Plant Pathol. J. 32(4) : 363-370 (2016)
http://dx.doi.org/10.5423/PPJ.NT.01.2016.0006
pISSN 1598-2254 eISSN 2093-9280

Note Open Access

The Plant Pathology Journal
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Antibacterial Activity of Cinnamaldehyde and Estragole Extracted from Plant Essential Oils against *Pseudomonas syringae* pv. *actinidiae* Causing Bacterial Canker Disease in Kiwifruit

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(Received on January 4, 2016; Revised on February 24, 2016; Accepted on March 2, 2016)

***Pseudomonas syringae* pv. *actinidiae* (Psa) causes bacterial canker disease in kiwifruit. Antibacterial activity of plant essential oils (PEOs) originating from 49 plant species were tested against Psa by a vapor diffusion and a liquid culture assays. The five PEOs from *Pimenta racemosa*, *P. dioica*, *Melaleuca linariifolia*, *M. cajuputii*, and *Cinnamomum cassia* efficiently inhibited Psa growth by either assays. Among their major components, estragole, eugenol, and methyl eugenol showed significant antibacterial activity by only the liquid culture assay, while cinnamaldehyde exhibited antibacterial activity by both assays. The minimum inhibitory concentrations (MICs) of estragole and cinnamaldehyde by the liquid culture assay were 1,250 and 2,500 ppm, respectively. The MIC of cinnamaldehyde by the vapor diffusion assay was 5,000 ppm. Based on the formation of clear zones or the decrease of optical density caused by these compounds, they might kill the bacterial cells and this feature might be useful for managing the bacterial canker disease in kiwifruit.**

Keywords **:** cinnamaldehyde, estragole, liquid culture assay, *Pseudomonas syringae* pv. *actinidiae*, vapor diffusion assay

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Kiwifruit is an edible berry fruit belonging to the genus *Actinidia*, which contains more than 70 species such as *Actinidia deliciosa* (green kiwifruit) and *Actinidia chinensis* (yellow kiwifruit) (Garcia et al., 2012). While it is native to China, kiwifruit has become commercially important to many countries after the introduction of *A. deliciosa* to New Zealand around 1930 (Ferguson and Stanley, 2003). Since the 1990s, kiwifruit cultivation has become popular in Korea. Bacterial canker disease caused by *Pseudomonas syringae* pv. *actinidiae* (Psa) is one of the most devastating diseases in kiwifruit-growing orchards worldwide, and has led to huge economic losses (Scortichini et al., 2012), since first reported in Japan in 1989 (Takikawa et al., 1989). The symptoms of bacterial canker disease are browning buds, withering flowers, dark brown spots with yellow halos on the leaves, bleeding cankers with reddish exudates on the twigs, leaders and trunks, and collapsing fruits (Balestra et al., 2009). The most well-known methods used for the prevention of Psa are the use of antibiotics and cupric compounds. However, major kiwifruit-cultivating countries such as Italy and New Zealand have prohibited the use of antibiotics to prevent Psa (Di Lallo et al., 2014; Han et al., 2003). In addition, Psa has gained genes that help increase the resistance to antibiotics and copper (Cooksey, 1994). As the amount of these chemical-resistant phytopathogenic bacteria increases, the importance of developing new agricultural control methods continues to grow.

Various plant essential oils (PEOs) have been studied for a long time, and several reports have shown that PEOs have antimicrobial activities against many microorganisms, mostly *in vitro* (Lopez et al., 2005). It has become apparent that PEOs are good sources of natural compounds that can be used to manage plant diseases in an

