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Antimicrobial Properties of Cold-Tolerant Eucalyptus Species against Phyto-pathogenic Fungi and Food-Borne Bacterial Pathogens

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Methanol extracts of three cold-tolerant eucalyptus trees - *Eucalyptus darlympleana, E. gunnii* and *E. unigera* were screened for antimicrobial activity against twenty two phyto-pathogenic fungi and six food-borne bacterial pathogens. *E. unigera* showed the antagonistic activity against all the tested pathogens. Among the tested fungal pathogens, *Pythium* species were highly sensitive to the leaf extracts. Especially, *P. vanterpoolii*, a causal agent of leaf blight in creeping bentgrass (*Agrostis palustris*), was completely inhibited by the extracts. The eucalyptus extracts were also effective in inhibiting the fungal growth of *Botrytis cinerea* and *Phomopsis sp.* isolated from the lesions of kiwifruit soft rot during post-harvest storage. *Escherichia coli* O-157 was less sensitive to the inhibition than the other bacterial pathogens tested. It was likely that Gram positive bacteria-*Bacillus subtilis* and *Streptococcus mutans* were more sensitive to the eucalyptus extracts than Gram negative bacteria-*Escherichia coli, Salmonella enteritidis* and *Peudomonas aeruginosa.* Our findings suggest that the cold-tolerant eucalyptus species have antimicrobial properties that can serve the development of novel fungitoxic agents or food preservatives.

Keywords : eucalyptus darlympleana, 'eucalyptus gunnii, eucalyptus unigera, pythium vanterpoolii, antimicrobial activity, kiwifruit soft rot.

Synthetic fungicides have been used for the control of plant diseases and the reduction of post-harvest decay of fruits and vegetables. However, many microbial pathogens have begun to develop resistance to the most widely used chemicals, so there is a need to develop new fungicides with improved performance and less potential environmental impact. Antimicrobial substances originated from plant have been extensively studied for their use as an agrochemical with highly selective activity against some plant pathogens or as lead molecules for the synthesis of new chemical fungicides (Muller-Riebau et al., 1995; Pai and Platt, 1995; Kim et al., 1996; Mohamed et al., 1996; Bae et al., 1997).

In recent years, there has been a dramatic increase in the number of reported cases of food-borne illness (Monitor, 1996). Consequently, there is considerable interest in ways to stop this upward trend and reduce the incidence of food poisoning. Due to negative consumer perceptions of artificial preservatives, attention is shifting towards alternatives that consumers perceive as natural products such as plant extracts. It is well established that plant extracts have antimicrobial properties against food poisoning bacteria (Paster et al., 1990; Lis-Balchin and Deans, 1997; Smith-Palmer et al., 1998).

Eucalyptus trees are among the most important hardwood forestry crops world-wide and provide a major source of pulp wood for high quality paper production, leaf material for distillation of essential oils, timber for construction and fuel (Turnbull, 1991). In addition, the trees have been known to produce several natural substances having antagonistic activities against several pathogenic microorganisms (Dwivedi and Dubey, 1986; Mireku and Wilkes, 1988; Singh and Agrawal, 1988; Sharma and Jandaik, 1995; Pattnaik, et al., 1996). Since low temperature is considered as an important environmental factor limiting the productivity of the plants (Leborgen et al., 1995), the antimicrobial properties of cold-tolerant eucalytus species have been rarely reviewed. This study examined the antimicrobial properties of cold-tolerant eucalyptus trees - *E. darlympleana, E. gunnii* and *E. unigera* against several phyto-pathogenic fungi and food-borne bacterial pathogens.

Three-year-old trees of *E. darlympleana, E. gunnii* and *E. unigera* were used for the investigation. The trees were prove to be highly cold-tolerant and successfully grown under the low temperature of the last two winter seasons in Korea (Lee, 1997). The leaves of the eucalyptus trees were collected in the experiment plots of RIST (Research Institute of Industrial Science and Technology) located at Kwangyang, southern part of Korea in September 1999. The air-dried leaves were placed in a dry oven at 60°C for 2 days. The dried leaves were ground and kept in a refrigera-

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tor at 4°C for the further examination.

