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Note

Anti-oomycete Activity of Furanocoumarins from Seeds of *Psoralea corylifolia* against *Phytophthora infestans*

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In the course of a searching natural antifungal compounds from plant seeds, we found that the methanol extract of *Psoralea corylifolia* seeds showed potent control efficacy against tomato late blight caused by *Phytophthora infestans* and wheat leaf rust *Puccinia recondita*. Under bioassay-guided purification, we isolated two furanocoumarins, psoralen and isopsoralen, with anti-oomycete activity against *P. infestans*. By 1-day protective application, both compounds strongly reduced the disease development of *P. infestans* on tomato seedlings, but hardly controlled development of leaf rust on wheat seedlings. This is the first report on the anti-oomycete activity of *P. corylifolia* as well as that of psoralen and isopsoralen.

Keywords : antifungal activity, isopsoralen, *Psoralea corylifolia*, *Phytophthora infestans*, psoralen

Phytophthora infestans (Mont.) de Bary, previously classified to the Kingdom Fungi, belongs to Pythiaceae, Pythiales, Oomycetes, Oomycota, Chromista. This oomycete causing late blight disease of potato and tomato is one of the most destructive pathogens with a worldwide distribution. Unlike most *Phytophthora* species, which causes soil-borne root-rotting disease, *P. infestans* is a specialized pathogen, primarily causing disease on the foliage, stems, potato tubers, and tomato fruits via airborne asexual sporangia during the growing season (Shattock, 2002). Chemical control remains the main measure to reduce the incidence of the disease in most crops, especially those grown under greenhouse conditions. On a worldwide basis, fungicidal control of *P. infestans* accounts for one-fourth of the total annual expenditure for fungicides on all crops (Erwin and Ribeiro, 1996). However, fungicide-resistant isolates have developed rapidly and have become widespread worldwide (Cohen and Reuveni, 1983; Gisi et al., 2000; Ishii et al., 2001; Shattock, 2002). In addition, the demand for organically grown foods is rapidly increasing because of concerns about human health and environmental quality. Biocontrol using plant-derived natural products is regarded as an 'environmentally friendly' alternative to synthetic fungicides for the protection of tomato and potato against late blight caused by *P. infestans*.

Psoralea corylifolia L. (Leguminosae) is widely distributed from India to Southeast Asian countries. The dry seeds of the plant, Malaytea scurfpea, named Pagoji or Bogolji in Korea, have been used to treat a wide range of diseases and conditions as a traditional medicine in Korea, China, and other countries. The seed-oil is used externally for the treatment of leucodermy, psoriasis, and leprosy in Indian folk medicine (Kondo et al., 1990). The seed extract containing coumarins, flavonoids, and meroterpene phenols was found to exert anti-oxidative, antimicrobial, anti-inflammatory, anti-platelet, anti-tumor, anti-mutagenic, and insect hormonal activities (Bapat et al., 2005; Haraguchi et al., 2002; Katsura et al., 2001; Khatune et al., 2004; Sun et al., 1998; Tsai et al., 1996; Wang et al., 2001). Recently, a simple flavonoid 4'-methoxy flavone from this plant was reported to display antifungal activity against dermatophytic fungi such as *Trichophyton rubrum* and *Trichophyton mentagrophytes* (Rajendra Prasad et al., 2004), and a few coumarins from this plant also showed antibacterial activities (Khatune et al., 2004). To the best of our knowledge, anti-oomycete activity of this plant as well as its metabolites has not been performed.

Plant seeds contain rich nutrients as well as antimicrobial compounds, which have been considered very important for plant defense during germination (Chen et al., 1999; Garcia-Olmedo et al., 1998). In order to search natural antifungal compounds from plant seeds, we tested the *in vivo* antifungal activity of various plant fruits (seeds) against plant pathogenic fungi (Choi et al., 2006). In this research, the methanol extract of *P. corylifolia* fruits showed remarkable control efficacy against tomato late blight caused by *P. infestans*. We then isolated two furanocoumarin compounds from the plant by bioassay-guided phytochemical investigation and determined their chemical structures by instrumental analyses. In this study, we report the isolation and identification of anti-oomycete compounds from methanol

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extract of *P. corylifolia* seeds. In addition, the disease control efficacy of the purified compounds was examined against five phytopathogenic fungi as well as *P. infestans*.

