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Screening for Antagonistic Natural Materials Against *Alternaria alternata*

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*Alternaria alternata*에 항균력이 있는 천연물 조사

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ABSTRACT : Of 79 plant materials tested, 7 showed antagonistic effects on *Alternaria alternata*, 11 were uncertain in the antifungal activity, and the others showed no antifungal activity against the fungal growth. Of the 7 antagonistic plant materials, *Cinnamomum loureirii* bark (cinnamon) was most highly antagonistic, and *Schisandra chinensis* fruit and *Cnidium officinale* root were moderately antagonistic to the fungus, from which active antifungal materials were separated into diethyl ether, *n*-butanol and diethyl ether, respectively. In cinnamon, visible inhibition zones were formed by the dilutions less than 1,000 × of the diethyl fraction, which was similar to the inhibitory efficacy of polyoxin. As components of cinnamon, cinnamaldehyde had much more powerful antagonistic efficacy to *A. alternata* than cinnamic acid, suggesting cinnamaldehyde may be the main material responsible for the antagonistic activity.

Key words : ***Alternaria alternata*, natural materials, antagonistic activity, cinnamon, cinnamaldehyde.**

Alternaria species are widely distributed in the agroecosystem. Some species cause plant diseases in a variety of host plants, and others grow saprophytically on diseased plant parts and increase disease severity. Many species produce toxins and contaminate food and feed, deteriorating the products and giving possible hazards to man and animal owing to their toxins. In the genus *Alternaria*, *A. alternata* is the most common species that is encountered on agricultural products; for example, the major *Alternaria* species isolated from red pepper fruits with black mold and sesame seeds is *A. alternata* (1). *A. alternata* is also known to be a toxic fungus that can produce various toxins including tenuazonic acid (1, 2).

Fungicides can successfully control plant diseases and reduce postharvest decay of fruits and vegetables. However, inappropriate use of fungicides may bring about unfavorable problems in environments and contamination of food products by the fungicide residues. Especially in case of fungicide application during storage and transportation of food products, chemical residue may be delivered directly to consumers before its decomposition. Use of less-toxic and rapid-decomposing chemicals or alternative control methods such as biological control are to be needed to reduce the hazards.

There are many edible plant materials and medicinal herbs that are known to be antagonistic to microorganisms (4~9). Therefore, in this study, various plant materials were screened for antagonistic efficacy against *A. alternata* to select possible candidates for the development of less-harmful and safe protectants for storage food products.

MATERIALS AND METHODS

Test fungus. The fungal isolate used in this study was obtained from a ginseng leaf with alternaria blight, and identified as *Alternaria alternata* (3). The fungus was grown on potato-dextrose agar (PDA) medium for

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about 10 days. Fungal spore suspension was made by pouring 20 ml of sterilized water into a 9-cm-d plate and by smearing the surface of the fungal culture medium. About 2 ml of spore suspension was put into 200 ml of hot (about 45°C) PDA (added with 0.1% lactic acid) before pouring into the 9-cm-d plates.

Screening for antagonistic plant materials. A total of 79 dried plant materials collected from Oriental medicine shops were screened for antagonism to *A. alternata*. Plant materials were ground and extracted with 5-fold volume (relative to weight) of ethanol overnight. Paper discs (8 mm in diameter) were soaked with each of the ethanol extracts, dried overnight, and placed on the center of PDA plates seeded with the *A. alternata* spores as described above. Three to five days later, inhibition of the fungal growth around paper discs was examined. Four replications were used for each plant material.

Separation of antifungal materials. Of the 79 plant materials tested in this experiment, 7 showed antifungal activity to *A. alternata*, and of these, 3 more powerful antifungal materials (bark of *Cinnamomum loureirii* Nees (CC), root of *Cnidium officinale* Makin (CR) and fruit of *Schisandra chinensis* Baillon (SF)) than others were used for separation of antifungal materials. A hundred grams of each dried plant material was extracted in 2 l of methanol, and evaporated to dryness. The methanol extracts of plant materials were separated in separation funnels by using 100 ml diethyl ether and 100 ml H_2O. The water layer was separated again by using the equal volume of chloroform to that of the water layer, and then the resulting water layer was further separated by the same method as above with *n*-butanol. Each layered solution was evaporated to dryness, and ethanol was added to each extract to make 100 ml solution. Paper discs (8 mm in diameter) were soaked with each of the ethanol solutions, dried overnight, and placed on the center of PDA plates seeded with *A. alternata* spores. Inhibition of the fungal growth was examined as described above.