Table 1. Plant species and parts for plant essential oils used in this study

Plant species	Plant part	Yield* or Sources† (%)
Acorus gramineus	Root	0.07
Ammi visnaga	Flowering plant	Oshadhi
Amomum kravanh	Seed	5.5
Anethum graveolens	Seed	Jin Amore
Artemisia capillaris	Whole plant	0.21
Asarum sieboldii	Root	0.45
Cacalia ainsliaeflora	Fruit	0.89
Carum carvi	Seed	Oshadhi
Chamaecyparis obtuse	Leaf	0.49
Chamaecyparis pisifera	Leaf	0.50
Chenopodium ambrosioides	Whole plant	0.44
Cinnamomum cassia	Bark	0.17
Coriandrum sativum	Herb	Oshadhi
Coriandrum sativum	Fruit	Oshadhi
Cuminum cyminum	Seed	Jin Aromatics
Eucalyptus citriodora	Leaf	G.R. Davis
Eucalyptus dives	Leaf	G.R. Davis
Eucalyptus globulus	Leaf	G.R. Davis
Eucalyptus polybractea	Leaf	G.R. Davis
Eucalyptus radiate	Leaf	G.R. Davis
Eucalyptus smithii	Leaf	G.R. Davis
Juniperus chinensis	Leaf	0.35
Juniperus chinensis var. globosa	Leaf	0.31
Juniperus chinensis var. kaizuka	Leaf	0.23
Juniperus chinensis var. sargentii	Leaf	0.35
Juniperus chinensis var. sargentii	Leaf	0.32
Juniperus rigida	Leaf	0.14
Kaempferia galangal	Root	0.36
Kunzea ericoides	Leaf	Oshadhi
Leptospermum petersonii	Leaf	G.R. Davis
Leptospermum scoparium	Leaf	Oshadhi
Melaleuca cajuputii	Leaf	Jin Amore
Melaleuca dissitiflora	Leaf	G.R. Davis
Melaleuca linariifolia	Leaf	G.R. Davis
Melaleuca quinquenervia	Leaf	G.R. Davis
Melaleuca uncinata	Leaf	G.R. Davis
Myristica fragrans	Fruit	2.9
Myrtle communis	Flowering plant	Oshadhi
Paeonia suffruticosa	Root	0.54
Pimenta dioica	Berries	Jin Amore
Pimenta racemose	Leaf	Jin Aromatics
Pinus densiflora	Leaf	0.12
Pinus koraiensis	Leaf	0.33
Pinus parviflora	Leaf	0.12
Platycladus orientalis	Leaf	0.08
Pseudolarix amabilis	Leaf	0.21
Saussurea lappa	Root	0.15
Schizonepeta tenuifolia	Whole plant	0.39
Thuja orientalis f. *sieboldii*	Leaf	0.16

*All numbers indicate yield (w/w).
†Company names to purchase oils; Osahdhi (Weinstrasse, Bühl/Baden, Germany), G.R. Davis (Riverstone, NSW, Australia), Jin Amore (New York, NY, USA) and Jin Aromatics (Anyang, Korea).

environmentally friendly way. For instance, Wilson et al. (1997) showed that essential oils from species of *Allium* and *Capsicum* have antifungal activities against *Botrytis cinerea*, which causes gray mold on grapes, strawberries, and tomatoes. In the case of plant-pathogenic bacteria, essential oils extracted from the fruits of *Cuminum cyminum* L. and *Carum carvi* L. were observed to have antibacterial activities against the genera *Clavibacter*, *Erwinia*, *Xanthomonas*, *Ralstonia*, and *Agrobacterium*, which cause various plant diseases (Iacobellis et al., 2005). Kotan et al. (2010) demonstrated that the essential oils of *Satureja spicigera* and *Thymus fallax* inhibit bacterial growth of the plant-pathogenic bacteria of *Clavibacter michiganensis* subsp. *michiganensis*, *Enterobacter intermedius*, *Erwinia* sp., *Pseudomonas* sp., and *Xanthomonas* sp., which cause diseases in tomatoes, potatoes, lettuce, apricots, cabbage, and so on. Recent studies have shown that some PEOs inhibited the growth of antibiotic-resistant pathogens (Mulyaningsih et al., 2010). In this study, the antibacterial activities of PEOs from forty-nine species against Psa were examined in order to determine how effective these PEOs might be for managing bacterial canker disease in kiwifruit.

The strains of Psa used in this study were obtained from Dr. Young Jin Koh at Sunchon National University. KBE9 and YCS3 strains of Psa, classified as biovar 2, and the strain SYS1, classified as biovar 3, were used in this study (Koh et al., 2010, 2012). These were cultured at 26°C on tryptic soy agar (TSA) or broth (TSB). And the forty-nine species of PEOs used in this experiment are listed in Table 1. Commercially available essential oils were purchased from G.R. Davis (Riverstone, NSW, Australia), Jin Aromatics (Anyang, Korea), Jin Amore (New York, NY, USA), and Osahdhi (Weinstrasse, Bühl/Baden, Germany). Other PEOs were extracted by using steam-distillation. Coniferous plant species were collected at Hongneung arboretum in Seoul, and medicinal plant species were purchased at Kyungdong medicinal market in Seoul. In brief, plant parts (about 900–3,220 g for each sample), which have been used as aroma sources or have been known to contain oils, were powdered in a blender and then diluted with distilled water (800 ml) in a 2 l flask and steam distilled (100°C). Yields of essential oils extracted via steam distillation in the laboratory are given in Table 1.

The statistical analysis was performed using the SAS software, version 5.1 (SAS Institute, Cary, NC, USA), and showed the validity of the results obtained in this study. All of the screening assays were repeated three times and analyzed by Duncan's multiple range test ($P < 0.05$, n = 3).

