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# *In Vivo* Antifungal Activities of 67 Plant Fruit Extracts Against Six Plant Pathogenic Fungi

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**Abstract** Methanol extracts of fruits of 67 plants were screened for *in vivo* antifungal activity against *Magnaporthe grisea*, *Corticium sasaki*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia recondita*, and *Blumeria graminis* f. sp. *hordei*. Among them, 13 plant extracts (3,000 μg/ml) showed more than 90% disease-control efficacy against at least one of six plant diseases. Specifically, the extracts of *Aleurites fordii*, *Angelica dahurica*, *Camellia japonica*, *Chamaecyparis pisifera*, *Pittosporum tobira*, and *Styrax japonica* controlled more than 90% of the development of rice blast at 1,000 μg/ml. Extracts of both *S. japonica* and *A. dahurica* fruits at 333 μg/ml concentration displayed strong antifungal activity against *M. grisea* on rice seedlings.

**Key words:** *In vivo* antifungal activity, *Angelica dahurica*, *Styrax japonica*, plant disease

The economic losses due to pre- and post-harvest fungal diseases in crops amount to 5–50%. Over the past several decades, synthetic fungicides have been used for control of plant pathogenic fungi. However, the awareness of the negative impact of chemical fungicides on humans and the environment has initiated the search for more friendly alternatives.

Many of the earliest pesticides were extracts of plants, and several plants were exploited more widely as sources of commercial insecticides. However, since the 1940s, synthetic agrochemicals have largely replaced plant-derived products as the key commercial insecticides. Nevertheless, it is now reversed, as it becomes evident that plant natural products still have enormous potential to inspire and influence modern agrochemical research. It is estimated that there are at least 250,000 different species of plant in the world. However, it is also estimated that only 10% of plant species have been examined chemically until 1993 [2], indicating an enormous scope for further work.

Recently, many studies on the use of plant extracts, essential oils, and pure active principles against plant pathogenic fungi have been conducted [4, 6, 13, 15, 18, 20]. Wilson *et al.* [23] found that extracts of *Allium* and *Capsicum* species were highly active against *Botrytis cinerea*, and various essential oils such as carvone, citral, cinnamaldehyde, and cuminaldehyde have also been used to control post-harvest diseases of tulip, potato, rice, and papaya [5, 16, 17, 22]. Kim *et al.* [9] reported that extracts of *Achyranthes japonica* and *Rumex crispus* effectively controlled the development of cucumber powdery mildew caused by *Podosphaera xanthii* in greenhouse trials. They also isolated three antifungal substances from the roots of *R. crispus* and identified them as nepodin, chrysophanol, and parietin [3]. In the present study, we examined the *in vivo* antifungal efficacy of 67 plant-fruit extracts against six plant pathogenic fungi on plant seedling under growth-chamber conditions, to develop environmental-friendly fungicides from plant resources.

A total of 67 plant-fruit samples were collected at various locations in Korea. The botanical name and family of the plant samples belong to 59 genera and 40 families. Fresh fruits (300 g) were macerated using a Waring blender (Dynamics Corporation of America, New Hartford, CT, U.S.A.) for 1 min and extracted with 600 ml of methanol (MeOH) for 2 h. The suspension was filtered through Whatman No. 2 filter paper and the marc was rinsed with 200 ml of MeOH, followed by filtration. The filtrate was concentrated *in vacuo* using a rotary evaporator.

In order to select samples with potent antifungal activity, the MeOH extracts (3,000 μg/ml) were tested *in vivo* for

