Isolation of Antifungal Compounds from *Gardenia jasminoides*

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**Abstract**: *Gardenia jasminoides* E. (Rubiaceae) methanol extracts showed the highest level of antifungal activity against *Pleurotus ostreatus*, a wood-rotting fungus, compared to five other methanol plants extracts; *Thuja orientalis* L. (Cupressaceae), *Datura innoxia* (Solanaeace), *Ligustirum japonicum* T. (Oleaceae), *Jupenr tus chinensis* var. *procmumrs* (Cupressaceae) and *Mallotus japonica* M. (Euphorbiaceae) and selected for further analysis. Two antifungal compounds were isolated from *n*-butanol and ethyl acetate solubles in the methanol extracts of *Gardenia jasminoides* leaves and stems by bioassay-guided fractionation, using *Pleurotus ostreatus*. The antifungal compounds found for the first time in *Gardenia jasminoides* against *Pleurotus ostreatus* were identified as genipin and geniposide based on instrumental analyses. Both also had potent inhibitory effects on two plant pathogenic fungi; *Fusarium oxysporum* and *Corynespora cassiicola*.

**Key words**: Antifungal activity, *Gardenia jasminoides*, iridoed, wood-rotting fungi, plant pathogenic fungi

**INTRODUCTION**

Gardenia has been traditionally used as a folk medicine for centuries in Asian countries. *Gardenia* is a genus of about 250 species of flowering plants in the family Rubiaceae, which is native to the tropical and subtropical regions of Africa, Southern Asia, Australasia and Oceania. *Gardenia jasminoides* Ellis (Rubiaceae) is an evergreen shrub, which grows to a height of 2 to 6 feet, depending on the variety, while its spread is about the same (Al-Juboorly et al., 1998). *Gardenia jasminoides* has been included in traditional medicinal formulations for the treatment of inflammation, jaundice, headache, edema, fever, hepatic disorders and hypertension (Aburada et al., 1976; Miyasita, 1976; Tseng et al., 95). *Gardenia jasminoides* has demonstrated effective pharmacological actions, such as protective activity against oxidative damage, as well as cytotoxic, anti-inflammatory and lytic effects (Tseng et al., 1995; Jagedeevaran et al., 2000; Koo et al., 2004, 2006). The compound, geniposide, gardenoside, crocin, crocetin and gardenin have been found in *G. jasminoides* (Oshima et al., 1988; Wei et al., 2008).

Fungi are one of the most harmful groups of plant pathogens, causing damage to agricultural crops, forest trees, wood and wood-based products (Punja and Ukhede, 2003). Fungi also pose a threat to humans in that they produce mycotoxins, poisonous chemical compounds, as well as cause contamination and spoilage of foods and feeds (Katta et al., 1995). However, some fungi are also beneficial to humans, being used in the agricultural and pharmaceutical industries and in biotechnological processes such as biopulping, bioremediation and so on. Several chemicals, so called fungicides, have been used to prevent and kill fungi in various environments. Though, they are efficient in their actions, they have the potential to disrupt environments; for instance they are toxic to humans and animals, accumulate in soil and water and cause resistance to build up in pathogen populations, which limits their application. Recent studies showed that synthetic fungicides have several disadvantages; significant nephrotoxicity in the case of amphotecin B (Georgopapadou and Walsh, 994), problematic drug-drug interactions (Von-Moltke et al., 1996), fungal resistance (Carledge et al., 1997) and adverse effects of griseofulvin and azole derivatives on human health, such as gastrointestinal inflammation, hepatotoxicity and respiratory disorders (Mackay-Wiggan et al., 2002). Many antifungal compounds have been reported to exist in plant tissues as factors in the resistance of plants to pathogen infections. Despite the various uses of Gardenia, antifungal activities against wood-rotting and agricultural pathogenic fungi have not been reported. Therefore, this

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study deals with not only the isolation and identification of antifungal compounds from *G. jasminoides* against *Pleurotus ostreatus*, a wood-rotting fungus, but also the activities of these compounds against two fungi pathogenic to plants, *Fusarium oxysporum* and *Corynespora cassicola*.

