | 169.94 ± 7.17a | 2278.96 ± 222.12a | 4.53 |
| Cinnamic acid | 0.81 ± 0.19 | 852.36 ± 28.46a | 32,231.97 ± 7532.75b | 0.41 |
| 3,4-Dimethoxybenzoic acid | 0.61 ± 0.19 | 1032.87 ± 66.07b | 127,826.08 ± 1213.11c | 0.22 |
| Vanillin | 0.75 ± 0.21 | 2846.76 ± 131.14b | 144,759.28 ± 17,673c | 1.56 |

Means within a column followed by the same letter of each time exposure are not significantly different at the 0.05 level by Tukey's test

**Table 2 The $LC_{50}$ and $LC_{90}$ values (ppm) of compounds isolated from *Maerua siamensis* against 3rd instars of *Aedes aegypti* after 48 h of exposure**

| Compounds | Slope | $LC_{50} \pm SE$ (ppm) | $LC_{90} \pm SE$ (ppm) | $\chi^2$ |
|---|---|---|---|---|
| Capparilosides A | 0.89 ± 0.37 | 71.14 ± 5.41a | 1980.51 ± 182.71a | 1.48 |
| Capparilosides B | 0.48 ± 0.24 | 1.56 ± 0.16b | 1980.51 ± 32.66a | 1.48 |
| Glochidone | 1.57 ± 0.46 | 158.70 ± 45.78c | 1045.70 ± 46.05b | 3.62 |
| Lupeol | 1.46 ± 0.57 | 58.87 ± 4.02a | 443.32 ± 15.54c | 1.56 |
| Chrysoeriol | 2.17 ± 0.34 | 77.55 ± 15.72a | 302.46 ± 3.52c | 0.25 |
| Cinnamic acid | 0.70 ± 0.22 | 22.79 ± 3.98b | 1581.11 ± 149.88d | 0.48 |
| 3,4-Dimethoxybenzoic acid | 0.81 ± 0.20 | 72.54 ± 3.06a | 2732.78 ± 216.78e | 0.34 |
| Vanillin | 0.98 ± 0.20 | 130.82 ± 3.71c | 2690.07 ± 151.79e | 0.78 |

Means within a column followed by the same letter of each time exposure are not significantly different at the 0.05 level by Tukey's test

glochidone were moderately toxic ($LC_{50}$ = 130.82 and 158.70 ppm, respectively).

Insects are well known to use detoxification enzyme to develop resistance to insecticides by increasing the level of enzymes. For example, Fonseca-González et al. [27] describe high levels of both cytochrome P450 monooxygenases and non-specific esterases in some of the fenitrothion and pyrethroid-resistant *Ae. aegypti* populations in Cambodia. Kasai et al. [28] also suggest that cytochrome P450 monooxygenases play an important role in resistance development for *Ae. aegypti* to pyrethroids. Additionally, Dusfour et al. [29] describe the regulation of cytochrome P450 genes and carboxylesterases in all three populations of *Ae. aegypti* from three French overseas territories worldwide. Thus, studies on the level of detoxification enzymes may be necessary to estimate trends in resistance to new compounds. However, in the present study, all compounds showed no significant effects on carboxylesterase enzyme (CEs) activity compared with controls. Compounds significantly inhibited

**Table 3 Carboxylesterase activity (nM para phenyl acetate/mg protein/min) and glutathione-*s*-transferase activity (CDNB product/mg protein/min) of surviving 3rd instar *Aedes aegypti* after treatment with pure compounds from *Maerua siamensis* for 24 h**

| Compounds | Carboxylesterase activity[a] | CF[b] | Glutathione-*s*-transferase activity[a] | CF[b] |
|---|---|---|---|---|
| Control | 0.030 ± 0.002a | – | 0.321 ± 0.121f | – |
| Capparilosides A | 0.031 ± 0.001a | 0.97 | 0.204 ± 0.014b | 1.57 |
| Capparilosides B | 0.029 ± 0.001a | 1.03 | 0.156 ± 0.005a | 2.06 |
| Glochidone | 0.031 ± 0.001a | 0.97 | 0.282 ± 0.006d | 1.14 |
| Lupeol | 0.032 ± 0.001b | 0.94 | 0.343 ± 0.023 g | 0.94 |
| Chrysoeriol | 0.031 ± 0.001a | 0.97 | 0.280 ± 0.009d | 1.15 |
| Cinnamic acid | 0.029 ± 0.001a | 1.03 | 0.207 ± 0.002c | 1.55 |
| 3,4-Dimethoxybenzoic acid | 0.031 ± 0.001a | 0.97 | 0.291 ± 0.076e | 1.10 |
| Vanillin | 0.032 ± 0.001b | 0.94 | 0.493 ± 0.289 h | 0.65 |

[a] Means within a column followed by the same letter are not significantly different at the 0.05 level by Tukey's test

[b] CF is a correction factor = (enzyme activity of control)/(enzyme activity of treatment)

glutathione-*s*-transferase activities between 1.10- and 2.60-fold, which could explain the mortality in mosquito larvae to the compounds.

## Conclusions

In conclusion, cappariloside A and cappariloside B are good potential larvicide agents. They show larvicidal activity against *Ae aegypti* larvae with $LC_{50} = 71.14$ and 99.79 ppm at 24 and 48 h, respectively.

#### Authors' contributions

SN collected samples from Nakhonsawan Campus, Mahidol University, Nakhonsawan, Thailand. SN, VB, and NK designed the experiment. SN, NK, NW, and DR performed the experiments, analysed data and wrote the paper. SN, VB, NK, and PM reviewed and checked all the details. All authors read and approved the final manuscript.

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#### Competing interests

The authors declare that they have no competing interests.

#### Availability of data and materials

All data are shown in Tables 1 and 2.

#### Consent for publication

This research has been confirmed for publication in the journal.

#### Ethics approval and consent to participate

Not applicable.

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