The ground samples (100 g) were 3 times extracted with methanol (totaling 1000 ml) with continuous shaking for 24 h at 25°C. The extract was filtered and the filtrate was evaporated under reduced pressure at a temperature not exceeding 55°C. The dark green viscous residue was reconstituted in methanol (50 ml) and was used for the evaluation of antimicrobial activity.

Twenty-two phyto-pathogenic fungal isolates were used to examine antagonistic effects of the plant extracts in this study (Table 1). The fungal isolates were grown on potato dextrose agar (PDA; Difco, Detroit, USA). Freshly transferred isolates were incubated at 25°C about 5 days and then used to examine the antifungal activities of the extracts. Six food-borne pathogenic bacteria strains - *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Staphylococcus aureus* and *Streptococcus mutans*- maintained at Department of Microbiology, Kyengsang National University were used to evaluate the antibacterial activities of the extracts. The bacterial strains were grown on nutrient agar (NA; Difco, Detroit, USA) at 30°C.

Antagonistic activity of the eucalyptus extracts against phyto-pathogenic fungi was estimated using the agar well method on PDA. Two wells (8 mm in diameter) placed 2 cm apart on either side of the center of PDA plate were prepared by a cork borer. Sterile filter paper (8 mm in diameter) was placed on the bottom of each well and the extract (100 μl) or methanol (control) was poured into the well and then left in a clean bench at room temperature until the solvent was completely dried. Antifungal substances can diffuse through the agar medium during drying. Agar plug (6 mm in diameter) cut from the margin of actively growing cultures of the 22 phyto-pathogenic fungi was placed at the center of the same plate. Inhibition of the fungal growth was examined by measuring the length of mycelial mat from the center of the plate 5 days after incubation at 25°C.

The disc diffusion method on nutrient agar was also employed to investigate antagonistic effects of the extracts on the growth of food-borne pathogenic bacteria. To prepare seed inoculum, each strain of the bacteria was inoculated into a 250 ml Erlenmeyer flask containing nutrient broth (100 ml). After 1-day incubation at 30°C and 180 rpm, each bacterial suspension (100 μl) was smeared evenly on fresh nutrient agar and then paper discs (8 mm in diameter) containing the reconstituted methanol extract (100 μl) were placed on the plate. Paper disc containing only methanol (100 μl) as a control was also placed on the same plate. Antibacterial activity of the extracts was evaluated by measuring the diameter of clear zone around the paper discs 24 h after incubation at 30°C.

The eucalyptus extracts were effective in inhibiting mycelial growth of the phyto-pathogenic fungi tested (Table 1). *Pythium* species, one of the most important soil-borne fungal pathogens, were extremely sensitive to the eucalyptus extracts. Especially, no mycelial growth of *P. vanterpoolii*, a causal agent of leaf blight in creeping bentgrass (*Agrostis palustris*), was induced by the eucalyptus extracts. Other fungal pathogens such as *P. aphanidermatum, P. graminicola, P. ultimum* and *Rhizoctonia solani* were also known to cause severe damage to creeping grass, Kentucky bluegrass (*Poa pratensis*) and zoysia grasses (*Zoysia japonica* and *Z. maltrella*) (Kim and Park, 1999). Recently, there is a great concern in Korea about over-use of chemical fungicides to prevent the grasses from several soil-borne pathogenic fungi on many golf courses and consequently heavy contamination of water bodies with toxic compounds originated from the fungicides threatening public health and ecosystem. This result suggests that antifungal activity of the eucalyptus extracts against soil-borne fungal pathogens of the grasses could provide environmen-

Table 1. Antifungal activity of the leaf extracts of cold-tolerant eucalyptus species against phyto-pathogenic fungi