**MATERIALS AND METHODS**

This research was carried out in the Faculty of Agriculture, Ehime University, Japan during 2005-2007 in an effort to isolate antifungal compounds from *G. jasminoides*.

**Chemicals:** Authentic geniposide and genipin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The silica gel used for column chromatography was Wakogel C-200 (Wako Pure Chemical Industries Ltd., Osaka, Japan). TLC (Thin layer chromatography) aluminum sheets (0.25 mm) (Silica gel 60 F254, 20×20 cm) and PLC (preparative thin layer chromatography) glass plates (2 mm) (Silica gel 60 F254, 20×20 cm) were obtained from Merck (Darmstadt, Germany). All solvents were of the highest purity or High Performance Liquid Chromatography (HPLC) grade.

**Apparatus:** Melting points were measured on a Yanaco micro melting point apparatus (Yanaco Co., Ltd., Kyoto, Japan) and were uncorrected. Ultraviolet (UV) spectra were measured with a Shimadzu UV-VIS 1200 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The HPLC was conducted with a Shimadzu system consisting of a LC-10ADvp pump equipped with a CTO-10 Avp column oven, SPD-10 Avp UV-visible spectrophotometric detector and Frac 10 A fraction collector; peak analysis and assignment were conducted using Class-LC10/M10, and an Inertisil ODS-3 (5 μm 4.6×250 mm) column was applied. Mass spectra were recorded on a Shimadzu GC-MS QP 5050A (Shimadzu Corp., Kyoto, Japan) at an electron energy of 70 eV with a direct inlet; 1H NMR and 13C NMR spectra were recorded on a JEOL JNM-EX-400 (JEOL, Ltd., Tokyo, Japan) at 400 and 100 MHz, respectively. Chemical shifts are expressed as δ in ppm and TMS was used as the internal standard. The coupling constant (J) was recorded in Hz.

**Plant materials:** Six plants, *Thuja orientalis* L. (Cupressaceae), *Datura innoxia* (Solanaceae), *Ligustrum japonicum* T. (Oleaceae), *Juniperus chinensis* var. procumbens (Cupressaceae), *Gardenia jasminoides* E. (Rubicaceae) and *Mallotus japonica* M. (Euphorbiaceae), were collected in November 2005, on the outskirts of Matsuyama City, Japan.

**Test fungi:** Three fungi were used in this study, *Pleurotus ostreatus*, *Fusarium oxysporum* (NBRC 31630) and *Corynespora cassicola* (NBRC 30049). *Pleurotus ostreatus* was isolated from Matsuyama City, Japan. The other two fungi were purchased from the National Institute of Technology and Evaluation (NITE), Kisarazu, Chiba, Japan.

**Fungal cultures:** Cultures of *P. ostreatus*, *C. cassicola* and *F. oxysporum* were grown on a PDA (potato dextrose agar) medium (200 g of potato, 20 g of glucose, 20 g of agar powder per liter, pH 5.60) on a Petri dish at 25°C for 10 days. All cultures were maintained at 25°C for a certain period to enable them to grow well. Subcultures of the fungi were conducted every 30 days during the experiments.

**Antifungal assay:** The dilution antifungal method was used for antifungal assays as described by Bajpai *et al.* (2008). Sterile potato dextrose agar and methanol extracts from 2 g of plant or the respective amount of methanol extracts were dissolved in acetone or water and placed in a 90 mm Petri dish. Control plates contained medium and solvents only. Mycelial plugs were punched out with a 5-mm diameter cork borer from the test fungi grown on each medium and placed at the center of each Petri dish. Inoculated agar plates were incubated at 25°C for 6 days in the dark room condition. Antifungal activity was determined based on inhibition using the formula:

\[
\text{Inhibition} \% = \left(1 - \frac{T}{C}\right) \times 100
\]

where, T is hyphal extension in treated plates and C is hyphal extension in the control.

