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Fungicidal Activity of Oriental Medicinal Plant Extracts against Plant Pathogenic Fungi

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Methanol extracts from 53 species of oriental medicinal plants in 34 families were tested for their fungicidal activities against *Pyricularia grisea, Rhizoctonia solani, Phytophthora capsici, Phytophthora infestans, Collectotrichum dematium, Botryospaeria dothidea, Fusarium oxysporum* f. sp. *cucumerinum, Botrytis cinerea, Puccinia recondita, and Erysiphe graminis.* In *in vitro* study using impregnated paper disc method, the efficacy varied with both plant pathogen and plant species tested. Methanol extracts of *Asarum sieboldii* roots, *Sinomenium acutum* roots, *Pinus densiflora* leaves, *Rheum undulatum* root barks, *Coptis japonica* roots, and *Phellodendron amurense* barks showed potent fungicidal activities against the various pathogens when treated with 10 mg/disc. In a whole plant test, methanol extracts of *P. densiflora* leaves and roots and *C. japonica* roots were highly effective against a variety of plant pathogens. As a naturally occurring fungicide, *P. densiflora*- and *C. japonica*-derived materials could be useful as new fungicidal products against various plant diseases induced by plant pathogenic fungi.

Key words : *fungicidal activity, oriental medicinal plant,* Pinus densiflora, Coptis japonica, *phytopathogenic fungi.*

The economic losses due to pre- and post-harvest fungal diseases in crops may be 5~50%, or even higher in developing countries.[1,2] Over the past decades, various attempts to control plant diseases have been taken for effective eradication or prevention through the development of synthetic fungicides. Although effective, their continued or repeated use has disrupted biological control system by natural enemies and widespread development of resistance to various types of fungicides,[3-5] toxicity to nontarget organisms, and human health and environmental concerns.[5,6] Decreasing efficacy and increasing concern over adverse environmental effects of the earlier types of fungicides have highlighted the need for the development of new types of selective control alternatives or of methods of crop protection without, or with reduced, use of conventional fungicides.

Plant extracts or phytochemicals may be an alternative to currently used disease control agents because they constitute a rich source of bioactive chemicals.[7-10] Since these are often active against a limited number of species including specific target diseases, are biodegradable to nontoxic products, and potentially suitable for integrated use, they could lead to the development of new classes of possibly safer disease control agents. Therefore, much efforts have been focused on plant materials for potentially useful products as commercial fungicides or as lead compounds.[11-14] Although pharmacological and epidemiological investigations have demonstrated that oriental medicinal plants have anticancer, antimicrobial and antiviral activities,[8,15] little work has been done to manage plant diseases or their damages using these plants.

In the laboratory and greenhouse studies described herein, we assessed *in vitro* and *in vivo* fungicidal activities of methanol extracts from 53 species of oriental medicinal plants against 10 important plant pathogenic fungi.

Materials and Methods

Plant pathogens and culture conditions. Ten plant pathogenic fungi were used in this study: *Pyricularia grisea*; *Rhizoctonia solani*; *Phytophthora capsici*; *Phytophthora*

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Abbreviations: CV, control value; DAI, days after incubation; PDA, potato dextrose agar; RH relative humidity.

infestans; *Collectotrichum dematium*; *Botryospaeria dothidea*; *Fusarium oxysporum* f. sp. *cucumerinum*; *Botrytis cinerea*; *Puccinia recondita*; and *Erysiphe graminis*. Stock cultures of all strains were routinely maintained on PDA slants and kept for stock at 4°C.

Plant materials and sample preparation. The oriental medicinal plants used in this study are randomly or anecdotally selected (Table 1). They were dried in an oven at 60°C for 2 days and finely powdered using a blender. Each sample (100 g) was extracted twice with 500 ml methanol at room temperature and filtered (Toyo filter paper No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated *in vacuo* at 35°C using a rotary vacuum evaporator. The yield of each methanol extraction is given in Table 1.

