Short Communication

Synergistic effects of cinnamaldehyde in combination with eugenol against wood decay fungi

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Abstract

The combined effects of using cinnamaldehyde with catechin, quercetin or eugenol against wood decay fungi were examined by comparing their isoeffective concentrations to that of individual compound. Among all combinations, cinnamaldehyde with eugenol revealed the strongest synergy against Laetiporus sulphureus. The synergism was due to the interference of fungal cell wall synthesis and cell wall destruction plus radical scavenging effect. Results also suggested that antioxidant with fungicidal effect might be a better candidate than pure antioxidant for the system of fungicide/antioxidant.

Keywords: Antifungal activity; Antioxidant; Synergy; Cinnamaldehyde; Eugenol

1. Introduction

Wood is widely used for a variety of purposes in both indoor and outdoor applications, but as a natural organic material, wood is susceptible degraded by many organisms. To counter fungal decay, wood products are often treated with preservatives. The traditional wood preservatives such as creosote and inorganic preservative “chromate copper arsenate” (CCA-type C) are highly effective in protecting wood, however, they have been strictly limited their use due to their toxicity, causing serious environmental hazards. Hence, developing the environment-friendly wood preservatives to replace the traditional wood preservatives becomes an imperative issue.

It has also been postulated that wood extractives can protect heartwood via at least three different mechanisms, namely fungicidal activity, free radical scavenging/antioxidation and metal chelation (Schultz and Nicholas, 2000). Schultz and Nicholas (2002) reported that combining butylated hydroxytoluene (BHT) or propyl gallate, with various organic biocides could enhance the antifungal effects. Recently, Mabicka et al. (2005) demonstrated that using 2-HPNO with either Irganox 1076 or EDTA resulted in significant synergism against a white-rot fungus. Study conducted by Hsu et al. (2007) also showed that the antifungal activities of octyl gallate were synergistically enhanced by cinnamaldehyde against various wood decay fungi.

Cinnamaldehyde, a major constituent of cinnamon essential oils, occurs naturally in the bark and leaves of cinnamon trees of the genus Cinnamomun. It has been proven to have strong antifungal activities against wide variety of wood decay fungi (Wang et al., 2005; Cheng et al., 2006), and is a potential candidate for effective and environmentally-benign wood preservatives. As for antioxidants, catechin and quercetin have been reported to process some antifungal activities (Hirasawa and Takada, 2004). Furthermore, eugenol has been demonstrated as an excellent fungicide against wood decay fungi (Wang et al., 2005).

To our best knowledge, there is no report on the antifungal activities against wood decay fungi using the combination of cinnamaldehyde with natural antioxidants such as cate-
ch, quercetin or eugenol. Therefore, the combining effects of using cinnamaldehyde with catechin, quercetin or eugenol against wood decay fungi were investigated in this study.

2. Methods

2.1. Fungal strains

The fungal strains used were white-rot fungus, Lenzites betulina (BCRC 35296) and brown-rot fungus, Laetiporus sulphureus (BCRC 35305). They were obtained from the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (Taiwan).

2.2. Chemicals

1-Diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma Chemical Co. (USA). Cinnamaldehyde, eugenol, catechin and quercetin were purchased from ACROS (Belgium).

2.3. Free radical scavenging activity

The scavenging activity of DPPH free radical was determined using the method reported by Guo et al. (2001). Antioxidants over a range of concentrations dissolved in ethanol (50 μl) were mixed with 1000 μl of 0.1 mM DPPH–ethanol solution and 450 μl of 50 mM Tris–HCl buffer (pH 7.4). Pure ethanol (50 μl) was used as the control for this experiment. After 30 min of incubation at ambient temperature, the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm of resulting solution. The lower the absorbance at 517 nm represents the higher DPPH scavenging activity. Ascorbic acid was used as the positive control and the percentage of DPPH scavenging activity is expressed by the following equation:

\[
\% \text{ Inhibition} = \left(1 - \frac{\text{absorbance of test sample}}{\text{absorbance of control}}\right) \times 100
\]

2.4. Antifungal assays

Antifungal assays were performed as described previously (Chang et al., 2000) with slight modifications. Cinnamaldehyde, catechin, quercetin and eugenol were dissolved in ethanol. Solutions of serial concentrations of chemicals were mixed with sterilized potato dextrose agar (PDA) in Petri dish (9 cm dia.) containing 15 ml agar. After inoculating the mycelia of fungus onto the center of agar, the dishes were incubated in the dark at 26 ± 2 °C and 70% relative humidity. When the mycelium of fungi reached the edges of the control dishes, the lowest concentration of the test compounds in which no recovery of microorganism was observed.