Phyto-pathogenic fungi	Inhibition rate[a] (%)		
	E. darlympleana	*E. gunnii*	*E. unigera*
Bipolaris coicis	37.0	33.5	44.3
Botryosphaeria dothidia	23.3	22.8	21.7
Botrytis cinerea	36.0	32.3	42.0
Colletotrichum gloeosporioides	20.7	17.9	31.4
Colletotrichum coccodes	23.1	19.1	29.7
Cercospora kikuchii	18.5	12.4	20.0
Fusarium graminearum	16.8	22.7	30.6
Fusarium moniliforme	32.0	33.6	40.2
Fusarium oxysporum	28.8	27.4	31.8
Fusarium solani	25.8	30.1	33.0
Pestalotiopsis longiseta	18.6	15.5	19.6
Phomopsis soje	26.9	18.3	31.3
Phomopsis sp.	42.0	35.3	52.0
Magnaporthe grisea	40.9	37.9	44.4
Pythium aphanidermatum	66.2	69.5	72.2
Pythium graminicola	63.5	64.9	71.9
Pythium myritylum	47.0	48.3	59.9
Pythium spinosum	65.5	59.6	68.5
Pythium ultimum	67.9	63.0	69.3
Pythium vanterpoolii	100.0	100.0	100.0
Rhizoctonia solani	41.0	39.7	48.0
Sclerotinia sclerotiorum	17.6	20.8	20.0

[a]Inhibition rates were calculated by the following equation; (1-dt/dc) × 100, where dt is the average diameter of fungal colony treated with the extracts and dc is the average diameter of fungal colony treated with methanol (control). Each value represents a mean of ten replicates.

Table 2. Antibacterial activity of the leaf extracts of cold-tolerant eucalyptus species against food-borne bacterial pathogens

Food-borne bacterial pathogens	Diameter of inhibition zone (mm)		
	E. darlympleana	*E. gunnii*	*E. unigera*
Bacillus subtilis	18.76	18.12	20.61
Escherichia coli	17.06	16.91	18.85
Escherichia coli O-157	12.52	–	11.74
Salmonella anterotidis	16.91	16.91	19.67
Streptococcus mutans	17.18	17.24	20.06
Peudomonas aeruginosa	17.51	13.52	15.36

The diameter of paper discs (8 mm) containing 100 μl methanol extract of the eucalyptus extracts was included in the clear zone. The diameter was measured 24 h after smearing with bacterial suspensions. Each value represents a mean of ten replicates

tally friendly alternative to chemical fungicides for managing the pathogens.

The pathogenic fungal isolates of *Botrytis cinerea* and *Phomopsis* sp. obtained from the soft rot lesions of kiwifruit were effectively inhibited by the extracts. During the last few years, the outbreaks of kiwifruit soft rot disease during the post-harvest storage were frequently reported in Korea (Koh, 1995). Considered limited application of chemical fungicides to the fruits during the storage, it seems appropriate that biological control of the pathogens with use of eucalyptus extract could be a supplemental control strategy.

Six fungal pathogens of *Bipolaris coicis, Botrytis cinerea, Fusarium moniliforme, Magnaporthe grisea, Phompsis* sp. and *Rhizoctonia solani* were also severely affected by the extract of *E. unigera* at the inhibition rates over 40%. Moderate inhibitions of mycelial growth were also found in *Colletotrichum gloeosporiodes, C. coccodes, Fusarium grminearum, F. oxysporum, F. solani* and *Phomopsis soje.* Among the tested eucalyptus species, *E. unigera* was likely to have the most pronounced antifungal activity against the phyto-pathogenic fungi tested.

Six food-borne pathogenic bacteria were also inhibited by the eucalyptus extracts. *Escherichia coli* O-157 was less sensitive to the inhibition than the other bacterial pathogens tested (Table 2). The leaf extracts of *E. unigera* also exhibited the strongest antagonistic activity against the bacterial pathogens. It was likely that Gram positive bacteria- *B. subtilis* and *Strep. mutans* were more sensitive to the extract of *E. unigera* than Gram negative bacteria-*E. coli, Sal. anteritidis* and *P. aeruginosa.*

This study clearly demonstrated that the cold-tolerant eucalyptus trees were effective in inhibiting fungal or bacterial growth. Especially, *E. unigera* exhibited strong antimicrobial activity against phyto-pathogenic fungi and food-borne bacterial pathogens. Therefore, the cold-tolerant eucalyptus trees can serve as a domestic source of pulp wood, essential oil and natural substances having biological activity such as antimicrobial properties. Further research will concentrate on elucidating biologically active compounds which are effective against pathogenic fungi and bacteria.