\(IC_{50}\) was defined as the lowest concentration of isolated compounds, which inhibited 50% of fungal growth.

**Statistical analysis:** All data are given as the Means±SD of three measurements. Statistical analysis was performed using SPSS 15 Software for Windows. The deviation of each experimental value was considered significant at p<0.01.

**Extraction of plants:** The leaves and stems (100 g) of 6 plants were dried, cut into small pieces and extracted twice with methanol at 60°C for 8 h. The methanol solution was concentrated with a rotary evaporator under reduced pressure to give the methanol extracts. For the isolation of antifungal compounds, the dried leaves and stems of *G. jasminoides* (1.0 kg) were extracted twice with methanol at 60°C for 8 h. The extract solution was concentrated with a rotary evaporator under reduced pressure to give the methanol extracts (272.6 g).
Isolation of compound 1 from n-butanol solubles in the methanol extracts of G. jasminoides: The methanol extracts of G. jasminoides (40 g) were suspended in water and then partitioned with n-hexane, chloroform, ethyl acetate and n-butanol, respectively, to give each of the solubles (n-hexane solubles (55.3 mg), chloroform solubles (266 mg), ethyl acetate solubles (5.5 g) and n-butanol solubles (24.1 g)). A portion of the n-butanol solubles (20 g) was separated by chromatography with a silica gel column (gradient of n-hexane-chloroform-methanol). The fractions collected were first checked by TLC and separated into 16 fractions (B.1-B.16). Spots were detected under a UV lamp (254 nm), or by spraying with anisaldehyde-sulfuric acid reagent or by heating after spraying with concentrated sulfuric acid. The antifungal assay against P. ostreatus showed fraction B.2 (3.5 g) was most active. The activity was 50% against P. ostreatus. Part of fraction B.2 (3 g) was further fractionated using silica gel column chromatography (gradient of 2-50% CHCl₃-MeOH) into 7 fractions (B.2.1-B.2.7). The most active fraction, B.2.6 (334 mg), with 10% activity against P. ostreatus was subjected to preparative thin layer chromatography and separated into three fractions (B.2.6.1-B.2.6.3). The most active fraction, B.2.6.2 (186 mg), with 38% activity against P. ostreatus, was separated using preparative thin layer chromatography by elution with n-hexane: CHCl₃: MeOH = 2: 5: 3 and gave two fractions, B.2.6.2.1 and B.2.6.2.2. The most active fraction, B.2.6.2.1 (108 mg), with 20% activity against P. ostreatus, was obtained. An active compound (Compound 1) was isolated from fraction B.2.6.2.1 by crystallization from acetone to yield white crystals (92 mg), with a melting point (m.p.) of 159-160°C.

Isolation of antifungal compound 2 from ethyl acetate solubles in the methanol extracts of G. jasminoides: The ethyl acetate solubles (20 g) were separated by chromatography with a silica gel column (gradient of n-hexane-chloroform-methanol). The fractions collected were firstly checked by TLC and separated into 10 fractions (E.1-E.10). The antifungal assay against P. ostreatus showed that fraction E.3 (1.8 g) was most active. A part of fraction E.3 (1.5 g) with 20% activity against P. ostreatus was further fractionated using silica gel column chromatography with a gradient of chloroform-methanol into 8 fractions (E.3.1-E.3.8). The most active fraction, E.3.8 (541 mg), with 12% activity against P. ostreatus, was further separated by silica gel column chromatography into five fractions (E.3.8.1-E.3.8.5). The most active fraction E.3.8.1 (132 mg), with 15% activity against P. ostreatus, was further separated using preparative thin layer chromatography by elution with chloroform-methanol (4:1) into 4 fractions (E.3.8.1.1-E.3.8.1.4). The most active fraction (E.3.8.1.1) (78 mg), with 12% activity against P. ostreatus, was further separated by preparative thin layer chromatography into three fractions (E.3.8.1.1.1-E.3.8.1.1.3). The most active fraction (E.3.8.1.1.1) (43 mg), with 17% activity against P. ostreatus, was obtained. An active compound (compound 2) was isolated from fraction E.3.8.1.1.1 by crystallization from diethyl ether and methanol to yield a white crystal, with m.p. of 117-118°C.

