Larvicidal activity of tectoquinone isolated from red heartwood-type *Cryptomeria japonica* against two mosquito species

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Abstract

Mosquito larvicidal activities of methanolic extracts from different plant parts of red heartwood-type *Cryptomeria japonica* D. Don against the fourth-instar larvae of *Aedes aegypti* and *Aedes albopictus* were examined. Results of mosquito larvicidal tests demonstrated that the *n*-hexane fraction of *C. japonica* sapwood methanolic extract had an excellent inhibitory effect against the larvae of *A. aegypti* and *A. albopictus* and its LC₅₀ values were 2.4 and 3.3 µg/ml, respectively, in 24 h. Following the bioactivity-guided fractionation procedure, the active constituent isolated from *C. japonica* sapwood was characterized as tectoquinone by spectroscopic analyses. The LC₅₀ values of tectoquinone against *A. aegypti* and *A. albopictus* in 24 h were 3.3 and 5.4 µg/ml, respectively. In addition, comparisons of mosquito larvicidal activity of anthraquinone congeners demonstrated that anthraquinone skeleton with a methyl group at C-2 position, such as tectoquinone, exhibited the strongest mosquito larvicidal activity. Results of this study show that the methanolic extract of *C. japonica* sapwood may be considered as a potent source and tectoquinone as a new natural mosquito larvicidal agent.

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1. Introduction

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, and several diseases (James, 1992). Mosquitoes also cause allergic responses on humans that include local skin and systemic reactions such as angioedema (Peng et al., 1999). The yellow fever mosquitoes, *Aedes aegypti* and *Aedes albopictus*, are two main species of mosquitoes responsible for dengue fever in Taiwan, where the number of dengue fever cases has increased significantly in recent years. Control of the

mosquito larvae is frequently dependent on continued applications of organophosphates (chlorpyrifos, temephos, and fenthion) and insect growth regulators (diflubenzuron and methoprene) (Yang et al., 2002). Effective, repeated use of controlling agents has disrupted natural biological control systems and led to outbreaks of insect species showing pesticide resistance. It has also provoked undesirable effects, including toxicity to nontarget organisms and fostered environmental and human health concerns (Casida and Quistad, 2000; Lee et al., 2001; Yang et al., 2002). These problems have highlighted the need for the development of new strategies for selective mosquito larval control.

Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, since they

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constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in integrated pest management programs. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae and the recent examples are studied by Cheng et al. (2003, 2004), Jantan et al. (2005) and Yang et al. (2002, 2003).

The Japanese cedar, *Cryptomeria japonica* D. Don (“sugi” in Japanese), is a widely distributed conifer. *C. japonica* is well-known in Taiwan as one of the important plantation tree species because of its beautiful yellowish red to red heartwood (Chang et al., 1999, 2000; Chang and Cheng, 2001). Owing to its industrial importance, its chemical components have been investigated in numerous studies (Lee and Lin, 1986; Shieh et al., 1981; Nagahama et al., 2001; Su et al., 1994, 1995, 1996). Our previous studies have demonstrated that leaf essential oil from *C. japonica* has excellent antitermite (Cheng and Chang, 2002), antifungal (Cheng et al., 2005) and mosquito larvicidal (Cheng et al., 2003) activities. However, there has been no thorough investigation on larvicidal activity of the components of *C. japonica* extracts. Therefore, in this study we examine the mosquito larvicidal activity of the components isolated and the methanolic extracts of different parts of red heartwood-type *C. japonica* against the fourth-instar larvae of *A. aegypti* and *A. albopictus*. Additionally, the relationships of structure–mosquito larvicidal activity of anthraquinone congeners were also investigated.

2. Methods

2.1. Plant material

Wood, bark, and leaves of a 53 years old Japanese cedar tree (*C. japonica* D. Don), in the Taxodiaceae class, were collected in June 2001 from the Experimental Forest of National Taiwan University located in Nantou County in Central Taiwan. The species was identified and the voucher specimens (CJHO01, CJSO01, CJBO01, and CJLO01) including heartwood, sapwood, bark, and leaf, were deposited at the Laboratory of Wood Chemistry (School of Forestry and Resource Conservation, National Taiwan University).

