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

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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 CH₂Cl₂, EtOAc, *n*-BuOH and H₂O. Among the fractions, CH₂Cl₂ 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 SiO₂ 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 ¹H-NMR and ¹³C-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-Baňos 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

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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 – ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 – ¹H-NMR (DMSO-d₆, 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-CH₃); ¹³C-NMR (DMSO-d₆, 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 – ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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 – ¹H-NMR (DMSO-d₆, 400 MHz) δ: 6.90 (2H, s, H-2 & 6); ¹³C-NMR (DMSO-d₆, 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 – ¹H-NMR (DMSO-d₆, 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); ¹³C-NMR (DMSO-d₆, 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.

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



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-dihydroxyben-zoic acid.

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 3,4dihydroxybenzoic acid) were isolated from EtOAc and *n*-BuOH soluble fraction of *E. darlympleana* leaf extract (Fig. 1).

Table 1. Antifungal activit	v of crude extracts of Eucal	lyptus trees against	phytopathogenic	fungi causing	g soft rot decay	<i>i</i> 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

Dethogonic fungi	Inhibition rates of mycelial growth (%)					
r autogenic tungi	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)

		Diameter of mycelium mats (mm)* Concentrations (mg/l)					
Compounds	Pathogenic fungi						
	-	Control	10	100	1000	5000	
	Botrytis cinerea	41.19±2.15	41.05±1.82	42.13±2.16	42.24±1.88	41.23±1.71	
Quercetin	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-	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	
0-L-Mannoside	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	$42.10{\pm}1.74$	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	

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

*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 detec-

tible 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 daryImpleana* on spore germination of phytopathogenic fungi causing soft rot decay of kiwifruit

Compounds			Spore germination rate (%)*					
	Pathogenic fungi	Control	Concentrations (mg/l)					
		Colluor	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) $\times 100$

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, nonaqueous (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.

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