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Short Communication

Insecticidal Effects of *Tabebuia avellanedae*-derived Constituent and Its Analogues against *Nilaparvata lugens* and *Laodelphax striatellus*

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Insecticidal activities of an active constituent derived from the bark of *Tabebuia avellanedae* and its analogues were examined using a micro-topical application bioassay against the brown planthopper, *Nilaparvata lugens* and the small brown planthopper, *Laodelphax striatellus*. The active constituent of *T. avellanedae* bark was characterized by various chromatographic methods and identified as 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphtoquinone. On the basis of 48 h lethal concentration 50 (LD_{50}) values, the most toxic compound against *N. lugens* was 1,4-Naphtoquinone (0.042 μg/female), followed by 5,8-Dihydroxy-1,4-naphtoquinone (0.080 μg/female), 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphtoquinone (0.091 μg/female), and 2,3-Dichloro-1,4-naphtoquinone (0.186 μg/female). Similar results against *L. striatellus* were observed for 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphtoquinone and its analogues, except for 2,3-Dichloro-1,4-naphtoquinone and 5,8-Dihydroxy-1,4-naphtoquinone. These results indicate that *T. avellanedae* bark-derived material and its analogues have potential as new preventive agents for control of *N. lugens* and *L. striatellus*.

Key words: *Laodelphax striatellus*, micro-topical application bioassay, natural insecticide, *Nilaparvata lugens*, *Tabebuia avellanedae*

Some synthetic chemicals are used as insecticides and crop protectants not only to improve crop yield but also to control insect pests throughout the world. However, their continued use may cause negative effects on insects and their natural enemies, environmental impacts, residual toxicity, and human health hazards [Ahn *et al*., 1998; de Silva *et al*., 2008; Lee *et al*., 2010a]. Due to increasing problems associated with the use of synthetic chemical insecticides, there is a need for the discovery and development of new, ecologically safe alternatives such as plant extracts and phytochemicals.

Secondary compounds derived from plants may provide alternative sources for insect control, because they contain bioactive constituents, such as alkaloids, phenolics, and terpenoids [Yang *et al*., 2002]. These phytochemicals are selective against certain insect pests, biodegrade into non-toxic products, and have few or no harmful effects on non-target organisms. Studies have been undertaken on plant materials or phytochemicals as potential insecticides or as lead compounds [Ahn *et al*., 1998; Yang *et al*., 2002]. A number of herb families, the Annonaceae, Lamiaceae, Meliaceae, Piperaceae, and Rutaceae, may provide alternative sources of insecticides [Hummelbrunner and Isman, 2001; Akhtar and Isman, 2004]. Chemical studies on many herb families have revealed that these plants contain natural insecticides. *Origanum vulgare* is toxic to *Blatella germanica*, *Plutella xylostella*, and *Reticulitermes speratus* [Ahn *et al*., 1998]. Toosendanin, a limonoid derived from *Melia toosendan*, is toxic to three stored-product beetles, *Cryptolestes ferrugineus*, *Sitophilus oryzae*, and *Tribolium castaneum* [Xie *et al*., 1995]. In our previous study, monoterpene alcohols, aliphatic aldehydes, quinolines, naphthoquinones, and triketones from plant extracts of these families were shown to possess strong acaricidal and insecticidal activities against *Dermatophagoides* spp., *Sogetella furcifera*, *Tetranychus urticae*, and *Tyrophagus putrescentiae* [Jeong *et al*., 2008; Lee *et al*., 2010a; 2010b; Jeon and Lee, 2011].