To determine the PEOs that show antibacterial activity against Psa, PEOs were purchased or extracted from various plant species as listed in Table 1, and their antibacterial activity was determined by two different assays, the vapor diffusion assay and the liquid culture assay. For the vapor diffusion assay, Psa strains were streaked on a TSA medium plate (petri dish of 90 mm diameter) from outside to inside, as shown in Fig. 1A (Song et al., 2013). A filter paper disc (8 × 0.7 mm; Advantec MFS Inc., Tokyo, Japan) was placed at the center of the plate cover, and 10 µl of undiluted 100% PEOs (or extracted compounds) were dropped on the filter paper disc. The plate cover with the oil disc was covered with the streaked bottom medium plate, and the combined plate was kept upside-down. After sealing with parafilm, the plate was incubated for 48 hours at 26°C. The length of the clean zones from the center of plate to the bacterial colonies was measured as an indication of antibacterial activity. Among the forty-nine PEOs tested, three PEOs from *Melaleuca linariifolia*, *M. cajuputii*, and *Cinnamomum cassia* showed significant antibacterial activities against three Psa strains, as measured by the vapor diffusion assay (Fig. 1). The PEO from *C. cassia* showed the strongest antibacterial activity and its average inhibition zone length was between 1.2 and 2.2 cm. The strength of the antibacterial activities were not statistically different between the Psa strains because the inhibition lengths were statistically similar. For the PEOs coming from *M. linariifolia* and *M. cajuputii*, the average length of the inhibition zones were not larger than 1 cm. Other PEOs, like *C. sativum*, did not show significant antibacterial activity against Psa.

For the liquid culture assay, as previously described (Song et al., 2013), the bacterial pre-cultures were prepared in liquid TSB at 26°C overnight. These were diluted to 1/100 in TSB, and cultured at 26°C until a value of 0.5 for the optical density measured at a wavelength of 600 nm (OD_{600}; equivalent to 5×10^8 colony-forming unit/ml) was obtained, as measured with NanoDrop (Thermo Scientific, Wilmington, DE, USA). The PEOs (or extracted compounds) were diluted to 10% dimethyl sulfoxide (DMSO) for 5,000 ppm as a final concentration. The 180 µl of bacterial suspension were mixed with 20 µl of each PEO or each compound at the 96 well plate. The negative control for this assay was the bacterial culture that was amended with the same amount of 10% DMSO only. Blank wells were also measured through liquid TSB. The plates were kept shaking at 120 rpm and 26°C. Bacterial growth was determined at OD_{600} using TECAN Infinite M200 (Tecan Group Inc., Männedorf, Switzerland), and was measured at 0, 1, 2, 4, 8, 16, and 24 hours after treatment with the PEOs (or extracted components). Among

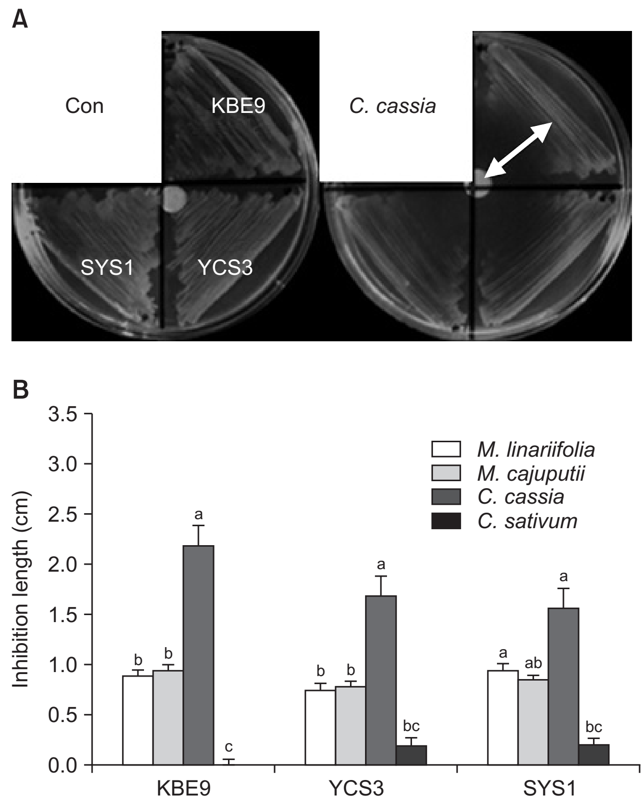
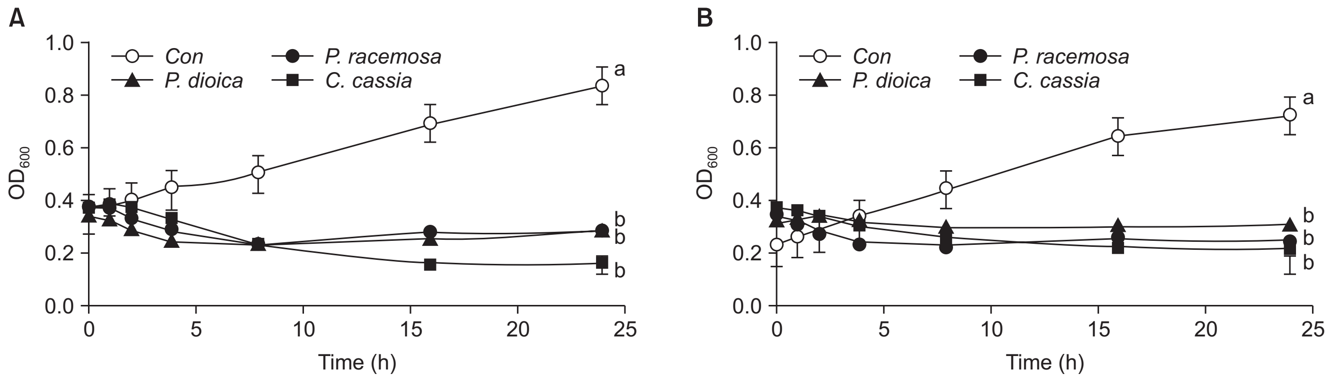


