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In vivo fungicidal activity of medicinal plant extracts against six phytopathogenic fungi

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Abstract
Methanol extracts from 27 medicinal plant species were tested at concentrations of 0.5, 1 and 2 mg/mL for their *in vivo* fungicidal activities against six phytopathogenic fungi. Their efficacy varied with plant pathogen, tissue sampled and plant species. Very strong fungicidal activity was produced by extracts of *Boswellia carterii, Saussurea lappa, Glycyrrhiza uralensis, Piper nigrum*, *Rheum coreanum, Lysimachia foenum-graecum*, *Evodia officinalis*, *Santalum album* and *Curcuma longa* at 2 mg/mL. At 1 mg/mL, *S. album*, *P. nigrum* and *L. foenum-graecum* showed potent fungicidal activity against *Blumeria graminis* f. sp. *hordei*, *Puccinia recondita* and *Magnaporthe grisea*, respectively. *Lysimachia foenum-graecum* exhibited strong fungicidal activity against *M. grisea* at 0.5 mg/mL.

Keywords: *Medicinal plants, fungicidal activity, phytopathogenic fungi,* in vivo *bioassay,* Lysimachia foenum-graecum, Santalum album, Piper nigrum

1. Introduction

Economic losses due to pre- and post-harvest diseases in crops may be 5 – 50%, or even higher in developing countries (Oerke et al. 1994). Over the past several decades, various attempts to control plant diseases have been made at eradication or prevention through the development of synthetic fungicides. Although the latter are effective, their continued or repeated application has disrupted biological control by natural enemies and led to outbreaks in diseases, widespread development of resistance to various types of fungicides (Georgopoulos 1987), toxicity to non-target organisms and environmental problems (Brown 1978; Hayes and Laws 1991). Decreasing efficacy and increasing concern over the adverse environmental effects of earlier types of fungicides have brought about the need for the development of new types of selective control alternatives and crop protection methods without, or with reduced, use of conventional fungicides.

Some plants may be alternatives to currently used disease control agents, since they constitute a rich source of bioactive chemicals (Swain 1977; Wink 1993). Because these chemicals are often active against a limited number of species, including the specific target species, are biodegradable to nontoxic products and are potentially suitable for integrated use, they could be developed as new classes of possibly safer disease control agents. Therefore, much effort has focused on plant materials for potentially useful products as commercial fungicides or as lead compounds (Balandrin 1985; Miyakado 1986; Benner 1993; Hedin et al. 1997).

In greenhouse studies we assessed the fungicidal activity of 27 medicinal plant extracts towards six phytopathogenic fungi which caused serious damage to crops in Korea.

2. Materials and methods

2.1. Fungal strain and culture conditions

The six phytopathogenic fungi used in this study were *Blumeria graminis* f. sp. *hordei*, *Botrytis cinerea*, *Magnaporthe grisea*, *Phytophthora infestans*, *Puccinia recondita* and *Thanatephorus cucumeris*. *Magnaporthe grisea*, *T. cucumeris*, *B. cinerea*, and *B. graminis* f. sp. *hordei* were supplied by the KRICT (Korea Research Institute of Chemical Technology). *Phytophthora infestans* and *P. recondita* were supplied by the Kangneung National University and Incheon University, respectively. Except for *P. recondita* and *B. graminis* f. sp. *hordei* which are unable to grow on artificial media, the other fungi were routinely maintained on potato dextrose agar (PDA) and V-8 agar slants, and were kept for stock at 4°C. *Puccinia recondita* and *B. graminis* f. sp. *hordei* were maintained on wheat and barley, respectively, as host plants.

2.2. Plant material and sample preparation

A total of 27 medicinal plant species frequently used for oriental medicine were selected and are

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listed in Table I. They were purchased from a 'Boeun' medicinal herb shop at Kyungdong Market. They were powdered using a blender. Each sample (500 g) was extracted two times with 5 L of methanol at room temperature and filtered (Toyo filter paper No. 2, Toyo Roshi). The combined filtrate was concentrated *in vacuo* at 35°C using a rotary vacuum evaporator.