Tabebuia avellanedae, a member of the Bignoniaceae, is a tree found in Central and South America that is reported to possess antibacterial, anticancer, antifungal,

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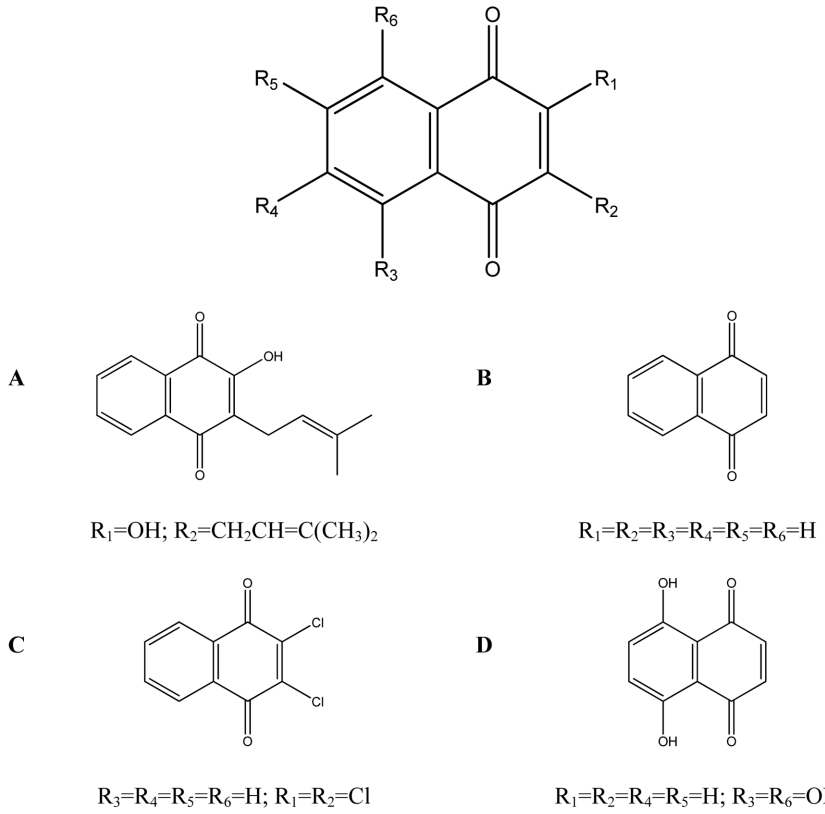

Fig. 1. Chemical structures of the isolated compound and its analogues. (A) 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone, (B) 1,4-Naphthoquinone, (C) 2,3-Dichloro-1,4-naphthoquinone, (D) 5,8-Dihydroxy-1,4-naphthoquinone.

anti-inflammatory, antioxidant, astringent, diuretic, and laxative properties [Park *et al*., 2004; Byeon *et al*., 2008]. The major constituents identified in the extracts and essential oil of *Tabebuia* spp. include benzaldehyde derivatives, benzoic acid, cumarins, flavonoids, furanonaphthoquinones, glycosides, naphthoquinones, and quinones [Warashina *et al*., 2004; Park *et al*., 2006; Byeon *et al*., 2008]. However, relatively little work has been done to evaluate the insecticidal activities of materials derived from *Tabebuia* spp. against the brown planthoppers, *Nilaparvata lugens* and *Laodelphax striatellus*. Therefore, in the present study, the active constituent of *T. avellanedae* bark against *N. lugens* and *L. striatellus* was isolated and identified by various spectroscopic techniques.

1,4-Naphthoquinone (1,4-NQ), 2,3-Dichloro-1,4-naphthoquinone (2,3-Dichloro-1,4-NQ), and 5,8-Dihydroxy-1,4-naphthoquinone (5,8-Dihydroxy-1,4-NQ) were supplied by Sigma-Aldrich (St. Louis, MO). All chemicals used in this study were of reagent grade. The bark of *T. avellanedae* was purchased from Raintree Nutrition, Inc. (Carson, NV). Finely powdered bark of *T. avellanedae* was homogenized with a grinder after washing, extracted twice with methanol (10 L) at room temperature for 48 h, and then filtered (Toyo filter paper No. 2, Toyo Roshi, Tokyo, Japan) *in vacuo*. The filtrate was concentrated and combined *in vacuo* at 45°C, using a rotary vacuum evaporator (EYELA auto jack NAJ-100, Tokyo, Japan). The methanol extract (850 g) was suspended in distilled water and sequentially divided into hexane (153 g), chloroform (94 g), ethyl acetate (113 g), butanol (220 g), and water-soluble (268 g) fractions for the bioassay. Solvent fractions were concentrated using rotary vacuum evaporation at 45°C. The water-soluble fraction was freeze-dried.