***In vitro* bioassay.** The fungicidal activities of test samples against seven pathogens used were determined using the paper disc method on PDA in Petri dish (9-cm diameter). One or two plugs of each subcultured fungal colony were homogeneously mixed with 10 ml of sterile potato dextrose broth. Inoculation was done by spreading 0.3 ml of the suspended fungal mycelia of *B. dothidea, R. solani, C. dematium, P. grisea, F. oxysporum* f. sp. *cucumerinum, B. cinerea,* and *P. capsici* on to sterile PDA.

The most important factor in *in vitro* screening for bioactive substances may be the starting dose or concentration. In a preliminary test, a dose of 10 mg/disc of a plant extract did not cause any problem with solubility and allowed detection of minor active phytochemicals. A sample (10 mg) in methanol solution (200 μl) was applied by syringe (0.5 ml) to a paper disc (Advantec, 8-mm diameter and 1-mm thick, Toyo Roshi). After evaporation of solvents, the paper discs were placed on the agar surface inoculated with each pathogen. All plates were incubated for 5 days at 26±1°C. Controls received methanol.

The fungicidal activity was determined as the diameter (mm) of the clear inhibition zones surrounding the discs. All tests were replicated three times.

***In vivo* bioassay.** The fungicidal activities of test samples against six pathogens used were determined by whole plant method. The plant samples were tested at rates of 2.5, 5.0 and 10 mg/pot. Test samples suspended in distilled water with Tween-20 (Junsei Chemical, Japan) added at the rate of 250 mg/liter were used. Fifty ml of each test sample solution was sprayed onto two pots on the turntable at the same time. After evaporation in a greenhouse for 1 day, each pathogen was inoculated into test samples. Controls received Triton X-100 solution. All treatments were replicated three times.

In a test with rice blast caused by *P. grisea,* rice plants with 2 leaf stage growth (three plants/pot) were sprayed with each test solution. Treated plants were inoculated with a suspension of conidia in distilled water (1×10^6 spores/ml) and kept in a chamber (25°C) for 24 hr under

Table 1. Oriental medicinal plant species tested.

Plant species	Family name	Tissue sampled[a]	Yield[b] (%)
Achyranthes japonica	Amaranthaceae	R	19
Rhus chinensis	Anacardiaceae	G	50
Angelica dahurica	Apiaceae	R	12
Angelica gigas		R	12
Anthriscus sylvestris		R	21
Bupleurum falcatum		R	15
Ledebouriella seseloides		R	17
Oenanthe decumbens		St	10
Torilis japonica		Fr	3
Acanthopanax sessiliflorus	Araliaceae	L	23
Aralia continentalis		B	12
Kalopanax pictum		B	10
Asarum sieboldii	Aristolochiaceae	R	13
Lithospermum erythrorhizon	Borraginaceae	St	28
Sinapis allba	Brassicaceae	R	17
Codonopsis pilosula	Campanulaceae	R	6
Platycodon grandiflorum		R	12
Kochia scoparia	Chenopodiaceae	Fr	7
Arctium lappa	Compositae	Fr	15
Artemisia messerschmidtiana		St	10
Atractylodes japonica		RB	8
Carthamus tinctorius		Fl	19
Cuscuta japonica	Convolvulaceae	Se	6
Scirpus fluviatilis	Cyperaceae	RB	9
Equisetum hyemale	Equisetaceae	Wp	5
Eucommia ulmoides	Eucommiaceae	L	11
Astragalus membranaceus	Fabaceae	R	10
Pueraria thunbergiana		R	21
Gentiana scabra var. *buergeri*	Gentianaceae	R	29
Imperata cylindrica var. *koenigii*	Gramineae	R	22
Belamcanda chinensis	Iridaceae	RB	20
Juncus effusus	Juncaceae	B	3
Schizonepeta tenuifolia var. *japonica*	Labiatae	Wp	6
Akebia quinata	Ladizabalaceae	Hw	9
Sinomenium acutum	Menispermaceae	R	3
Morus alba	Moraceae	R	3
Fraxinus rhynchophylla	Oleaceae	L	35
Bletilla striata	Orchidaceae	T	6
Gastrodia elata		RB	10
Pinus densiflora	Pinaceae	L	33
Pleuropterus multilorus	Polygonaceae	T	14
Rheum undulatum		RB	50
Ganoderma lucidum	Polyporaceae	Wp	19
Aconitum psudo-laeve var. *erectum*	Ranunculaceae	R	13
Clematis florida		R	19
Coptis japonica		R	21
Zizyphus jujuba	Rhamnaceae	Fr	29
Chaenomeles sinensis	Rosaceae	Fr	37
Crategus maximowiczii		Fr	39
Phellodendron amurense	Rutaceae	B	15
Poncirus trifoliata		Fr	32
Lycium chinensis	Solanaceae	Fr	20
Typha orientalis	Typaceae	P	10