2.2. Chemicals

Anthraquinone, 3-methyl-1,6,8-trihydroxyanthraquinone (emodin), 1,2-di-hydroxyanthraquinone (alizarin), 2-hydroxymethylantraquinone, 1-hydroxyanthraquinone, and anthraquinone-2-carboxylic acid were obtained from TCI Chemical Corp. (Japan). Chlorpyrifos (*O,O*-diethyl-*O*-3,5,6-trichloro-2-pyridyl phosphorothioate) was purchased from Sigma Chemical Corp. (USA). All other chemicals were of reagent grade.

2.3. Mosquito larvae

Larvae of *A. aegypti* and *A. albopictus* from the Kaohsiung strain were reared in the Department of Parasitology, Chang-Gung University at 27 °C with a photoperiod of 12 h light and 12 h dark and 80 ± 10% RH. A 10% yeast suspension was used as food source.

2.4. Extraction and isolation

C. japonica heartwood (4.1 kg), sapwood (2.4 kg), bark (1.6 kg), and leaf (1.8 kg) chips were prepared from freshly cut tree and air-dried samples were extracted twice with methanol at room temperature for 7 days and then filtered. The combined filtrate was concentrated under vacuum at 40 °C to yield 9.14%, 0.54%, 12.73%, and 12.11% (according to the weight of the dried samples), respectively. The methanolic extract of sapwood (13.0 g) was sequentially partitioned into *n*-hexane (4.4 g), chloroform (2.4 g), ethyl acetate (4.1 g), and methanol (1.4 g) portions for subsequent bioassay. The organic solvent portions were concentrated to dryness under reduced pressure at 40 °C.

The active *n*-hexane fraction (1.6 g) was divided into 11 subfractions (SH1–SH11) using a silica gel column (Merck 70–230 mesh, 4.0 g) and successively eluted with a stepwise gradient of *n*-hexane/ethyl acetate (from 100/0 to 0/100 by volume). During this step, the active SH3 subfraction (139 mg) of the *n*-hexane fraction showed a strong larvicidal activity (100% mortality) against *A. aegypti* and *A. albopictus* at 12.5 µg/ml. Ferruginol (**1**, 16 mg) and tectoquinone (**2**, 45 mg) were isolated and purified from the active SH3 subfraction by semi-preparative HPLC (Hitachi model L-7150 pump equipped with a L7490 refractive index detector) with a Zorbax sil column (250 mm × 9.4 mm i.d., 5 µm). The mobile phase was hexane/ethyl acetate/dichloromethane (80:15:5, v/v/v), at a flow rate of 2.0 ml/min and a detection wavelength of 254 nm. Structural determination of the active compound was made by spectral analysis. ¹H and ¹³C NMR spectra were recorded with a Bruker DMX500 spectrometer at 500 MHz and chemical shifts were given in ppm. Fourier transform infrared (FT-IR) spectra were recorded on a Bio-Rad FTS-40 instrument and mass spectra (MS) were obtained on a Finnigan MAT-95 S mass spectrometer.

2.5. Mosquito larvicidal bioassay

The method of Rafikali and Nair (2001) was modified and employed to conduct mosquito larvicidal activity test. Ten fourth-instar mosquito larvae were placed in 24.5 ml of degassed distilled water, followed by addition of 500 µl DMSO solution containing the test samples in a 30-ml cup, with gentle shaking to ensure a homogeneous test solution, and each cup was left at ambient temperature. Concentrations of 400, 200, 100, 50, and 25 µg/ml of extracts were tested and each compound was tested at 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 µg/ml.

The control was prepared with 24.5 ml of degassed distilled water and 500 μ l of DMSO solution. Each test was replicated four times. For comparison, commercial chlorpyrifos, an organophosphorus pesticide was used as a positive control. The toxicity of chlorpyrifos was determined at 6.25, 3.125, 1.56, 0.78, and 0.39 μ g/ml.

Larvicidal activity was evaluated 24 h after treatment. Larvae were considered to be dead if appendages did not move when prodded with a wooden dowel. The percentage of mortality was corrected for control mortality using Abbott's formula and the results were plotted on log/probability paper using the method of Finney (1971). Toxicity and effectiveness were reported as LC_{50} and LC_{90} , which represent the concentrations in μ g/ml with 50% and 90% larvae mortality in 24 h, respectively.

