Short Communication

Antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi

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Abstract

In this study, the antifungal activities of cinnamaldehyde and eugenol congeners against white-rot fungus *Lenzites betulina* and brown-rot fungus *Laetiporus sulphureus* were evaluated and the relationships between the antifungal activity and the chemical structures were also examined. Results from antifungal tests revealed that cinnamaldehyde, α -methyl cinnamaldehyde, (*E*)-2-methylcinnamic acid, eugenol and isoeugenol exhibited strong antifungal activity against all fungi tested. Results derived from the chemical structure-antifungal activity relationship study suggested that compounds with an aldehyde group or an acid group, a conjugated double bond and a length of CH chain outside the ring affect their antifungal properties. Furthermore, the presence of the methyl moiety in the *ortho* position may have a considerable influence on the inhibitory action against *L. betulina* and *L. sulphureus*. In addition, the lipophilicity may play, in part, a crucial role in determining the toxicity of phenylpropenes.

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1. Introduction

Biodegradation of wood by fungi is a serious problem for both wooden structures and forest management. Basidiomycete fungi of the genera *Trametes*, *Lenzites*, and *Laetiporus* are recognized worldwide as major wood decay fungi (Wu et al., 2005). Failure to control them can result in tremendous economic and resource losses. To extend the service life, wood products are therefore often treated with preservatives such as creosote, pentachlorophenol and inorganic arsenicals. However, these agents will be phased out in the near future due to their potential adverse impact on the environment. Therefore, the search for safer phytochemicals with lower environmental and mammalian

toxicity is a major concern and imperative (Schultz and Nicholas, 2000; Wedge et al., 2000).

Various plant materials are believed to have antifungal property and many essential oils have been reported to have antifungal activities. Cinnamaldehyde, a major constituent of cinnamon essential oils, occurs naturally in the bark and leaves of cinnamon trees of the genus *Cinnamomum*. It has been proven to have strong antifungal activities against a wide variety of wood decay fungi (Wang et al., 2005a; Cheng et al., 2006). Eugenol is a major phenolic component of clove oil and has been demonstrated as an excellent fungicide against wood decay fungi (Wang et al., 2005a). Moreover, several naturally occurring compounds such as α -cadinol (Chang et al., 1999), T-muurolol, T-cadinol, γ -cadinene (Kondo and Imamura, 1986; Chang et al., 2000), cryptomeridiol (Morita et al., 1997), chamaecynone (Wang et al., 2005b), and ferruginol (Rudman, 1965) have been reported to be effective against wood decay fungi. To the best of our knowledge, no antifungal

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activities of the congeners of both cinnamaldehyde and eugenol have been reported. Therefore, this paper describes a laboratory study to examine the toxicity of the congeners of both cinnamaldehyde and eugenol against wood decay fungi and their structure–activity relationships were addressed.

2. Methods

2.1. Fungal strains

The wood decay fungi were obtained from the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (Taiwan). The fungal strains used in experiments were as follows: one white-rot fungi [*Lenzites betulina* (BCRC 35296)] and one brown-rot fungi [*Laetiporus sulphureus* (BCRC 35305)].

2.2. Chemicals

Benzaldehyde (**1**), 4-hydroxybenzaldehyde (**2**), cinnamaldehyde (**3**), benzenepropanal (**4**), (*E*)-cinnamic acid (**6**), cinnamyl alcohol (**7**), methyl cinnamate (**9**), cinnamyl acetate (**10**), (*E*)-2-methylcinnamic acid (**11**), (*E*)-3-methylcinnamic acid (**12**), (*E*)-4-methylcinnamic acid (**13**), (*E*)-2-hydroxycinnamic acid (**14**), (*E*)-3-hydroxycinnamic acid (**15**), (*E*)-4-hydroxycinnamic acid (**16**), and eugenol (**17**) were purchased from Acros (Belgium). 2-Methyl-3-phenyl-2-propen-1-ol (**8**) was purchased from Sigma Chemical Co. (USA). α -Methyl cinnamaldehyde (**5**), acetyleugenol (**18**), isoeugenol (**19**), and methyleugenol (**20**) were purchased from TCI (Japan). Commercial fungicide didecyl dimethyl ammonium chloride (DDAC) was used as a positive control.