[a]B, bark; Fl, flower; Fr, fruit; G, gall; Hw, heartwood; L, leaf; P, pollen; R, root; RB, root bark; Se, seed; St, stem; T, tuber; and Wp, whole plant.
[b](Dried weight of methanol extract/dried weight of sample)×100.

100% RH. Treated and control plants were then held in a lighted chamber (26±2°C and 85% RH) for 5 days and rated on the disease severity. For rice sheath blight caused by *R. solani*, each test solution was sprayed onto rice plants with 3 leaf stage growth (three plants/pot). The plants were inoculated by injecting inoculum at the base of the rice plants. Inoculum was made by incubating mycelial plugs in wheat bran medium at 25°C for 7 days and macerated into mixer. Treated and control plants were held in a lighted chamber (28°C) for 5 days. With cucumber gray mold caused by *B. cinerea*, cucumber plants with the first leaf stage growth (one plant/pot) were sprayed with each test solution. The cucumber was inoculated with conidia (1×10^6 spores/m*l*) of *B. cinerea* incubated on PDA medium at 20°C for 15 days by leaf spray and then placed in a chamber (20°C) for 4~5 days. For tomato late blight caused by *P. infestans*, each test solution was sprayed onto tomato plants with 2 leaf stage growth (two plants/pot). The plants were inoculated with a suspension of 1×10^5 zoosporangia/ml incubated on V-8 juice agar medium at 20°C for 14 days. They were kept in a chamber (18°C) for 4 days and then disease ratings were made. For wheat leaf rust caused by *P. recondita*, wheat plants with the first leaf stage growth (four plants/pot) were sprayed with each test solution. The plants were sprayed with a suspension (60 mg/100 ml of 250 ppm Tween-20) of uredospores colonized on the second leaf of wheat and then placed in a moist chamber. One day after inoculation, plants were held in a growth chamber (20°C and 70% RH). The fungicidal activities of the test samples was made on 10 DAI. For barley powdery mildew caused by *E. graminis*, barley plants with fully expended first leaf (four plants/pot) were sprayed with a suspension of a test material. Treated plants were dusted with conidia of *E. graminis* formed on the primary leaf of barley and held in a chamber (20°C). The disease severity was rated on 10 DAI.

The control effect of test samples against each plant disease was evaluated with CV calculated by the formula CV (%)=[(A-B)/A]×100, where A and B represent the disease area on the untreated and treated plants, respectively.

Results

***In vitro* fungicidal activity.** The fungicidal activities of test samples varied with both plant species and pathogen used (Table 2). In a test with *P. grisea*, 22 plant species among the 53 plant extracts tested showed fungicidal activity. Of these, methanol extracts of *Pinus densiflora* (Pinaceae) leaves and *Coptis japonica* (Ranunculaceae) roots showed the most potent fungicidal activities (++++), with inhibition diameters (i.d.) of 43 and 41 mm, respectively. Strong activity (+++) was obtained from extracts of 20 plant samples. The other 31 plant species revealed weak or little activity.

Extracts from *C. japonica* roots revealed significant fungicidal activity against *P. capsici* with inhibition diameters of 27 mm. Strong fungicidal activity was produced from extracts of *Artemisia messerschmidtiana* (Compositae) stems, *Phellodendron amurense* (Rutaceae) barks, *Gastrodia elata* (Orchidaceae) root barks and *Rheum undulatum* (Polygonaceae) root barks. Moderate activity was observed from extracts of 14 plant samples.

