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Article

Insecticidal Properties of Euphorbiaceae: *Sebastiania corniculata*-derived 8-Hydroxyquinoline and Its Derivatives against Three Planthopper Species (Hemiptera: Delphacidae)

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This study examined the insecticidal effects of *Sebastiania corniculata* materials against *Laodelphax striatellus*, *Nilaparvata lugens*, and *Sogatella furcifera*. Based on the lethal dose$_{50}$ (LD_{50}) values, the chloroform fraction of *S. corniculata* showed the most potential activity against *L. striatellus* (1.09 μg/female), *N. lugens* (4.46 μg/female), and *S. furcifera* (2.32 μg/female). Therefore, we purified the active component of the chloroform fraction, using various chromatographies, and identified it as 8-hydroxyquinoline. To establish the structure-activity relationships, we tested 8-hydroxyquinoline and its derivatives against the 3 planthopper species. Based on 48 h LD_{50} values, isoquinoline and 6-methoxyquinoline were the most effective against the 3 species. 8-Hydroxyquinoline, 4-methylquinoline, 6-methylquinoline, and 8-hydroxy-2-methylquinoline showed high insecticidal effects. These results indicate that changing the nitrogen atom's position in quinoline's pyridine ring plays an important role in the insecticidal effects. We found a high correlation between the introduction of a functional group into the quinoline structure and toxicity against the 3 planthoppers. Our results indicate these insecticidal effects seem to require quinoline derivatives containing hydroxyl (R_4 position), methyl (R_2 and R_3 position), and methoxy (R_3 position) groups and a structural isomer of quinoline and suggest that these derivatives may be useful as new preventative agents against the damage caused by a wide range of pests in rice farming areas.

Key words: 8-hydroxyquinoline, insecticidal effects, LD_{50} values, micro-topical application bioassay, planthopper, quinoline derivatives, *Sebastiania corniculata*

Planthoppers are common rice insect pests, especially in many parts of Asia. The brown planthopper (BPH), *Nilaparvata lugens* Stål, small brown planthopper (SBPH), *Laodelphax striatellus* Fallén and white-backed planthopper, *Sogatella furcifera* Horvath (WBPH), all belonging to the Family Delphacidae (Homoptera), are three of the major rice-infesting pests in subtropical and temperate areas [Endo *et al*., 2002; Senthil-Nathan *et al*., 2009]. These pests cause direct damage by sucking on and depleting the plant's nutrients. They also cause indirect damage, by transmitting several viral diseases, such as rice black-streaked dwarf virus (RBSDV) and rice stripe virus (RSV) [Senthil-Nathan *et al*., 2009; Duan *et al*., 2010]. This causes a decline in grain quality and serious yield losses in many rice varieties [Zhang *et al*., 2008]. Planthopper control has mainly depended on synthetic insecticides, such as imidacloprid, methamidophos, and BHC (benzene hexachloride) [Endo *et al*., 2002; Senthil-Nathan *et al*., 2009]. Although synthetic insecticides were useful for controlling planthoppers in the past, their constant use has led to enhanced pest resistance, environmental pollution, mortality among the pests' natural enemies, and potentially serious ill effects on mammals in the same environment [de Silva *et al*., 2008; Duan *et al*., 2010]. Hence, many studies have investigated the effects of plant-derived insecticides, such as allelochemicals and secondary metabolites, in search of plants that may be useful sources of chemicals that are bioactive against planthoppers in the rice ecosystem.

Plant-derived extracts and phytochemicals, such as alkaloids, flavonoids, quinones, and terpenoids, are widely distributed in nature. In the Euphorbiaceae family, the *Sebasticania corniculata* (*S. corniculata*) is a

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perennial herb distributed through tropical America. It has yielded several phenolic compounds, triterpenoids, and steroids [Machado *et al*., 2005; Macias-Rubalcava *et al*., 2007]. Previous studies have investigated phytochemicals containing biologically active elements, such as antiviral [Kott *et al*., 1999], antimicrobial [Khera *et al*., 2003; Kim *et al*., 2006; Jeon *et al*., 2009], antispasmodic [Yunes *et al*., 1990], and antinociceptive effects [Luzzi *et al*., 2000]. However, relatively few studies have evaluated *S. corniculata* extracts' insecticidal effects against planthoppers. Therefore, this study evaluated *S. corniculata* for its insecticidal effects against three species of planthoppers, in an attempt to identify new, natural insecticides.