Fig. 1. Antibacterial activity of three plant essential oils (PEOs) by the vapor diffusion assay against *Pseudomonas syringae* pv. *Actinidiae* (Psa). (A) Actual plate images of inhibition zones for the three strains of Psa (KBE9, YCS3, and SYS1) 24 hours after treatment with 10 µl of the indicated PEOs on a paper disc. (B) Measurements of the inhibition zone lengths as indicated in Fig. 1A. The three PEOs among the forty-nine species showed significant antibacterial activity with about three of the strains of Psa. The letters at the top of the error bars are the results from Duncan's multiple range test ($P < 0.05$, n = 3). Each error bar represents a standard error. Con, control; *C. cassia*, *Cinnamomum cassia*; *M. linariifolia*, *Melaleuca linariifolia*; *M. cajuputii, Melaleuca cajuputii*; *C. sativum*, *Cinnamomum sativum*.



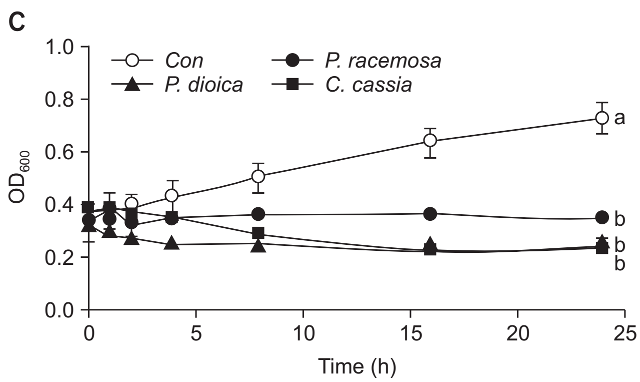


Fig. 2. Antibacterial activity of three plant essential oils (PEOs) by the liquid culture assay against *Pseudomonas syringae* pv. *Actinidiae* (Psa). Bacterial growth, determined as OD_{600} values, was measured at the indicated time points after PEO treatment. Three indicated PEOs among forty-nine species showed significant antibacterial activity against the Psa strains (A, KBE9; B, YCS3; C, SYS1). The letters to the right of the numbers indicate the results from Duncan's multiple range test ($P < 0.05$, n = 3). Each error bar represents a standard error. OD_{600}, the optical density measured at a wavelength of 600 nm; Con, control; *P. dioica*, *Pimenta dioica*; *P. racemosa*, *Pimenta racemosa*; *C. cassia*, *Cinnamomum cassia*.

Table 2. List and composition ratio of separated components of three plant essential oils

Components	Composition ratio (%)		
	Cinnamomum cassia	*Pimenta racemosa*	*Pimenta dioica*
Cinnamadehyde	90.00		
Eugenol		46.25	86.44
Myrcene		25.88	
4-Allylphenol		10.49	
β-Caryophyllene		0.38	7.70
Limonene		3.10	0.18
Linalool		2.61	
Methyl eugenol		0.45	3.87
α-Pinene		0.36	
6-Methyl-5-hepten-2-one		0.93	
1.8-Cineole		1.05	0.10
α-Phellandrene		0.13	
ρ-Cymene		0.75	
α-Humulene			0.99
Terpinen-4-ol		0.65	
α-Terpineol		0.33	
Estragole		0.14	
Geraniol		0.15	
Copaene		0.26	
Sum	90.00	93.55	99.28

the PEOs tested, the *C. cassia* PEO showed the strongest antibacterial activity measured by the liquid culture assay because it reduced bacterial concentration by up to about OD_{600} 0.1 (Fig. 2) and made bacterial cultures appear clear 24 hours after treatment. PEOs from *Pimenta racemosa* and *P. dioica* also showed significant antibacterial activity as measured by the liquid culture assay. These results indicate that five PEOs can inhibit growth of Psa strains, regardless of biovar 2 or 3, *in vitro*. Further, the results indicate that the assay method used might affect the efficiency in finding the effective PEOs against Psa.