2.3. Antifungal assays

One white-rot fungus, *L. betulina* (CCRC 35296) and one brown-rot fungus, *L. sulphureus* (CCRC 35305), were used in this experiment. The method of Chang et al. (1999) was employed for antifungal evaluation of cinnamaldehyde and eugenol congeners. Briefly, 100, 50, 25, and 12.5 μ g/ml of compounds were added to sterilize potato dextrose agar (PDA) in 9-cm Petri dishes. After transferring the mycelium of two fungi strains, the testing dishes were incubated at 26 ± 2 °C, 70% relative humidity. When the mycelium of fungi reached the edges of control dishes (without compounds added) after approximately 10–14 days, the antifungal indices were calculated. Each test was repeated four times, and the data averaged. The IC_{50} values (the concentration in μ g/ml that inhibited 50% of the mycelium of fungi growth) were calculated by probit analysis. Didecyl dimethyl ammonium chloride (DDAC), a commercially available fungicide, was used as a reference compound. The formula of antifungal indices was shown as follows:

$$\text{Antifungal index (\%)} = (1 - D_a/D_b) \times 100$$

where D_a is the diameter of the growth zone in the experimental dish (cm) and D_b is the diameter of the growth zone in the control dish (cm).

2.4. Statistical analyses

All results were expressed as mean $\pm$ SD ($n = 4$). The significance of difference was calculated using SAS Scheffé's test, and results with $P < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Antifungal activity of cinnamaldehyde and its congeners

In our previous studies, cinnamaldehyde (**3**) is the strongest antifungal compound in the leaf essential oils of *Cinnamomum osmophloeum* (Wang et al., 2005a; Cheng et al., 2006). Thus, to examine the structure–activity relationships of cinnamaldehyde, 15 compounds whose chemical structures are similar to cinnamaldehyde were selected for this study. These compounds are benzaldehyde (**1**), 4-hydroxybenzaldehyde (**2**), benzenepropanal (**4**), α -methyl cinnamaldehyde (**5**), (*E*)-cinnamic acid (**6**), cinnamyl alcohol (**7**), 2-methyl-3-phenyl-2-propen-1-ol (**8**), methyl cinnamate (**9**), cinnamyl acetate (**10**), (*E*)-2-methylcinnamic acid (**11**), (*E*)-3-methylcinnamic acid (**12**), (*E*)-4-methylcinnamic acid (**13**), (*E*)-2-hydroxy-cinnamic acid (**14**), (*E*)-3-hydroxycinnamic acid (**15**), and (*E*)-4-hydroxycinnamic acid (**16**).

Fig. 1 presents the results of antifungal activity of these compounds against white-rot fungus *L. betulina* and

Fig. 1. Antifungal activities of cinnamaldehyde and its congeners against white-rot fungus *L. betulina* and brown-rot fungus *L. sulphureus* (concentration used is 100 μ g/ml).

brown-rot fungus *L. sulphureus* at the concentration of 100 µg/ml. Results from antifungal tests revealed that compounds **3**, **5**, **6**, and **11–13** were the most active against *L. sulphureus* amongst the 16 compounds tested, with antifungal indices higher than 59.3%; while compounds **1–2**, **4**, **7–10**, and **14–16** were the least active, with antifungal indices lower than 8.1%. Furthermore, compounds **3** and **11** inhibited completely the growth of *L. sulphureus* at the concentration of 100 µg/ml. Similarly, compounds **3**, **5**, **6**, and **11–13** also exhibited strong antifungal action with antifungal indices of 100.0% against *L. betulina* at the concentration of 100 µg/ml, while compounds **1–2**, **4**, **7–10**, and **14–16** did not express antifungal activities at the same concentration, with antifungal indices lower than 9.7%.

