Plant Pathol. J. 24(3) : 322-327 (2008)

The Plant Pathology Journal

Antifungal Activity of Eucalyptus-Derived Phenolics Against Postharvest Pathogens of Kiwifruits

Soon-Ok Oh[1,2], Jung A Kim[2], Hae-Sook Jeon[2], Jong Cheol Park[3], Young Jin Koh[4]*, Hyun Hur[5] and Jae-Seoun Hur[2]*

[1]*Kunming Institute of Botany, Chinese Academy of Sciences, Heilongtan, Kunming, Yunnan 650204, China*

[2]*Korean Lichen Research Institute,* [3]*Department of Oriental Medicine Resources, and* [4]*Department of Plant Medicine, Sunchon National University, Suncheon 540-742, Korea*

[5]*Department of Biology, Dongguk University, Seoul 100-715, Korea*

(Received on May 5, 2008; Accepted on July 21, 2008)

Antifungal activities of natural substrances from *Eucalyptus darlympleana*, *E. globules*, *E. gunnii* and *E. unigera* were evaluated against postharvest pathogens of kiwifruits, *Botrytis cinerea*, *Botryosphaeria dothidea*, and *Diaporthe actinidiae*, to screen effective natural substances as an alternative to chemical fungicides. Methanol extract of the *Eucalyptus* trees showed strong antagonistic activity against the pathogenic fungi. Among them, *E. unigera* and *E. darlympleana* effectively inhibited mycelial growth of the pathogens. For chemical identification of the antifungal substances, the methanol extract of *E. darlympleana* leaves was successively partitioned with CH_2Cl_2, EtOAc, *n*-BuOH and H_2O. Among the fractions, CH_2Cl_2 and *n*-BuOH showed strong inhibitory activity of mycelial growth of the fungi. Five compounds were isolated from EtOAc and *n*-BuOH fractions subjected to SiO_2 column chromatography. Two phenolic compounds (gallic acid and 3,4-dihydroxybenzoic acid) and three flavonoid compounds (quercetin, quercetin-3-O-α-L-rhamnoside, quercetin-3-O-β-D-glucoside) were identified by ^{1}H-NMR and ^{13}C-NMR spectroscopy. Among them, only gallic acid was found to be effective in mycelial growth and spore germination of *B. cinerea* at relatively high concentrations. The results suggest that gallic acid can be a safer and more acceptable alternative to current synthetic fungicides controlling soft rot decay of kiwifruit during postharvest storage.

Keywords **:** biofungicide, bioresource, natural substances, postharvest storage, soft rot decay

Storage rots cause serious economic losses in kiwifruit (Chinese gooseberry, *Actinidia deliciosa*). *Botryosphaeria dothidea*, *Botrytis cinerea* and *Diaporthe actinidiae* were identified as major fungal pathogens causing soft rot decay during postharvest storage in Korea (Lee et al., 2001). Current control methods against these fungi include preharvest sprays with fungicides. Postharvest dips in fungicides are also effective, but there are problems of persistent residues (Pyke et al., 1994). Prolonged and intensive use of fungicides may also create a selection pressure for fungicide-resistant pathogens (Pak et al., 1990). Curing during pre-storage period is commercially applied to control the pathogens (Bautista-Baños et al., 1995), but success is sometimes variable, possibly due to inadequate control of factors, such as relative humidity during the curing process (Bautista-Baños et al., 1997). Experimental control methods such as hot water dips, application of natural coatings, and biological control have been attempted, but all these methods have their shortcomings (Cheah et al., 1992).

There is interest in the use of natural products as safer and more acceptable alternatives to current fungicides. Antimicrobial substances originated from plant have been extensively studied for their use as an agrochemical with highly selective activity against several plant pathogens or as leading molecules for the synthesis of new chemical fungicides (Ahn et al., 2005; Blima et al., 2008; Delaquis et al., 2002; Ramezani et al., 2002; Sacchetti et al., 2005; Schelz et al., 2006). Eucalyptus trees have been known to produce several natural substances having antagonistic activities against several pathogenic microorganisms (Delaquis et al., 2002; Pattnaik et al., 1996; Ramezani et al., 2002; Schelz et al., 2006). This study examined the antimicrobial properties of compounds isolated from several eucalyptus trees against the fungal pathogens causing kiwifruit soft rot decay during postharvest storage.

