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Larvicidal Activity of the Active Constituent Isolated from *Tabebuia avellanedae* Bark and Structurally Related Derivatives against Three Mosquito Species

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ABSTRACT: Mosquito larvicidal activities of active constituent isolated from *Tabebuia avellanedae* bark and its structurally related derivatives were examined against the fourth instar larvae of *Aedes aegypti*, *Culex pipiens pallens*, and *Ochlerotatus togoi*. The insecticidal constituent of *T. avellanedae* bark was isolated by chromatographic techniques and identified as 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione. On the basis of the 50% lethal concentration (LC_{50}) values against *C. pipiens pallens* larvae, the most toxic compound was 1,4-naphthalenedione (1.26 mg/L), followed by 1,2-naphthalenedione (1.43 mg/L), 1,4-naphthalenediol (3.20 mg/L), 2-chloro-3-pyrrolidino-1,4-naphthalenedione (5.11 mg/L), 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione (8.30 mg/L), and 2-chloro-3-morpholino-1,4-naphthalenedione (12.98 mg/L). Similar results against *A. aegypti* and *O. togoi* larvae were observed for 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione and its derivatives. According to the LC_{50} values against three mosquito species, these compounds were less toxic than pirimiphos-methyl. Nonetheless, naturally occurring *T. avaellenedae* bark-derived materials could be useful as a natural mosquito control agent.

KEYWORDS: *Aedes aegypti, Culex pipiens pallens, mosquito larvicidal activity, Ochlerotatus togoi, Tabebuia avellanedae*

INTRODUCTION

Mosquitoes are infamous as major vectors for the transmission of several disease, such as dengue hemorrhagic fever, filariasis, Japanese encephalitis, malaria, schistosomiasis, and yellow fever, which are nowadays among the greatest human health problems in the world.[1−3] These also cause allergic responses in humans, including local skin and systemic reactions, such as angioedema.[4] The yellow fever mosquitoes, *Aedes aegypti* (L.) and *Ochlerotatus togoi* (Theobald), and northern house mosquito, *Culex pipiens pallens* (Coquillett), affect many millions of people all over the world.[5] Control of mosquito populations has been principally through the use of organophosphates, such as chlorpyrifos, fenthion, and temephos, and insect-growth regulators, such as difluenzuron and methoprene, which are still the most effective synthetic pesticides.[5] However, continued use of these chemicals for vector control gives rise to potential problems, such as disrupted natural biological control systems, leading to outbreaks of mosquito species, widespread development of resistance, and adverse effects on the environment through the contamination of soil, water, and air.[2,6] Therefore, these problems necessitate the development of new strategies for the selective control of mosquito larvae.[7]

Plant-derived extracts may be alternative sources of mosquito larval control agents,[5−8] because their secondary metabolites include a rich source of bioactive compounds, which are biodegradable into nontoxic products, and are potentially appropriate for use with pest management systems.[4,9] Red Lapacho (*Tabebuia avellanedae* Lorentz ex Griseb.; family Bignoniaceae) is an evergreen, canopy tree, with purple flowers, widespread in tropical rain forests throughout Central and South America, and is a component of traditional medicines.[10−12] The bioactive chemicals of *T. avellanedae* inner bark was isolated, including anthraquinones, benzaldehyde derivatives, benzoic acid derivatives, coumarins, flavonoids, iridoids, and naphthoquinones.[11] Furthermore, the biologically active components in *T. avellanedae* have been reported to have antibacterial, anticoagulation, antifungal, anti-inflammatory, antitumor, astringent, and diuretic activities.[11,12] Despite these biological effects, relatively few studies have been conducted to evaluate the mosquito larvicidal activities against larvae from *A. aegypti*, *C. pipiens pallens*, and *O. togoi*. In this study, we evaluated the mosquito larvicidal activity of the active component isolated from *T. avellanedae* bark against larvae from *A. aegypti*, *C. pipiens pallens*, and *O. togoi* and compared the results to the effects of its derivatives and synthetic larvicidal agent.

MATERIALS AND METHODS

Chemicals. Seven compounds examined in this study were as follows: 2-chloro-3-morpholino-1,4-naphthalenedione (97% purity), 2-chloro-3-pyrrolidino-1,4-naphthalenedione (98% purity), 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione (98% purity), 1,4-naphthalenedione (97% purity), 1,2-naphthalenedione (97% purity), and Triton X-100, which were purchased from Sigma-Aldrich (St. Louis, MO). 1,4-Naphthalenediol (95% purity) and pirimiphos-methyl (99.5% purity) were provided from Fluka Chemical Crop (Milwaukee, WI). All other chemicals were of reagent grade and commercially available.