PEOs are mixtures of many compounds, and their antibacterial activities generally come from particular components. To find the particular components responsible for antibacterial activities, the components of the three PEOs originating from *C. cassia*, *P. racemosa*, and *P. dioica*, which showed the greatest antibacterial activity against Psa strains as measured by either the vapor diffusion assay or the liquid culture assay, were separated. The components found in the PEOs and their corresponding composition ratios are listed in Table 2. All of these components were tested by the vapor diffusion assay and the liquid culture assay in order to find the compound(s) responsible for the antibacterial activity against Psa. Cinnamaldehyde, which is the major component in *C. cassia*, showed significant antibacterial activities in the vapor diffusion assay against Psa strains (Fig. 3). The length of the inhibitory zone averaged between 2.33 and 3.13 cm. Cinnamaldehyde also inhibited bacterial growth in the liquid culture assay (Fig. 4). Eugenol, which is the major component of both PEOs originated from *P. racemosa* and *P.*

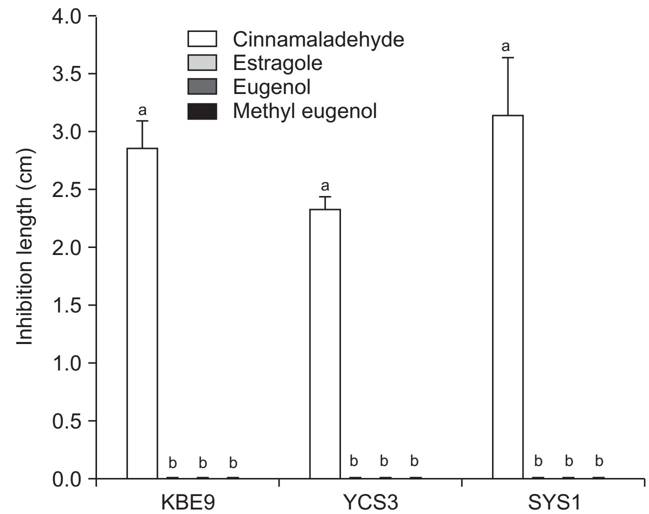
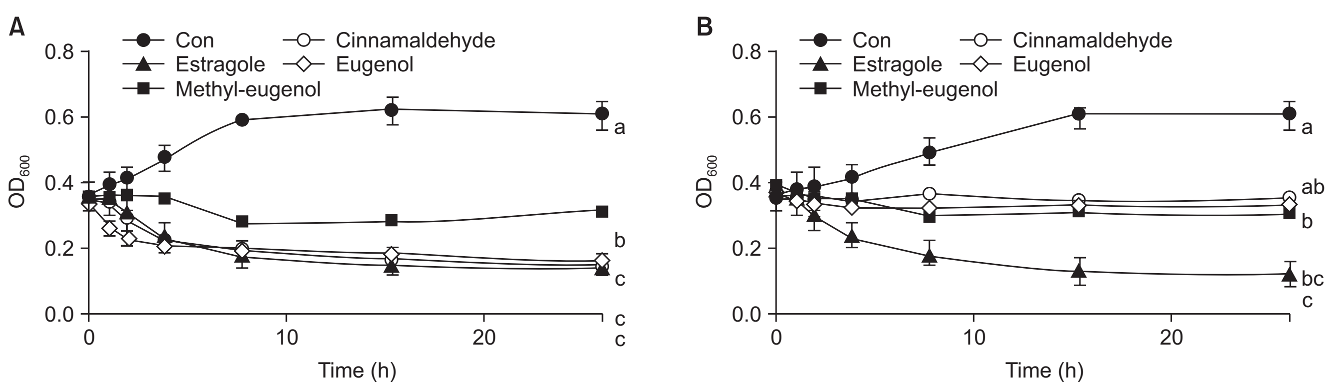


Fig. 3. Antibacterial activity of cinnamaldehyde and three phenolic compounds by the vapor diffusion assay against *Pseudomonas syringae* pv. *actinidiae*. The assays were performed as described in Fig. 1. The letters at the top of the error bars show the results from Duncan's multiple range test ($P < 0.05$, n = 3). Each error bar represents a standard error.