2.6. Statistical analyses

All results were expressed as mean $\pm$ SD ($n = 4$). The percentages of mortality were determined and transformed to arcsine square root values for analysis of variance (ANOVA). The Scheffe's test was utilized to analyze for significant differences among the test extracts and compounds against the two strains of mosquito larvae. Results with $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Mosquito larvicidal activity of methanolic extracts from different plant parts

To evaluate the mosquito larvicidal activities of the methanolic extracts of different plant parts of *C. japonica*, fourth-instar larvae of yellow fever mosquito (*A. aegypti* and *A. albopictus*) were used. Results from Figs. 1 and 2 showed that the larval mortality of the methanolic extracts of *C. japonica* heartwood, bark, and leaf extracts did not exceed 50.0% at the concentration of 400 μ g/ml, indicating no significant toxicity to *A. aegypti* and *A. albopictus*, while

Fig. 1. Mosquito larvicidal activity of methanolic extracts of different parts of *C. japonica* at different concentrations against fourth-instar larvae of *A. aegypti*. Each experiment was performed four times and the data averaged ($n = 4$). Numbers followed by different letters (a–e) are significantly different at the level of $p < 0.05$ according to the Scheffe's test.

Fig. 2. Mosquito larvicidal activity of methanolic extracts of different parts of *C. japonica* at different concentrations against fourth-instar larvae of *A. albopictus*. Each experiment was performed four times and the data averaged ($n = 4$). Numbers followed by different letters (a–e) are significantly different at the level of $p < 0.05$ according to the Scheffe's test.

the sapwood caused 100% larval mortality of *A. aegypti* and *A. albopictus* in 24 h at 400 μ g/ml concentration.

From the comparisons of the LC_{50} and LC_{90} values of the methanolic extract of the different plant parts of *C. japonica* (Table 1), the methanolic extract of the sapwood showed an excellent toxicity, the LC_{50} values being 11.5 and 15.8 μ g/ml with corresponding LC_{90} values of 23.4 and 62.4 μ g/ml, respectively. Araújo et al. (2003) also found that *Hyptis martiusii* leaf essential oils induced 100% mortality of *A. aegypti* larvae after 1 day but it takes a very high dose of 500 mg/l. In other investigation, Cavalcanti et al. (2004) reported that the larvicidal activity of essential oils from Brazilian plants with LC_{50} values against the larvae of *A. aegypti* that ranged from 60 to 69 μ g/ml. Comparing these values with that obtained in this study reveals that the methanolic extract of *C. japonica* sapwood has an excellent mosquito larvicidal effect.

3.2. Mosquito larvicidal activity of different fractions from methanolic extract of sapwood

When fractions obtained from the methanolic extract of *C. japonica* sapwood were bioassayed, significant differences in toxicity to mosquito larvae were observed (Table 2). As shown in Table 2, except for the *n*-hexane fraction, all the test samples showed no significant toxicity against

Table 1

Lethal concentrations (LC, μ g/ml) of methanolic extracts of different plant parts of *C. japonica* against fourth-instar larvae of *A. aegypti* and *A. albopictus* in 24 h

Parts	LC_{50}		LC_{90}	
	<i>A. aegypti</i>	<i>A. albopictus</i>	<i>A. aegypti</i>	<i>A. albopictus</i>
Heartwood	>400.0	>400.0	>400.0	>400.0
Sapwood	11.5	15.8	23.4	62.4
Bark	>400.0	>400.0	>400.0	>400.0
Leaf	>400.0	>400.0	>400.0	>400.0
Chlorpyrifos ^a	1.1	1.8	2.5	2.8

^a Positive control.