2.3. In vivo *bioassay*

The plant samples were tested at concentrations of 2, 1 and 0.5 mg/mL (dried plant extract/distilled water, w/v). Appropriate amounts of the test samples in 0.45 mL of dimethyl sulfoxide (DMSO) were suspended in distilled water with Tween 20 at a concentration of 250 mg/L. Each suspension was sprayed onto the test plants. After evaporation in a greenhouse for 1 day, each pathogen was inoculated into the treated test plants. All treatments were replicated three times. Controls received DMSO-Tween 20 solution. Test plants rice (*Oryza sativa*), tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*), barley (*Hordeum sativum*) and wheat (*Triticum aestivum*) were reared in pots (Ø 4.5 cm) for 1–3 weeks at 25 ± 5°C.

In a test with rice blast (RCB) caused by *M. grisea*, 'Chucheongbyeo' rice plants in the second leaf stage (three plants/pot) were sprayed with each test solution. The plants were inoculated with conidia in distilled water (1×10^6 spores/mL) and kept in a dew chamber (25°C) for 1 day under 100% relative humidity (RH). Treated and control plants were then placed into a growth chamber (26 ± 2°C and 85% RH) for 5 days, after which time the disease severity was rated.

For rice sheath blight (RSB) caused by *T. cucumeris*, each test solution was sprayed onto 'Chucheongbyeo' rice plants in the fourth leaf stage (three plants/pot). Plants were inoculated by pouring inoculum onto the base of the rice plants. Inoculum of *T. cucumeris* was made by incubating mycelial plugs in wheat bran medium at 25°C for 7 days, and was macerated at a rate of 500 g/L distilled water

Table I. Plant species tested.

Plant species	Family name	Tissue sampled	Yield (%)[1]	Main or bioactive compounds	Reference
Acorus gramineus Soland.	Araceae	Root	9.5	β-Asarone	Park et al. (2003)
Acorus calamus var. *angustata* Bess.		Root	10.1	β-Asarone	Schmidt and Streloke (1994)
Boswellia carterii Birdwood	Burseraceae	Resin	88.8	Boswellic acid	Jing et al. (1999)
Artemisia vulgaris L.	Asteraceae	Whole plant	6.6	Thujone	Judžentiene and Buzelytė (2006)
Saussurea lappa Clarke		Root	30.9	Costunolide	Jeong et al. (2002)
Juniperus chinensis L.	Cupressaceae	Wood	5.4	Nootkatin and Hinokitiol	Nakatsuka and Hirose (1955)
Dioscorea batata Decne.	Dioscoreaceae	Root	2.4	Batasin, Dioscin	Korean Pharmacognosy Association (2000)
Illicium verum Hook. f.	Illiciaceae	Fruit	4.9	Anethole	Minakshi et al. (2002)
Agastache rugosa (Fisch. et Meyer) O. Kuntze.	Labiatae	Whole plant	9.5	Estragole	Shin (2004)
Schizonepeta tenuifolia Briq.		Whole plant	8.1	Pulegone	Park et al. (2006)
Machilus thunbergii Siebold & Zucc.	Lauraceae	Root	5.8	Machilin A–E	Shimomura et al. (1988)
Gledisia japonica var. *koraiensis* Nak.	Fabaceae	Fruit	17.3	–	–
Glycyrrhiza uralensis Fisch.		Root	21.9	Licochalcone, glycyrrhizin	Korean Pharmacognosy Association (2000)
Eugenia aromatica Merr. et Perry	Myrtaceae	Flower bud	37.8	Eugenol	Korean Pharmacognosy Association (2000)
Paeonia moutan Sims.	Paeoniaceae	Bark	18.6	Paeonol	Ishiguro et al. (2006)
Piper nigrum L.	Piperaceae	Fruit	10.0	Amides	Park et al. (2002)
Rheum coreanum Nak.	Polygonaceae	Root	41.6	Sennoside	Korean Pharmacognosy Association (2000)
Lysimachia foenum-graecum Hance	Primulaceae	Whole plant	9.0	Triterpene glycosides	Shen et al. (2005)
Evodia officinalis Dode	Rutaceae	Fruit	9.5	Evodamine	Hwang et al. (2001)
Zanthoxylum piperitum DC.		Fruit	28.3	Sanshool I–IV	Korean Pharmacognosy Association (2000)
Santalum album L.	Santalaceae	Wood	9.5	Santalol	Korean Pharmacognosy Association (2000)
Stemona japonica Miq.	Stemonaceae	Root	15.2	Stemonine	Pilli et al. (2000)
Aquilaria sinensis Gilg.	Thymelaeaceae	Wood	6.6	Chromone deratives	Yagura et al. (2005)
Angelica dahurica Benth. et Hook.	Apiaceae	Root	17.7	Coumarins	Kwon et al. (1997)
Cnidium officinale Makino		Root	10.0	Phthalides	Choi et al. (2001)
Nardostachys chinensis Batal.	Valerianaceae	Root	12.9	Calarene	Han et al. (2000)
Curcuma longa L.	Zingiberaceae	Root	10.0	Curcumin	Apisariyakul et al. (1995)