**Compound 1:** White crystals, m.p. 159-160°C (lit. m.p. 161-162°C, Tsai et al., 2002) UV λmax (MeOH) nm (log ε): 244 (4.16), [α]D +10.00 (0.5; MeOH) (lit. [α]D +7.5, Endo and Taguchi, 1973), EIMS m/z (rel.int.) 388 (M+) (0.3), 370 (1), 352 (0.6), 284 (1), 256 (1), 226 (12), 209 (38), 208 (36), 176 (47), 148 (100), 119 (19), 91 (26), 73 (39). 1H-NMR (400 MHz, CDCl₃) δ: 2.09 (1H, d, J = 16.8, 6.7 Hz, H-6), 2.72 (1H, t, J = 8.4 Hz, H-6), 2.82 (1H, dd, J = 16.8, 6.7 Hz, H-5), 3.15 (1H, d, J = 7.8 Hz H-9), 3.21 (1H, t, J = 8.7 Hz, H-4), 3.23 (1H, t, J = 9.5 Hz, H-3'), 3.31 (1H, d, J = 8.8 Hz, H-6'), 3.77 (1H, m, H-5'), 3.63 (1H, dd, J = 11.1 Hz, 2.2 Hz, H-2'), 3.70 (3H, s, H-12), 3.85 (1H, d, J = 11.7 Hz, H-6'), 4.17 (1H, d, J = 7.4 Hz, H-10), 4.30 (1H, d, J = 14.6 Hz, H-10'), 4.70 (1H, d, J = 7.4 Hz, H-1'), 5.16 (1H, d, J = 7.9 Hz, H-1), 5.79 (1H, bs, H-7), 5.70 (1H, s, H-3'). 13C-NMR (100 MHz, CDCl₃) δ: 37.4 (C-12), 40.5 (C-6), 47.8 (C-9), 52.5 (C-5), 62.2 (C-6'), 63.5 (C-10), 72.4 (C-4'), 75.7 (C-3'), 78.2 (C-2'), 79.2 (C-5'), 99.0 (C-1), 110.1 (C-1'), 113.4 (C-4), 129.1 (C-7), 145.6 (C-8), 154.2 (C-3), 170.3 (C-11).

**Acetate of compound 1:** A mixture of compound 1 (5 mg) and acetic anhydride (20 μL) in pyridine (20 μL) was kept at room temperature overnight. The mixture was then diluted with water in an ice-bath and stood for 1-2 h. A white precipitate of compound 1 pentaacetate was filtered with suction and dried in a vacuum dryer to give a white crystal (8.2 mg), m.p. 132-133°C after recrystallization from methanol. (lit. m.p. 135-136°C, Endo and Taguchi, 1973). EIMS m/z (rel.int.) 598 (M+), 583 (0.3), 460 (0.3), 376 (0.2), 331 (88), 271 (3), 169 (100), 127 (11), 109 (39), 91 (5), 59 (2). The MS spectrum of compound 1 penta-acetate coincided with that of the authentic geriposide penta-acetate prepared from geriposide by acetylation with acetic anhydride and pyridine. The mixed-melting point test of compound 1 penta-acetate and authentic geriposide penta-acetate was unaltered.

**Compound 2:** White crystals, m.p. 117-118°C, (lit. m.p. 120-121°C, Koo et al., 2004) UV λmax (CHCl₃) nm (log ε): 258 (3.83), [α]D +133.99 (0.5; CHCl₃) (lit. [α]D +109,
Acetate of compound 2: A mixture of compound 2 (5 mg) and acetic anhydride (20 μL) in pyridine (20 μL) was kept at room temperature overnight. The mixture was then diluted with water in an ice-bath and stood for 1-2 h and extracted twice with ethyl acetate. The ethyl acetate solution was washed with aq. NaHCO₃ followed by a 1N HCl solution and dried over anhydrous sodium sulfate. After evaporation of the solution, oily products of compound 2 diacetate were obtained. The acetate was purified by column chromatography to give colorless oily products (6.8 mg). EIMS m/z (rel.int.) 310 (M⁺0.1), 309(0.5), 250(30), 206(27), 190(88), 162(71), 148(100), 147(92), 120(45), 119(48), 91(56), 78(58), 77(46), 65(15), 59(37). The EI MS spectra of compound 2 diacetate coincided with those of authentic gentinip diacetate prepared by acetylation with acetic anhydride and pyridine.