Materials and Methods

Plant materials. Five to seven-year-old trees of *Eucalyptus darlympleana*, *Eucalyptus globules*, *Eucalyptus gunnii*, and

*Corresponding authors.
YJ Koh. Phone) +82-61-750-3865, Fax) +82-61-750-3865,
E-mail) jshur1@sunchon.ac.kr
JS Hur. Phone) +82-61-750-3383, Fax) +82-61-750-3308,
E-mail) jshur1@sunchon.ac.kr

Eucalyptus unigera were used for the investigation. Young leaves of the trees were collected in the experiment plots of RIST (Research Institute of Industrial Science and technology) located at Gwangyang, southern part of Korea in August, 2003. The air-dried leaves were placed in a dry oven at 65°C for 3 days. The dried leaves were ground and kept in a refrigerator at 4°C for the further examination.

Plant pathogens. Three fungal isolates causing kiwifruits soft rot decay, *Botrytis cinerea*, *Botryosphaeria dothidea*, and *Diaporthe actinidiae* (*Phomopsis mali*), were isolated directly from the infected fruits and maintained at Plant Pathology Lab., Department of Plant medicine, Sunchon National University. Identification of these isolates was previously reported (Lee et al., 2001). The fungal isolates were grown on potato dextrose agar (PDA). Freshly transferred isolates were incubated at 25°C for 5 days before the tests.

Preparation of methanol extracts and their antifungal activity. The ground samples (100 g) of the *Eucalyptus* leaves were extracted three times with 500 ml MeOH. The extract was filtered and the filtrate was evaporated under reduced pressure at a temperature not exceeding 55°C. The concentrates were resolved with 30 ml MeOH for the measurement of antifungal activity. Antifungal activity of the crude extracts was evaluated against the pathogenic fungi by agar well method. Single well (8 mm in diameter) was prepared at the center of the potato dextrose agar plate. A sterile filter paper (8 mm in diameter) was placed on the bottom of the well and 200 μl of the crude extracts or methanol (control) was dripped into the well and then left in a clean bench for 1 hr until the solvent was completely evaporated. Agar plug (8 mm in diameter) cut from the margin of actively growing cultures of the fungi was placed on the filter paper in the well. Inhibition of fungal growth was examined by measuring the diameter of mycelium mat on the plate after 5 day incubation at 25°C.

Extraction, isolation and identification of phenolic compounds. The ground leaves of *E. darlympleana* (1 kg) were refluxed with MeOH. The extract was filtered with a Whatman No. 1 and the filtrate was successively partitioned with organic solvents of the different polarities to afford CH_2Cl_2, ethyl acetate (EtOAc), *n*-BuOH and aqueous fractions, respectively. Antifungal activity of each fraction was examined with agar well method described above. The EtOAc and *n*-BuOH soluble fractions were further purified by repeated silica gel and Sephadex LH-20 column chromatography. Details in purification and chemical identification were previously reported (Park et al., 2004).

Quercetin – ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.64 (1H, d, J = 2.4 Hz, H-2'), 7.53 (1H, dd, J = 2.4 & 8.4 Hz, H-6'), 6.85 (1H, d, J = 8.4 Hz, H-5'), 6.38 (1H, d, J = 2.0 Hz, H-8), 6.16 (1H, d, J = 2.0 Hz, H-6); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 175.5 (C-4), 163.5 (C-7), 160.4 (C-5), 155.8 (C-9), 147.4 (C-4'), 146.5 (C-2), 144.8 (C-3'), 135.5 (C-3), 121.7 (C-1'), 119.8 (C-6'), 115.4 (C-5'), 114.6 (C-2'), 102.8 (C-10), 98.0 (C-6), 93.2 (C-8).