[1](Weight of crude methanol extract/weight of dried test material) × 100.

with a mixer. Treated and control plants were placed into a lighted chamber (28°C) for 10 days, and then the disease severity was rated.

For cucumber gray mold (CGM) caused by *B. cinerea*, 'Hausbackdadagi' cucumber plants in the first leaf stage (one plant/pot) were sprayed with each test solution. The cucumber plant was inoculated with conidia (5×10^5 spores/mL) of *B. cinerea* and then placed into a dew chamber (20°C) for 4 days, after which time the disease severity was rated.

For tomato late blight (TLB) caused by *P. infestans*, each test solution was sprayed onto 'Seokwang' tomato plants in the second leaf stage (one plants/pot). The plants were inoculated with a suspension of 1×10^5 zoosporangia/mL made from 14-day-old culture on V-8 juice agar at 20°C. They were placed into a chamber (18°C) for 5 days, and then the disease severity was rated.

For wheat leaf rust (WLR) caused by *P. recondita*, 'Chokwang' wheat plants in the first leaf stage (five plants/pot) were sprayed with each test solution. The plants were dusted with a suspension of uredospores colonized on their second leaf and then placed into a moist chamber. One day after inoculation, the plants were held in a growth chamber (22°C and 70% RH). The disease severity was measured 10 days after inoculation.

For barley powdery mildew (BPM) caused by *B. graminis* f. sp. *hordei*, healthy young 'Allbori' barley plants with a fully expanded first leaf (four plants/pot) were sprayed with a suspension of each test material. Treated plants were dusted with conidia of *B. graminis* formed on the primary leaf of barley and held in a growth chamber (20°C and 50–60% RH). The disease severity was rated on 10 days after inoculation.

2.4. Data analysis

The test samples' fungicidal activity was indicated by a control value (CV) calculated by the formula CV $(\%) = [(A - B)/A] \times 100$, where A represents the disease area on untreated plants and B represents the disease area on treated plants. Disease area was based on a percentage scale on a leaf or sheath which was inoculated one day after the test compound's application. Disease area was measured with Samsung MW-200B1 Color Image Microscope System. The control value was transformed to arcsine square root values for ANOVA. Treatment means were compared and separated by Scheffe's test (SAS 1999).

3. Results

The *in vivo* fungicidal activity of the test samples against six plant pathogens when treated with 2 mg/mL is shown in Table II. In a test with *M. grisea*, very strong fungicidal activities (>90%) were produced from methanol extracts of *P. nigrum*, *R. coreanum*, *L. foenum-graecum*, and *E. officinalis*. Extracts from *G. uralensis* and *P. moutan* showed strong fungicidal activities (80–90%). Extracts of *N. chinensis* and *C. longa* showed moderate activity (60–80%). The other samples exhibited activities <60%.

With *T. cucumeris*, *P. nigrum* showed weak activity, and the other samples showed no fungicidal activity.