Materials and Methods

Chemicals. 6-Hydroxyquinoline, isoquinoline, 4-methylquinoline, and 6-methylquinoline were purchased from Sigma-Aldrich (St. Louis, MO). 2-Hydroxyquinoline, 4-hydroxyquinoline, 8-hydroxy-2-methylquinoline, and 6-methoxyquinoline were supplied by Fluka (Milwaukee, WI, U.S.A). 2-Methylquinoline was purchased from Wako Chemical Co. (Tokyo, Japan). All experimental chemicals were of reagent grade.

Plant preparation. The *S. corniculata* (6 kg) was purchased from Prof. Sang-Hyun Lee (Forestry Department, Chonbuk National University, South Korea). We ground the samples in a blender, extracted them twice with methanol (15 L) at room temperature for 2 days, and then filtered them (Toyofilter paper No. 2, Toyo Roshi, Japan) in vacuum. Then we concentrated the combined filtrates, in vacuum, at 45°C, using a rotary vacuum evaporator. We sequentially divided the concentrated material (20 g) into *n*-hexane (1.3 g), chloroform (3.8 g), ethyl acetate (3.2 g), butanol (3.4 g), and water-soluble (7.5 g) fractions for subsequent bioassay. Next, via rotary evaporation (EYELA autojack NAJ-100, Japan), we concentrated the solvent fractions. Finally, we freeze-dried the water fraction.

Planthoppers. Our bioassays examined 3 species of planthoppers. Colonies of *Nilaparvata lugens* (*N. lugens*) and *Sogatella furcifera* (*S. furcifera*) were obtained from Jeonbuk province. *Laodelphax striatellus* (*L. striatellus*) was collected from Chungnam province, South Korea. We maintained the planthopper cultures, without exposure to any known insecticides, in laboratory cages at 26±1°C, with a 16 h light/8 h dark photoperiod and 60-80% relative humidity (RH).

Bioassay. We employed the micro-topical application technique reported by Nagata *et al*. [1979] the toxicity bioassay. Our test insects were macropterous adult females, 3-5 days old. First, we lightly anaesthetized the test insects with carbon dioxide, and then applied a 0.2 μL (0.1 μL for *S. furcifera*) droplet of one of the compounds, dissolved in acetone, methanol, and dichloromethane, topically to each insect's middle-abdomen, using a hand microapplicator (Burkard Manufacturing Co., Ltd., Rickmansworth, UK). We tested each compound on 20 planthoppers per test, and we repeated each test 3 times. We treated control planthoppers with 0.1 μL of solvent, alone. After the applications, we reared the planthoppers on a rice seedling in a glass cylinder (3×20 cm) and observed the insects' mortality at 24 and 48 h.