Quercetin 3-O-α-L-rhamnoside – ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.54 (1H, d, J = 2.0 Hz, H-2'), 7.54 (2H, dd, J = 2.0 & 8.4 Hz, H-6'), 6.81 (1H, d, J = 8.4 Hz, H-5'), 6.38 (1H, d, J = 1.9 Hz, H-8), 6.18 (1H, d, J = 1.9 Hz, H-6), 5.24 (1H, J = 1.4 Hz, anomeric H), 0.80 (3H, d, J = 5.5 Hz, rha-CH_3); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 178.2 (C-4), 164.8 (C-7), 161.8 (C-5), 157.8 (C-2), 157.0 (C-9), 149.0 (C-4'), 145.7 (C-3'), 134.8 (C-3), 121.7 (C-6'), 121.3 (C-1'), 116.3 (C-2'), 116.5 (C-5'), 104.7 (C-10), 102.4 (C-1"), 99.3 (C-6), 94.2 (C-8), 71.9 (C-4"), 71.3 (C-5"), 71.1 (C-3"), 70.8 (C-2"), 18.3 (C-6").

Quercetin 3-O-β-D-glucoside – ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.26 (1H, d, J = 2.0 Hz, H-2'), 7.26 (1H, dd, J = 2.0 & 8.4 Hz, H-6'), 6.84 (1H, d, J = 8.4 Hz, H-5'), 6.36 (1H, d, J = 2.0 Hz, H-6), 6.18 (1H, d, J = 2.0 Hz, H-8), 5.23 (1d, J = 7.7 Hz, anomeric H); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 177.9 (C-4), 164.6 (C-7), 161.8 (C-5), 156.8 (C-2), 156.7 (C-9), 149.0 (C-4'), 145.3 (C-3'), 133.9 (C-3), 122.2 (C-6'), 121.8 (C-1'), 116.8 (C-5'), 115.8 (C-2'), 104.6 (C-10), 101.5 (C-1"), 99.3 (C-6), 94.2 (C-8), 78.3 (C-5"), 77.2 (C-3"), 74.8 (C-2"), 70.7 (C-4"), 61.7 (C-6").

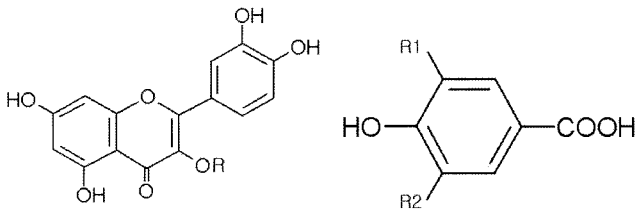
Gallic acid – ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 6.90 (2H, s, H-2 & 6); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 167.2 (C-7), 145.2 (C-3 & 5), 137.8 (C-4), 120.3 (C-1), 108.5 (C-2 & 6).

3,4-dihydroxybenzoic acid – ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.31 (1H, d, J = 2.0 Hz, H-2), 7.26 (1H, dd, J = 2.0 & 8.0 Hz, H-6), 6.77 (1H, d, J=8.0 Hz, H-5); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 167.1 (C-7), 149.8 (C-4), 144.7 (C-3), 121.8 (C-1), 121.5 (C-6), 116.4 (C-2), 115.0 (C-5).

Antifungal activity of phenolic compounds. Antifungal activity of the compounds purified from the extracts was examined against aerial mycelium growth of the fungi using the agar well method on PDA plates. Stock solution was prepared by dissolving the compound (10 mg) in 2 ml of DMSO solution (5% in distilled water) and then diluted to 10 to 5,000 μg/ml. Single well (8 mm in diameter) was prepared at the center of the plate. A sterile filter paper (8 mm in diameter) was placed on the bottom of the well and 100 μl of the diluted solutions of the phenolics or 5% DMSO solution (control) was dripped into the well and then left in a clean bench. An agar plug (8 mm in diameter) cut from the margin of actively growing cultures of the fungi was placed on the filter paper in the well. Inhibition of

fungal growth was examined by measuring the length of mycelium mat from the center of the plate after 5 days incubation at 25°C.