Table 2

Lethal concentrations (LC, $\mu\text{g/ml}$) of four fractions of the methanolic extract of *C. japonica* sapwood against fourth-instar larvae of *A. aegypti* and *A. albopictus* in 24 h

Fractions	LC ₅₀		LC ₉₀	
	<i>A. aegypti</i>	<i>A. albopictus</i>	<i>A. aegypti</i>	<i>A. albopictus</i>
Methanol	>12.5	>12.5	>12.5	>12.5
Ethyl acetate	>12.5	>12.5	>12.5	>12.5
Chloroform	>12.5	>12.5	>12.5	>12.5
<i>n</i> -Hexane	2.4	3.3	8.6	>12.5
Chlorpyrifos ^a	1.1	1.8	2.5	2.8

^a Positive control.

both *A. aegypti* and *A. albopictus* larvae in 24 h. The *n*-hexane fraction showed an excellent toxicity, with LC₅₀ values of 2.4 $\mu\text{g/ml}$ (LC₉₀ = 8.6 $\mu\text{g/ml}$) and 3.3 $\mu\text{g/ml}$ (LC₉₀ > 12.5 $\mu\text{g/ml}$), respectively. When incubation was extended to 48 h, the LC₅₀ values were 2.0 and 0.9 $\mu\text{g/ml}$ with corresponding LC₉₀ values of 8.1 and 8.3 $\mu\text{g/ml}$, respectively (data not shown). Thus, we continued to analyze the mosquito larvicidal compounds in the *n*-hexane fraction of *C. japonica* sapwood extracts. *n*-Hexane fraction was divided into 11 subfractions (SH1–SH11) with open column chromatograph.

Table 3 presents the LC₅₀ and LC₉₀ values of subfractions SH1–SH11 of the *n*-hexane fraction. Compared with other subfractions, subfractions SH2 and SH3 had excellent toxicity against both *A. aegypti* and *A. albopictus* larvae. It was clear that subfraction SH3 exhibited the best larvicidal activities, with LC₅₀ values of 0.2 $\mu\text{g/ml}$ (LC₉₀ = 0.9 $\mu\text{g/ml}$) and 0.4 $\mu\text{g/ml}$ (LC₉₀ = 0.9 $\mu\text{g/ml}$), respectively. The LC₅₀ values of a well-known commercial pesticide used as a positive control in this study, chlorpyrifos, were 1.1 and 1.8 $\mu\text{g/ml}$, respectively (Table 3). Comparison of the LC₅₀ with those of subfraction SH3 indicated that this subfraction has excellent mosquito larvicidal effects. Accordingly, we continued to isolate compounds from subfraction SH3 using HPLC.

Table 3

Lethal concentrations (LC, $\mu\text{g/ml}$) of subfractions SH1–SH11 against fourth-instar larvae of *A. aegypti* and *A. albopictus* in 24 h

Fractions	LC ₅₀		LC ₉₀	
	<i>A. aegypti</i>	<i>A. albopictus</i>	<i>A. aegypti</i>	<i>A. albopictus</i>
SH1	>12.5	>12.5	>12.5	>12.5
SH2	2.6	10.0	12.0	63.1
SH3	0.2	0.4	0.9	0.9
SH4	>12.5	>12.5	>12.5	>12.5
SH5	>12.5	>12.5	>12.5	>12.5
SH6	>12.5	>12.5	>12.5	>12.5
SH7	>12.5	>12.5	>12.5	>12.5
SH8	>12.5	>12.5	>12.5	>12.5
SH9	>12.5	>12.5	>12.5	>12.5
SH10	>12.5	>12.5	>12.5	>12.5
SH11	>12.5	>12.5	>12.5	>12.5
Chlorpyrifos ^a	1.1	1.8	2.5	2.8

^a Positive control.

3.3. Identification of active compounds

Bioassay-guided fractionation of the subfraction SH3 afforded two active constituents identified by instrumental analyses, including MS, IR, and NMR, and by comparisons with published data (Lin et al., 1975; Wang et al., 2002; Itokawa et al., 1991; Danielsen, 1996). The constituents were characterized as ferruginol (**1**) and tectoquinone (**2**). They were identified on the basis of the following evidence.

