

# Insecticidal toxicities of naphthoquinone and its structural derivatives

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**Abstract** Insecticidal toxicities of bioactive constituent from *Diospyros kaki* roots and its structural derivatives were evaluated against the larvae of *Aedes aegypti*, *Culex pipiens pallens*, and *Ochlerotatus togoi*. Bioactive constituent of *D. kaki* roots was isolated by some chromatographic methods and identified as 5-hydroxy-2-methyl-1,4-naphthoquinone. Based on the LC<sub>50</sub> values of 5-hydroxy-2-methyl-1,4-naphthoquinone structural derivatives, 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (0.89, 0.80, and 1.04 µg/mL) had the most potent insecticidal activity, followed by 2,3-dibromo-1,4-naphthoquinone (1.32, 1.28, and 1.94 µg/mL), 5,8-dihydroxy-1,4-naphthoquinone (3.57, 3.34, and 5.04 µg/mL), 2,3-dichloro-1,4-naphthoquinone (4.76, 3.89, and 5.33 µg/mL), 5-hydroxy-2-methyl-1,4-naphthoquinone (5.66, 5.43, and 6.09 µg/mL), and 2,2'-bi(3-hydroxy-1,4-naphthoquinone) (33.93, 32.82, and 36.17 µg/mL) against *Ae. aegypti*, *Cx. pipiens pallens*, and *Oc. togoi*, respectively. In this regard, 5-hydroxy-2-methyl-1,4-naphthoquinone and its structural derivatives could be suitable as insecticidal agents to control the larvae of the three mosquito species.

**Keywords** *Aedes aegypti* · *Culex pipiens pallens* · Naphthoquinone derivatives · Insecticidal toxicities · *Ochlerotatus togoi*

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## Introduction

Mosquito is the most important mediator with respect to human disease and global distribution (James 1992). They function as vectors for various diseases, which are dengue fever, encephalitis, and filariasis (Rajeswary and Govindarajan 2013). These diseases are accompanied by economic losses, particularly in tropical and subtropical countries (James 1992). Synthetic mosquito larvicides, such as growth regulators (methoprene) and organophosphates (chlorpyrifos and temephos), have been used for several decades to control the larvae of mosquitoes (Yang et al. 2002). Despite their effectiveness, continuous use of synthetic larvicides has caused undesirable effects, such as reducing biological control by natural enemies, development of larvicide resistance, and toxicity to non-target and beneficial organisms (Park and Shin 2005). The problems have been needed for the new development of safer insecticides for the larvae of mosquitoes (Yang et al. 2002).

Plant substances may be an alternative source of insecticides and larvicides because they constitute the rich materials of biological compounds (Lee 2002; Lee et al. 2013; Yang et al. 2013). In particular, a variety of plant species with chemical defense mechanisms against herbivores could provide a new source of control agents against a wide spectrum of insect vectors (Kim et al. 2002; Yang et al. 2002). Various studies have focused on essential oils, phytochemicals, and plant extracts, such as mosquito control agents (Tabanca et al. 2013). Therefore, it is established that natural products derived from plants have the potential as alternatives to conventional repellents, insecticides, and larvicides (Ali et al. 2013; Tabanca et al. 2013). *Diospyros kaki* (Ebenaceae) is native to Korea, China, and Japan and 90 % of its production occurs in

eastern Asia (Sunity and Himanshu 2011). *D. kaki* has been continuously used as traditional medicine because of its active compounds, such as carotenoids, epicatechin, galocatechin, and polyphenols (George and Redpath 2008; Sunity and Himanshu 2011). *D. kaki* roots contain abundant phenolic compounds, including anthocyanidin, chlorogenic acid, quinones, syringic acid, and tannin (Sattar et al. 1992; Suzuki et al. 2005). Phenolic compounds possess biological effects, such as anticarcinogenic, antimutagenic, insecticidal, and radioprotective activities (Bachrach and Wang 2002; Lee and Lee 2008; Pu et al. 2013). Although various studies have reported on the compounds derived from *D. kaki* roots, the insecticidal activity of an active compound from *D. kaki* roots against mosquito larvae has not been reported previously. In this regard, the objective of the present study is to isolate the bioactive compound from *D. kaki* roots and evaluate the insecticidal toxicities of the bioactive compound and its structural derivatives against the three types of the mosquito species.