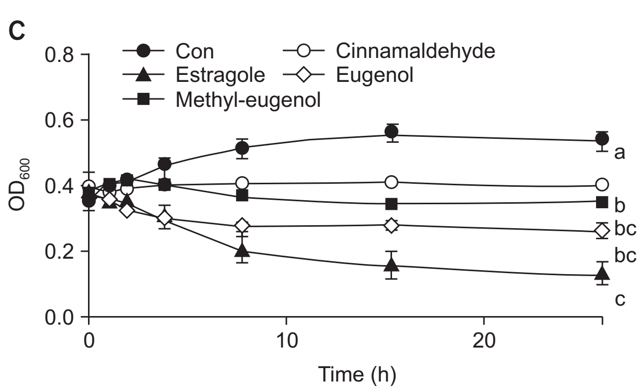


Fig. 4. Antibacterial activity of cinnamaldehyde and three phenolic compounds by the liquid culture assay against *Pseudomonas syringae* pv. *Actinidiae* (Psa). The assays were performed as described in Fig. 2. The four indicated species of separated components showed significant antibacterial activity against the Psa strains (A, KBE9; B, YCS3; C, SYS1). The letters to the right of the numbers show the results from Duncan's multiple range test ($P < 0.05$, n = 2). Each error bar represents a standard error. OD_{600}, the optical density measured at a wavelength of 600 nm; Con, control.

dioica, showed significant antibacterial activity against Psa strains in the liquid culture assay. Methyl eugenol, which is present in both PEOs (Table 2), also showed significant antibacterial activity against Psa in the liquid culture assay. Interestingly, although estragole is a very minor component of the *P. racemose* PEO, it showed the strongest activity in the liquid culture assay regardless of Psa strains (Fig. 4). None of the other components showed antibacterial activity against Psa strains.

The minimum inhibitory concentration (MIC) is important for the application of PEOs and their components exhibiting antibacterial activity against Psa. Thus, the MICs of cinnamaldehyde and estragole were determined. The PEO components showing effective antibacterial activities were diluted twofold with 10% DMSO and were used for the vapor diffusion assay (10,000, 5,000, 2,500, 1,250, 625, and 312.5 ppm) or the liquid culture assay (2,500, 1,250, 625, 312.5, and 78.125 ppm). The lowest concentrations of the compounds showing antibacterial activity were determined as the MIC in both assays. The amount of cinnamaldehyde serially diluted was halved, starting from 10,000 down to 312.5 ppm, and the samples were tested by the vapor diffusion assay. Although 625, 1,250, or 2,500 ppm samples of cinnamaldehyde showed some activity, they were not significantly different from the control (Table 3). However, the 5,000 ppm solution was the lowest concentration to appear in the inhibition zone, which was significantly different from the control; this indicated that the 5,000 ppm concentration was the MIC of cinnamaldehyde, according to the vapor diffusion assay (Table 3). The MIC of cinnamaldehyde was also measured by the liquid culture assay, and its concentration was similarly serially diluted from 2,500 to 78.125 ppm. As shown in Table 4, the 1,250 ppm solution of cinnamaldehyde was the lowest concentration that consistently showed significant antibacterial activity against all Psa strains in the liquid culture assay. The same assay was performed with serially diluted estragole, which indicated

Table 3. Minimum inhibitory concentration of the cinnamaldehyde in the vapor diffusion assay against Psa strains

Concentration of cinnamaldehyde (ppm)	The length of inhibition zone (mm)		
	KBE9	YCS3	SYS1
Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
312.5	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
625	0.17 ± 0.24^{c}	0.17 ± 0.17^{cd}	0.00 ± 0.00^{c}
1,250	0.40 ± 0.22^{bc}	0.30 ± 0.08^{c}	0.00 ± 0.00^{c}
2,500	0.32 ± 0.32^{bc}	0.10 ± 0.08^{bc}	0.10 ± 0.14^{bc}
5,000	0.70 ± 0.08^{b}	0.60 ± 0.08^{b}	0.30 ± 0.24^{b}
10,000	1.23 ± 0.09^{a}	1.17 ± 0.12^{a}	1.10 ± 0.16^{a}

Values are presented as mean ± standard deviation.
Different letters show results from Duncan's multiple range test ($P < 0.05$, n = 3).