For spore germination test, the compounds were also dissolved in DMSO solution (5% in potato dextrose broth) and diluted to 10 to 5,000 μg/ml. Conidia of the fungi were harvested by flooding 2-weeks-old plates with 10 ml distilled water. The conidia were collected by centrifugation of the spore suspension at 5,000 rpm for 10 min and resuspended in the diluted solutions. Several droplets (100 μl) of the conidia suspensions (approximately 10^5 conidia/ml) were placed on cavity slide glass (Fujiwara glass & Co., Japan). The glasses were sealed in a moistened box, and incubated at 25°C for 24 hr. The percentages of germinated and germinating conidia were determined by the direct microscopic examination of 100 conidia with 10 replicates.



	R		R1	R2
1)	H	4)	OH	H
2)	α-L-rhamnoside	5)	OH	OH
3)	β-D-glucoside			

Fig. 1. Chemical structures isolated from the leaves of *Eucalyptus darlympleana*. 1) quercetin; 2) quercetin-3-O-α-L-rhamnoside; 3) quercetin-3-O-β-D-glucoside; 4) gallic acid; 5) 3,4-dihydroxybenzoic acid.

Results

Antifungal activity of crude extracts of *Eucalyptus* trees against postharvest pathogens of kiwifruits. Crude extracts of all the tested *Eucalyptus* trees showed strong antifungal activity against the soft rot decay fungi of *B. dothidea*, *B. cinerea* and *D. actinidae* during postharvest storage of kiwifruits (Table 1). Among the pathogenic fungi, *D. actinidiae* was highly sensitive to all the extracts. *E. unigera* and *E. darlympleana* effectively inhibited mycelial growth of the pathogens. However, *E. gunii* and *E. globulous* had no inhibitory effect on mycelial growth of *B. dothidea*.

Identification of phenolic compounds from *E. darlympleana*. *E. darlympleana* was used for further examination of antifungal substances against the pathogens. Among the differential fractions of methanol extract, *n*-BuOH fraction was most effective in mycelial growth inhibition of all the pathogens (Table 2). Chloroform fraction was also effectively inhibited mycelial growth of the pathogens. Ethyl acetate fraction showed moderate antifungal activity against *B. dothidea*, but no noticeable activity was found against *B. cinera* and *D. actinidiae*.

For chemical identification of antifungal substances, EtOAc and *n*-BuOH soluble fraction of the leaf extract were investigated. Three flavonoid compounds (quercetin, quercetin-3-O-α-L-rhamnoside, quercetin-3-O-β-D-glucoside) and two phenolic compounds (gallic acid and 3,4-dihydroxybenzoic acid) were isolated from EtOAc and *n*-BuOH soluble fraction of *E. darlympleana* leaf extract (Fig. 1).

Table 1. Antifungal activity of crude extracts of Eucalyptus trees against phytopathogenic fungi causing soft rot decay of kiwifruit

Pathogenic fungi	*E. unigera*	*E. darlympleana*	*E. gunnii*	*E. globulus*
Botryosphaeria dothidea	+++	+++	−	−
Botrytis cinerea	++	+	+	+
Diaporthe actinidiae	+++	++	++	+++

−: no inhibition; +: inhibition rate of mycelial growth less than 50%, compared with control; ++: 50% < inhibition rate < 80%; +++: inhibition rates > 80%

Table 2. Antifungal activities of different solvent fractions of *Eucalyptus darlympleana* against phytopathogenic fungi causing soft rot decay of kiwifruit

Pathogenic fungi	Inhibition rates of mycelial growth (%)			
	Chloroform	Ethyl acetate	Butanol	Aqueous
Botryosphaeria dothidea	52.95±2.65	39.69±3.78	49.18±3.41	−
Botrytis cinera	20.20±0.78	−	39.03±2.26	−
Diaporthe actinidiae	21.52±1.47	−	51.79±3.18	16.44±1.25

Data are means of five replicates and were calculated by the following equation:
Inhibition rates (%) = (1−mycelial dia. control/mycelial dia. of treatment)×100
−: no detectible inhibition (less than 10% inhibition rate)

Table 3. Antifungal activities of the compounds isolated from *Eucalyptus darylmpleana* against aerial mycelium growth of phytopathogenic fungi causing soft rot decay of kiwifruit.