## Materials and methods

### Chemicals

2,2'-Bi(3-hydroxy-1,4-naphthoquinone) and pirimiphos-methyl were purchased from Fluka (Switzerland). 2,3-Dibromo-1,4-naphthoquinone, 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone, 2,3-dichloro-1,4-naphthoquinone, and 5,8-dihydroxy-1,4-naphthoquinone were provided by Sigma-Aldrich (USA).

### Sample preparation

*Diospyros kaki* roots were supplied from the Jeonju market in Korea during spring 2012 and dried at 30 °C. After washing, the roots (2 kg) were homogenized with a grinder and then extracted with methanol in an incubator at 25 °C for 48 h. The methanol extract (628 g, yield 31.4 %) was filtered and consecutively divided with hexane, chloroform, ethyl acetate, butanol, and distilled water. Five fractions were concentrated at 45 °C using a vacuum evaporator and the distilled water fraction was freeze-dried.

### Isolation and identification

Chromatographic methods were used to isolate the insecticidal compound of the chloroform fraction partitioned from the methanol extract of *D. kaki* roots (Lee and Lee 2008). The chloroform fraction (9 g) was loaded on an open column, containing a silica gel (70–230 mesh, 500 × 701 mm; Merck, USA) and sequentially eluted with

a mixture of organic solvents consisting of hexane:chloroform (3:7, 1:9, and 0:10, v:v). All fractions were analyzed using thin layer chromatography and the fractions with similar thin layer chromatography patterns were combined. As a result, six fractions (DK1–DK6) were produced. The active DK1 fraction (4.4 g) was again chromatographed using a silica gel column and a mixed organic solvent consisting of hexane:chloroform (3:7, v:v), and six fractions (DK11–DK16) were obtained. The active DK11 fraction (1.2 g) was isolated using prep HPLC (JAI Co., Ltd., Japan), following the analytical conditions: JAI GS column (GS310 500 + GS310 500 mm, 20 × 500 mm, Japan); mixed organic solvents consisting of hexane:chloroform:isopropanol (30:70:2, v:v:v); flow rate of 5 mL/min; and UV detection at an absorbance of 268 nm. As a result, five fractions (DK111–DK115) were obtained. The active DK114 fraction (940 mg) was chromatographed on a JAI W column (W 253 500 mm, 20 × 500 mm) under the same conditions. Finally, the active DK1144 fraction (380 mg) was isolated. The structure of the DK1144 fraction was measured by spectroscopic methods. <sup>1</sup>H and <sup>13</sup>C distortionless enhancement by polarization transfer (DEPT) nuclear magnetic resonance spectra were documented with the JNM-ECA 600 spectrometer (JEOL Ltd., Japan) using CDCl<sub>3</sub> at 600 and 150 MHz, respectively. Tetramethylsilane served as the chemical shifts, and internal standards are given in delta (δ, parts per million). A spectrum of absorbance was obtained using an ultraviolet spectrometer (model: DR 4000, HACH, Korea).

### Mosquitoes and bioassay

The mosquito species were *Ae. aegypti*, *Cx. pipiens pallens*, and *Oc. togoi* supplied from Seoul National University in 2012. Each larva was reared in a plastic tray (200 × 350 × 70 mm) and was provided with a sterile diet consisting of chick chow powder: yeast (4:1, v:v). The mosquito adults were supplied with a blood from the mouse and 10 % sucrose solution. The mosquito larvae and adults were maintained on a light:dark photoperiod (16:8 h) at 28 °C and 70 % relative humidity. The insecticidal activities of active component and its structural derivatives against the larvae of the three mosquito species were conducted using the standard method modified by WHO (2009). Twenty-fourth-instar larvae were placed in distilled water (24.5 mL), containing an each sample (0.5 mL), was added in a 30 mL cup. For comparison, pirimiphos-methyl, which is one of the commercial insecticides, was used as the positive control. The negative control was prepared from distilled water (24.5 mL) and dimethyl sulfoxide solution (0.5 mL), which were added with mosquito larvae. All treatments were replicated five