Antifungal activity of dilutions of CC. CC which showed the strongest antifungal activity against *A. alternata* was examined for the antifungal activity of the dilutions. Diethyl ether extracts of CC obtained as described above, which had antifungal materials, were diluted with ethanol to 10 to 10^5 times (w/v). Paper discs (8 mm in diameter) were soaked with each diluted solution, dried, and placed on *A. alternata*-seeded PDA medium to examine antifungal activity.

Antifungal activity of major CC components. The components of CC, cinnamaldehyde, cinnamic acid and cinnamyl alcohol, were dissolved in ethanol (in case of cinnamaldehyde in hexane+ethanol), diluting to 10^{-5} to 10^{-1} (w/v). Paper discs (8 mm in diameter) were soaked with each of the solutions or polyoxin, dried overnight, and placed on the center of PDA plates seeded with *A. alternata* spores. Inhibition of the fungal growth by the cinnamon components was examined as described above, and compared with that by polyoxin.

RESULTS

Screening for antagonistic plant materials. Of 79 plant materials tested in this experiment, 7 obviously showed antagonistic effects on *A. alternata* growth on PDA medium, forming clear inhibition zones around the paper discs treated with the extracts (Fig. 1), 11 were uncertain in the antifungal activity, and the others showed no antifungal activity against *A. alternata* (Table 1). Of the 7 antagonistic plant materials, CC was most highly antagonistic, (almost completely inhibiting the fungal growth) (Fig. 1), SF and CR were moderately antagonistic, and rhizome of *Atractylodes japonica*, rhizome of *Coptis japonica*, root of *Gly-*

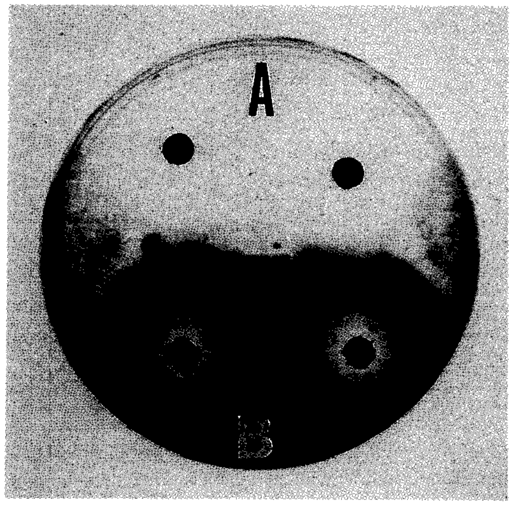

Fig. 1. Antagonistic effects of *Cinnamomum loureirii* bark (A) and *Schisandra chinensis* fruit on the growth of *Alternata alternata* as indicated by the inhibition zones formed around the paper discs treated with the ethanol extracts of the plant materials.