IC₅₀ values of the 16 compounds against *L. betulina* and *L. sulphureus* were further determined and the results obtained are shown in Table 1. In terms of the IC₅₀ values, the antifungal activity of compounds **3**, **5**, **6**, and **11–13** against *L. sulphureus* is ranked as cinnamaldehyde (**3**) (35.3 µg/ml) > (*E*)-2-methylcinnamic acid (**11**) (39.2 µg/ml) > (*E*)-4-methylcinnamic acid (**13**) (58.5 µg/ml) > (*E*)-3-methylcinnamic acid (**12**) (66.7 µg/ml) > α-methyl cinnamaldehyde (**5**) (83.5 µg/ml) > (*E*)-cinnamic acid (**6**) (87.4 µg/ml). On the other hand, the order of IC₅₀ values against *L. betulina* are α-methyl cinnamaldehyde (**5**) (22.0 µg/ml) > (*E*)-2-methylcinnamic acid (**11**) (47.2 µg/ml) > (*E*)-3-methylcinnamic acid (**12**) (49.3 µg/ml) > (*E*)-cinnamic acid (**6**) (55.8 µg/ml) > (*E*)-4-methylcinnamic acid (**13**) (58.2 µg/ml) > cinnamaldehyde (**3**) (58.9 µg/ml). These results indicated that having an aldehyde group or an acid group, a conjugated double bond and a length of CH chain outside the ring, i.e. cinnamaldehyde or α-methyl cinnamaldehyde, possesses much stronger antifungal activity. A similar observation was also noted in our previous studies on the antibacterial (Chang et al.,

2001), antitermitic (Chang and Cheng, 2002), antimosquito (Cheng et al., 2004), and antifungal (Lee et al., 2005; Wang et al., 2005a) activities of *C. osmophloeum*. A different finding was observed in the other bioactivity assay. Kim et al. (2004) have evaluated the acaricidal activity of cinnamaldehyde and its congeners against *Tyrophagus putrescentiae*, their results indicated that a conjugated double bond and a length of CH chain outside the ring appear not to be responsible for *T. putrescentiae* toxicity. In addition, on the basis of the antifungal activity of the seven cinnamic acid derivatives, the hydroxyl group on the cinnamic acid structure has no significant influence on antifungal activity, but the presence of the methyl group in the *ortho* position may have a considerable influence on the inhibitory action against *L. betulina* and *L. sulphureus* (Table 1). The results differ from those reported by Kim et al. (2004), who suggested that the presence of the hydroxyl moiety in the *ortho* position may have a considerable influence on the *T. putrescentiae* toxicity. We assume that the discrepancy might be due to the different mechanisms involved in the inhibition of fungi and mites.

3.2. Antifungal activity of eugenol and its congeners

According to our previous studies (Wang et al., 2005a; Yen and Chang, 2008), eugenol has been demonstrated as an excellent fungicide against wood decay fungi. To our best knowledge, antifungal activity of the eugenol congeners against wood decay fungi has not been reported, so this property appears to be worthy of evaluation. To examine the structure–activity relationships of eugenol (**17**), three compounds whose chemical structures are similar to eugenol were selected for the study. These compounds were acetyleugenol (**18**), isoeugenol (**19**), and methyleugenol (**20**). The antifungal activities of these compounds against both white-rot fungus (*L. betulina*) and brown-rot fungus (*L. sulphureus*) at the concentration of 100 µg/ml are shown in Fig. 2.