Sample Preparation. The bark of *T. avellanedae* was purchased from Raintree Nutrition, Inc. (Carson, NV). A voucher specimen was authenticated by Prof. Jeong-Moon Kim and deposited in the

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Table 1. Lavicidal Activities of Various Solvent Fractions Obtained from the Methanol Extract of *T. avellanedae* Bark against Fourth Instar Larvae of Three Mosquito Species

fraction	concentration (mg/L)	mortality (%, mean ± SE)[a]		
		A. aegypti	*C. pipiens pallens*	*O. togoi*
methanol extract	200	100 a	100 a	100 a
	100	100 a	100 a	100 a
	50	42.7 ± 1.8 bc	58.9 ± 2.1 b	40.5 ± 1.4 bc
	25	18.6 ± 1.5 c	20.3 ± 1.7 c	11.8 ± 0.9 cd
hexane fraction	100	0 d	0 d	0 d
chloroform fraction	100	100 a	100 a	100 a
	50	89.2 ± 2.1 ab	95.1 ± 1.8 a	87.7 ± 1.9 b
	25	39.7 ± 1.7 c	51.2 ± 1.5 bc	30.8 ± 1.4 c
ethyl acetate fraction	100	28.0 ± 3.2 c	36.2 ± 2.4 c	20.7 ± 1.7 c
butanol fraction	100	0 d	0 d	0 d
water-soluble fraction	100	0 d	0 d	0 d

[a]Means within a column followed by the same letter are not significantly different (p = 0.05; Scheffe's test).

herbarium at the Department of Landscape Architecture, College of Agriculture, Chonbuk National University, Jeonju, South Korea. The bark (5 kg) was air-dried, ground to a fine powder, and extracted twice with methanol (10 L) at room temperature for 2 days, after which it was filtered (Toyofilter Paper No. 2, Toyo Roshi, Tokyo, Japan) under vacuum. The extract was concentrated and combined *in vacuo* at 45 °C, using a rotary vacuum evaporator (EYELA Autojack NAJ-100, Tokyo, Japan). The methanol extract (862 g) of *T. avellanedae* was sequentially partitioned into hexane (155 g), chloroform (101 g), ethyl acetate (115 g), butanol (218 g), and water-soluble (270 g) fractions for the bioassays. The organic solvent fractions were concentrated to dryness by rotary evaporation at 45 °C, whereas the water-soluble fraction was freeze-dried (Bondiro, Ilshin Biobase, Yangju, Korea).

Isolation and Identification. The chloroform (20 g) fraction was separated on a silica gel column (Merck, 70−230 mesh, 600 g, 550 mm inner diameter × 700 mm, Rahway, NJ) and was then continuously eluted using a stepwise gradient of chloroform/methanol (9:1, 8:2, 7:3, and 0:10, v/v) to give six fractions (T1−T6). Each column fraction was analyzed by thin-layer chromatography (TLC) to identify similar TLC patterns. The T2 fraction (7.2 g) exhibited larvicidal activity against target mosquitoes, and hence, this fraction was exposed to further separation on a column (Merck, 70−230 mesh, 600 g, 550 mm inner diameter × 700 mm, Rahway, NJ) and eluted with chloroform/methanol (3:7, 2:8, 1:9, and 0:10, v/v) to yield the active fraction T22. The T22 fraction (522 mg) was then isolated by preparative high-performance liquid chromatography (HPLC, Japan Analytical Industry Co., Ltd., Tokyo, Japan) using a JAI GS series column (GS310 500 mm + GS310 500 mm). The mobile phase used for HPLC was hexane/chloroform (3:7, v/v), which was applied at a flow rate of 3.5 mL/min, and ultraviolet (UV) detection was applied at 255 nm. The resulting active T223 fraction (117 mg) was further separated using a JAI W series column (W252 500 mm + W253 500 mm) and chloroform (100%) under the same conditions. The final active compound T2232 (32.9 mg) was isolated, and the structure of the active component was determined by various instrumental analyses. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained with a JNM-ECA 600 spectrometer (JEOL, Ltd., Tokyo, Japan; 600 and 150 MHz) using $CDCl_3$, with tetramethylsilane (TMS) as an internal standard. Furthermore, to determine the connection between protons (H) and carbons (C), one-dimensional (1D) NMR spectra [distortionless enhancement by polarization transfer (DEPT) NMR] and two-dimensional (2D) NMR spectra [^{1}H−^{1}H correlation spectroscopy (COSY) and heteronuclear multiple-quantum coherence (HMQC)] were recorded under identical conditions. The ultraviolet−visible (UV−vis) absorption spectra were obtained with a UV spectrometer (DR 4000 spectrophotometer, HACH, Loveland, CO).