Table 1. Antifungal efficacy of plant materials against *Alternaria alternata*

Family	Species	Plant parts used	Chinese name	Antifungal efficacy[a]
Alismataceae	*Alisma orientale*	Root	澤瀉	–
Amaranthaceae	*Achyranthes japonica*	Root	牛膝	–
Araceae	*Arisaema amurense*	Rhizome	天南星	–
Araliaceae	*Aralia continentalis*	Root	獨活	–
	Panax ginseng	Root	人蔘	–
	Pinellia ternata	Corm	半夏	–
Aristolochiaceae	*Asiasarum sieboldii*	Root	細辛	±
Campanulaceae	*Adenophora remotiflora*	Root	薺尼	–
	Platycodon grandiflorum	Root	桔梗	–
Caprifoliaceae	*Lonicera japonica*	Flower	金銀花	–
Compositae	*Arctium lappa*	Seed	牛蒡子	–
	Artemisia asiatica	Leaf	艾葉	–
	Artemisia capillaris	Leaf	茵蔯	–
	Artemisia japonica	Rhizome	白朮	+
	Carthamus tinctorius	Flower	紅花	–
	Chrysanthemum indicum	Flower	甘菊	–
	Saussurea lappa	Root	木香	+
Cornaceae	*Cornus officinalis*	Fruit	山茱萸	±
Curcubitaceae	*Trichosanthes kirilowii*	Root	瓜樓根	–
Cyperaceae	*Cyperus rotundus*	Root	香附子	±
	Scirpus flaviatilis	Rhizome	三稜	–
Dioscoreaceae	*Dioscorea japonica*	Root	山藥	–
Ephedraceae	*Ephedra sinica*	Stem	麻黄	±
Gentianaceae	*Gentiana scabra*	Root	龍膽	–
Graminae	*Phyllostachys bambusoides*	Endothelium	竹茹	±
Illiciaceae	*Illicium verum*	Fruit	大茴香	–
Labiatae	*Aniosomelis indica*	Leaf	藿香	–
	Leonurus sibiricus	Whole plant	益母草	–
	Mentha arvensis	Root	薄荷	–
	Perilla furthescens	Leaf	蘇葉	±
	Phlomis umbrosa	Root	續斷	–
	Salivia miltiorrhiza	Root	丹蔘	–
	Schizonepeta tenuifolia	Whole plant	荊芥	–
	Scutellaria baicalensis	Root	黄芩	±
Laliaceae	*Anemarrhena asphodeloides*	Root & stem	知母	–
Lardizabalaceae	*Akebia quinata*	Stem	木通	–
Lauraceae	*Cinnamomum loureirii*	Bark	桂皮(肉桂)	+++
	Machilus thunbergii	Bark	厚朴	–
Leguminosae	*Glycyrrhiza uralensis*	Root	甘草	+
	Pueraria thumbergiana	Root	葛根	–
Liliaceae	*Asparagus cochichinensis*	Root	天門冬	±
	Fritillaria cirrhosa	Bulb	貝母	–
	Liriope platyphylla	Root tuber	麥門冬	–
Magnoliaceae	*Magnolia kobus*	Flower bud	辛荑	–
Menispermaceae	*Sinomenium acutum*	Root & stem	防已	–
Moraceae	*Morus alba*	Root bark	桑白皮	–
Paeoniaceae	*Paeonia albiflora*	Root	芍藥	–
	Paeonia moutan	Root bark	牧丹皮	±
Palme	*Areca dicksonii*	Pericarp	腹皮	–
Plantaginaceae	*Plantago asiatica*	Seed	車前子	–

Table 1. Continued

Family	Species	Plant parts used	Chinese name	Antifungal efficacy[a]
Polygalaceae	*Polygala tenuifolia*	Root	遠志	−
Polygalaceae	*Rheum coreanum*	Root	大黃	−
Polyporaceae	*Poria cocos*	Fungus	茯苓	−
	Polyporus umbellatus	Fungus	猪苓	−
Ranunculariaceae	*Cimicifuga heracleifolia*	Rhizome	升麻	−
	Coptis japonica	Root	黃連	+
Rhamnaceae	*Zizyphus jujuba*	Fruit	大棗	−
Rosaceae	*Chaenomeles sinensis*	Fruit	木瓜	−
	Prunus armeniaca	Seed	杏仁	−
	Prunus persica	Seed	桃仁	−
Rubiaceaei	*Gardenia jasminoides*	Fruit	梔子	−
Rutaceae	*Poncirus trifoliata*	Fruit	枳實	−
Schisandraceae	*Schisandra chinensis*	Fruit	五味子	++
Scrophurariaceae	*Rehmannia glutinosa*	Root	地黃	−
	Scrophularia buergeriana	Root	玄蔘	−
Solanaceae	*Lycium chinense*	Root bark	地骨皮	±
	Lycium chinensis	Fruit	枸杞子	−
Umbelliferae	*Angelica dahurica*	Root	白芷	−
	Angelica gigas	Root	當歸	−
	Anthriscus sylvestris	Root	前胡	−
	Bupleurum falcatum	Root	柴胡	−
	Cnidium officinale	Root & stem	川芎	++
	Ostericum koreanum	Root	羌活	-
	Siler divaricatum	Root	防風	−
Zingiberaceae	*Alipinia oxphylla*	Fruit	益智仁	−
	Amomum xanthioides	Seed	縮砂	±
	Zingiber officinale	Root	乾薑	−