RESULTS

Screening of antifungal activity from six plant methanol extracts: Six plants were tested for their antifungal activity. The antifungal activity of each methanol extract from T. orientalis, D. innoxia, L. japonicum, J. chinensis, G. jasminoides and M. japonica against P. ostreatus was 14.5, 4, 10, 7.49 and 7% respectively as shown in Fig. 1. The antifungal assays showed that G. jasminoides had the highest level of activity among the plants tested and selected for isolation, therefore the other five plant extracts were excluded from further analysis.

Isolation of antifungal compounds from G. jasminoides: The G. jasminoides methanol extracts were suspended in water and then partitioned with n-hexane, chloroform, ethyl acetate and n-butanol, respectively to give each soluble. Each soluble was subjected to antifungal assays. The assays against P. ostreatus confirmed that n-butanol was more active than the others, as shown in Table 1. In addition, the amounts of n-hexane and chloroform solubles were small and Thin Layer Chromatography (TLC) profiles of n-butanol and ethyl acetate solubles were similar. So, n-butanol and ethyl acetate solubles were used for further separation of the antifungal compounds.

Antifungal assay-guided fractionation of the n-butanol solubles by silica gel Column Chromatography (CC) and elution with n-hexane-chloroform-methanol gave one fraction (B.2) with 50% activity. Repeated column chromatography of fraction B.2 over silica gel and preparative thin layer chromatography resulted in the isolation of a fraction (B.2.6.2.1) with 20% activity (Fig. 2). Compound 1 was isolated as white crystals from fraction B.2.6.2.1 by recrystallization from acetone in a yield of 0.3% of the methanol extracts.

Antifungal assay-guided fractionation of the ethyl acetate solubles by silica gel column chromatography and elution with n-hexane-chloroform-methanol gave one fraction (E.3) with 20% activity. Repeated column chromatography of fraction E.3 over silica gel and preparative thin layer chromatography resulted in the isolation of a fraction (E.8.1.1.1) with 17% activity (Fig. 3). Compound 2 was isolated as white crystals from fraction E.8.1.1.1 by recrystallization from diethyl ether and methanol in a yield of 0.03% of the methanol extracts.

Identification of antifungal compounds from G. jasminoides: Compound 1 was a white crystal, with a melting point of 159-160°C. The reddish brown
Fig. 2: The antifungal n-butanol solubles in the methanol extracts of G. jasminoides active against P. ostreatus. Bars indicate SD

Fig. 3: The antifungal ethyl acetate solubles in the methanol extracts of G. jasminoides active against P. ostreatus. Bars indicate SD