Isolation and identification. The assay described above detected potent insecticidal activity in the chloroform fraction (12 g). We sequentially chromatographed this fraction on a silica gel column (Merck 70-230 mesh, 800 g, 6.0 i.d.×85 cm, Rahway, NJ), and continuously eluted it using a stepwise gradient of chloroform/methanol (100:0, 90:10, 80:20, 70:30, 60:40 and 50:50, v/v). The active fraction (C3) showed the most insecticidal activity against the 3 planthopper species. We further chromatographed this fraction on a silica gel column and eluted it with chloroform/methanol (5:1, v/v), analyzing the column fractions via thin layer chromatography (TLC) and pooling fractions with similar TLC patterns. Then, we chromatographed the active fraction (C33, 2.8 g) on a Sephadex LH-20 column (Pharamacia) by the chloroform/acetone/methanol (25:2:2, v/v) giving 6 fractions (C331-C333). To purify the biologically active fraction (C332, 279 mg), we used a Japanese, analytical-industry recycling preparative, high-performance liquid chromatography method (HPLC, LC-908W-C60, JAI, Tokyo, Japan), for separating the active constituent, and then examined the eluates for insecticidal activity. The first column was a JAI GS Series Column (GS310 50 cm +GS310 50 cm, 21.5 mm i.d.×50 cm L, Japan Analytical Industry Co., Ltd., Japan), using chloroform/acetone (20:2, v/v), with a flow rate of 10 mL/min and detection at 254 nm. Due to fraction C3323's activity (188 mg), it was further chromatographed on a JAI W Series Column (W-253 50 cm+W-252 50 cm, 20.0 mm i.d.×50 cm L, Japan Analytical Industry Co., Ltd., Tokyo, Japan) under the identical conditions described above. Finally, we isolated the active principle (C33232, 84 mg) by assessing the activities of the eluates and determined its structure based on spectroscopic analyses, as follows. We recorded the ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra in chloroform, using a JNM-ECA600 spectrometer at 600 and 150 MHz (with tetramethylsilane (TMS) as an internal standard), respectively, and expressing the chemical shifts in δ (ppm). Using a ^{1}H-^{1}H correlation spectrum, as well as a ^{13}C-^{1}H correlation spectrum, we

acquired unambiguous ^{1}H and ^{13}C-NMR chemical shifts. UV spectra were obtained in methanol, using a Waters 490 spectrometer (Waters, Boston, MA) with EI-Mass spectra obtained using a JEOL JMS-DX 30 spectrometer (JEOL, Tokyo, Japan).

Statistical analysis. As stated, we determined the mortalities at 24 and 48 h after treatment, considering a given planthopper dead if appendages did not move when we prodded it with an insect pin. Lethal dose$_{50}$ (LD_{50}) values were calculated based on a standard probit analysis [SAS, 1990].

Results and Discussion

Table 1 shows the insecticidal effects of materials derived from *S. corniculata* against *L. striatellus, N. lugens,* and *S. furcifera,* which we examined using micro-topical application bioassay (Table 1). When we examined the methanol extract of *S. corniculata*, it showed a clear dose-response relationship in all three species. The LD_{50} values of the *S. corniculata* extract were 11.15, 15.64, and 13.89 μg/female against *L. striatellus*, *N. lugens,* and *S. furcifera*, respectively. In particular, the chloroform fraction of the *S. corniculata* methanol extract showed the highest potential activity against *L. striatellus* (1.09 μg/female), *N. lugens* (4.46 μg/female) and *S. furcifera* (2.32 μg/female), respectively. However, we observed zero or weak inhibitory activity against the 3 species from the hexane, ethyl acetate, butanol, and water fractions.

Due to the insecticidal activity of the chloroform fraction, we purified the active compound using a silica gel column and high performance liquid chromatography (HPLC). Structural determination of the isolate was made via spectroscopic analyses, including UV, electron impact mass spectrometry (EI-MS), ^{1}H-NMR, ^{13}C-NMR and 2D-NMR (^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and DEPT) and by direct comparison with an authentic reference compound. We identified the active compound as 8-hydroxyquinoline based on the following evidence: 8-hydroxyquinoline (C_9H_7NO, MW, 145); EI-MS (70 eV) *m/z* (% relative intensity): M^+ 145 (100), 144 (2), 117 (85), 116 (14), 90 (7), 89 (6); ^{1}H-NMR (CD_3OD, 400 MHz); 8.75-8.76 (1H, *m*, *J*=5.84 Hz, H-2), 8.20-8.22 (1H, *m*, *J*=10 Hz, H-4), 7.42-7.46 (1H, *m*, *J*=17.8 Hz, H-5), 7.38-7.40 (1H, *d*, *J*=7.6 Hz, H-3), 7.32-7.34 (1H, *m*, *J*=9.52 Hz, H-6), 7.07-7.10 (1H, *m*, *J*=8.8 Hz, H-7), 5.42 (OH, *s*, H-8); ^{13}C-NMR (CD_3OD, 100 MHz); 152.7 (C-8), 150.3 (C-2), 138.8 (C-9), 135.3 (C-4), 129.0 (C-10), 126.2 (C-6), 121.3 (C-3), 120.3 (C-5), 112.0 (C-7).