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antifungal activity against the following six plant diseases: rice blast (*Magnaporthe grisea*), rice sheath blight (*Corticium sasaki*), tomato gray mold (*Botrytis cinerea*), tomato late blight (*Phytophthora infestans*), wheat leaf rust (*Puccinia recondita*), and barley powdery mildew (*Blumeria graminis* f. sp. *hordei*). Rice (*Oryza sativa* L., cv. Nakdong), tomato (*Lycopersicon esculentum* Mill., cv. Seokwang), barley (*Hordeum sativum* Jessen, cv. Dongbori), and wheat (*Triticum aestivum* L., cv. Chokwang) plants were grown in plastic pots (4.5-cm diameter) in the greenhouse at 25±5°C for 1 to 4 weeks. The MeOH extracts of the plants were dissolved in MeOH and added to Tween 20 (250 μg/ml) solution. The final concentration of MeOH was 5% (v/v). The plant seedlings were sprayed with the solution until run-off. Control plants were sprayed with Tween 20 solution containing 5% MeOH. After 24 h, the treated plant seedlings were inoculated with spores or mycelial suspensions of one of six plant pathogenic fungi, followed by rating disease symptoms at 3–7 days after inoculation [8]. Pots were arranged as a randomized complete block with three replicates per treatment. Experiments were conducted twice in a growth chamber, and the mean value of six estimates for each extract was converted into percentage fungal control.

Thirteen (19%) out of 67 plant-fruit extracts displayed disease control activity of more than 90% against at least

**Table 1.** *In vivo* antifungal activity of various fruit extracts against six plant pathogens[a].

| Family | Plant | Control value (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | RCB | RSB | TGM | TLB | WLR | BPM |
| Aceraceae | *Acer tegmentosum* | 83 | 0 | 0 | 0 | 0 | 0 |
| Actinidiaceae | *Actinidia arguta* | 13 | 0 | 17 | 0 | 3 | 58 |
| Aquifoliaceae | *Ilex cornuta* | 0 | 25 | 0 | 0 | 0 | 0 |
| Araliaceae | *Acanthopanax sessiliflorus* | 25 | 6 | 17 | 0 | 0 | 8 |
| | *Aralia elata* | 75 | 0 | 0 | 0 | 0 | 0 |
| Berberidaceae | *Nandina domestica* | 58 | 0 | 0 | 0 | 33 | 0 |
| | *Berberis poiretii* | 38 | 0 | 8 | 0 | 0 | 0 |
| Buxaceae | *Buxus microphylla* var. *koreana* | 13 | 6 | 8 | 6 | 0 | 33 |
| Caprifoliaceae | *Viburnum awabuki* | 13 | 0 | 25 | 0 | 3 | 0 |
| | *Viburnum sargentii* | 70 | 0 | 0 | 31 | 0 | 0 |
| Celastraceae | *Euonymus alatus* for. *ciliato-dentatus* | 38 | 0 | 0 | 0 | 13 | 0 |
| | *Euonymus japonicus* | 0 | 6 | 0 | 0 | 20 | 50 |
| | *Euonymus sieboldiana* | 70 | 0 | 8 | 6 | 27 | 0 |
| Convolvulaceae | *Pharbitis nil* | 38 | 0 | 8 | 0 | 3 | 0 |
| Companulaceae | *Platycodon grandiflorum* | 0 | 0 | 42 | 0 | 0 | 8 |
| Cornaceae | *Cornus controversa* | 13 | 0 | 79 | 0 | 3 | 0 |
| | *Cornus officnalis* | 58 | 0 | 8 | 0 | 0 | 0 |
| | *Cornus walteri* | 0 | 0 | 79 | 25 | 3 | 0 |
| Cucurbitaceae | *Momordica charantia* | 0 | 0 | 0 | 0 | 0 | 0 |
| Cupressaceae | *Chamaecyparis pisifera* | 95 | 100 | 33 | 78 | 73 | 0 |
| | *Thuja orientalis* | 75 | 0 | 8 | 0 | 27 | 0 |
| Eucommiaceae | *Eucommia ulmoides* | 0 | 29 | 50 | 25 | 0 | 0 |
| Euphobiaceae | *Aleurites fordii* | 95 | 0 | 0 | 0 | 73 | 0 |
| | *Daphniphyllum macropodum* | 0 | 0 | 8 | 0 | 0 | 0 |
| | *Mallotus japonicus* | 38 | 0 | 8 | 19 | 0 | 0 |
| | *Ricinus communis* | 0 | 0 | 8 | 0 | 0 | 0 |
| Fagaceae | *Castanopsis cuspidate* var. *sieboldii* | 0 | 0 | 0 | 6 | 0 | 0 |
| | *Castanopsis cuspidate* var. *thunbergii* | 0 | 0 | 42 | 0 | 3 | 0 |
| Hipocastanaceae | *Aesculus turbinata* | 95 | 0 | 0 | 0 | 3 | 0 |
| Iridaceae | *Belamcanda chinensis* | 0 | 0 | 71 | 0 | 0 | 0 |
| Lardizabalaceae | *Stauntonia hexaphylla* | 13 | 0 | 0 | 0 | 3 | 0 |
| Leguminosae | *Amorpha fruticosa* | 95 | 18 | 17 | 98 | 83 | 0 |
| | *Cassia tora* | 93 | 0 | 0 | 6 | 3 | 58 |
| Magnoliaceae | *Kadsura japonica* | 58 | 0 | 25 | 0 | 20 | 0 |
| Moraceae | *Broussonetia papyrifera* | 13 | 5 | 42 | 6 | 0 | 0 |
| Mysinaceae | *Ardisia crenata* | 90 | 0 | 0 | 0 | 0 | 8 |