Table 4. Minimum inhibitory concentrations of the estragole and cinnamaldehyde in the liquid culture assay against Psa strains

Concen/ration of compounds (ppm)	The OD_{600} value 24 h after treatment					
	Estragole			Cinnamaldehyde		
	KBE9	YCS3	SYS1	KBE9	YCS3	SYS1
Control	0.67 ± 0.01^{ab}	0.62 ± 0.00^{a}	0.45 ± 0.00^{a}	0.70 ± 0.02^{a}	0.66 ± 0.07^{a}	0.36 ± 0.05^{b}
78.1	0.66 ± 0.01^{ab}	0.61 ± 0.00^{a}	0.43 ± 0.00^{a}	0.7 ± 0.00^{ab}	0.63 ± 0.00^{ab}	0.43 ± 0.00^{ab}
156.2	0.67 ± 0.02^{ab}	0.61 ± 0.01^{a}	0.44 ± 0.01^{a}	0.7 ± 0.01^{ab}	0.63 ± 0.01^{ab}	0.43 ± 0.06^{a}
312.5	0.68 ± 0.01^{ab}	0.62 ± 0.00^{a}	0.44 ± 0.00^{a}	0.7 ± 0.01^{ab}	0.62 ± 0.01^{ab}	0.43 ± 0.0^{ab}
625	0.71 ± 0.02^{a}	0.62 ± 0.01^{a}	0.45 ± 0.00^{a}	0.68 ± 0.01^{b}	0.58 ± 0.01^{b}	0.39 ± 0.00^{ab}
1,250	0.64 ± 0.00^{ab}	0.55 ± 0.06^{b}	0.37 ± 0.05^{b}	0.52 ± 0.01^{c}	0.1 ± 0.00^{c}	0.18 ± 0.01^{c}
2,500	0.62 ± 0.01^{b}	0.46 ± 0.03^{c}	0.17 ± 0.01^{c}	0.17 ± 0.01^{d}	0.09 ± 0.00^{c}	0.11 ± 0.00^{d}

Values are presented as mean ± standard deviation.
OD_{600}, the optical density measured at a wavelength of 600 nm.
Different letters show results from Duncan's multiple range test ($P < 0.05$, n = 3).

that the lowest concentration of estragole to show activity was 2,500 ppm (Table 4).

Cinnamaldehyde, the yellow aroma liquid, has been widely studied and used by many scientists because of its antimicrobial activity against diverse microorganisms (Ooi et al., 2006). That study also showed great antibacterial activity for cinnamaldehyde against eight bacterial species. The antibacterial mechanism of cinnamaldehyde has also been studied in (Di Pasqua et al., 2006; Gill and Holley, 2004). The target of cinnamaldehyde is mainly the bacterial membrane; the contact of cinnamaldehyde with the bacterial membrane might cause the loss of membrane functionality or cause the loss of channel proteins in the membrane, resulting in death of bacterial cells. Thus, bacteria could not grow around filter paper containing certain PEOs in the vapor diffusion assay and moreover, their growth in the liquid culture was decreased. Estragole is a phenylpropene, a plant secondary metabolite. The study of antibacterial activity of estragole is comparatively less than that of cinnamaldehyde, but nonetheless it has been shown to carry antibacterial activity (Shahat et al., 2011). The antibacterial activity of estragole was stronger in the liquid culture assay than that of cinnamaldehyde, but showed weaker activity than that of cinnamaldehyde when using the vapor diffusion assay. Eugenol can be found in PEOs originating from clove, cinnamon, allspice and basil (Catherine et al., 2012). It has been shown to have both an antioxidant and antimicrobial activity (Devi et al., 2010), but very little is known about its mode of action against the target microorganisms. Moreover, although the structure of methyl eugenol is similar to eugenol, its antibacterial activity has not been well determined yet in comparison to eugenol. In this study, methyl eugenol has been shown to have antibacterial activity against Psa strains, similar to eugenol, for the first time.

To determine why certain compounds only showed antibacterial activity in either the vapor diffusion assay or the liquid culture assay, their structures, vapor pressure, boiling points, and solubility in water were studied based on information presented in previous studies. However, these physical characteristics of those compounds could not explain the different activities exhibited when using different assays. Thus, the underlying mechanisms of this feature remains to be determined.

Acknowledgments

This work was supported by the Technology Commercialization Support Program (No. 113044-3) of the Korea Institute of Planning & Evaluation for Technology in Food, Agriculture Forestry & Fisheries and also by "Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ00932602)," Rural Development Administration, Republic of Korea.

References

Balestra, G. M., Mazzaglia, A., Quattrucci, A., Renzi, M. and Rossetti, A. 2009. Current status of bacterial canker spread on kiwifruit in Italy. *Australas. Plant Dis. Notes* 4:34-36.

Catherine, A. A., Deepika, H. and Negi, P. S. 2012. Antibacterial activity of eugenol and peppermint oil in model food systems. *J. Essent. Oil Res.* 24:481-486.

Cooksey, D. A. 1994. Molecular mechanisms of copper resistance and accumulation in bacteria. *FEMS Microbiol. Rev.* 14:381-386.