The dried seeds of *P. corylifolia* were purchased in September 2007 at Shinheung Oriental Drugstore, Daegu, Korea. A voucher specimen was deposited at the School of Biotechnology, Yeungnam University. Psoraleae Semen (1 kg) was percolated in 1.5 L of *n*-hexane at room temperature to remove fats. The defatted materials were extracted five times with methanol at room temperature to give an extract (352 g). The methanol extract was suspended in distilled water and successively partitioned with *n*-hexane, chloroform, ethyl acetate, and 1-butanol to yield 50 g, 125 g, 45 g, and 50 g fractions, respectively. Of the four solvent-soluble fractions, the chloroform fraction was the most active. The $CHCl_3$ fraction was subjected to silica gel column with the elution of a hexane-ethyl acetate solvent gradient, which gave 20 subfractions (Fr. C1-C20). According to an *in vivo* assay against *P. infestans*, the 13th fraction (Fr. C13) and 15th fraction (Fr. C15) with potent activity were chemically investigated to elucidate the compounds to exhibit the bioactivity. Fr. C13 (240 mg) and Fr. C15 (400 mg) were dissolved in a mixture of *n*-hexane + ethyl acetate and a few drops of methanol and then placed at room temperature for 24 h, which afforded colorless crystals, compounds **1** (85 mg) and **2** (120 mg), respectively.

The ^{1}H-NMR spectrum of compound **1** showed characteristic signals for coumarin at δ 6.36 and 7.78 (each 1H, d, J = 9.6 Hz) due to the H-3 and H-4, respectively, in the α-pyrone ring. Two proton signals at δ 6.82 (1H, d, J = 2.4 Hz) and 7.68 (1H, dd, J = 0.9, 2.4 Hz) were indicative of a furan ring. Two singlet signals at δ 7.67 and 7.46 ascribed to H-5 and H-8, respectively, indicated that **1** has a linear furanocoumarin skeleton. In the EI-MS spectrum of **1**, a molecular ion peak appeared at *m/z* 186 and the base peak appeared at *m/z* 158 due to the elimination of a CO group. Based on the analysis of the above ^{1}H-NMR and EI-MS data together with the ^{13}C-NMR data, **1** was assigned to be psoralen, which was reported from *P. corylifolia*. The spectral data for **1** agreed with those previously reported in the literature (Guo et al., 2005).

EI-MS, ^{13}C-NMR and DEPT data for compound **2** indicated the molecular formula $C_{11}H_6O_3$ (nine unsaturations). The ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1**. However, two singlet proton signals at δ 7.67 and 7.46 corresponding with H-5 and H-8 in **1**, respectively, were missing in **2**. Instead, two *ortho*-coupled proton signals at δ 7.34 and 7.40 appeared with 8.5 Hz of coupling constants, indicating that **2** has an angular furanocoumarin skeleton. On the basis of ^{1}H-NMR, ^{13}C-NMR, and EI-MS data, the structure of **2** was assigned as isopsoralen (**2**) (Guo et al., 2005). Thus, the chemical structures of two anti-oomycete compounds were determined to be psoralen and isopsoralen by mass and NMR spectral data (Guo et al., 2005) (Fig. 1).

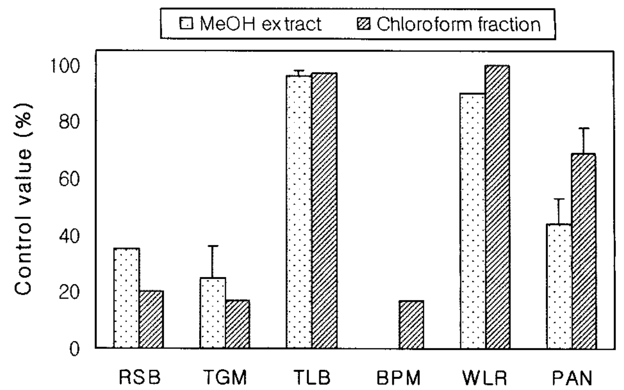

Fig. 1. *In vivo* antifungal activity of the methanol extract (2,000 μg/ml) from *Psoralea corylifolia* seeds and its chloroform-soluble fraction (2,000 μg/ml) against six phytopathogenic fungi. Seedlings were inoculated with spores or mycelial suspensions of the test organisms 1 day after spraying with the chemical solutions. RSB, rice sheath blight; TGM, tomato gray mold; TLB, tomato late blight; BPM, barley powdery mildew; WLR, wheat leaf rust; and PAN, red pepper anthracnose. The disease severity of untreated control plants was 90% for RSB, 60% for TGM, 65% for TLB, 15% for WLR, 30% for BPM, and 40% for PAN.