In a test with *B. cinerea*, very strong fungicidal activity was produced from extracts of *L. foenum-graecum*. Strong fungicidal activity was observed from extracts of *R. coreanum*. Moderate activity was observed from extracts of *A. rugosa*, *G. uralensis*, *P. nigrum*, *E. officinalis* and *N. chinensis*. The other plant species revealed weak or no fungicidal activity.

With *P. infestans*, over 90% CV was obtained with extracts of *P. nigrum*. Strong antifungal activity was obtained in extracts of *S. lappa*, *A. rugosa*, *L. foenum-graecum* and *C. longa*. Extracts of *B. carterii*, *A. sinensis* and *N. chinensis* showed moderate activity. The other plant species revealed weak or no antifungal activity.

In a test with *P. recondita*, extracts from *B. carterii*, *S. lappa*, *G. uralensis*, *P. nigrum*, *R. coreanum*, *L. foenum-graecum*, *E. officinalis*, *S. album*, and *C. longa* revealed very strong fungicidal activity whereas strong activity was observed with extracts of *J. chinensis, A. rugosa* and *M. thunbergii*. Moderate activity was observed in extracts of *E. aromatica*, *P. moutan, Z. piperitum, A. sinensis, C. officinale* and *N. chinensis*. The other plant samples showed weak or no antifungal activity.

The results from *B. graminis* f. sp. *hordei* showed that *S. album* exhibited very strong antifungal activity, and extract of *B. carterii*, *I. verum*, *A. rugosa*, *P. nigrum* and *L. foenum-graecum* showed potent fungicidal activity. Extract of *J. chinensis* showed moderate activity. The other samples showed weak or no antifungal activity. Of all the plants suspensions tested, those showing very strong fungicidal activity were diluted to reduce their concentration (1 and 0.5 mg/mL) and tested again (Table III). At 1 mg/mL, extracts of *L. foenum-graecum* exhibited significant fungicidal activity toward *M. grisea*, and *P. nigrum* showed very strong fungicidal activity toward *P. recondita*. Extract from *S. album* revealed highly effective antifungal activity against *B. graminis* f. sp. *hordei*. Extracts from *P. nigrum* and *L. foenum-graecum* showed strong antifungal activity toward *P. infestans* and *P. recondite*, respectively. At 0.5 mg/mL, *L. foenum-graecum* showed very strong fungicidal activity against *M. grisea*. Strong activity was observed with extracts of *S. album* and *L. foenum-graecum* against *B. graminis* f. sp. *hordei* and *P. recondita*, respectively.

4. Discussion

In the greenhouse studies with methanol extracts from 27 plant samples, the responses varied with

Table II. Fungicidal activity of plant extracts against six plant pathogenic fungi.[1]