[a] Degree of antifungal efficacy: + : weak antagonistic (< 1 mm inhibition zone), ++ : moderately antagonistic (1~4 mm inhibition zone), +++ : strong antagonistic (> 4 mm inhibition zone), − : not antagonistic, and ± : uncertain antagonistic (formation of no clear inhibitiion zone but loose mycelium formation).

cyrrhiza uralensis, and root of *Saussurea lappa* were weakly antagonistic to *A. alternata*. Plant materials with uncertain antagonistic efficacies, forming no definite inhibition zones but forming less mycelium around the treated paper discs, were seed of *Amomum xanthioides*, root of *Asiasarum sieboldii*, stem of *Asparagus cochichinensis*, fruit of *Cornus officinalis*, root of *Cyperus rotundus*, stem of *Ephedra sinica*, root bark of *Lycium chinense*, root bark of *Paeonia moutan*, leaf of *Perilla furtescens*, endothelium of *Phyllostachys bambusoides*, and root of *Scatellaria baicalensis*.

Separation of antifungal materials. Solubilities of antifungal materials in the three plant materials with moderate or strong antagonistic efficacy against *A. alternata* were examined. The results are shown in Table 2. In CC and CR, antifungal activities were only shown in the diethyl ether fractions, while their water fractions had no evident antagonistic effects. On the other hand, no antifungal activity was observed in the diethyl ether fraction of SF, and the antifungal materials appeared to be more soluble in water than in chloroform. In SF, the strongest antifungal activity was shown in the *n*-butanol fraction.

Antifungal activities of dilutions of CC. In CC, a visible inhibition zone was formed at the 1,000 x dilution, and slight inhibition of the fungal growth was noted at the 2,000 × dilution by the indication of loose mycelial formation around the treated paper discs, while no antifungal activity was noted above the 2,000 × dilution (Table 3). The inhibition zones formed by the 1,000 × dilution of polyoxin were similar to those by the 500 × CC dilution, but the 100 × dilution of po-

Table 2. Solubility of antifungal materials against *Alternaria alternata* in different solvents

Plant material[a]	1st separation[b]		2nd separation[c]		3rd separation[d]	
	Diethyl ether	Water	Chloroform	Water	*n*-Butanol	Water
CC	+[e]	–				
CR	+	–				
SF	–	+	±	+	+	–

[a] CC : bark of *Cinnamonium loureirii*, CR : rhizome of *Cnidium officinale*, and SF : fruit of *Schisandra officinale*.
[b] Ethanol extracts of the plant materials were separated in separation funnels containing equal volumes of diethyl ether and water.
[c] The water layer of the 1st separation was separated by chloroform and water.
[d] The water layer of 2nd separation was separated by *n*-butanol and water.
[e] + : clear inhibition zone formed, – : no inhibition zone formed, and ± : not clear inhibition zone but loose mycelium formed around treated paper discs.

Table 3. Antifungal activity of dilutions of the bark of *Cinnamomum loureirii* aginst *Alternaria alternata*

Dilution (×)	Antifungal activity[a]	
	Diethyl ether fraction of CC	Polyoxin
100	+++	+++
200	+++	NT
500	++	NT
1,000	+	++
2,000	±	NT
5,000	–	NT

[a] Distance between the margins of paper discs and inhibition zones were measured: + : weakly antagonistic (< 1 mm inhibitiion zone), ++ : moderately antagonistic (1~4 mm inhibition zone), +++ : strongly antagonistic (> 4 mm inhibition zone), – : not antagonistic, and ± : uncertain antagonistic (formation of no clear inhibition zone but loose mycelium formation).

Table 4. Antifungal activity of dilutions of the components of the bark of *Cinnamomum loureirii* aginst *Alternaria alternata*

Chemical	Concentration (%)			
	0.01	0.05	0.1	1.0
Cinnamaldehyde	–[a]	–	+	+++
Cinnamyl alcohol	–	–	±	++
Cinnamic acid	–	–	–	+

[a] Degree of antifungal efficacy: + : weak antagonistic (< 1 mm inhibition zone), ++ : moderately antagonistic (1~4 mm inhibition zone), +++ : strong antagonistic (> 4 mm inhibition zone), – : not antagonistic, and ± : uncertain antagonistic (formation of no clear inhibition zone but loose mycelium formation).

lyoxin was similar to the 200 × dilution of CC in the size of the inhibition zone.