To isolate the active compound of the chloroform fraction derived from the *T. avellanedae* bark, the chloroform fraction (10 g) was loaded on a silica gel column (Merck 70-230 meth, 600 g, 5.5 i.d.×50 cm, Rahway, NJ), and continuously eluted with a gradient step of chloroform/methanol (90:10, 80:20, 70:30, and 100, v/v). The separated fractions were analyzed via thin layer chromatography (TLC). Fractions showing similar patterns were pooled. In this step, six fractions (C1-6) were obtained and used in bioassay against *N. lugens* and *L. striatellus*. The C2 fraction (3.7 g) showed strong activity against *N. lugens* and *L. striatellus*. Therefore, the active C2 fraction was re-chromatographed on a silica gel column, which was successively eluted with chloroform/methanol (70:30, 80:20, 90:10, and 100%, v/v). Among the five fractions (C21-25), C22 fraction (260 mg)

showed the strongest insecticidal activity against *N. lugens* and *L. striatellus*. To isolate the insecticidal constituent of C22 fraction, it was further purified via prep HPLC (Recycling Preparative HPLC, Japan Analytical Industry Co., Tokyo, Japan). The column used was a JAI GS Series Column (GS310 50 cm+GS310 50 cm×2, 21.5 i.d.×500 mm L; Tokyo, Japan) with hexane/chloroform (30:70, v/v) as the mobile phase at a flow rate of 5 mL/min and UV detection (255 nm). In this step, six fractions (C221-226) were obtained and bioassayed. The active C223 fraction (58 mg) was subjected to further chromatography using a JAI W Series Column (W253 50 cm+W252 50 cm×2, 20.0 i.d.×500 mm L), using chloroform (100%) as the mobile phase at a flow rate of 3.5 mL/min, with detection at 255 nm. Finally, the active C2232 (16.75 mg) was isolated successfully on a single peak. The chemical structure of the isolated compound was determined by spectroscopic analysis. ^{1}H- and ^{13}C-NMR spectra were recorded in deuteriochloroform ($CDCl_3$), using a JNM-ECA600 spectrometer (Tokyo, Japan), at 600 and 150 MHz [with tetra methyl silane (TMS) as an internal standard], respectively, with chemical shifts reported in parts per million (δ), and coupling constants (*J*). Additionally, UV visible absorption spectrum was obtained in chloroform, using a Waters 490 spectrometer (Boston, MA). Electron ionization mass spectrometer (EI-MS) spectra were obtained with a JEOL JMS-DX 30 spectrometer (JEOL, Tokyo, Japan).