Plant species	Control value (mean ± S.E., %)					
	RCB[2]	RSB	CGM	TLB	WLR	BPM
Acorus gramineus	41 ± 2.8abcd[3]	15 ± 0.5bc	36 ± 1.7efgh	6gh	33.0 ± 1.5efg	0e
Acorus calamus var. *angustata*	15 ± 0.5cd	10 ± 2.0bc	51.3 ± 1.3cdefg	5 ± 1.0gh	32 ± 2.0efg	0e
Boswellia carterii	0d	30ab	60 ± 3.4bcdef	72 ± 2.0abcd	92 ± 1.5abc	90 ± 2.8a
Artemisia vulgaris	16 ± 1.7cd	9.3 ± 1.3bc	60 ± 0.5bcdef	32 ± 2.0efg	52 ± 2.0def	25 ± 8.6cd
Saussurea lappa	33 ± 2.3bcd	0c	31.3 ± 1.3fghi	83.6 ± 3.1ab	100a	0e
Juniperus chinensis.	14 ± 4.0d	0c	20ghi	20 ± 2.8fgh	86 ± 3.4abcd	80 ± 5.0a
Dioscorea batatas	0d	15 ± 2.8bc	0i	0h	32 ± 1.0egf	11.3 ± 1.3de
Illicium verum	16 ± 1.1cd	0c	34 ± 4.0efgh	53 ± 1.7cde	20 ± 0.5fg	90 ± 1.1a
Agastache rugosa	33 ± 7.5bcd	23.3 ± 3.3abc	64.6 ± 7.6abcde	82 ± 2.0ab	90 ± 5.0abcd	82 ± 1.1a
Schizonepeta tenuifolia	0d	0c	34 ± 4.0efgh	0h	53 ± 1.7cdef	0e
Machilus thunbergii	41 ± 3.4abcd	0c	34 ± 2.0efgh	0h	86 ± 3.4abcd	54.6 ± 4.6b
Gledisia japonica var. *koraiensis*	0d	0c	52 ± 2.3cdefg	0h	34.3 ± 2.1efg	48.3 ± 1.6bc
Glycyrrhiza uralensis	82.6 ± 1.7ab	0c	80 ± 1.1abc	60bcde	92 ± 2.0ab	0e
Eugenia aromatica	0d	0c	28 ± 4.6fghi	0h	80 ± 2.8abcd	25 ± 5.0cd
Paeonia moutan	86 ± 3.4ab	0c	34 ± 4.0efgh	0h	66 ± 9.2abcde	25 ± 2.8cd
Piper nigrum	96 ± 2.0a	45 ± 5.0a	80abc	100a	100a	82 ± 2.0a
Rheum coreanum	93 ± 1.7ab	0c	85 ± 2.8ab	21.3 ± 1.3fgh	91 ± 2.3ab	0e
Lysimachia foenum-graecum	100a	26.6 ± 1.6ab	94 ± 3.4a	88 ± 4.1ab	100a	82 ± 1.1a
Evodia officinalis	91 ± 2.0ab	0c	72 ± 2.0abcd	46 ± 3.4def	93 ± 1.7abcd	0e
Zanthoxylum piperitum	0d	30 ± 2.8ab	0i	46 ± 6.0def	66.6 ± 4.4abcde	0e
Santalum album	16 ± 6.0	25 ± 2.8ab	52 ± 7.0cdefg	46 ± 3.4def	93 ± 3.0ab	100a
Stemona japonica	33 ± 3.0bcd	0c	50.3 ± 1.7cdefg	0h	0g	0e
Aquilaria sinensis	0d	10bc	36 ± 1.7efgh	65 ± 5.0bcd	80 ± 1.1abcd	0e
Angelica dahurica	0d	0c	11.3 ± 1.3hi	0h	0g	0e
Cnidium officinale	0d	0c	34.3 ± 4.3efgh	5.3 ± 1.2gh	80abcd	0e
Nardostachys chinensis	80 ± 5.7ab	30 ± 10ab	80 ± 4.0abc	72.6 ± 2.6abcd	66 ± 2.3abcd	0e
Curcuma longa	76 ± 2.8abc	20 ± 0.5bc	44 ± 4.0defgh	82 ± 1.5ab	96 ± 1.1ab	0e

[1]2 mg/mL treatment. [2]RCB, *Magnaporthe grisea*; RSB, *Thanatephorus cucumeris*; CGM, *Botrytis cinerea*; TLB, *Phytophthora infestans*; WLR, *Puccinia recondita*; and BPM, *Blumeria graminis* f. sp. *hordei*. [3]Means within a column followed by same letters are not significantly different ($P = 0.05$, Scheffe's test).

Table III. Fungicidal activity of plant extracts against five plant pathogenic fungi.