**Table 1.** Continued.

| Family | Plant | Control value (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | RCB | RSB | TGM | TLB | WLR | BPM |
| Oleaceae | *Chionanthus retusus* | 70 | 0 | 33 | 0 | 10 | 0 |
| | *Fraxinus mandshurica* | 70 | 0 | 8 | 0 | 0 | 0 |
| | *Ligustrum lucidum* | 25 | 0 | 58 | 38 | 0 | 0 |
| Phytolaccaceae | *Phytolacca americana* | 0 | 0 | 17 | 0 | 27 | 0 |
| Pittosporaceae | *Pittosporum tobira* | 100 | 5 | 67 | 0 | 3 | 0 |
| Ranunculaceae | *Paeonia suffruticosa* | 75 | 0 | 42 | 6 | 0 | 8 |
| Rhamnaceae | *Hovenia dulicis* | 0 | 0 | 0 | 0 | 0 | 0 |
| Rosaceae | *Agrimonia pilosa* | 83 | 0 | 42 | 69 | 0 | 0 |
| | *Rhaphiolepis umbellata* | 58 | 0 | 58 | 0 | 0 | 0 |
| | *Rhodotypos scandens* | 13 | 0 | 0 | 0 | 3 | 0 |
| | *Rosa multiflora* | 13 | 0 | 0 | 0 | 0 | 0 |
| | *Rosa rugosa* | 58 | 0 | 0 | 0 | 0 | 0 |
| | *Sanguisorba officinalis* | 80 | 0 | 71 | 31 | 20 | 0 |
| | *Sorbus alnifolia* | 0 | 0 | 25 | 0 | 20 | 0 |
| Rubiaceae | *Paederia scandens* | 13 | 0 | 33 | 0 | 0 | 0 |
| Saururaceae | *Sarurus chinensis* | 70 | 0 | 8 | 69 | 0 | 0 |
| Saxifragaceae | *Ribes fasciculatum* var. *chinense* | 0 | 0 | 0 | 0 | 0 | 0 |
| Scrophulariaceae | *Rehmannia glutinosa* | 38 | 0 | 17 | 0 | 0 | 0 |
| | *Scrophularia buergeriana* | 25 | 0 | 0 | 0 | 3 | 0 |
| Staphyleaceae | *Staphylea bumalda* | 25 | 0 | 8 | 0 | 0 | 8 |
| Styraceae | *Pterostyrax hispida* | 13 | 53 | 58 | 0 | 13 | 0 |
| | *Styrax japonica* | 100 | 12 | 17 | 6 | 3 | 0 |
| | *Styrax obassina* | 95 | 5 | 0 | 0 | 27 | 0 |
| Taxaceae | *Taxus cuspidata* | 13 | 0 | 50 | 0 | 0 | 0 |
| | *Torreya nucifera* | 70 | 0 | 8 | 0 | 0 | 0 |
| Theaceae | *Camellia japonica* | 95 | 15 | 88 | 0 | 3 | 0 |
| | *Ternstroemia japonica* | 100 | 29 | 0 | 0 | 20 | 0 |
| Umbelliferae | *Angelica dahurica* | 100 | 15 | 0 | 0 | 93 | 83 |
| | *Saposhnikovia sesseloides* | 95 | 0 | 8 | 0 | 90 | 50 |
| Verbenaceae | *Callicarpa japonica* | 13 | 0 | 0 | 0 | 0 | 25 |
| Vitaceae | *Parthenocissus quinquefolia* | 38 | 29 | 42 | 0 | 3 | 0 |