2.5. Statistical analyses

All results were expressed as mean ± SE (n = 5). The significance of difference among individual mean was determined by Scheffe’s multiple comparison procedure in SPSS. Results with P < 0.05 were considered to be statistically significant difference.

3. Results and discussion

3.1. Free radical scavenging activity of antioxidants

The free radical has been proposed to be one of the decay mechanisms by fungi (Schultz and Nicholas, 2002). Therefore, the DPPH assay was used to evaluate the relationship between radical scavenging and antifungal activities. The free radical scavenging activities of cinnamaldehyde, catechin, quercetin and eugenol were determined by DPPH assay. The EC_{50} values of cinnamaldehyde, catechin, quercetin and eugenol were >500, 5.4, 31.5 and 10.3 μg/ml, respectively. Catechin showed the strongest free radical scavenging performance among the test compounds and only slightly less than the control using (−)-ascorbic acid (EC_{50} = 3.1 μg/ml). On the other hand, cinnamaldehyde had the lowest free radical scavenging activity.

3.2. Antifungal activity of individual compound

The antifungal activities of test compounds were first examined at the concentration of 100 μg/ml, and the results are shown in Table 1. Among all compounds tested, the commercial fungicide, propiconazole, was the most effective and completely inhibited the growth of L. betulina and L. sulphureus at the concentration of 1 μg/ml. Cinnamaldehyde and eugenol also exhibited strong antifungal activities with antifungal index of 100% against both L. betulina and L. sulphureus, while catechin and quercetin did not express antifungal activities at the same concentration. The IC_{50} and IC_{90} values for individual
compounds were further determined, and the results obtained for cinnamaldehyde, eugenol, catechin and quercetin against two wood decay fungi are shown in Table 2. The IC₅₀ values of cinnamaldehyde were 0.65 and 0.23 mM against L. betulina and L. sulphureus, respectively, and the observations were in accordance with the results reported previously (Wang et al., 2005; Hsu et al., 2007). Among these three antioxidants, only eugenol showed excellent antifungal activities against L. betulina and L. sulphureus with IC₅₀ of 0.37 and 0.25 mM, respectively. On the contrary, catechin and quercetin revealed very limited inhibitory effects against L. betulina and L. sulphureus. As for IC₉₀, the similar results were found that both cinnamaldehyde and eugenol exhibited much stronger antifungal activities against L. betulina and L. sulphureus than those of catechin and quercetin. Although catechin and quercetin have been reported to have antifungal effects against Candida albicans (Hirasawa and Takada, 2004), our results clearly indicated that quercetin or catechin alone had almost no antifungal activities against wood decay fungi, and there was also no correlation between the free radical scavenging capabilities and their antifungal properties, indicating that the radical scavenging properties of test compounds might not directly contribute to their antifungal activities.

### Table 2

IC₅₀ and IC₉₀ values of test compounds and in combinations with cinnamaldehyde against wood decay fungi

<table>
<thead>
<tr>
<th>Compounds</th>
<th>L. betulina (IC₅₀ (mM))</th>
<th>L. sulphureus (IC₅₀ (mM))</th>
<th>L. betulina (IC₉₀ (mM))</th>
<th>L. sulphureus (IC₉₀ (mM))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ ± SE</td>
<td>IC₉₀ ± SE</td>
<td>IC₅₀ ± SE</td>
<td>IC₉₀ ± SE</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>0.65 ± 0.03ᵇ</td>
<td>0.72 ± 0.06ᵇ</td>
<td>0.23 ± 0.02ᵇ</td>
<td>0.53 ± 0.02ᵇ</td>
</tr>
<tr>
<td>Eugenol</td>
<td>0.37 ± 0.02ᵃ</td>
<td>0.65 ± 0.05ᵃ</td>
<td>0.25 ± 0.03ᵇ</td>
<td>0.52 ± 0.01ᵇ</td>
</tr>
<tr>
<td>Catechin</td>
<td>&gt;100ᵃ</td>
<td>&gt;100ᵃ</td>
<td>40 ± 0.12ᵃ</td>
<td>80 ± 0.14ᵇ</td>
</tr>
<tr>
<td>Quercetin</td>
<td>&gt;100ᵃ</td>
<td>&gt;100ᵃ</td>
<td>64 ± 0.25ᵇ</td>
<td>&gt;100ᵇ</td>
</tr>
<tr>
<td>Cin. + eugenol</td>
<td>0.38 ± 0.02ᵃ</td>
<td>0.63 ± 0.04ᵃ</td>
<td>0.18 ± 0.01ᵇ</td>
<td>0.37 ± 0.02ᵇ</td>
</tr>
<tr>
<td>Cin. + catechin</td>
<td>1.22 ± 0.04ᵇ</td>
<td>1.40 ± 0.09ᵇ</td>
<td>0.23 ± 0.04ᵇ</td>
<td>0.52 ± 0.04ᵇ</td>
</tr>
<tr>
<td>Cin. + quercetin</td>
<td>1.44 ± 0.06ᵇ</td>
<td>1.65 ± 0.07ᵇ</td>
<td>0.26 ± 0.02ᵇ</td>
<td>0.53 ± 0.03ᵇ</td>
</tr>
</tbody>
</table>