times and were incubated for 24 h at 28 °C. Percent mortalities were investigated and transformed to arcsine square root values for variance analysis with SPSS statistical software (version 18.0, SPSS Inc., USA). Treatment means were compared with Scheffe's test ( $p < 0.05$ ). Means values of untransformed data are reported.  $LC_{50}$  values in all treatments were calculated by probit analysis.

## Results and discussion

The insecticidal activity of the methanol extract from *D. kaki* roots against the larvae of the three mosquito species is shown in Table 1. The *D. kaki* extract exhibited 100 % mortality against *Cx. pipiens pallens*, *Ae. aegypti*, and *Oc. togoi* at 100 µg/mL. Based on the 50 and 25 µg/mL concentrations, the methanol extract of *D. kaki* roots had potent larvicidal activity against *Cx. pipiens pallens* (85.7 and 40.3 % mortality), followed by *Ae. aegypti* (72.7 and 28.6 % mortality) and *Oc. togoi* (60.5 and 9.8 % mortality). A previous study reported that plants are particularly suitable for crop protection because of the presence of defense chemicals with more than one mode of action (Jiang et al. 2002). Furthermore, the methanol extract (600 g) of *D. kaki* roots was partitioned using the polarity of several solvent systems to obtain the five fractions of hexane (22 g, yield 3.7 %), chloroform (67 g, yield 11.2 %), ethyl acetate (188 g, yield 31.3 %), butanol

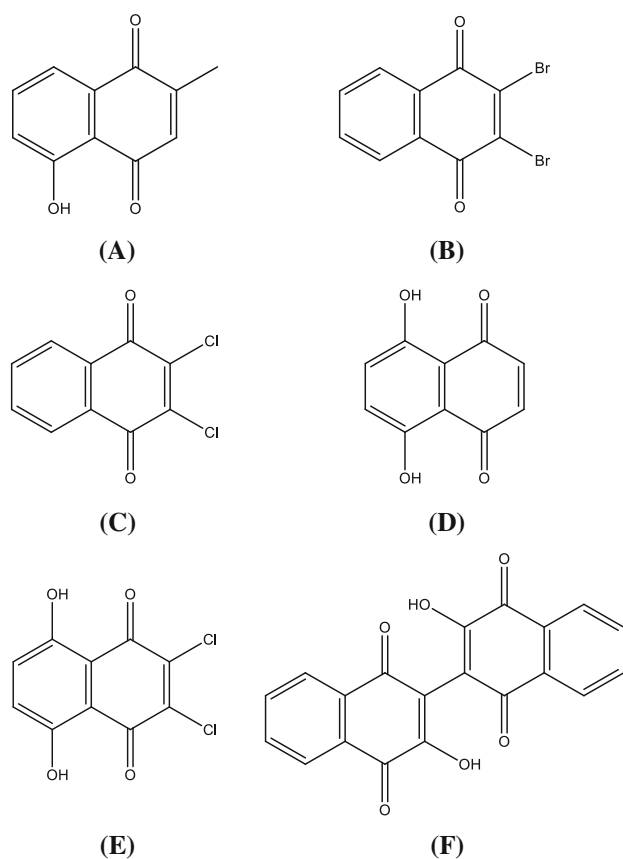
(109 g, yield 18.2 %), and distilled water (83 g, yield 13.8 %). The chloroform fraction possessed excellent larvicidal toxicities (86.5, 100, and 69.5 % mortality at 50 µg/mL) against *Cx. pipiens pallens*, *Ae. aegypti*, and *Oc. togoi*, respectively. Therefore, the chloroform fraction partitioned from the *D. kaki* extract was isolated using some chromatographic methods to identify bioactive compound.