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References

Bae, E. Y., Shin, E. J., Lee, D. H., Koh, Y. J. and Kim, J. 1997. Antifungal Kaempferol-3-O-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside from leaves of *Phytolacca americana* L. *Kor. J. Plant. Pathol.* 13:371-376. (in Korean)

Dwivedi, R. S. and Dubey, R. C. 1986. Effects of volatile and non-volatile fractions of tow medicinal plants on germination of *Marcrophomina phaseolina* sclerotia. *Trans. Br. Mycol. Soc.* 87:326-327.

Kim, J. W. and Park, E. W. 1999. Occurrence and pathogenicity of *Pythium* species isolated from leaf blight symptoms of turfgrasses at golf courses in Korea. *Plant Pathol. J.* 15:112-117. (in Korean)

Kim, Y, U., Yu, Y. H. and Ohh, S. H. 1996. Screening for antagonistic natural materials against *Alternaria alternata. Kor. J. Plant Pathol.* 12:66-71. (in Korean)

Koh, Y. J. 1995. Economically important diseases of kiwifruit. *Plant Dis. Agri.* 1:3-13. (in Korean)

Leborgen, N., Teulieres, C., Travert, S., Rols, M.-P., Teissie, J. and Boudet, A. M. 1995. Introduction of specific carbohydrates into *Eucalyptus gunnii* cells increases their freezing tolerance. *Eur. J. Biochem.* 229:710-717.

Lee, C. I. 1997. Selection of landscaping trees for steel-making factory. POSCO Research Report. Research Institute of Industrial Science and Technology, Pohang, Korea. (in Korean)

Lis-Balchin, M. and Deans, S. G. 1997. Bioactivity of selected plant essential oils against *Listeria monocytogens. J. Appl. Microbiol.* 82:759-762.

Mireku, E and Wilkes, J. 1988. Production of phenols in the sapwood of *Eucalytus maculata* after wounding and infection. *Eur. J. For. Pathol.* 18:121-127.

Mohamed, S., Saka, S., El-Sharkawy, S. H., Ali, A. M. and Muid, S. 1996. Antimycotic screening of 58 Malaysian plants against plant pathogens. *Pestic. Sci.* 47:259-264.

Monitor. 1996. Production and Health. National Statistics. Government Statistical Service. London: Official for National Statistics.

Muller-Riebau, F., Berger, B. and Yegen, O. 1995. Chemical composition and fungitoxic properties to phyto-pathogenic fungi of essential oils of selected aromatic plants growing wild in Turkey. *J. Agric. Food Chem.* 43:2262-2266.

Pai, S. T. and Platt, M. W. 1995. Antifungal effects of *Allium sativum* (garlic) extracts against the *Aspergillus* species involved in otomycosis. *Lett. Appl. Microbiol.* 20:14-18.

Paster, N., Juven, B. J. and Shaaya, E. 1990. Inhibitory effect of oregano and thyme essential oils on moulds and food borne bacteria. *Lett. Appl. Microbiol.* 11:33-37.

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

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.

Singh, B. C. and Agrawal, S. C. 1988. Efficacy of odoriferous organic compounds of the growth of Keratinophilic fungi. *Curr. Sci.* 57:807-809.

Smith-Palmer, A., Stewart, J. and Fyfe, L. 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Lett. Appl. Microbol.* 26: 118-122.

Turnbull, J. W. 1991. Future use of Eucalyptus: opportunities and problems. In: *IUFRO symposium on Intensive Forestry: the role of eucalyptus*. Vol. I. pp. 2-29. Durban, South Africa.