Compounds	Pathogenic fungi	Diameter of mycelium mats (mm)*				
		Concentrations (mg/l)				
		Control	10	100	1000	5000
Quercetin	*Botrytis cinerea*	41.19±2.15	41.05±1.82	42.13±2.16	42.24±1.88	41.23±1.71
	Botryosphaeria dothidea	42.08±1.67	41.67±1.95	41.67±1.68	41.69±1.96	44.06±3.25
	Diaporthe actinidiae	43.22±2.28	41.69±1.54	42.31±2.23	42.14±2.16	41.65±1.71
Quercetin-3-O-α-L-rhamnoside	*Botrytis cinerea*	41.19±2.15	43.54±2.95	44.21±3.42	42.51±2.15	42.02±2.08
	Botryosphaeria dothidea	42.08±1.67	41.93±1.27	42.53±2.76	42.97±2.83	42.35±2.69
	Diaporthe actinidiae	43.22±2.28	41.85±1.73	40.77±1.81	41.12±1.39	41.03±1.52
Quercetin-3-O-β-D-glucoside	*Botrytis cinerea*	41.19±2.15	40.36±1.41	40.52±1.79	43.20±3.65	40.51±1.28
	Botryosphaeria dothidea	42.08±1.67	42.43±1.92	42.08±1.65	42.10±1.74	41.18±1.34
	Diaporthe actinidiae	43.22±2.28	42.35±2.44	42.29±2.81	42.06±1.58	40.41±1.84
Gallic acid	*Botrytis cinerea*	41.19±2.15	41.56±1.64	41.85±2.37	**18.75±0.58**	**6.08±0.83**
	Botryosphaeria dothidea	42.08±1.67	41.82±2.19	41.12±1.09	41.40±2.75	40.12±2.64
	Diaporthe actinidiae	43.22±2.28	43.51±3.32	42.19±2.52	42.32±2.41	40.86±2.21
3,4-dihydroxybenzoic acid	*Botrytis cinerea*	41.19±2.15	42.38±3.29	42.60±2.11	41.42±1.97	40.89±2.56
	Botryosphaeria dothidea	42.08±1.67	42.16±1.65	42.29±1.49	41.67±2.13	41.97±1.82
	Diaporthe actinidiae	43.22±2.28	42.20±2.38	42.12±2.41	43.15±3.28	41.16±1.78

*Data are means of five replicates

Among the isolated compounds, gallic acid significantly inhibited aerial mycelium growth of *B. cinerea* more than 54% and 85% at the concentration of 1,000 mg/l and 5,000 mg/l, respectively, compared to control. However, no detectible effect of gallic acid on mycelium growth was found in *B. dothidia* and *D. actinidiae* (Table 3). Mycelial growth of the pathogens was not inhibited by other compounds of 3,4-dihydroxybenzoic acid, quercetin, quercetin-3-O-rhamno-

Table 4. Antifungal activities of the compounds isolated from *Eucalyptus darylmpleana* on spore germination of phytopathogenic fungi causing soft rot decay of kiwifruit

Compounds	Pathogenic fungi	Spore germination rate (%)*				
		Control	Concentrations (mg/l)			
			10	100	1000	5000
Quercetin	*Botrytis cinerea*	94.3±2.6	91.3±2.1	95.7±3.8	92.3±2.2	87.6±1.2
	Botryosphaeria dothidea	54.2±3.2	48.2±5.1	55.7±4.6	44.2±2.8	41.8±2.4
	Diaporthe actinidiae	95.4±1.6	88.3±2.3	90.1±2.7	96.5±3.2	81.0±2.1
Quercetin-3-O-α-L-rhamnoside	*Botrytis cinerea*	94.3±2.6	89.4±2.7	95.8±3.3	94.7±3.1	92.1±2.8
	Botryosphaeria dothidea	54.2±1.6	55.2±2.7	45.3±1.7	55.8±4.3	44.7±2.1
	Diaporthe actinidiae	95.4±1.6	95.8±1.8	93.1±2.4	94.1±2.6	93.7±2.3
Quercetin-3-O-β-D-glucoside	*Botrytis cinerea*	94.3±2.6	93.3±2.3	96.1±2.9	95.6±2.8	92.2±2.7
	Botryosphaeria dothidea	57.2±2.8	47.8±4.4	53.6±3.2	51.4±2.9	47.3±2.6
	Diaporthe actinidiae	95.4±1.6	94.1±2.9	91.1±2.7	91.7±3.4	88.2±2.3
Gallic acid	*Botrytis cinerea*	94.3±2.6	95.2±1.8	93.7±1.5	85.7±2.3	**0.0**
	Botryosphaeria dothidea	55.9±2.3	56.1±2.7	55.8±2.6	55.3±1.8	**16.2±0.5**
	Diaporthe actinidiae	95.4±1.6	95.8±2.1	94.8±2.4	84.2±1.4	**38.6±1.6**
3,4-dihydroxybenzoic acid	*Botrytis cinerea*	94.3±2.6	93.8±1.8	92.3±2.2	90.7±1.7	90.7±1.6
	Botryosphaeria dothidea	55.4±1.9	52.4±1.6	46.5±2.7	55.6±4.6	42.3±2.1
	Diaporthe actinidiae	95.4±1.6	87.7±1.8	89.2±3.3	61.4±2.5	**0.0**