Silica gel column and prep HPLC were conducted to isolate the insecticidal compound of the chloroform fraction. The DK1144 fraction was isolated as the bioactive compound in *D. kaki* roots and the DK1144 structure was identified by some spectroscopic methods, such as  $^1H$ ,  $^{13}C$ , and DEPT NMR spectra. The DK1144 fraction was characterized as 5-hydroxy-2-methyl-1,4-naphthoquinone ( $C_{11}H_8O_3$ ) (Fig. 1A); EI-MS (70 eV)  $m/z$   $M^+$  188 (100), 173 (17), 160 (19), 131 (18), 120 (11), 92 (9), 63 (5);  $^1H$  NMR ( $CDCl_3$ , 600 MHz)  $\delta$  2.086–2.090 (d, 3H,  $J = 1.6$  Hz), 6.693–6.697 (d, 1H,  $J = 1.6$  Hz), 7.124–7.148 (m, 1H,  $J = 9.6$  Hz), 7.470–7.488 (d, 1H,  $J = 7.2$  Hz), 7.508–7.523 (d, 1H,  $J = 6.0$  Hz);  $^{13}C$  NMR ( $CDCl_3$ , 150 MHz)  $\delta$  16.54 (CH), 113.98 (C), 118.16 (CH), 123.02 (CH), 130.89 (C), 134.28 (CH), 134.94 (CH), 148.44 (C), 159.96 (C), 183.54 (C), 189.03 (C). The analytical result of 5-hydroxy-2-methyl-1,4-naphthoquinone was consistent with the NMR data of previously reported study (Lee and Lee 2008).

The insecticidal toxicity of 5-hydroxy-2-methyl-1,4-naphthoquinone was evaluated against the three mosquito

**Table 1** Insecticidal toxicities of the methanol extract from *D. kaki* roots and the five fractions against the three mosquito larvae

Materials	Concentration (µg/mL)	Mortality (mean $\pm$ SE %)		
		<i>Ae. aegypti</i>	<i>Cx. pipiens pallens</i>	<i>Oc. togoi</i>
Methanol extract	100	100	100	100
	50	72.7 $\pm$ 1.6	85.7 $\pm$ 2.0	60.5 $\pm$ 1.5
	25	28.6 $\pm$ 1.6	40.3 $\pm$ 1.8	9.8 $\pm$ 1.2
Hexane fraction	100	0	0	0
	50	0	0	0
	25	0	0	0
Chloroform fraction	100	100	100	100
	50	86.5 $\pm$ 2.5	100	69.5 $\pm$ 1.8
	25	31.5 $\pm$ 1.5	56.4 $\pm$ 2.2	14.7 $\pm$ 2.4
Ethyl acetate fraction	100	15.5 $\pm$ 1.7	31.4 $\pm$ 2.5	4.5 $\pm$ 1.5
	50	0	0	0
	25	0	0	0
Butanol fraction	100	25.4 $\pm$ 2.2	40.5 $\pm$ 1.6	10.4 $\pm$ 2.3
	50	0	0	0
	25	0	0	0
Distilled water fraction	100	0	0	0
	50	0	0	0
	25	0	0	0



**Fig. 1** Structures of 5-hydroxy-2-methyl-1,4-naphthoquinone derivatives: (A) 5-hydroxy-2-methyl-1,4-naphthoquinone, (B) 2,3-dibromo-1,4-naphthoquinone, (C) 2,3-dichloro-1,4-naphthoquinone, (D) 5,8-dihydroxy-1,4-naphthoquinone, (E) 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone, (F) 2,2'-bi(3-hydroxy-1,4-naphthoquinone)

larvae (Table 2). The  $LC_{50}$  values of 5-hydroxy-2-methyl-1,4-naphthoquinone (A) were 5.66, 5.43, and 6.09  $\mu\text{g}/\text{mL}$  against *Ae. aegypti*, *Cx. pipiens pallens*, and *Oc. togoi*, respectively. The larvicidal toxicity of 5-hydroxy-2-methyl-1,4-naphthoquinone (A) has not been reported, and these findings indicate that the sensitivity of 5-hydroxy-2-methyl-1,4-naphthoquinone (A) against *Cx. pipiens pallens* was the highest among the three mosquito species. According to previous study, the resistance of natural substances derived from plants against *Ae. aegypti* and *Oc. togoi* was higher than that of a biomaterial against *Cx. pipiens pallens* because of the influence of phytochemicals (pellitorine, guineensine, and retrofractamide A) and biochemical factors (detoxifying enzyme, acetylcholinesterase, and penetration of sodium channel) (Yang et al. 2013; Kim et al. 2008).