Plant species	Conc. (mg/mL)	Control value (mean ± S.E., %)				
		RCB[1]	CGM	TLB	WLR	BPM
Boswellia carterii	1.0	–[2]	0c	15 ± 1.7bc	32.3 ± 1.3d	0f
Saussurea lappa	1.0	–	–	15 ± 2.5bc	53 ± 3.0bc	–
Illicium verum	1.0	–	–	0c	–	16 ± 6.0e
Agastache rugosa	1.0	–	48 ± 4.6a	15 ± 5.0bc	0e	0f
Glycyrrhiza uralensis	1.0	0d[3]	47 ± 1.7ab	0c	0e	–
Piper nigrum	1.0	0d	47 ± 1.1ab	88 ± 1.1a	94 ± 2.0a	41.3 ± 1.3c
	0.5	–	10.6 ± 0.3c	73 ± 1.1a	42 ± 2.0cd	25 ± 1.7de
Rheum coreanum	1.0	0d	47 ± 4.0ab	–	0e	–
Lysimachia foenum-graecum	1.0	100a	70 ± 2.8a	15 ± 5.0bc	88 ± 2.0a	33 ± 1.1cd
	0.5	90 ± 1.1b	0c	0c	86.6 ± 0.8a	–
Evodia officinalis	1.0	16 ± 1.1c	0c	0c	66 ± 6.0b	–
Santalum album	1.0	–	22.6 ± 1.6bc	0c	10 ± 2.3e	99 ± 0.5a
	0.5	–	–	–	–	80 ± 2.8b
Curcuma longa	1.0	0d	11 ± 2.3b	24.3 ± 4.3b	45.6 ± 0.6cd	–

[1]See Table II. [2]Not tested. [3]Means within a column followed by same letters are not significantly different ($P = 0.05$, Scheffe's test).

plant species, plant tissue and pathogen used. The plants belonging to the families Burseraceae, Asteraceae, Fabaceae, Piperaceae, Polygonaceae, Primulaceae, Rutaceae, Santalaceae and Zingiberaceae showed very strong fungicidal activity. Jacobson (1989) pointed out that the most promising botanicals as sources of novel plant-based pesticides for use at present and in the future are species of the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae and Canellaceae.

Various compounds including phenolics, terpenoids and alkaloids exist in plants. These compounds jointly or independently contribute to the generation of biological activities. These phytochemicals act in many ways on various types of disease complexes, and may be applied to the plants in the same way as other agricultural chemicals. They are being considered as potential alternatives to synthetic fungicides, or as lead compounds for new classes of synthetic fungicides, such as podoblastin produced by *Podophyllum peltatum*. However, the use of crude plant extracts instead of a purified or synthetic compound may result in beneficial effects beyond mere phytopathogenic fungi control and thus may convey additional economic benefit. The cost of crude versus synthetic materials is a function of the complexity involved in the manufacture of each one. Crude extracts also have the potential for synergism among individual components.

Current control of plant diseases is primarily based on repeated or continued applications of fungicides. However, their extensive use for decades has led to widespread development of resistance. Therefore, more emphasis has to be given to the need for selective plant disease control agents for use in IPM. For the development of new types of fungicides and effective control of fungicide resistant pathogens, novel target sites of fungicidal action have been extensively studied (Köller 1992). Additionally, certain plant-derived materials are found to be highly effective against fungicide-resistant pathogens. For example, natural compounds such as cinnamaldehyde and salicylaldehyde were effective against four strains of thiabendazole-resistant *Fusarium sambucinum* (Vaughn and Spencer 1994). Recently, Ahn et al. (2005) found that methyl gallate and gallic acid isolated from *Galla rhois* acted on a cAMP-related signaling pathway regulating appressorium formation in *M. grisea*. However, the exact antifungal mode of action of active plant extracts in our study is unclear.

Our study found that methanol extracts of *L. foenum-graecum* and *P. nigrum* were the most effective among all the test plant against plant pathogens. *Lysimachia foenum-graecum* has been commonly used as a perfume and a pest repellent. Shen et al. (2005) reported five triterpene glycosides and their effect on the arachidonic acid metabolizing enzyme. However, there has been no report on the antifungal activity of *L. foenum-graecum*. *Piper nigrum* is a widely used spice, and its bioactivity has been well studied. Park et al. (2002) isolated insecticidal amides from the fruit of *P. nigrum*. Singh et al. (2004) reported the antifungal activity of volatile oil and acetone extract of *P. nigrum* against *F. graminearum*, *P. viridcatum* and *A. ochraceus*.

Based upon our results, plants extracts might be useful products for developing new types of fungicides, or as biorational management agents for controlling plant pathogens on crops, although their effects on natural enemies, crops, or the environment have not been fully investigated.

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