With *C. dematium*, the most significant fungicidal activity was obtained in extracts from *C. japonica* roots. Extracts of *Torilis japonica* (Apiaceae) fruits revealed strong activity. The other plant species were ineffective.

The results from *R. solani* showed that extracts of roots from *Asarum sieboldii* (Aristolochiaceae) and *C. japonica* revealed moderate fungicidal activity. However, the other 50 plant samples exhibited little or no fungicidal activity.

In tests with *B. dothidea*, extracts from *C. japonica* roots exhibited the most potent fungicidal activity (i.d. 31 mm). Strong fungicidal activity was produced from the extracts of *P. amurense* barks. The other plant species were ineffective.

With *F. oxysporum* f. sp. *cucumerinum*, extracts of *R. undulatum* root barks and *C. japonica* roots showed strong fungicidal activities whereas moderate activity was obtained in extracts of *Carthamus tinctorius* (Compositae) flowers, *Sinomenium acutum* (Menispermaceae) roots, *Crategus maximowiczii* (Rosaceae) fruits and *P. amurense* barks.

Extracts from *A. sieboldii* roots was highly effective against *B. cinerea* with inhibition zone of 40 mm. The other plant species showed little or no fungicidal activity.

***In vivo* fungicidal activity.** Because of potent fungicidal activity of extracts from *P. densiflora* leaves and *C. japonica* roots against seven pathogens *in vitro, in vivo* study was performed using whole plant method. These plant extracts were diluted to provide lower concentrations for titration studies, and the results are presented in Table 3. At rates of 5.0 and 10.0 mg/pot, extracts of *P. densiflora* roots and leaves exhibited significant fungicidal activities against *P. grisea, R. solani, B. cinerea, P. recondita* and *E. graminis*. Extracts from *C. japonica* roots revealed highly effective against *B. cinerea, P. recondita,* and *E. graminis*. However, weak or little fungicidal activity was obtained in these plant extraxcts at 2.5 mg/pot.

Discussion

In the *in vitro* and *in vivo* studies with oriental medicinal plant extracts, the fungicidal activity was plant species- and plant pathogen-dependent. The plants belonging to the families, Pinaceae, Ranunculaceae Compositae, Rutaceae, Orchidaceae, Polygonaceae, Apiaceae, Aristolochiaceae Menispermaceae, and Rosaceae revealed strong fungicidal activity. Jaconson[16] pointed out that the most

Table 2. *In vitro* fungicidal activities of test plant extracts toward plant pathogenic fungi, impregnated paper disc method, 10 mg/disc, 5 days.

Plant species[a]	Fungicidal activity[b]						
	Pg[c]	Pc	Cd	Rs	Bd	Fo	Bc
K. pictum	+++[d]	++	-	-	-	-	-
A. sieboldii	+++	-	-	++	++	-	++++
S. allba	-	-	-	-	-	-	-
C. pilosula	+++	-	-	-	-	-	-
A. japonica	-	++	-	-	-	-	-
C. tinctorius	-	-	-	-	-	++	-
A. messerschmidtiana	+++	+++	-	-	-	-	-
C. japonica	+++	-	-	-	-	-	-
S. fluviatilis	+++	+	-	-	-	-	-
G. scabra var. *buergeri*	+++	++	-	-	-	-	-
S. tenuifolia var. *japonica*	+++	++	-	-	-	-	-
B. chinensis	+++	++	-	-	-	-	-
P. thunbergiana	-	++	-	-	-	-	-
S. acutum	+++	++	-	-	-	++	-
M. alba	+++	-	-	-	-	-	-
G. elata	+++	+++	-	-	-	-	-
B. striata	+++	++	-	-	-	-	-
P. densiflora	++++	++	+	+++	+++	++	++++
R. undulatum	+++	+++	-	-	-	+++	-
A. psudo-laeve var. *erectum*	-	+	-	-	-	-	-
C. florida	-	++	-	-	-	-	-
C. japonica	++++	++++	++++	++	++++	+++	++++
C. maximowiczii	-	-	-	-	-	++	-
A. membranaceus	+++	++	-	-	-	-	-
P. trifoliata	-	++	-	-	-	-	-
P. amurense	+++	+++	-	-	+++	++	-
Z. jujuba	+++	-	-	-	-	-	-
L. chinensis	+++	++	-	-	-	-	-
A. dahurica	+++	++	-	-	-	-	-
O. javanica	+++	-	-	-	-	-	-
T. japonica	-	-	+++	-	-	-	-