Mosquitoes. The colonies of *A. aegypti*, *C. pipiens pallens*, and *O. togoi* were obtained in 2012 from the Seoul National University, Seoul, South Korea. The collected larvae were reared in plastic trays (20 × 35 × 7 cm) and fed a sterilized diet (80:20 mix of chick chow powder/yeast). Adult mosquitoes were maintained on a 10% aqueous sucrose solution and blood from a live mouse. Mosquitoes were held at 28 ± 2 °C, with a light/dark photoperiod of 16:8 h at 70 ± 5% relative humidity (RH).

Bioassay. A direct-contact toxicity bioassay slightly modified from the method described by Jang et al.[13] was used to evaluate the larvicidal toxicity of test samples to fourth instar larvae of *A. aegypti*, *C. pipiens pallens*, and *O. togoi*. Concentrations of the test samples were prepared by a serial dilution of a stock solution of the samples in acetone. Each sample in acetone was suspended in distilled water with Triton X-100 added at 100 μL/L. Batches of 20 early fourth instar larvae of *A. aegypti*, *C. pipiens pallens*, and *O. togoi* were separately put into the paper cups (250 mL) containing each test solution (200 mL) using a pipet. The toxicity of each sample was determined with various concentrations ranging from 200 to 0.06 mg/L. Controls received acetone/Triton X-100 solutions. Treated and control larvae were held under the same conditions mentioned earlier. Larvicidal activity was evaluated 24 h after treatment. Mosquito larvae were considered to be dead if they did not move when prodded with a wooden dowel pin. All treatments were replicated 4 times. No mortality was observed within any of the control groups.

Data Analysis. The percentage of mortality was determined and transformed to arcsine square root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at p = 0.05. Means [±standard error (SE)] of untransformed data are reported. The LC_{50} values were calculated using probit analysis.[14]

RESULTS AND DISCUSSION

The larvicidal activities of the methanol extract extracted from *T. avellanedae* bark against *A. aegypti*, *C. pipiens pallens*, and *O. togoi* are observed in Table 1. The methanol extract of *T. avellanedae* bark possessed mosquito larvicidal activity against *A. aegypti*, *C. pipiens pallens*, and *O. togoi*, producing 100% mortality at 100 mg/L. *T. avellanedae* bark extract showed a clear dose−response relationship against three mosquito species. Table 1 also shows the larvicidal activities of the five fractions (hexane, chloroform, ethyl acetate, butanol, and water-soluble portion) separated from the methanol extract of *T. avellanedae* bark. Significant differences in larvicidal effect in five fractions of the *T. avellanedae* bark extract were observed, and active fractions were applied to further isolation. On the basis of the mortality of those five fractions against *A. aegypti*, *C. pipiens pallens*, and *O. togoi*, the chloroform fraction had strong larvicidal activity, while the other fractions had weak or no toxicity, at a concentration of 100 mg/L. There was no

Table 2. Larvicidal Activities of the Constituent Isolated from *T. avellanedae* Bark and Its Derivatives against Fourth Instar Larvae of Three Mosquito Species