The quinoline structure is present in the most important class of heterocyclic aromatic organic compounds, found in many synthetic and natural products having a wide range of pharmacological activities, including antibacterial, anticancer, antifungal, anti-inflammatory, anti-obesity, and antiviral [Yunes *et al*., 1990; Kott *et al*., 1999; Khera *et al*., 2003; Kim *et al*., 2006; Jeon *et al*., 2009;]. It has been used in food and medicinal chemistry as a starting material for numerous pharmacologically-activity compounds [de Souza *et al*., 2009; Jeon *et al*., 2009; Lee and Lee, 2009]. Furthermore, 8-hydroxyquinoline and its derivatives are important constituents of a variety of pharmaceutically-important compound classes, including antibacterial, anticancer, antifungal, and antimicrobial activities [Kim *et al*., 2006; Jeon *et al*., 2009]. Researchers are still investigating novel compounds of this type [Zeng *et al*., 2006]. In our previous study, we also found that the antimicrobial activities of 8-hydroxyquinoline and its derivatives showed high growth-inhibiting activity against human intestinal bacteria [Kim *et al*., 2006; Jeon *et al*., 2009; Lee and Lee, 2009]. However, in spite of its many pharmacological activities, relatively few studies have focused on the insecticidal effects of quinoline derivatives against these

Table 1. Insecticidal properties of various fractions obtained from the methanol extract of *S. corniculata* against *L. striatellus*, *N. lugens,* and *S. furcifera*, using micro-topical application bioassay

Fraction	Planthoppers	LD_{50} values (μg/female)[a]	95% Confidence limit[b]
Methanol extract	*L. striatellus*	11.15	10.94-11.32
	N. lugens	15.64	14.95-16.86
	S. furcifera	13.89	13.24-14.36.
Hexane fraction	*L. striatellus*	-[c]	-
	N. lugens	-	-
	S. furcifera	-	-
Chloroform fraction	*L. striatellus*	**1.09**	**0.86-1.43**
	N. lugens	**4.46**	**4.07-4.89**
	S. furcifera	**2.32**	**1.89-2.67**
Ethyl acetate fraction	*L. striatellus*	59.89	58.26-61.33
	N. lugens	78.23	77.29-80.13
	S. furcifera	75.57	73.19-77.86
Butanol fraction	*L. striatellus*	-	-
	N. lugens	-	-
	S. furcifera	-	-
Water fraction	*L. striatellus*	-	-
	N. lugens	-	-
	S. furcifera	-	-

[a]LD_{50} values (48 h mortality) calculated by probit analysis.
[b]95% confidence limits in parentheses were based on 3 replication assays.
[c]No activity.

Table 2. LD_{50} values of quinoline derivatives against the 3 planthopper species