Colonies of susceptible planthoppers, *N. lugens* and *L. striatellus*, were obtained from the National Academy of Agricultural Science, RDA, Suwon, Korea, and maintained in a laboratory without external exposure to any known insecticides. The planthoppers were bred in plastic containers (40×40×30 cm) and reared on rice (*Oryzae sativa* L.) seedlings (7-10 days after germination). They were maintained in the laboratory at 25±1°C, with a light:dark photoperiod of 16:8 h at 65-75% relative humidity (RH). Only adults were used for the insecticidal bioassay. Insecticidal activities of the active compound isolated from the *T. avellanedae* bark and its derivatives against *N. lugens* and *L. striatellus* were determined by a micro-topical toxicity bioassay using a hand microapplicator (Burkard Manufacturing Co., Rickmansworth, England). The micro-topical application technique described by Nagata *et al*. [1979] and Lee *et al*. [2010a] was used with slight modification. Each test material was diluted into a series of concentrations with acetone and applied to 3- to 5-day-old macropterous adults. Thirty insects were lightly anaesthetized with carbon dioxide. Subsequently, 0.2 μL (0.1 μL for *L. striatellus*) droplet of each sample solution was applied topically with a hand microapplicator to the mid-abdomen of each insect. Control planthoppers (*N. lugens* and *L. striatellus* adults) were treated with acetone only. The treated planthoppers were maintained with rice seedlings in a glass tube at 25±1°C, 65-75% relative humidity, and a light:dark photoperiod of 16:8 h. Mortality was observed at 48 h post- treatment. To determine mortality, all treatments were replicated three times. According to SAS (version 6), the LD_{50} (lethal dosage needed to kill 50% of *N. lugens* and *L. striatellus* adults) value estimated using an ANOVA.

Insecticidal activities of various fractions obtained from *T. avellanedae* bark extract against *N. lugens* and *L. striatellus* were evaluated via micro-topical application bioassay (Table 1). When the methanol extract of *T. avellanedae* bark was examined against *N. lugens* and *L. striatellus*, significant dose-response relationships were detected between toxicity and the two insect species. The LD_{50} values of methanol extracts were 11.06 and 27.37 μg/female against *N. lugens* and *L. striatellus*, respectively. In particular, the chloroform fraction of the methanol extract derived from *T. avellanedae* barks revealed the highest potential insecticidal activities against *N. lugens* (5.53 μg/female) and *L. striatellus* (9.73 μg/female). However, weak or no insecticidal activities were shown by the ethyl acetate, hexane, butanol, and water-soluble fractions (Table 1).

Due to the potential insecticidal activity of the chloroform fraction, this fraction was subjected to a silica gel column and high performance liquid chromatography (HPLC). Chemical structural determination of the isolated compound was performed by various spectroscopic analyses, such as UV, EI-MS, ^{1}H-NMR and ^{13}C-NMR,

Table 1. Insecticidal activities of various fractions obtained from the methanol extract of *T. avellanedae* bark against *N. lugens* and *L. striatellus*, using micro-topical application bioassay

Fraction	LD_{50} values (μg/female)[a] (95% confidence limits)[b]	
	N. lugens	*L. striatellus*
Methanol extract	11.06 (10.255-12.309)	27.37 (24.337-29.131)
Hexane fraction	-[c]	-
Chloroform fraction	5.53 (5.203-5.778)	9.73 (9.460-9.893)
Ethyl acetate fraction	73.06 (72.885-75.008)	159.32 (159.012-161.588)
Butanol fraction	-	-
Water fraction	-	-

[a]LD_{50} values (48 h mortality) calculated by probit analysis.
[b]95% confidence limits in parentheses are based on three replication assays.
[c]No activity.

Table 2. LD_{50} values of isolated compound and its analogues against *N. lugens* and *L. striatellus*

Compound	LD_{50} values (μg/female)[a] (95% confidence limits)[b]	
	N. lugens	*L. striatellus*
2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphtoquinone	0.091 (0.0783-0.1034)	0.243 (0.2128-0.2779)
1,4-Naphthoquinone	0.042 (0.0308-0.0612)	0.152 (0.1446-0.1703)
2,3-Dichloro-1,4-naphthoquinone	0.186 (0.1655-0.1981)	-[c]
5,8-Dihydroxy-1,4-naphthoquinone	0.080 (0.0641-0.0967)	-

[a]LD_{50} values (48 h mortality) calculated by probit analysis.
[b]95% confidence limits in parentheses are based on three replication assays.
[c]No activity.