compound	mosquito	mortality (%, mean ± SE)[a] 10.0 mg/L	5.0 mg/L	2.5 mg/L	1.0 mg/L
2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione	*A. aegypti*	56.5 ± 1.4 bc	32.3 ± 2.5 c	12.3 ± 2.1 de	0 e
	C. pipiens pallens	67.5 ± 1.8 b	45.0 ± 1.6 c	17.5 ± 1.4 d	7.5 ± 2.1 de
	O. togoi	51.9 ± 2.1 bc	28.4 ± 1.8 cd	9.7 ± 1.1 de	0 e
1,4-naphthalenedione	*A. aegypti*	100 a	100 a	60.3 ± 2.6 b	40.6 ± 2.3 c
	C. pipiens pallens	100 a	100 a	73.5 ± 1.6 b	48.1 ± 1.8 c
	O. togoi	100 a	100 a	54.1 ± 1.5 bc	37.7 ± 1.9 c
1,2-naphthalenedione	*A. aegypti*	73.2 ± 2.5 b	63.5 ± 2.1 b	54.6 ± 1.5 bc	39.6 ± 1.5 c
	C. pipiens pallens	86.5 ± 1.7 b	72.2 ± 2.4 b	63.4 ± 1.7 bc	46.1 ± 1.4 c
	O. togoi	72.1 ± 2.0 b	57.5 ± 1.8 bc	52.6 ± 1.6 c	31.1 ± 1.9 cd
1,4-naphthalenediol	*A. aegypti*	100 a	72.3 ± 2.1 a	25.1 ± 2.5 cd	0 e
	C. pipiens pallens	100 a	85.6 ± 2.7 a	35.7 ± 2.4 c	12.2 ± 1.1 d
	O. togoi	100 a	68.8 ± 1.8 b	22.3 ± 2.1 d	5.3 ± 1.3 de
2-chloro-3-pyrrolidino-1,4-naphthalenedione	*A. aegypti*	80.4 ± 2.0 b	46.3 ± 2.1 c	13.8 ± 1.1 d	0 e
	C. pipiens pallens	87.2 ± 1.8 ab	48.1 ± 1.7 c	14.2 ± 1.4 d	0 e
	O. togoi	69.6 ± 1.5 b	32.2 ± 1.6 c	8.5 ± 0.9 d	0 e
2-chloro-3-morpholino-1,4-naphthalenedione	*A. aegypti*	31.1 ± 1.8 c	8.9 ± 1.1 d	0 e	0 e
	C. pipiens pallens	35.4 ± 2.1 c	11.2 ± 0.7 d	0 e	0 e
	O. togoi	19.8 ± 0.8 cd	5.5 ± 0.7 de	0 e	0 e

[a]Means within a column followed by the same letter are not significantly different (p = 0.05; Scheffe's test).

mortality in negative controls for any mosquito species in this study.

Because of the potent larvicidal activities of the chloroform fraction obtained from the methanol extract of *T. avellanedae* bark, the active constituent of the chloroform fraction was isolated. Bioassay-guided fractionation of the chloroform fraction yielded an active component, which was identified by various spectroscopic analyses, including electron impact ionization−mass spectrometry (EI−MS) and 1D NMR (^{1}H and ^{13}C NMR), using direct comparison to authentic reference compounds. Furthermore, together with the conjugation of proton and carbon, the chemical formula of the active constituent was deduced to isolate from *T. avellanedae*, according to the measurements of 1D (DEPT NMR) and 2D (^{1}H−^{1}H COSY and HMQC) NMR. The larvicidal active compound was characterized as 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione ($C_{15}H_{14}O_3$; MW, 242.3); EI−MS (70 eV) m/z (percent relative intensity), M^+ 227 (100), 199 (31), 181 (22), 159 (13), 128 (25), 105 (26); ^{1}H NMR ($CDCl_3$, 600 MHz, ppm) δ, 1.65 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 3.29 (d, J = 7.0 Hz, CH_2), 5.21 (m, 1H, CH), 7.29 (s, 1H, OH), 7.62−8.21 (m, 4H, Ar−H); ^{13}C NMR ($CDCl_3$, 150 MHz, ppm) δ, 184.3, 181.5, 152.5, 134.8, 133.8, 133.0, 132.8, 129.4, 126.5, 126.0, 123.1, 120.5, 25.4, 22.5, 17.8. The isolation and spectral analyses of 2-hydroxyl-3-(3-methyl-2-butenyl)-1,4-naphthalenedione from *T. avellanedae* bark were largely consistent with those of early studies.[12,15]

To evaluate the larvicidal toxicity of 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione isolated from *T. avellanedae* bark and five structurally related compounds (1,4-naphthalenedione, 1,2-naphthalenedione, 1,4-naphthalenediol, 2-chloro-3-pyrrolidino-1,4-naphthalenedione, and 2-chloro-3-morpholino-1,4-naphthalenedione) against fourth instar larvae from *A. aegypti*, *C. pipiens pallens*, and *O. togoi*, the direct-contact toxicity bioassay was examined (Table 2 and Figure 1). When the larvicidal activities against fourth instar larvae from *A. aegypti*, *C. pipiens pallens*, and *O. togoi* were evaluated, 1,4-naphthalenedione gave the highest mortality (100% at 5.0 mg/L), followed by 1,2-naphthalenedione (54.6, 63.4, and 52.6% at 2.5 mg/L), 1,4-naphthalenediol (25.1, 35.7, and 22.3% at 2.5 mg/L), 2-chloro-3-pyrrolidino-1,4-naphthalenedione (13.8, 14.2, and 8.5% at 2.5 mg/L), 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione (32.3, 45.0, and 28.4% at 5.0 mg/L), and 2-chloro-3-morpholino-1,4-naphthalenedione (8.9, 11.2, and 5.5% at 5.0 mg/L), respectively. The results of this study indicate that the larvicidal activity of *T. avellanedae* bark extract could be mostly attributed to 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione against three mosquito species.