*Data are means of ten replicates and were calculated by the following equation:
Spore germination rate (%) = (germinated spores/total 100 spores)×100

side and quercetin-3-O-glucoside at all.

Gallic acid also inhibited spore germination in a similar manner with aerial mycelium growth inhibition (Table 4). Spore germination of *B. cinerea* was only sensitive to gallic acid and completely inhibited at the concentration of 5,000 mg/l. Spore germination of *B. dothidea* was also sensitive to gallic acid with more than 70% of inhibition rate at the concentration of 5,000 mg/l, compared with control. Although gallic acid significantly inhibited spore germination of *D. actinidiae*, 3,4-dihydroxybenzoic acid more effectively inhibited spore germination of *D. actinidiae* than gallic acid. Complete inhibition was found at the concentration of 5,000 mg/l of 3,4-dihydroxybenzoic acid.

Discussion

This study provides information on antifungal activity of *Eucalyptus* tree extracts against soft rot decay fungi of kiwifruits during postharvest storage. Methanol extracts of *Eucalyptus* leaves exhibited strong antifungal activity against *B. dothidea*, *B. cinerea* and *D. actinidae*. Strength and spectrum of antifungal activity of the extracts varied in *Eucalyptus* tree species and in fungal species. *E. unigera* and *E. darlympleana* were suggested to be a potent resource for the development of natural antifungal substances. For further investigation, *E. darlympleana* was selected for the purpose because of its cold-resistance, which is critical factor for the growth of *Eucalyptus* tree in Korea. The tree is highly cold tolerant and successfully grows under the low temperature of winter seasons in Korea (Hur et al., 2000).

There have been many reports demonstrating that *Eucalyptus* extracts have been inhibited the growth of several fungi in vitro and in vivo (Blima et al., 2008; Ramezani et al., 2002). However, most studies examined antifungal or antimicrobial activity of volatile essential oils of *Eucalyptus* leaves rather than methanolic and aqueous extracts (Delaquis et al., 2002; Sacchetti et al., 2005; Schelz et al., 2006). In agreement with previous reports, non-aqueous (chloroform) fraction of *E. darlympleana* extract effectively inhibited the fungal growth. In general, aqueous extracts show less antimicrobial activity (Zhang and Lewis, 1997). However, significantly high antifungal activity was found in hydrophilic fraction (butanol and aqueous) of the extract, which result have not been reported in *Eucalyptus* plants. Therefore, chemical identification of the fraction was attempted to elucidate antifungal substances as a natural compound.

Two phenolic compounds (gallic acid and 3,4-dihydroxybenzoic acid) and three flavonoid compounds (quercetin, quercetin-3-O-α-L-rhamnoside, quercetin-3-O-β-D-glucoside) were isolated. Among them, only gallic acid was found to be effective in mycelial growth and sporulation of *B. cinerea* at relatively high concentrations. Spore germination of the tested pathogenic fungi was more sensitive to the phenolic compound than mycelial growth. The inhibitory effect of spore germination is a fact that increases the efficacy of the use of the phenolic compounds for disease control. If a fungus has a certain degree of mycelium growth in the phenolic treatment, the spore germination reduction has an additional impact in relation to mycelial development of the fungi. Since *B. cinerea* is major fungal pathogen causing serious economic losses in kiwifruit industry (Poole and McLeod, 1992) and was found to be sensitive to the compound, gallic acid as a natural product can be safer and more acceptable alternative to current fungicides controlling stem-end rot of kiwifruit.