colorization on spraying with concentrated sulfuric acid and dark brown coloration on spraying with anisaldehyde-sulfuric acid reagents on the TLC plate indicated the presence of a terpenoid having a glucose moiety. The EI mass (MS) spectrum of compound 1 showed a molecular ion peak at m/z 388, corresponding with the molecular formula C_{19}H_{32}O_{11}. The MS spectrum of compound 1 was characteristic of a monoterpene with a base peak at m/z 148 matched to an iridoid moiety [M'-glucose-CH_{2}OCOH]. Several important fragments ions, at m/z 209, 208 and 176, were also observed. From the analysis of the mass spectrum, compound 1 was suggested to be an iridoid compound having a glucose and methoxy carbonyl moiety. The UV spectrum of compound 1 showed maximum absorbance at 244 nm, characteristic of an iridoid compound (Franzyk et al., 1997). Acetylation of compound 1 with acetic anhydride in pyridine gave crystalline penta-acetate, with a m.p. of 132-133°C. The EI MS spectrum of acetate showed molecular ion peak at m/z 598, with the molecular formula C_{27}H_{36}O_{16}. In the 'H-NMR spectrum of compound 1, signals from seven glucoseyl protons (H-1', H-6', H-2', H-5', H-6', H-3' and H-4') at 4.70, 3.85, 3.63, 3.37, 3.36, 3.23 and 3.21 ppm; two cyclic ethylene protons (H-3 and H-7) at 7.50 and 5.79 ppm, two hydroxymethylene protons (H-10 and H-10) at 4.30 and 4.17 ppm; three cyclic methine protons (H-1, H-9 and H-5) at 5.16, 3.15 and 2.82 ppm, two cyclic methylene protons (H-6 and H-6) at 2.09 and 2.07 and one methoxy group (H-12) at 3.70 ppm, respectively, were observed. In the 13C-NMR spectrum, the carbon signal of two cyclic ethylene groups (C-3 and C-4 and C-8 and C-7) at 154.2, 113.4 and 145.6, 129.1 ppm, one α-carbonyl group (C-11) at 170.3 ppm, one methoxyl group (C-12) at 37.4 ppm, three methine carbons (C-1, C-5, and C-9) at 99.0, 52.5 and 47.8 ppm, one cyclic methylene group (C-6) at 40.50 ppm, six glucosyl carbons (C-1', C-5', C-2', C-3', C-4', and C-6') at 101.1, 79.2, 78.7, 75.7, 72.4 and 62.2 ppm and one hydroxymethyl group (C-10) at 63.5 ppm, respectively, were observed. The 'H-NMR and 13C-NMR spectra of compound 1 and its acetate derivatives strongly indicated compound 1 to be geniposide. The 'H-NMR and 13C-NMR spectra of the isolated compound 1 coincided with those of authentic geniposide. The mixed melting point test of compound 1 and the authentic sample was not depressed. Therefore, compound 1 was identified as geniposide.

Compound 2 was composed of white crystals, with a melting point of 117-118°C. The reddish pink to brown coloration on spraying with concentrated sulfuric acid on the TLC plate indicated the presence of terpenoid. The EI MS spectrum of compound 2 showed a molecular ion peak at m/z 226 corresponding to the molecular formula C_{19}H_{32}O_{11}. The UV spectrum of compound 2 showed maximum absorption at 258 nm which is characteristic of an iridoid compound. Acetylation of compound 2 with acetic anhydride in pyridine yielded an oily diacetate. The EIMS spectrum of the acetylated compound showed a molecular ion peak at m/z 310 which corresponded to the molecular formula C_{21}H_{34}O_{12}. In the 'H-NMR spectrum of compound 2, signals from two cyclic ethylene protons (H-3 and H-7) at 7.53 and 4.84 ppm, one hydroxymethylene (H-10) group at 4.31 ppm; three methine protons (H-1, H-9 and H-5) at 5.89, 3.23 and 2.91 ppm; one methoxyl group (H-12) at 3.74 ppm and two methylene protons were observed (H-6 and H-6) were observed at 2.55 and 2.09 ppm, respectively. In the 13C-NMR spectrum, the carbon signals of two cyclic ethylene groups (C-3 and C-4 and C-8 and C-7) at 152.3, 110.9 ppm and 141.9, 130.9 ppm; one α-carbonyl group (C-11) at 167.7 ppm; one methoxyl group (C-12) at 36.7 ppm; three methane groups (C-1, C-5
had higher antifungal activity against *P. ostreatus* as well as two other fungi tested, *F. oxysporum* and *C. cassicola*, than genipin. Both genipin and geniposide had better antifungal activity against *P. ostreatus* than *F. oxysporum* and *C. cassicola*. Geniposide had similar antifungal activity against *F. oxysporum* (16%) and *C. cassicola* (17%). Genipin demonstrated much lower activity against *C. cassicola* (10%), however, activity increase significantly against *F. oxysporum* (15%) and *P. ostreatus* (18%). The IC₅₀ values of geniposide and genipin in the three fungi are shown in Table 2. Geniposide and genipin demonstrated dose-dependent inhibition of the fungus. Geniposide had a potent antifungal activity; it had IC₅₀<100 μg mL⁻¹ concentrations against all plant pathogenic fungi tested. On the contrary, genipin showed IC₅₀<100 μg mL⁻¹ only against *P. ostreatus*.