Ferruginol (**1**) Yellow oil; EI-MS, m/z 286 ($\text{C}_{20}\text{H}_{30}\text{O}$); IR (KBr) ν_{max} 3370 (O–H), 1612, 1501, 1440 (aromatic ring) cm^{-1} ; ^1H NMR (500 MHz, CD_3OD): δ 0.89 (3H, s, H-18), 0.93 (3H, s, H-19), 1.15 (3H, s, H-20), 1.22 (3H, d, $J = 7.0$ Hz, H-16), 1.29 (3H, d, $J = 7.0$ Hz, H-17), 2.77 (1H, ddd, $J = 17.0, 10.5, 7.0$ Hz, H-7a), 2.81 (1H, ddd, $J = 2.0, 6.5, 17.0$ Hz, H-7b), 3.11 (sept, $J = 7.0$ Hz, H-15), 6.62 (1H, s, H-11), 6.81 (1H, s, H-14); ^{13}C NMR (125 MHz, CD_3OD): δ 19.12 (C-6), 19.18 (C-2), 21.46 (C-19), 22.50 (C-16), 22.67 (C-17), 24.60 (C-20), 26.47 (C-15), 29.60 (C-7), 33.17 (C-18), 33.23 (C-4), 37.27 (C-10), 38.65 (C-1), 41.57 (C-3), 50.23 (C-5), 110.86 (C-11), 126.47 (C-14), 126.27 (C-8), 131.69 (C-13), 148.16 (C-9), 150.95 (C-12).

Tectoquinone (**2**) Yellow solid; EI-MS, m/z 222 ($\text{C}_{15}\text{H}_{10}\text{O}_2$); IR (KBr) ν_{max} 1674, 1593 (C=O) cm^{-1} ; ^1H NMR (500 MHz, CD_3OD): δ 2.50 (3H, s, H-15), 7.56 (1H, d, $J = 7.9$ Hz, H-3), 7.76 (1H, m, H-6), 7.78 (1H, m, H-7), 8.17 (1H, d, $J = 7.9$ Hz, H-4), 8.27 (1H, m, H-5), 8.29 (1H, m, H-8); ^{13}C NMR (125 MHz, CD_3OD): δ 21.91 (C-15), 127.12 (C-8), 127.14 (C-5), 127.42 (C-4), 127.48 (C-1), 131.26 (C-14), 133.37 (C-13), 133.54 (C-11), 133.57 (C-12), 133.91 (C-7), 134.03 (C-6), 134.93 (C-3), 145.28 (C-2), 182.99 (C-10), 183.43 (C-9).

3.4. Mosquito larvicidal activity of pure compounds

The larvicidal activity of compounds **1–2** against both *A. aegypti* and *A. albopictus* larvae is given in Table 4. The LC₅₀ and LC₉₀ values of **2** on *A. aegypti* and *A. albopictus* larvae in 24 h were 3.3 $\mu\text{g/ml}$ (LC₉₀ = 8.8 $\mu\text{g/ml}$) and 5.4 $\mu\text{g/ml}$ (LC₉₀ = 26.9 $\mu\text{g/ml}$), respectively. Compound **1** revealed weak or no activity, no LC₅₀ value was determined in this range of concentration (LC₅₀ > 100.0 $\mu\text{g/ml}$). However, these compounds isolated were slightly less active than chlorpyrifos (LC₅₀ = 1.3 $\mu\text{g/ml}$ for *A. aegypti*

Table 4

LC₅₀ and LC₉₀ values ($\mu\text{g/ml}$) of two compounds from *C. japonica* against *A. aegypti* and *A. albopictus* larvae in 24 h

Compounds	<i>A. aegypti</i>		<i>A. albopictus</i>	
	LC ₅₀	LC ₉₀	LC ₅₀	LC ₉₀
Ferruginol	>100.0	>100.0	>100.0	>100.0
Tectoquinone	3.3	8.8	5.4	26.9
Chlorpyrifos ^a	1.3	3.4	1.1	2.3

^a Positive control.

larvae; $LC_{50} = 1.1 \mu\text{g/ml}$ for *A. albopictus* larvae). No mortality was observed in the controls.

It has been reported that 1 α -acetoxy-3 α -propanoyloxyvilasinin, 1 α ,7 α ,11 β -triacetoxy-4 α -carbomethoxy-12 α -(2-methylpropanoyloxy)-14 β ,15 β -epoxy-havanensin, 1 α ,11 β -diacetoxy-4 α -carbomethoxy-7 α -hydroxy-12 α -(2-methylpropanoyloxy)-15-oxohavanensin from *Turraea wakefieldii* and *Turraea floribunda* were effective against third-instar larvae of *Anopheles gambiae* with LD_{50} values of 7.1, 4.0, and 3.6 ppm, respectively (Ndung'u et al., 2004). Jang et al. (2005) also found that β -thujaplicin from *Chamaecyparis obtusa* leaves was effective against fourth-instar larvae of *A. aegypti*, *Ochlerotatus togoi*, and *Culex pipiens* with LC_{50} values of 2.91, 2.60, and 1.33 ppm, respectively. In our previous study, cinnamaldehyde, cinnamyl acetate, and eugenol all had excellent larvicidal effect against *A. aegypti* larvae in 24 h with LC_{50} values of 29, 33, and 33 $\mu\text{g/ml}$, respectively (Cheng et al., 2004). Results of the present study suggest that tectoquinone is a potential natural mosquito larvicide.