Compounds	LD_{50} values (μg/female)[a] (95% Confidence limit)[b] (Slope±SE)		
	L. striatellus (Small brown planthopper)	*N. lugens* (Brown planthopper)	*S. furcifera* (White-backed planthopper)
Quinoline	-[c]	-	-
Isoquinoline	**0.0352** (0.0323-0.0369) (0.47±0.22)	**0.0862** (0.0835-0.0896) (1.6±0.25)	**0.0694** (0.0661-0.0732) (1.1±0.23)
2-Hydroxyquinoline	-	-	-
4-Hydroxyquinoline	-	-	-
6-Hydroxyquinoline	-	-	-
8-Hydroxyquinoline	**0.0546** (0.0515-0.0578) (0.72±0.23)	**0.2684** (0.2327-0.2887) (1.3±0.23)	**0.2159** (0.2032-0.2243) (1.4±0.25)
2-Methylquinoline	-	-	-
4-Methylquinoline	**0.0827** (0.0795-0.0854) (1.2±0.25)	**0.1628** (0.1585-0.1659) (1.2±0.24)	**0.1482** (0.1426-0.1529) (1.3±0.21)
6-Methylquinoline	**0.0547** (0.0513-0.0579) (1.0±0.23)	**0.1209** (0.1183-0.1246) (1.5±0.25)	**0.1143** (0.1117-0.1186) (1.3±0.26)
8-Hydroxy-2-methylquinoline	**0.1586** (0.1357-0.1761) (1.6±0.24)	**0.1316** (0.1266-0.1351) (1.7±0.27)	**0.1256** (0.1219-0.1286) (1.2±0.22)
6-Methoxyquinoline	**0.0462** (0.0415-0.0488) (1.1±0.23)	**0.0945** (0.0911-0.0972) (1.5±0.25)	**0.0757** (0.0721-0.0793) (1.4±0.26)

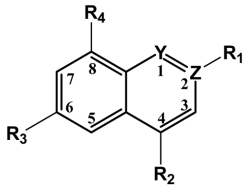
[a]LD_{50} values (48 h mortality) calculated by probit analysis.
[b]95% confidence limits in parentheses were based on three replication assays.
[c]No activity.

3 planthopper species. Therefore, we evaluated the insecticidal effects of 8-hydroxyquinoline derived from *S. corniculata,* aiming to develop safer and more effective insecticides for planthopper control. Furthermore, we described the structure-activity relationships for the quinoline derivatives containing functional radicals, such as hydroxyl-, methyl- and methoxyl-groups (Table 3).

To evaluate the relationships between quinoline derivatives and toxicity against the 3 planthopper species, we compared LD_{50} values, estimated by micro-topical application bioassay (Table 2). Based on 48 h LD_{50} values, the most toxic compound was isoquinoline (0.0352 μg/female) against *L. striatellus*, followed by 6-methoxyquinoline (0.0462 μg/female), 8-hydroxyquinoline (0.0546 μg/female), 6-methylquinoline (0.0547 μg/female), 4-methylquinoline (0.0827 μg/female), and 8-hydroxy-2-methylquinoline (0.1586 μg/female). Against *N. lugens*, isoquinoline (0.0862 μg/female) was, again, the most active compound, followed by 6-methoxyquinoline (0.0862 μg/female), 6-methylquinoline (0.1209 μg/female), 8-hydroxy-2-methylquinoline (0.1316 μg/female), 4-methylquinoline (0.1628 μg/female), and 8-hydroxyquinoline (0.2684 μg/female). According to the LD_{50} values, quinoline derivative's insecticidal effects on *S. furcifera* ranked similarly to *N. lugens,* but quinoline derivative's activity against *S. furcifera* was slightly lower than against *N. lugens*. As a result, isoquinoline and 6-methoxyquinoline were the most effective against all 3 planthopper species. However, we observed no insecticidal activity in 2-hydroxyquinoline, 4-hydroxyquinoline, 6-hydroxyquinoline, 2-methylquinoline, and quinoline.

To establish a structure-activity relationship, and to ascertain the role of functional groups for the quinoline derivatives' insecticidal effects, we evaluated the compounds based on their active radicals (R_1, R_2, R_3, and R_4), by comparing the LD_{50} values (Table 3). This analysis