Gallic acid has been extracted from several plants and found to have strong antifungal activity against several phytopathogenic fungi (Ahn et al., 2005; Shukla et al., 1999). Gallic acid was evident in inhibition of spore germination and appressorium formation of rice blast fungus of *Magnaporthe grisea* (Ahn et al., 2005). Thus, the phenolic compound was suggested as a natural substance for the development of target-site specific biofungicide against fungicide-resistant phytopathogenic fungi. It was also reported that 3,4-dihydroxybenzoic acid isolated from *Cananga odorata* showed antifungal activity (Rahman et al., 2005).

Gallic acid has also been known to have strong antioxidant activity (Bisignano et al., 2000; Liu et al., 2000). Stem-end-rot caused by *B. cinerea* is the most important postharvest disease of kiwifruit (Poole and McLeod, 1992). In addition to direct spoilage of diseased tissue, fruit infected by the fungus produces small amounts of ethylene in cool storage. Ethylene released by infected fruit could cause further losses by softening adjacent sound fruits (Niklis et al., 1997; Qadir et al., 1997). Ethylene formation involved in senescence and pathogenesis process in cells is mediated by the oxidants triggered by several biological and environmental stresses (Yang and Hoffman, 1984; Mehlhorn and Wellburn, 1987; Regoli and Winston, 1999). It is speculated that gallic acid as a strong antioxidant agent can scavenge the oxidants and subsequently prevent ethylene production in infected fruit. This can delay fruit softening and reduced soft rot decay. Systemic investigation on the speculation is further needed.

In conclusion, demand for natural extracts is increasing in agrochemical industry in order to find effective alternatives to synthetic fungicides. In this context, gallic acid from *E. darlympleana* methanol extract gave interesting results, being one of the best performance compounds in terms of both mycelial growth and spore germination inhibition of soft rot decay fungi of kiwifruits during postharvest storage.

Acknowledgements

This work was supported by Rural Development Administration program (Grant 20050401033570), Republic of Korea.

References

Ahn, Y.-J., Lee, H.-S., Oh, H.-S., Kim, H.-T. and Lee, Y.-H. 2005. Antifungal activity and mode of action of Galla rhois-derived phenolics against phytopathogenic fungi. *Pest. Biochem. Physiol.* 81:105-112.

Bautista-Baŭos, S., Long, P. G. and Ganeshanandam, S. 1995. Physiological changes in kiwifruit during a curing period and incidence of *Botrytis cinerea* during storage. *Acta Hort.* 398:233-240.

Bautista-Baŭos, S., Long, P. G. and Ganesh, S. 1997. Curing of kiwifruit for control of postharvest infection by *Botrytis cinerea*. *Postharvest Biol. Technol.* 12:137-145.

Bisignano, G., Sanogo, R., Marino, A., Aqino, R., D'Angelo, V., German, O. M. P., De Pasquale, R. and Pizza, C. 2000. Antimicrobial activity of *Mitracarpus scaber* extract and isolated constituents. *Lett. Appl. Microbiol.* 30:105-108.

Bluma, R., Amaiden, M. R. and Etchverry, M. 2008. Screening of Argentine plant extracts: Impact on growth parameters and aflatoxin B_1 accumulation by *Aspergillus* section *Flavi*. *Int. J. Food Microbiol.* 122:114-125.

Cheah, L. H., de Silva, N., Irving, D. E., Hunt, A. W. and Tate, K. G. 1992. Hot water dips for control of *Botrytis cinerea* storage rot in kiwifruit. *Acta Hort.* 297:605-609.

Delaquis, P. J., Stanich, K., Girard, B. and Mazza, G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol.* 74:101-109.

Hur, J.-S., Ahn, S. Y., Koh, Y. J. and Lee, C. I. 2000. Antimicrobial properties of cold-tolerant *Eucalyptus* species against phyto-pathogenic fungi and food-borne bacterial pathogens. *Plant Pathol. J.* 16:286-289.