The structural toxicity relationships of 5-hydroxy-2-methyl-1,4-naphthoquinone (A) and its structural derivatives, including 2,2'-bi(3-hydroxy-1,4-naphthoquinone) (F), 2,3-dibromo-1,4-naphthoquinone (B), 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (E), 2,3-dichloro-1,4-

**Table 2** Insecticidal toxicities of 5-hydroxy-2-methyl-1,4-naphthoquinone derivatives and commercial insecticide against the three mosquito larvae

Compounds	Mosquito species	$LC_{50}$ ( $\mu\text{g}/\text{mL}$ )	95 % confidence interval
5-Hydroxy-2-methyl-1,4-naphthoquinone	<i>Ae. aegypti</i>	5.66	5.60–5.78
	<i>Cx. pipiens pallens</i>	5.43	5.33–5.51
	<i>Oc. togoi</i>	6.09	5.98–6.18
2,3-Dibromo-1,4-naphthoquinone	<i>Ae. aegypti</i>	1.32	1.27–1.44
	<i>Cx. pipiens pallens</i>	1.28	1.20–1.40
	<i>Oc. togoi</i>	1.94	1.88–2.06
2,3-Dichloro-1,4-naphthoquinone	<i>Ae. aegypti</i>	4.76	4.71–7.93
	<i>Cx. pipiens pallens</i>	3.89	3.78–3.99
	<i>Oc. togoi</i>	5.33	5.29–5.39
5,8-Dihydroxy-1,4-naphthoquinone	<i>Ae. aegypti</i>	3.57	3.51–3.63
	<i>Cx. pipiens pallens</i>	3.34	3.27–3.40
	<i>Oc. togoi</i>	5.04	4.96–5.11
2,3-Dichloro-5,8-dihydroxy-1,4-naphthoquinone	<i>Ae. aegypti</i>	0.89	0.81–0.92
	<i>Cx. pipiens pallens</i>	0.80	0.74–0.87
	<i>Oc. togoi</i>	1.04	0.98–1.09
2,2'-Bi(3-hydroxy-1,4-naphthoquinone)	<i>Ae. aegypti</i>	33.93	33.81–34.05
	<i>Cx. pipiens pallens</i>	32.82	32.62–33.05
	<i>Oc. togoi</i>	36.17	35.96–36.31
Pirimiphos-methyl	<i>Ae. aegypti</i>	0.18	0.15–0.22
	<i>Cx. pipiens pallens</i>	0.13	0.10–0.19
	<i>Oc. togoi</i>	0.24	0.18–0.28

naphthoquinone (C), and 5,8-dihydroxy-1,4-naphthoquinone (D) were determined (Table 2; Fig. 1). Based on the  $LC_{50}$  values of 5-hydroxy-2-methyl-1,4-naphthoquinone derivatives against *Ae. aegypti*, it was found that 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (E) (0.89  $\mu\text{g}/\text{mL}$ ) had the most potent larvicidal toxicity, followed by 2,3-dibromo-1,4-naphthoquinone (B) (1.32  $\mu\text{g}/\text{mL}$ ), 5,8-dihydroxy-1,4-naphthoquinone (D) (3.57  $\mu\text{g}/\text{mL}$ ), 2,3-dichloro-1,4-naphthoquinone (C) (4.76  $\mu\text{g}/\text{mL}$ ), and 2,2'-bi(3-hydroxy-1,4-naphthoquinone) (F) (33.93  $\mu\text{g}/\text{mL}$ ); 2,3-Dichloro-5,8-dihydroxy-1,4-naphthoquinone (E) (0.80  $\mu\text{g}/\text{mL}$ ) possessed the strongest larvicidal toxicity against *Cx. pipiens pallens*, followed by 2,3-dibromo-1,4-naphthoquinone (B) (1.28  $\mu\text{g}/\text{mL}$ ), 5,8-dihydroxy-1,4-naphthoquinone (D) (3.34  $\mu\text{g}/\text{mL}$ ), 2,3-dichloro-1,4-naphthoquinone (C) (3.89  $\mu\text{g}/\text{mL}$ ), and 2,2'-bi(3-hydroxy-1,4-naphthoquinone) (F) (32.82  $\mu\text{g}/\text{mL}$ ). In the case of *Oc. togoi*, 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (E)