[a]The MeOH extracts of plant fruits were dissolved in MeOH (5%) and Tween 20 (250 μg/ml), and then sprayed to run off the following seedlings at 3,000 μg/ml; rice (3-leaf stage), tomato (3-leaf stage), wheat (1-leaf stage), and barley (1-leaf stage). After 24 h, the treated plant seedlings were inoculated with spores or mycelial suspensions of the fungi. RCB, rice blast; RSB, rice sheath blight; TGM, tomato gray mold; TLB, tomato late blight; WLR, wheat leaf rust; BPM, barley powdery mildew.

one of six plant diseases (Table 1). Of the active extracts, four exhibited control values of more than 90% against two of the six plant diseases: extracts of both *Angelica dahurica* (Ad) and *Saposhnikovia sesseloides* (Ss), belonging to Umbelliferae, were active against rice blast and wheat leaf rust. The extract of *Chamaecyparis pisifera* (Cp) effectively controlled both rice blast and rice sheath blight, and the extract of *Amorpha fruticosa* (Ar) controlled both rice blast and tomato late blight. The MeOH extracts of plants such as *Aesculus turbinate*, *Aleurites fordii* (Af), *Ardisia crenata*, *Camellia japonica* (Cj), *Cassia tora*, *Pittosporum tobira* (Pt), *Styrax japonica* (Sj), *Styrax obassina*, and *Ternstroemia japonica* showed strong control efficacy against only rice blast out of the six plant diseases tested. Among 40 families tested, the extracts of plants belonging to 9 families displayed potent *in vivo* antifungal activity.

The thirteen active plant extracts were evaluated at concentrations of 1,000 μg/ml and 333 μg/ml for 1-day protective activity against each of the plant diseases. Among them, the 6 plant extracts, Ad, Af, Cj, Cp, Pt, and Sj, at 1,000 μg/ml controlled development of only rice blast by more than 90% in 1-day protective application (Table 2). Specifically, the extracts of Ad and Sj at 333 μg/ml showed disease control values of 95% and 87% against *M. grisea* on rice seedlings, respectively. The extract of Ad also exhibited control values of 67% against wheat leaf rust at concentration of 1,000 μg/ml and 43% at 333 μg/ml (Fig. 1C). The extract of Ar effectively controlled the development of *P. infestans* on tomato

**Table 2.** Protective efficacy of several plant extracts against rice blast.

| Plant Name | Concentration (μg/ml) | |
|---|---|---|
| | 1,000 | 333 |
| *Aesculus turbinata* | 17 | 5 |
| *Aleurites fordii* | 90 | 50 |
| *Amorpha fruticosa* | 10 | 0 |
| *Angelica dahurica* | 100 | 95 |
| *Ardisia crenata* | 10 | 0 |
| *Camellia japonica* | 92 | 64 |
| *Cassia tora* | 30 | 7 |
| *Chamaecyparis pisifera* | 98 | 67 |
| *Pittosporum tobira* | 93 | 66 |
| *Saposhnikovia seseloides* | 23 | 0 |
| *Styrax japonica* | 97 | 87 |
| *Styrax obassia* | 89 | 5 |
| *Ternstroemia japonica* | 72 | 12 |

seedlings (Fig. 1B). At lower concentrations, the extracts of Cp and Ss had only a little antifungal activity against rice sheath blight and wheat leaf rust (Figs. 1A, 1C).

The above results indicate that the methanol extract of Ad fruits has a potent protective activity against rice blast and wheat leaf rust. The dried root of *A. dahurica* Benth et Hook. is an important herbal medicine, used as an antipyretic and analgesic for cold, headaches, and toothaches [7, 14, 19]. Ryu *et al.* [21] reported that the methanol extract of Ad roots showed potent control activity against rice blast (*M. grisea*), tomato late blight (*P. infestans*), and barley powdery mildew (*B. graminis* f. sp. *hordei*), and the antifungal principles of *A. dahurica* roots were imperatorin and isoimperatorin.