Table 3. Structure-activity relationship of the quinoline derivatives



Compounds	R_1	R_2	R_3	R_4	Y	Z	LD_{50} values (μg/female)		
							L. striatellus	*N. lugens*	*S. furcifera*
Quinoline	**H**	**H**	**H**	**H**	**N**	**C**	**-**	**-**	**-**
Isoquinoline	H	H	H	H	**C**	**N**	**0.0352**	**0.0862**	**0.0694**
2-Hydroxyquinoline	**OH**	H	H	H	N	C	-	-	-
4-Hydroxyquinoline	H	**OH**	H	H	N	C	-	-	-
6-Hydroxyquinoline	H	H	**OH**	H	N	C	-	-	-
8-Hydroxyquinoline	H	H	H	**OH**	N	C	**0.0546**	**0.2684**	**0.2159**
2-Methylquinoline	**CH_3**	H	H	H	N	C	-	-	-
4-Methylquinoline	H	**CH_3**	H	H	N	C	**0.0827**	**0.1628**	**0.1482**
6-Methylquinoline	H	H	**CH_3**	H	N	C	**0.0547**	**0.1209**	**0.1143**
8-Hydroxy-2-methylquinoline	**CH_3**	H	H	**OH**	N	C	**0.1586**	**0.1316**	**0.1256**
6-Methoxyquinoline	H	H	**CH_3O**	H	N	C	**0.0462**	**0.0945**	**0.0757**

showed that, among the quinoline derivatives, isoquinoline, which is a structural isomer of quinoline, showed most toxicity against the 3 planthopper species. Isoquinoline and quinoline consist of a benzene ring fused to a pyridine ring. These results indicate that changing the position of the nitrogen atom in quinoline's pyridine ring plays an important role in insecticidal effects against these 3 planthopper species. Moreover, we also found a high correlation between the introduction of a functional group into the quinoline structure and toxicity against the 3 planthopper species. As shown in Table 3, introducing a hydroxyl group in the R_1, R_2, or R_3 positions in quinoline resulted in a compound with no insecticidal effects. However, introducing hydroxyl group at the R_4 position led to a dramatic increase in insecticidal effects. Furthermore, introduction of a methyl group into quinoline's R_1 position also showed no insecticidal effects, but the introduction of a methyl group at the R_2 or R_3 positions caused a significant increase in insecticidal effects. Interestingly, the structure-activity relationship between 8-hydroxyquinoline and 8-hydroxy-2-methylquinoline revealed that introducing a hydroxyl group into R_4 position of 2-methylquinoline produced a moderate increase in insecticidal effects against the 3 planthopper species. According to these results, a hydroxyl group in the R_4 position and bonded to quinoline's benzene ring is very important for increasing the insecticidal effects against the 3 planthopper species. Additionally, 6-methoxyquinoline (which has a methoxy group in the R_3 position) showed higher potential activity than did the other quinoline derivatives (except isoquinoline).

In this study, we evaluated the insecticidal effects of naturally occurring and synthetic quinoline derivatives on 3 planthopper species. Our results show that, of the quinoline derivatives, isoquinoline, 8-hydroxyquinoline, 4-methylquinoline, 6-methylquinoline, 8-hydroxy-2-methylquinoline, and 6-methoxyquinoline are the most promising, for use against these 3 species, due to the low doses required to produce high activity. Recently, many insecticides have been replaced with newer, safer agents due to the older agents' toxicity and tendencies to cause pest resistance. According to the Material Safety Data Sheet provided by Sigma-Aldrich [2010], the oral LD_{50} values of isoquinoline (615 mg/kg), 6-methyl-quinoline (800 mg/kg), 8-hydroxyquinoline (1,200 mg/kg), and 8-hydroxy-2-methylquinoline (2,250 mg/kg) indicate a low- to moderate acute toxicity to mammals. Moreover, quinoline derivatives have been widely used in medical drugs and the food industry for many years [Jeon *et al*., 2009; Lee and Lee, 2009]. The remarkable fact is that 8-hydroxyquinoline derived from natural sources may present a useful lead in the development of more potent insecticides, which might provide eco-friendly insect control agents for integrated pest management programs. For this reason, further studies are needed, to evaluate the expense and efficacy of these quinoline derivatives on a wide range of pests in rice farming areas, as well as to develop formulations with increased insecticidal potency and stability.

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