3.5. Mosquito larvicidal activity of anthraquinone congeners

The mosquito larvicidal activity of six available anthraquinones (anthraquinone, alizarin, 1-hydroxyanthraquinone, anthraquinone-2-carboxylic acid, 2-hydroxymethylanthraquinone, and emodin) which are structurally related to tectoquinone, was assessed. Table 5 presents the LC_{50} and LC_{90} values of these compounds against *A. aegypti* and *A. albopictus* larvae in 24 h. In the present study, no activity was observed for anthraquinone, alizarin and 1-hydroxyanthraquinone, their LC_{50} values were more than 25.0 $\mu\text{g/ml}$, indicating that the number of hydroxyl groups on the anthraquinone structure has no significant influence on mosquito larvicidal activity. This was similar to the result of Yang et al. (2003) who reported that alizarin, danthron, and quinizarin do not have mosquito larvicidal activity. Comparison of the LC_{50} and LC_{90} values of anthraquinone-2-carboxylic acid, 2-hydroxymethylanthraquinone, and tectoquinone against *A. aegypti* and *A. albopictus* larvae in 24 h revealed that tectoquinone had the strongest mosquito larvicidal activity.

Table 5
 LC_{50} and LC_{90} values ($\mu\text{g/ml}$) of anthraquinone congeners against *A. aegypti* and *A. albopictus* larvae in 24 h

Compounds	<i>A. aegypti</i>		<i>A. albopictus</i>	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Tectoquinone	3.3	8.8	5.4	>25.0
Anthraquinone	>25.0	>25.0	>25.0	>25.0
Alizarin	>25.0	>25.0	>25.0	>25.0
1-Hydroxyanthraquinone	>25.0	>25.0	>25.0	>25.0
Anthraquinone-2-carboxylic acid	16.3	25.0	17.9	>25.0
2-Hydroxymethylanthraquinone	15.4	23.7	17.0	>25.0
Emodin	5.3	19.1	4.2	17.7
Chlorpyrifos ^a	1.1	2.5	1.8	2.8

^a Positive control.

Morimoto et al. (2002) reported that a methyl group at the C-2 position of the anthraquinone, are important for antifeedant activity against the common cutworms. It is noted that anthraquinone with a methyl group has the best mosquito larvicidal activity, among the compounds tested.

The effect having of a methyl substituent on the anthraquinone structure on mosquito larvicidal activity can be also gleaned from the activity of emodin. It has LC_{50} values of 5.3 and 4.2 $\mu\text{g/ml}$ with corresponding LC_{90} values of 19.1 and 17.7 $\mu\text{g/ml}$ against *A. aegypti* and *A. albopictus* larvae in 24 h (Table 5), respectively. Yang et al. (2003) reported a similar result, that emodin had strong larvicidal effects against the larvae of *A. aegypti*, *O. togoi* and *C. pipiens pallens*, showing LC_{50} values of approximately 1.4, 1.9, and 2.2 mg/l, respectively. It is apparent that both tectoquinone and emodin with a methyl substituent showed strong mosquito larvicidal activities against *A. aegypti* and *A. albopictus* larvae.

4. Conclusions

This study demonstrated that the methanolic extract of *C. japonica* sapwood and tectoquinone have excellent mosquito larvicidal activities against both *A. aegypti* and *A. albopictus*. Thus, tectoquinone and the methanolic extract of *C. japonica* sapwood have potential to be developed as natural larvicidal agents. However, further investigations for the insecticidal action mode of tectoquinone, effects on nontarget organisms and field evaluation are needed. Moreover, these results could be useful in the research for selecting newer, more selective, biodegradable and natural larvicidal compounds.

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