The methanol extract of *P. corylifolia* seeds, its solvent-soluble fractions, and the purified compounds were tested *in vivo* for antifungal activity against the following six plant diseases: rice sheath blight (*Corticium sasaki* Matsu), tomato gray mold (*Botrytis cinerea* Pers.:Fr.), tomato late blight (*P. infestans*), barley powdery mildew (*Blumeria graminis* (DC.) Golovin ex Speer f. sp. *hordei*), wheat leaf rust (*Puccinia recondita* Rob ex Desm), and red pepper anthracnose (*Colletotrichum coccodes* Wallr.). The *in vivo* antifungal bioassays were performed as described previously (Kim et al., 2001; Cho et al., 2006). Rice (*Oryza sativa* L., cv. Nakdong), tomato (*Solanum lycopersicum* Mill., cv. Seokwang), barley (*Hordeum sativum* Jessen, cv. Dongbori), wheat (*Triticum aestivum* L., cv. Chokwang), and red pepper (*Capsicum annuum* L., cv. Bugang) plants were grown in plastic pots (4.5-cm diameter) in a greenhouse at 25±5°C for 1 to 4 weeks. The methanol extract and 1-butanol fraction were dissolved in methanol, and the other fractions obtained during the purification and purified compounds were diluted in acetone. Each solution was then

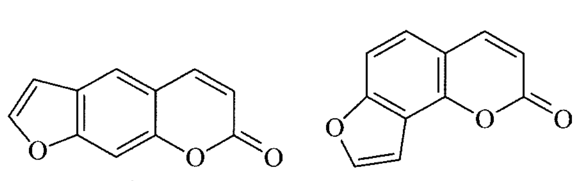

Fig. 2. Chemical structures of psoralen (**1**) and isopsoralen (**2**).

added to Tween 20 (250 μg/ml) solutions at final concentrations of 5% (v/v) for methanol and 10% (v/v) for acetone. The plant seedlings were sprayed with each solution until run-off. Control plants were sprayed with Tween 20 solution containing 5% methanol or 10% acetone. After 24 h, the treated plant seedlings were inoculated with spores or mycelial suspensions of one of the six plant pathogens, followed by rating disease symptoms 3-7 days after inoculation.

Pots were arranged as a randomized complete block with three replicates per treatment. Validamycin A for rice sheath blight, fludioxonil for tomato gray mold, dimethomorph for tomato late blight, flusilazole for wheat leaf rust, benomyl for barley powdery mildew, and dithianon for red pepper anthracnose were applied as positive controls. Experiments were conducted in a growth chamber.

The mean value of three estimates for each treatment was converted into percentage fungal control. The percentage of disease control was determined using the following equation: control value (%)=100[(A−B)/A], where A=the area of infection (%) on leaves or sheaths sprayed with Tween 20 solution alone and B=the area of infection (%) on treated leaves or sheaths.

As shown in Fig. 1, the methanol extract of *P. corylifolia* seeds exhibited control value of more than 90% against tomato late blight caused by *P. infestans* as well as wheat leaf rust caused by *P. recondita* at a concentration of 2,000 μg/ml. It also moderately reduced the development of anthracnose caused by *C. coccodes* on red pepper seedlings. The other plant diseases such as rice sheath blight, tomato gray mold, and barley powdery mildew were hardly controlled by the extract.

A few studies have demonstrated a moderate antifungal activity of the seeds of *P. corylifolia*, especially the essential oil, against skin disease fungi such as *Aspergillus candidus*, *Aspergillus fumigatus*, *Candida albicans*, and *T. mentagrophytes* (Grover and Rao, 1979; Sharma and Singh, 1979; Vaijayanthimala et al., 2000). In this study, we found that the methanol extract of these plant seeds exhibited potent anti-oomycete activity against *P. infestans* as well as antifungal activity against *P. recondita in vivo*.

Bioassay-guided isolation using an *in vivo* assay against

Table 1. *In vivo* antifungal activity of two furanocoumarins isolated from the *Psoralea corylifolia* seeds against six phytopathogenic fungi[a]

Chemical	Conc. (μg/ml)	Disease control (%)					
		RSB[b]	TGM	TLB	BPM	WLR	PAN
Psoralren	100	6±16[c]	7±10	29±20	13±11	13±9.4	8±0.0
	500	24±8.3	0±0.0	82±5.1	42±11	0±0.0	44±15
Isopsoralen	100	0±0.0	7±10	21±10	0±0.0	3±4.7	22±15
	500	0±8.3	36±10	84±8.1	0±0.0	33±0.0	89±3.1
Validamycin A	5	88±0.0	–[d]	–	–		–
	50	100	–	–	–		–
Fludioxonil	5	–	82±5.1	–	–		–
	50	–	100	–	–		–
Dimethomorph	2	–	–	85±1.0	–		–
	10	–	–	100	–		–
Benomyl	1	–	–	–	87±4.7	–	–
	100	–	–	–	100	–	–
Flusilazole	2	–	–	–	–	87±0.0	–
	10	–	–	–	–	100	–
Dithianon	10	–	–	–	–	–	73±6.3
	50	–	–	–	–	–	91±0.0