**DISCUSSION**

Screening of plants having antifungal activity showed that *G. jasminoides* methanol extracts had the most active antifungal activity against *P. ostreatus*. Antifungal monoterpene compounds from *G. jasminoides* were isolated for the first time from n-buthanol solubles and ethyl acetate solubles, respectively. Repeated silica gel column chromatography of n-buthanol and ethyl acetate solubles afforded two antifungal compounds. The compounds were identified as geniposide, an iridoid glucoside and genipin, the aglycone of geniposide, by instrumental analysis and comparison with authentic standards (melting point, MS, ¹H and ¹³C-NMR). Geniposide and genipin has been isolated from Gardenia species (Choi et al., 2001). However, there are no reports about the activity of genipin and geniposide against wood-rotting fungi. This is the first report that geniposide and genipin from *G. jasminoides* are antifungal compounds against wood-rotting fungi.

Both antifungal compounds isolated from *G. jasminoides* demonstrated a potent antifungal activity against *P. ostreatus* and two other fungi pathogenic to plants, i.e., *Corynespora cassicola* and *Fusarium oxysporum*. *C. cassicola* generates necrotic spots not only on leaves but also on stems, roots, flowers or fruits of more than 70 plants species from tropical and subtropical countries (Silva et al., 1995, 2000). *F. oxysporum* attacks plant root and causes vascular wilt, yellows, root rot and damping off (Benner, 1993).
The potential activities of these natural products are essentially dependent on their structure and physical properties. The significance of iridoid and terpenoid glycosides in pharmaceuticals has been reviewed according to their different activities. The antifungal mechanism of iridoid compounds is not yet clear. The presence of a lactone group in a compound may lead to specific reactivity with a sulphhydryl group (Hall et al., 1977). However, lactone groups act as inhibitory substances against several microorganisms due to their ability to penetrate microbial cells (Rai et al., 2003). The variation in sensitivity may be due to the permeability of the mycelia and spore wall to the different fungi tested. The antifungal activity of terpenoid glycosides may differ among compounds and conditions (Mares, 1987). Several mechanisms for antifungal activities of these compounds occur at the cellular level. First of all, terpenoid glycosides provide a chemical barrier that produces extracellular enzymes against fungi (Roldán-Arjona et al., 1999). Alternatively, the compounds had a direct effect on the cell membrane by modifying structural-functional properties (Anisimov et al., 2000). Other factors include the position of hydroxyl groups, attachment point of a sugar on an aglycone and number of sugar units and type of sugar linkage capable of changing the polarity of these compounds (Tokaï et al., 1988). In addition to the presence and amount of sugar, the configurations of glycosides have great importance (Chandel and Rastogi, 1980).

CONCLUSION

_Gardenia jasminoides_ was determined to have antifungal activity. Bioassay-guided fractionation of the leaves and stem extracts yielded two active compounds. The active antifungal compounds isolated from _G. jasminoides_ were identified as geniposide and genipin. Geniposide was obtained from _n_-butanol solubles while genipin was obtained from ethyl acetate solubles. Both compounds exhibited activity against the wood rotting fungus, _P. ostreatus_. Antifungal assays against two plant pathogenic fungi, _C. cassiicola_ and _F. oxysporum_, showed that geniposide and genipin also had antifungal activity. Genipin and geniposide demonstrated a dose dependent effect on selected fungi. These findings provide the first evidence of antifungal activity of geniposide and genipin isolated from _G. jasminoides_ against agricultural pathogens. The compounds may become bio fungicides, with no environmental side effects.

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REFERENCES