On the other hand, the extracts of Sj and Pt had a potent protective activity against only rice blast. *S. japonica* Sieb et Zucc. is a deciduous tree grown in the Southern areas of Korea and Japan. The pericarps of Sj are used as washing soap, cough medicine, and piscicidal agent [24]. The volatile substances extracted from Sj leaves showed antibacterial effect against foodborne bacteria such as *Bacillus cereus*, *Salmonella typhimurium*, and *Staphylococcus aureus* [10]. *P. tobira* AIT is a broadleaf evergreen shrub with small creamy white flowers and fragrant orange blossoms. The fruits of Pt are green and showy, turning to brown capsules that eventually split open, exposing orange seeds. A saponin mixture obtained from Pt leaves was reported to possess antibiotic activity, and three triterpenoid sapogenins, $R_1$-barrigenol, 21-*O*-angeloyl-$R_1$-barrigenol, and 21-*O*-angeloyl-barringtogenol C, have been isolated from the leaves [1, 25].

The extract of Cp fruits displayed a broad spectrum of antifungal activity against plant pathogenic fungi: *M. grisea* (rice blast), *C. sasaki* (rice sheath blight), *P. infestans* (tomato late blight), and *P. recondita* (wheat leaf rust). *C. pisifera* (Siebold & Zuccarini) Endlicher is a large evergreen tree with a straight trunk and open narrow pyramidal crown, with the bark being reddish-brown, fibrous, shreddy, and peeling in long thin strips. Kobayashi *et al.* [11, 12] have isolated diterpene carboxylic acid, pisiferic acid, and *O*-methylpisiferic acid from leaves and twigs of *C. pisifera* Endl. var. *plumosa* Beissn. as antibiotics against Gram-negative bacteria, and found them to have antifungal activity against *M. grisea*. To the best of our knowledge, this is the first report that the extracts of *S. japonica*, *P. tobira*, *C. japonica*, and *A. fordii* have a potent antifungal activity on rice blast. Their high efficacy against plant pathogenic fungi in a growth chamber led us to test them under glasshouse and field conditions. Further studies on the characterization of the antifungal substances from the active plant extracts are in progress.

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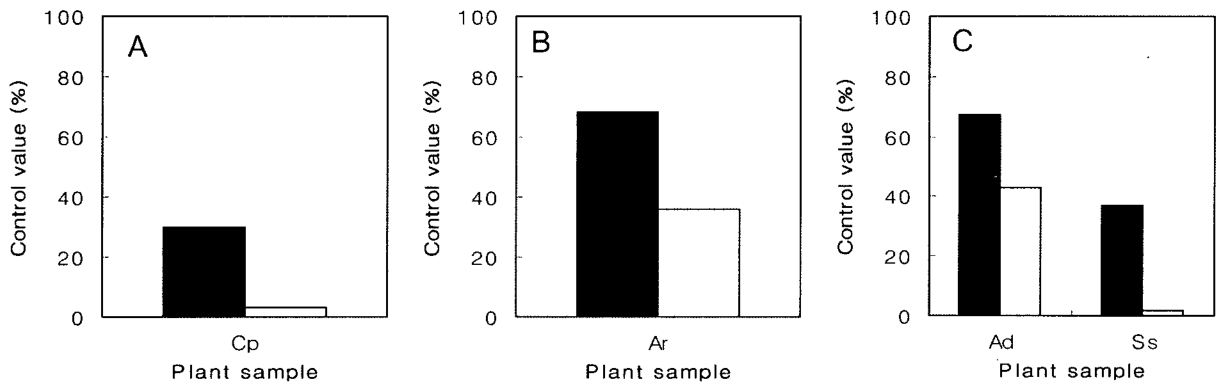


**Fig. 1.** Protective efficacy of several extracts at concentrations of 1,000 μg/ml (■) and 333 μg/ml (□) against rice sheath blight (**A**), tomato late blight (**B**), and wheat leaf rust (**C**) (Cp, *Chamaecyparis pisifera*; Ar, *Amorpha fruticosa*; Ad, *Angelica dahurica*; Ss, *Saposhnikovia seseloides*).

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