[a]The compounds were dissolved in acetone (10%) and Tween 20 (250 μg/ml), and they were then sprayed to run off the following seedlings: rice (4-leaf stage), tomato (3-leaf stage), barley (1-leaf stage), wheat (1-leaf stage), and red pepper (4-leaf stage). After 24 h, the treated plant seedlings were inoculated with spores or mycelial suspensions of the pathogens. The disease severity of untreated control plants was 85% for RSB, 70% for TGM, 70% for TLB, 15% for WLR, 30% for BPM, and 23% for PAN.

[b]RSB, rice sheath blight (*Corticium sasaki*); TGM, tomato gray mold (*Botrytis cinerea*); TLB, tomato late blight (*Phytophthora infestans*); BPM, barley powdery mildew (*Blumeria graminis* f. sp. *hordei*); WLR, wheat leaf rust (*Puccinia recondita*); and PAN, red pepper anthracnose (*Colletotrichum coccodes*).

[c]Each value represents the mean±standard deviation of three replicates.

[d]Not tested

P. infestans yielded two furanocoumarins, psoralen and isopsoralen. By 1-day protective application, psoralen (500 μg/ml) and isopsoralen (500 μg/ml) strongly suppressed the disease development of *P. infestans* on tomato seedlings (Table 1). Isopsoralen also exhibited potent *in vivo* antifungal activity against *C. coccodes* on red pepper plants. However, both compounds did not show control efficacy against wheat leaf rust (*P. recondita*), although the methanol extract of this plant displayed potent control efficacy against the rust disease. Further studies are required for isolation and characterization of the antifungal substances active to *P. recondita* from this plant.

Rajendra Prasad et al. (2004) isolated 4'-methoxy flavone from the methanolic extract of *P. corylifolia* seeds as the most active antifungal compound against dermatophytic fungi such as *T. rubrum*, *T. mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum gypseum*. In addition, Newton et al. (2002), reported antimycobacterial activity of bakuchiol purified from *P. corylifolia* seeds; it showed significant antimycobacterial activity (IC_{50}=15.79 μg/ml) against *Mycobacterium aurum*, a model species for the tuberculosis pathogen. In this study, we isolated psoralen and isopsoralen from the same material as the active compounds against tomato late blight caused by *P. infestans*. We also isolated bakuchiol from the chloroform fraction of the methanol extract of this plant by using standard chemical. However, the compound did not show control efficacy against any of the six plant diseases tested in this study (data not shown). These results indicate that each antimicrobial constituent of *P. corylifolia* seeds has a different antimicrobial spectrum.

Psoralen has been isolated from some antifungal plants including *Melicope borbonica* (Rutaceae), *Psychotria spectabilis* Steyerm (Rubiaceae), and *Zanthoxylum americanum* Mill. (Rutaceae) (Bafi-Yeboa et al., 2005; Benevides et al., 2004; Simonsen et al., 2004). However, antifungal activity of this compound was simply described against *Cladosporium cladosporioides* and *C. sphaerospermum* or not demonstrated. Psoralen was also found to be the most active component on patches of leucoderma (Qamaruddin et al., 2002). On the other hand, Sadari et al. (1999) reported that isopsoralen, also known as angelicin, and its synthetic derivatives showed antifungal activity against *C. albicans, Saccharomyces cerevisiae,* and *Aspergillus niger.* In these derivatives, the free 6-OH was found to be important for the antifungal activity. In addition, isopsoralen showed tranquilosedative, anticonvulsant, and central muscle relaxant activities in rats, mice and rabbits (Chandhoke and Ray Ghatak, 1975). To our knowledge, this is the first report of the anti-oomycete activity of psoralen and isopsoralen isolated from *P. corylifolia* seeds. In addition, they did not show phytotoxicity on some crops such as tomato, rice, wheat, barley, and red pepper (data not shown). The results in this study suggest that the methanolic extract of *P. corylifolia* seeds and two furanocoumarins, psoralen and isopsoralen, could be used as a biopesticides for the control of late blight on tomato and potato plants.

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