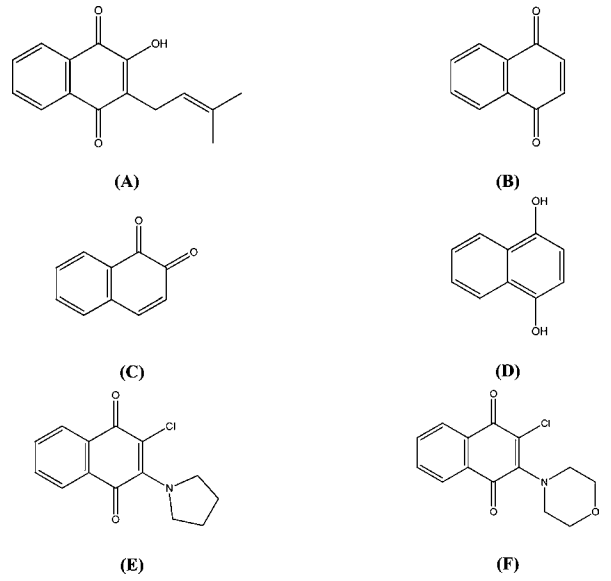

Figure 1. Chemical structures of 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione and its derivatives: (A) 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione, (B) 1,4-naphthalenedione, (C) 1,2-naphthalenedione, (D) 1,4-naphthalenediol, (E) 2-chloro-3-pyrrolidino-1,4-naphthalenedione, and (F) 2-chloro-3-morpholino-1,4-naphthalenedione.

Table 3. Larvicidal Toxicities of 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione, Its Derivatives, and Organophosphorus Insecticide against Fourth Instar Larvae of Three Mosquito Species

compound	mosquito species	LC_{50} (mg/L)	95% CL[a]
2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione	*A. aegypti*	8.83	8.79−8.87
	C. pipiens pallens	8.30	8.11−8.41
	O. togoi	9.49	9.32−9.67
1,4-naphthalenedione	*A. aegypti*	1.69	1.60−1.74
	C. pipiens pallens	1.26	1.21−1.31
	O. togoi	2.08	1.94−2.19
1,2-naphthalenedione	*A. aegypti*	1.79	1.39−1.48
	C. pipiens pallens	1.43	1.74−1.84
	O. togoi	2.19	2.06−2.30
1,4-naphthalenediol	*A. aegypti*	3.42	3.38−3.47
	C. pipiens pallens	3.20	3.16−3.25
	O. togoi	4.02	3.92−4.17
2-chloro-3-pyrrolidino-1,4-naphthalenedione	*A. aegypti*	5.68	5.47−5.79
	C. pipiens pallens	5.11	5.01−5.27
	O. togoi	6.23	6.06−6.44
2-chloro-3-morpholino-1,4-naphthalenedione	*A. aegypti*	13.88	13.74−14.11
	C. pipiens pallens	12.98	12.69−13.17
	O. togoi	15.36	15.15−15.49
pirimiphos-methyl[a]	*A. aegypti*	0.17	0.13−0.20
	C. pipiens pallens	0.12	0.09−0.15
	O. togoi	0.22	0.18−0.26

[a]Positive control.

The larvicidal activities of 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione and its five derivatives were compared to that of pirimiphos-methyl against fourth instar larvae of *A. aegypti*, *C. pipiens pallens*, and *O. togoi* using a direct-contact toxicity bioassay (Table 3). Pirimiphos-methyl was used as a positive control. On the basis of 24 h LC_{50} values, the most toxic compound against *C. pipiens pallens* was 1,4-naphthalenedione (1.26 mg/L), followed by 1,2-naphthalenedione (1.43 mg/L), 1,4-naphthalenediol (3.20 mg/L), 2-chloro-3-pyrrolidino-1,4-naphthalenedione (5.11 mg/L), 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione (8.30 mg/L), and 2-chloro-3-morpholino-1,4-naphthalenedione (12.98 mg/L). Similar results against *A. aegypti* and *O. togoi* larvae were observed with 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione and its derivatives. These results indicate that the most active analogue (1,4-naphthalenedione) had only 10 times less toxicity than pirimiphos-methyl as the positive control. However, naturally occurring *T. avellanedae* bark-derived materials and structurally related analogues could be useful as lead compounds and natural mosquito control agents to prevent the widespread resistance and adverse effects on the environments.