and by direct comparison with an authentic reference compound. The active compound was characterized as 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone ($C_{15}H_{14}O_3$, MW, 242); EI-MS (70 eV) *m/z* (% relative intensity): M^+ 227 (100), 199 (17), 181 (15), 159 (13), 128 (21), 105 (27), 77 (25); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 1.69 (s, 3H, CH_3), 1.79 (s, 3H, CH_3), 3.30 (d, *J*=7.0 Hz, 2H, CH_2), 5.21 (m, 1H, CH), 7.30 (s, 1H, OH), 7.68-8.11 (m, 4H, Ar-H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 181.7 (C-1), 152.7 (C-2), 123.4 (C-3), 184.5 (C-4), 126.0 (C-5), 134.8 (C-6), 132.8 (C-7), 125.9 (C-8), 129.4 (C-9), 132.9 (C-10), 22.6 (C-1'), 119.6 (C-2`), 133.8 (C-3'), 17.9 (C-4'), 25.7 (C-5'). The spectroscopic data of 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone matched with those previously reported for naphthoquinone isolated from *Tabebuia impetiginosa* [Park *et al*., 2006; Jeon and Lee, 2011].

Insecticidal activities of 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-NQ and its analogues against *N. lugens* and *L. striatellus* were examined by comparing the LD_{50} values using a micro-topical application method (Table 2). On the basis of 48 h LD_{50} values against *N. lugens*, 1,4-NQ (0.042 μg/female) was the most effective compound, followed by 5,8-Dihydroxy-1,4-NQ (0.080 μg/female), 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-NQ (0.091 μg/female), and 2,3-Dibromo-1,4-NQ (0.076 μg/female). Against *L. striateelus*, 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-NQ (0.243 μg/female) was the most active constituent, followed by 1,4-NQ (0.152 μg/female). However, no activity was observed for 2,3-Dichloro-1,4-NQ or 5,8-Dihydroxy-1,4-NQ, which indicates that there are species-specific differences in the planthoppers, with *N. lugens* being more susceptible to *T. avellanedae* extract as well as to the four naphthoquinone analogues of 2-Hydroxy-3-(3-methyl-2-butenyl)-1,4-NQ, 1,4-NQ, 2,3-Dichloro-1,4-NQ, and 5,8-Dihydroxy-1,4-NQ. Akhtar and Isman [2004] reported that even closely related species can show widely different susceptibilities to pure allelochemicals or to the same plant extracts.

The pharmacological and insecticidal activities of *Tabebuia* spp. are well known [Park *et al*., 2004; Byeon *et al*., 2008; Jeon and Lee, 2011]. *Tabebuia* spp. contains various compounds such as anthraquinones, benzaldehyde derivatives, coumarins, flavonoids, iridoids, and naphthoquinones [Park *et al*., 2004; Byeon *et al*., 2008]. These constituents exist in many medicinal plants and, jointly or independently, exhibit a variety of bioactivities [Tak *et al*., 2006]. Therefore, naturally occurring quinones and quinolines have attracted interest as insecticidal compounds that are effective against *D. farinae*, *D. pteronyssinus*, *L. striatellus*, *N. lugens*, *S. furcifera*, and *T. putrescenitae*, including 1,4-benzoquinone and 5-hydroxy-2-methyl-1,4-naphthoquinone from *Pyrus ussuriensis* and *Diospyros kaki* [Lee, 2007; Lee and Lee, 2008] and 8-Hydroxyquinoline from *Sebastiania corniculata* [Lee *et al*., 2010a]. In the present study, 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-NQ isolated from an extract of *T. avellanedae* bark and its analogues exhibited insecticidal activities against *N. lugens* and *L. striatellus*. These results indicate that 2-hydroxy-3-(3-methyl-2-butenyl)-1,4-NQ and its analogues are useful bioactive chemical compounds for managing planthoppers and may be suitable for use as lead compounds. Further studies should be conducted to investigate the safety issues of active compounds for human health, the insecticidal mode of action, and other formulations to improve insecticidal potency and stability.

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