(1.04 µg/mL) showed excellent larvicidal toxicity, followed by 2,3-dibromo-1,4-naphthoquinone (B) (1.94 µg/mL), 5,8-dihydroxy-1,4-naphthoquinone (D) (5.04 µg/mL), 2,3-dichloro-1,4-naphthoquinone (C) (5.33 µg/mL), and -2,2'-bi(3-hydroxy-1,4-naphthoquinone) (F) (36.17 µg/mL). The larvicidal toxicities of the 5-hydroxy-2-methyl-1,4-naphthoquinone structural derivatives were less than those of pirimiphos-methyl (0.18, 0.13, and 0.24 µg/mL) against *Ae. aegypti*, *Cx. pipiens pallens*, and *Oc. togoi*. Nevertheless, 5-hydroxy-2-methyl-1,4-naphthoquinone (A) and its structural derivatives could be suitable as natural insecticidal agents to control mosquito larvae.

2,3-Dibromo-1,4-naphthoquinone (B) possessed potent insecticidal toxicity against the three mosquito larvae when 1,4-naphthoquinone contained Br, Cl, and OH functional groups, followed by 5,8-dihydroxy-1,4-naphthoquinone (D) and 2,3-dichloro-1,4-naphthoquinone (C). However, when combined with an OH functional group on 2,3-dichloro-1,4-naphthoquinone (C), the larvicidal toxicities of 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (E) were approximately 5.35, 4.86, and 5.13 times more effective than those of 2,3-dichloro-1,4-naphthoquinone (C) against *Ae. aegypti*, *Cx. pipiens pallens*, and *Oc. togoi*, respectively. In contrast, when a CH<sub>3</sub> functional group was on 5-hydroxy-1,4-naphthoquinone, the insecticidal toxicities of 5-hydroxy-2-methyl-1,4-naphthoquinone (A) were less than those of 5,8-dihydroxy-1,4-naphthoquinone (D) against the three mosquito larvae. These findings indicate that the presence of some functional groups on 1,4-naphthoquinone influenced insecticidal toxicity against the *Cx. pipiens pallens*, *Ae. aegypti*, and *Oc. togoi* larvae. In particular, when the Cl and OH functional groups were placed on 1,4-naphthoquinone at the same time, insecticidal toxicity against the three mosquito larvae was the highest among compounds. In previous study, Ribeiro et al. (2009) have reported the insecticidal toxicities of 1,4-naphthoquinone structural derivatives against the *Ae. aegypti* larvae. Based on the LC<sub>50</sub> values against *Ae. aegypti*, it was found that 3-bromo-5-hydroxy-1,4-naphthoquinone (0.873 µg/mL) possessed the potent larvicidal toxicity, followed by 5-hydroxy-1,4-naphthoquinone (3.587 µg/mL), 3-bromo-5-methoxy-1,4-naphthoquinone (5.752 µg/mL), and 3-bromo-5-acetoxy-1,4-naphthoquinone (7.287 µg/mL). These findings indicated that 1,4-naphthoquinone structural derivatives, containing Br and OH functional groups on 1,4-naphthoquinone, had the potent insecticidal effects against the mosquito larvae. From these results, the present study suggest that 1,4-naphthoquinone structural derivatives are insecticidal agents against the mosquito larvae.

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