Changing the position of the carbonyl groups (C═O) in the naphthalene skeleton changes larvicidal activities against *A. aegypti*, *C. pipiens pallens*, and *O. togoi*. 1,4-Naphthalenedione (which have carbonyl groups at the *para* position) had higher larvicidal activity than 1,2-naphthalenedione (which have carbonyl groups at the *ortho* position). Interestingly, the structure−activity relationships between 1,4-naphthalenedione and 1,4-naphthalenediol (which have hydroxyl groups at the *para* position) exhibited that the introduction of the hydroxyl groups in the naphthalene skeleton produced a decrease in the larvicidal activity against *A. aegypti*, *C. pipiens pallens*, and *O. togoi*. Moreover, the introduction of different functional groups into the C_2 and C_3 positions in the 1,4-naphthalenedione skeleton decrease the larvicidal activities against *A. aegypti*, *C. pipiens pallens*, and *O. togoi*. This result shows that the presence of various functional groups in the 1,4-naphthalenedione skeleton decreases the larvicial activity of tested compounds. In addition, the fourth instar larvae from *C. pipiens pallens* were the most susceptible to the *T. avellanedae* bark-isolated compound and its derivatives than *A. aegypti* and *O. togoi*. Similarly, Park et al.[16] reported that the larvae from *A. aegypti* and *O. togoi* were more tolerant than *C. pipiens pallens* larvae to plant-based substances and their derivatives, including guineensine, pellitorine, pipercide, and retrofractamide A. These results indicate that the larvicidal toxicity of plant extract and phytochemicals can be influenced by the species of mosquito investigated. Moreover, different susceptibilities of the three mosquito larvae to the phytochemicals might be attributed to various differences in physiological or biochemical factors, such as the detoxifying enzyme effect, penetration, and target site (acetylcholinesterase or sodium channel) insensitivity.[17,18] However, the mosquito larvicidal mode of action of these compounds is not yet fully understood.[18]

In the previous studies, several naphthoquinone derivatives in plants had been investigated to possess larvicidal toxicity against various mosquito species and have been suggested as alternatives to the synthetic larvicides.[6,17] For example, it has been studies that the cordiaquinones derived from the *Cordia curassavica* and lapachol isolated from *Cybistax antisyphilitica* have potent larvicidal toxicity against larvae of *A. aegypti*.[19−21] Other naturally occurring mosquito larvicidal quinones are anthraquinones, such as emodin (6-methyl-1,3,8-trihydroxyanthraquinone) from *Cassia obtusifolia* and tectoquinone (2-methylanthraquinone) from *Cryptomeria japonica*.[1,4,5,21] The larvicidal activity of lapachol isolated from *C. antisyphilitica* stem wood against *A. aegypti* larvae (third instar) was reported by Rodrigues et al.[20] However, in this study, the larvicidal activities of lapachol isolated from *T. avellanedae* bark and its structurally related compounds against fourth instar larvae of *A. aegypti*, *C. pipiens pallens*, and *O. togoi* were investigated. Furthermore, the larvicidal activity of synthetic insecticide

(pirimiphos-methyl) for comparison was evaluated against three mosquito species, and the results were different from those of the previous study.[7,20] Differences in the toxicity of mosquito larval may be explained on the basis of biological factors, such as the instar sizes, life cycles, and food habits of mosquito species.

T. avellanedae is commonly used in the medicine and fragrance industries. Moreover, *T. avellanedae* is currently recognized by the U.S. Food and Drug Administration as generally regarded as safe (GRAS).[22] According to the Material Safety Data Sheet (MSDS),[23] the oral toxicity (LD_{50}) value of 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione was reported to be 487 mg/kg, suggesting a moderate acute toxicity to mammals. Results of the present study and previous reports indicate that 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione isolated from *T. avellanedae* bark and its derivatives could be useful in the development of mosquito larvicidal supplement agents against larvae of *A. aegypti*, *C. pipiens pallens*, and *O. togoi*. For this reason, further research should be conducted to evaluate safety issues associated with *T. avellanedae* bark-derived compounds and to assess their potential risks to human health and the environment, mosquito larvicidal mode of action, and development of formulations to increase larvicidal potency and stability.

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Notes

The authors declare no competing financial interest.

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