Results are mean ± SE (n = 5).

Means in column with different superscript letters are significantly different at alpha level of 0.05.

3.3. Combined effects of cinnamaldehyde with eugenol, catechin or quercetin

Cinnamaldehyde combined with natural antioxidants were studied to determine whether the combination of two compounds has synergistic, additive or antagonistic effects against wood decay fungi. The interaction was evaluated by comparing the isoeffective concentrations (IC₅₀ and IC₉₀) of test compounds and designated combinations. It is considered synergy when the isoeffective concentration of combination was significantly lower than those of compounds acting alone (Tallarida, 2001). Cinnamaldehyde with eugenol, catechin or quercetin were prepared at 1:1 ratio in molarities with serial concentrations for assaying, and the values of IC₅₀ and IC₉₀ were given in Table 2.

Significant synergy was observed on the combination of cinnamaldehyde with eugenol against L. sulphureus. The antifungal index of cinnamaldehyde against L. sulphureus at the concentration of 0.17 mM was 41%, and that of eugenol at the same concentration was 24%, while the antifungal index of combination using cinnamaldehyde and eugenol against L. sulphureus dramatically increased to 90%, indicating potenti of synergistic effect. The synergy was further confirmed by comparing their isoeffective concentrations. The values of IC₅₀ and IC₉₀ for the combination of cinnamaldehyde with eugenol against L. sulphureus were 0.18 and 0.37 mM, respectively, which were significantly lower than those of using either cinnamaldehyde or eugenol alone. However, only additive effect was found on the combination of cinnamaldehyde and eugenol against L. betulina with IC₅₀ (0.38 mM) and IC₉₀ (0.63 mM). In addition, the combinations of cinnamaldehyde with catechin or quercetin against L. sulphureus also exhibited additive effects, but both combinations showed marked antagonistic effects against L. betulina. The values of IC₅₀ and IC₉₀ for the combination of cinnamaldehyde with catechin against L. betulina were 1.22 and 1.40 mM, and against L. sulphureus were 0.23 and 0.52 mM, respectively. Among all samples tested, the strongest antagonistic effect was discovered on the combination of cinnamaldehyde and quercetin against L. betulina with IC₅₀.
examining the inhibitory effects of cinnamaldehyde on the synthesis of enzymes by cinnamaldehyde has been studied by susceptive to fungicides. Inhibition of fungal cell wall synthesis or altering the cell wall structure, resulting in dysfunctions of cell wall and increase of permeability to allow foreign particles entering fungal cell causing yeast death. The antifungal mechanisms of cinnamaldehyde and eugenol in wood decay fungi are very possible performing in the same way as they do in the yeast. Any action on the antifungal mechanisms of cinnamaldehyde and eugenol in wood decay fungi are very possible performing in the same way as they do in the yeast. Any action on the surface alternation induced by thymol and eugenol showed excellent antifungal properties, and strong synergy was also observed against L. sulphureus on the basis of IC50, IC90, MIC or MFC. The synergistic effect of cinnamaldehyde with eugenol could be attributed to the integrated actions of cell wall alteration, interference of cell wall synthesis, and the addition of radical scavenging. It also suggested that antioxidant with fungicidal effect might be a better candidate than pure antioxidant for the system of fungicide/antioxidant.

Acknowledgement

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References