Antifungal activity of major CC components. Cinnamaldehyde had much more powerful antagonistic efficacy to *A. alternata* than cinnamic acid (Table 4). Up to 0.05% no inhibition zone was formed by any of the chemicals. In 0.1%, cinnamaldehyde had definite inhibition zones, but cinnamic acid had no detectable antifungal activity. In 1.0%, cinnamaldehyde had the very large inhibition zone, but cinnamic acid showed weak antagonistic activity. The antagonistic effects of cinnamyl alcohol was between those of the two materials.

DISCUSSION

Powell and Ko (7) screened 57 plant species of 32 families, and found that 20 species were antagonistic to *Phytophthora palmivora*. Shirata and Takahashi (9) reported that 6 plants out of 23 species were antagonistic to some fungi and bacteria. In our study, out of 79 plant species, 7 plant materials were definitely antagonistic to *A. alternata*. The plant materials antagonistic to *A. alternata* are commonly used as ingredients in the oriental medicine, food, beverages and/ or tonics. *Glycyrrhiza uralensis* root is most frequently used in the oriental medicine, sometimes as a sweetener. Cinnamon is widely used as spicery for a fruit punch (made of honey, dried persimmons and fine nuts and cinnamon), food and tonics. *Schisandra chinensis* fruit is used as a tea. These aspects suggest that the plant materials may be safe in their use as protectants for food products. Especially cinnamon also had antagonistic effects on other fungi and bacteria (unpublished data), which suggests that artificial preservatives may be spared for health food, beverages and tonics containing those antagonistic materials, especially cinnamon.

Shirata and Takahashi (9) showed that bark pieces of mulberry shoot were strongly antagonistic to *Bipolaris leersiae*, but not to *Fusarium solani*. The mulberry shoot bark was not inhibitory to *A. alternata* in our study. *Paeonia suffruticosa* root bark was antagonistic to *Pseudomonas* and *Erwinia* species (unpublished data), but not definitely antagonistic to *A. alternata*. The above aspects suggest that the antagonistic activity of a plant material may vary depending upon microbial species. The antagonistic efficacy may also vary with origin and status of plant materials that may be associated with the composition and concentration of antagonistic components.

Of the antagonistic plant materials, SF (*S. chinensis* fruit), CR (*C. officinale* root) and CC (*C. loureirii* bark, cinnamon) had more powerful antagonistic efficacy to *A. alternata* than the others tested. For SF, the antagonistic materials were more soluble in water than diethyl ether, and were mostly separated into the butanol layer. However, CR and CC contained antagonistic materials soluble in diethyl ether, but not in water, suggesting that the active materials may be oil components. The dilutions of the CC ethyl fraction had inhibitory activities similar to those of cinnamaldehyde which is a major component of cinnamon oil. This suggests the antagonistic material of CC may be cinnamaldehyde.

In our study, the diethyl fraction of CC or cinnamaldehyde was not tested for the control of *A. alternata in situ* by using food products or for field crops. Although the CC diethyl fraction was inhibitory to the fungal growth as much as polyoxin, the *in situ* control efficacy may differ from the *in vitro* activity, and therefore, control efficacy for food products in storage or crops in fields should be evaluated before using the material for the control of Alternaria diseases. Combination of the material with fungicides also should be considered to enhance the control efficacy and to minimize fungicidal spray.

요 약

79 종의 식물재료의 *Alternaria alternata*에 대한 항균활성을 조사한 결과, 7 가지 식물재료가 항균활성이 있었고, 11 가지는 확실치 않았으며, 나머지 61 가지는 항균활성이 나타나지 않았다. *A. alternata*에 항균활성이 나타난 7 가지 식물재료 중 계피가 활성이 가장 높았으며, 오미자와 천궁이 중정도의 활성을 보였으며, 나머지는 약한 활성을 나타내었다. 항균 활성 성분은 계피와 천궁의 경우는 diethyl ether에 분리되었고, 오미자의 경우는 butanol에 분리되었다. 계피의 diethyl ether 추출물은 1,000 배액 이하에서 식별 가능한 항균활성이 나타났다. 계피의 성분으로 cinnamaldehyde가 가장 높은 활성을 보여, 이 물질이 계피의 주요 항균물질로 사료된다.

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