[a]Plants showing positive results only are presented.
[b]Very strong response ++++, zone diameter >30 mm; strong +++, zone diameter 21-30 mm; moderate +++, zone diameter 16-20 mm; weak +, zone diameter 10-15 mm; and little or no response -, zone diameter <10 mm.
[c]Pg, *Pyricularia grisea*; Pc, *Phytophthora capsici*; Cd, *Collectotrichum dematium*; Rs, *Rhizoctonia solani*; Bd, *Botryospaeria dothidea*; Fo, *Fusarium oxysporum*; and Bc, *Botrytis cinerea*.

Table 3. Control effect of oriental medicinal plant extracts on some crop diseases, whole plant test

Test material	Tissue sampled	Rate, mg/pot	Control value[a]					
			Pg[b]	Rs	Bc	Pi	Pr	Eg
P. densiflora	leaf	2.5	+	-	+	-	+	+
		5.0	+++	++	++	-	++	++
		10.0	+++	+++	++	+	+++	++
	root	2.5	+	-	-	-	+	+
		5.0	+++	++	++	+	++	++
		10.0	+++	++	+++	++	+++	++
C. japonica	root	2.5	-	-	+	-	-	++
		5.0	-	-	+	-	+++	+++
		10.0	-	+	+++	+	+++	+++

[a]Strong response +++, CV >80%; moderate ++, CV 61-80%; weak +, CV 40-60%; and little or no response -, CV<40%.
[b]Pg, *Pyricularia grisea*; Rs, *Rhizoctonia solani*; Bc, *Botrytis cinerea*; Pi, *Phytophthora infestans*; Pr; *Puccinia recondita*; and Eg, *Erysiphe graminis*.

promising botanical pesticides are in the families, Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, and Canellaceae. It has been also reported that Annonaceous plant species can be employed as safe, effective, economical, and environmentally friendly pesticides on the home garden, ornamental, and greenhouse.[17]

Since certain plant-derived extracts and phytochemicals act in many ways on various types of disease complex,

and may be applied to the plant in the same way as other agricultural chemicals, they are being considered as potenial alternatives for synthetic fungicides,[17,18] or lead compounds for new classes of synthetic fungicides such as podoblastin produced by *Podophyllum peltatum*.[14,17] Various compounds including phenolics, terpenoids, alkaloids and lignans exist in plants.[7-10] These compounds jointly or independently contribute to generation of biological activities. However, little information is available for fungicidal activities of oriental medicinal plants, although these plants have long been considered to have natural properties.[8]

In our *in vitro* study, methanol extracts of *A. sieboldii* roots, *S. acutum* roots, *P. densiflora* leaves, *R. undulatum* root barks, *C. japonica* roots, and *P. amurense* barks showed potent fungicidal activities against the various pathogens. Especially, the strong activity of *P. densiflora* leaves and roots and *C. japonica* roots *in vivo* confirms their superiority and usefulness as a potent fungicide against various plant pathogenic fungi tested. These plant species might form a new source for managing plant pathogens in field ecosystem, although their effects on natural enemies and environment remain unknown. Additionally, plant-derived materials are found to be highly effective on fungicide-resistant pathogens. For example, natural compounds such as cinnamaldehyde and salicylaldehyde were effective against four strains of *Fusarium sambucinum* resistant thiabendazole.[19]

Based upon our results and these earlier findings, fungicidal components from the oriental medicinal plants described might be useful products for developing new types of fungicides, or biorational management agents for controlling plant pathogens on crops, although their effects on natural enemies, vegetable qualities, or environment has not been fully investigated.

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