It was noticed that compounds **17** and **19** exhibited the strongest activity against both *L. sulphureus* and *L. betulina* amongst the four compounds tested. Both compounds **17** and **19** inhibited totally the growth of *L. betulina* (antifungal index = 100.0%) at the concentration of 100 µg/ml. As for *L. sulphureus*, however, compound **17** (antifungal index = 90.3%) showed a lower activity than compound **19** (antifungal index = 100.0%). The antifungal activity of all these compounds against *L. sulphureus* in term of the IC₅₀ values is ranked as isoeugenol (**19**) (27.6 µg/ml) > eugenol (**17**) (62.9 µg/ml) > acetyleugenol (**18**) (>100.0 µg/ml) = methyleugenol (**20**) (>100.0 µg/ml). On the other hand, the order of IC₅₀ values against *L. betulina* is eugenol (**17**) (36.9 µg/ml) > isoeugenol (**19**) (39.9 µg/ml) > acetyleugenol (**18**) (>100.0 µg/ml) = methyleugenol (**20**) (>100.0 µg/ml) (Table 2). These results indicated that lipophilicity may play, in part, a crucial role in determining the toxicity of phenylpropanes.

Table 1
IC₅₀ values (µg/ml) of cinnamaldehyde and its congeners against white-rot fungus *L. betulina* and brown-rot fungus *L. sulphureus*

Compounds	Fungi	
	<i>L. betulina</i>	<i>L. sulphureus</i>
1	>100.0	>100.0
2	>100.0	>100.0
3	58.9	35.3
4	>100.0	>100.0
5	22.0	83.5
6	55.8	87.4
7	>100.0	>100.0
8	>100.0	>100.0
9	>100.0	>100.0
10	>100.0	>100.0
11	47.2	39.2
12	49.3	66.7
13	58.2	58.5
14	>100.0	>100.0
15	>100.0	>100.0
16	>100.0	>100.0
DDAC	13.7	1.3

Fig. 2. Antifungal activities of eugenol and its congeners against white-rot fungus *L. betulina* and brown-rot fungus *L. sulphureus* (concentration used is 100 µg/ml).

Table 2
IC₅₀ values (µg/ml) of eugenol and its congeners against white-rot fungus *L. betulina* and brown-rot fungus *L. sulphureus*

Compounds	Fungi	
	<i>L. betulina</i>	<i>L. sulphureus</i>
17	36.9	62.9
18	>100.0	>100.0
19	39.9	27.6
20	>100.0	>100.0
DDAC	13.7	1.3

A similar finding was observed in other bioactivity assay. Kim et al. (2003) have evaluated the acaricidal activity of clove bud oil-derived eugenol and its congeners against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*. Their results indicated that the lipophilicity of the four phenylpropenes plays a crucial role in dust mite toxicity. Wu et al. (2005) studied the structure–activity relationship between cadinane-type sesquiterpene derivatives and antifungal activity against wood decay fungi. Their findings showed that the presence of an unsaturated double bond and oxygen-containing functional groups in the compounds plays a key role in their antifungal activity, and that the stereo configuration of cadinane-type sesquiterpenes also influences their antifungal activity.

4. Conclusions

With regard to environmental protection, the search for environmental friendly preservatives from highly durable tree species represents one approach to achieving protection of the wood without polluting the environment. In this study, we investigated the structure–activity relationships of cinnamaldehyde and eugenol congeners against wood decay fungi, which has not been reported previously. Cinnamaldehyde, α -methyl cinnamaldehyde, (*E*)-2-methylcin-

namic acid, eugenol, and isoeugenol showed good antifungal activities against all fungi tested. Clearly, the presence of an aldehyde group or an acid group, a conjugated double bond and a length of CH chain outside the ring has an influence on their antifungal properties. Furthermore, the presence of the methyl group in the *ortho* position may have a considerable influence on the inhibitory performance against *L. betulina* and *L. sulphureus*. In addition, the lipophilicity may play, in part, a crucial role in determining the toxicity of the phenylpropenes. Thus, understanding the influence of structures of both cinnamaldehyde and eugenol congeners on their antifungal actions could be helpful in designing and developing more effective wood preservatives.

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