Devi, K. P., Nisha, S. A., Sakthivel, R. and Pandian, S. K. 2010. Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *J. Ethnopharmacol.* 130:107-115.

Di Lallo, G., Evangelisti, M., Mancuso, F., Ferrante, P., Marcelletti, S., Tinari, A., Superti, F., Migliore, L., D'Addabbo, P., Frezza, D., Scortichini, M. and Thaller, M. C. 2014. Isolation and partial characterization of bacteriophages infecting *Pseudomonas syringae* pv. *actinidiae*, causal agent of kiwifruit bacterial canker. *J. Basic Microbiol.* 54:1210-1221.

Di Pasqua, R., Hoskins, N., Betts, G. and Mauriello, G. 2006. Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. *J. Agric. Food Chem.* 54:2745-2749.

Ferguson, A. R. and Stanley, R. 2003. Kiwifruit. In: *Encyclopedia of food sciences and nutrition*, eds. by B. Caballero, L. C. Trugo and P. M. Finglas, pp. 3425-3431. Academic Press, Waltham, MA, USA.

Garcia, C. V., Quek, S. Y., Stevenson, R. J. and Winz, R. A. 2012. Kiwifruit flavour: a review. *Trends Food Sci. Technol.* 24:82-91.

Gill, A. O. and Holley, R. A. 2004. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. *Appl. Environ. Microbiol.* 70:5750-5755.

Han, H. S., Nam, H. Y., Koh, Y. J., Hur, J. S. and Jung, J. S. 2003. Molecular bases of high-level streptomycin resistance in *Pseudomonas marginalis* and *Pseudomonas syringae* pv. *actinidiae*. *J. Microbiol.* 41:16-21.

Iacobellis, N. S., Lo Cantore, P., Capasso, F. and Senatore, F. 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. *J. Agric. Food Chem.* 53:57-61.

Koh, Y. J., Kim, G. H., Jung, J. S., Lee, Y. S. and Hur, J. S. 2010. Outbreak of bacterial canker on Hort16A (*Actinidia chinensis* Planchon) caused by *Pseudomonas syringae* pv. *actinidiae* in Korea. *N. Z. J. Crop Hortic. Sci.* 38:275-282.

Koh, Y. J., Kim, G. H., Koh H. S., Lee, Y. S., Kim, S. C. and Jung, J. S. 2012. Occurrence of a new type of *Pseudomonas syringae* pv. *actinidiae* strain of bacterial canker on kiwifruit in Korea. *Plant Pathol. J.* 28:423-427.

Kotan, R., Cakir, A., Dadasoglu, F., Aydin, T., Cakmakci, R., Ozer, H., Kordali, S., Mete, E. and Dikbas, N. 2010. Antibacterial activities of essential oils and extracts of *Turkish Achillea*, *Satureja* and *Thymus* species against plant pathogenic bacteria. *J. Sci. Food Agric*. 90:145-160.

Lopez, P., Sanchez, C., Batlle, R. and Nerin, C. 2005. Solid-and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. *J. Agric. Food Chem*. 53:6939-6946.

Mulyaningsih, S., Sporer, F., Zimmermann, S., Reichling, J. and Wink, M. 2010. Synergistic properties of the terpenoids aromadendrene and 1, 8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine* 17:1061-1066.

Ooi, L. S., Li, Y., Kam, S. L., Wang, H., Wong, E. Y. and Ooi, V. E. 2006. Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* Blume. *Am. J. Chin. Med*. 34:511-522.

Scortichini, M., Marcelletti, S., Ferrante, P., Petriccione, M. and Firrao, G. 2012. *Pseudomonas syringae* pv. *actinidiae*: a re-emerging, multi-faceted, pandemic pathogen. *Mol. Plant Pathol*. 13:631-640.

Shahat, A. A., Ibrahim, A. Y., Hendawy, S. F., Omer, E. A., Hammouda, F. M., Abdel-Rahman, F. H. and Saleh, M. A. 2011. Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. *Molecules* 16:1366-1377.

Song, Y. R., Park, I. K. and Oh, C. S. 2013. Two methods to test antibacterial activity of plant essential oils. *Research Collection of Institute of Life Sciences & Resources*, *Kyung Hee University* 32:61-65.

Takikawa, Y., Serizawa, S., Ichikawa, T., Tsuyumu, S. and Goto, M. 1989. *Pseudomonas syringae* pv. *actinidiae* pv. nov.: the causal bacterium of canker of kiwifruit in Japan. *Ann. Phytopathol. Soc. Jpn*. 55:437-444.

Wilson, C. L., Solar, J. M., El Ghaouth, A. and Wisniewski, M. E. 1997. Rapid evaluation of plant extracts and essential oils for antifungal activity against *Botrytis cinerea*. *Plant Dis*. 81:204-210.