Lee, J. G., Lee, D. H., Park, S. Y., Hur, J.-S. and Koh, Y. J. 2001 First report of *Diaporthe actinideae*, the causal organism of stem-end rot of kiwifruit in Korea. *Plant Pathol. J.* 110-113.

Liu, Z., Ma, L., Zhou, B., Yang, L. and Liu, Z. L. 2000. Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein. *Chem. Physics Lipids.* 106:53-63.

Mehlhorn, H. and Wellburn, A. R. 1987. Stress ethylene determines plant sensitivity to ozone. *Nature* 327:417-418.

Niklis, N., Sfakiotakis, E. and Thanassoulopoulos, C. C. 1997. Ethylene production by *Botrytis cinerea*, kiwifruit and *Botrytis* rotted kiwifruit under several storage temperatures. *Acta Hort.* 444:733-737.

Pak, H. A., Beever, R. E. and Laracy, E. P. 1990. Population dynamics of dicarboximide-resistant strains of *Botrytis cinerea* on grapevine in New Zealand. *Plant Pathol.* 39:501-509.

Park, J. C., Park, J. G., Hur, J.-S., Choi, M. R., Yoo, E. J., Kim, S. H., Son, J. C. and Kim, M. S. 2004. Inhibitory effects of methanol extract, phenolic acids and flavonoids from the leaves of *Eucalyptus darlympleana* against 1,1-Diphenyl-2-picrylhydrazyl radical. *Natual Product Sciences* 10:244-247.

Pattnaik, S., Subramanyam, V. R. and Kole, C. 1996. Antibacterial and antifungal activity of ten essential oils *in vitro*. *Microbios.* 86:237-246.

Poole, P. R. and McLeod, L. C. 1992. Inhibition of *Botrytis cinerea* infection in kiwifruit tissues. *Acta Hort.* 297:159-164.

Pyke, N. A., Manktelow, D., Elmer, P. and Tate, K. G. 1994. Post harvest dipping of kiwifruit iprodione to control stem end rot caused by *Botrytis cinerea*. *Newzland. J. Crop Hortic. Sci.* 22:81-86.

Qadir, A., Hewett, E. W. and Long, P. G. 1997. Ethylene production by *Botrytis cinerea*. *Postharvest Biol. Technol.* 11:85-91.

Rahman, M. M., Lopa, S. S., Sadik, G., Rashid, H.-O., Islam, R., Khondkar, P., Alam, A. H. M. K. and Rashid, M. 2005. Antibacterial and cytotoxic compounds from the bark of *Conaga odorata*. *Fitoterpia* 76:758-761.

Ramezani, H., Singh, H. P., Batish, D. R. and Kohli, R. K. 2002. Antifungal activity of the volatile oil of *Eucalyptus citriodora*. *Eitoterapia* 73:261-262.

Regoli, F. and Winston, G. W. 1999. Quantification of total oxidant scavenging capacity of antioxidants for peroxynitrite, peroxyl radicals, and hydroxyl radicals. *Toxicol. Appl. Pharmacol.* 156:96-105.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. and Bruni, R. 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in food. *Food Chem.* 91:621-632.

Schelz, Z., Molnar, J. and Hohmann, J. 2006. Antimicrobial and antiplasmid activities of essential oils. *Fitoterapia* 77:279-285.

Sharma, V. P. and Jandaik, C. L. 1995. Effect of some plant materials in controlling different moulds in *Agaricus bisporus* (Lange) Imbach. *Indian J. Mycol. Plant Pathol.* 24:183-185.

Shukla, Y. N., Srivastava, A., Kumar, S. and Kumar, S. 1999. Phytotoxic and antimicrobial constitutes of *Argyreia speciosa* and *Oenothera biennis*. *J. Ethnopharmacol.* 67:241-245.

Yang, S. F. and Hoffman, N. E. 1984. Ethylene biosynthesis and its regulation in higher plants. *Ann. Rev. Plant Physiol.* 35: 155-189.

Zhang, Y. and Lewis, K. 1997. Fabatins: new antimicrobial plant peptides. *FEMS Microbiol. Lett.* 149:59-64.