Evaluation of antifungal properties of octyl gallate and its synergy with cinnamaldehyde

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Abstract

The objective of this study was to investigate the possibility of using octyl gallate alone or with organic biocides as a preservative against wood decay fungi. Antifungal activities of three antioxidants, propyl gallate, octyl gallate and butylated hydroxytoluene (BHT) were tested against four wood decay fungi, *Lenzites betulina*, *Trametes versicolor*, *Gloeophyllum trabeum* and *Laetiporus sulphureus*. Octyl gallate was found to be the only active compound with IC₅₀ values of 0.47, 0.16, 0.24 and 0.04 mM against *L. betulina*, *T. versicolor*, *G. trabeum* and *L. sulphureus*, respectively. A synergistic effect was also found when octyl gallate was combined with cinnamaldehyde. Results obtained herein demonstrated that octyl gallate by itself exhibited an excellent antifungal property and enhanced protection was further observed by combining it with cinnamaldehyde.

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1. Introduction

Wood products are used extensively in both indoor and outdoor applications because of their appealing properties such as aesthetic appearance, low density, low thermal expansion and good mechanical strength. However, being a natural organic material, wood is susceptible to degradation caused by many organisms especially fungi, resulting in tremendous economic and resource losses. To extend the service life, wood products are therefore often treated with preservatives. A major problem of the wood preservatives such as creosote, pentachlorophenol and inorganic arsenicals is that they pose a serious threat to the environment. Because of this, environmentally benign organic preservatives for wood are urgently needed.

Such preservatives can be generated by studying the species of wood naturally resistant to fungi and by studying

the mechanism of fungal attack. Natural compounds extracted from various woods have been proven to have antifungal properties. Examples of such compounds are cinnamaldehyde (Wang et al., 2005; Cheng et al., 2006), α -cadinol (Chang et al., 1999), carvacrol (Kai, 1991), T-muurolol, T-cadinol, γ -cadinene (Kondo and Imamura, 1986), cryptomeridiol (Morita et al., 1997), tropolones (Hart, 1989), pinosylvins (Schultz et al., 1990), oxyresveratrol, dihydromorin (Schultz et al., 1995), gallic acid (Kishino et al., 1995) and ferruginol (Rudman, 1965), among many others.

Degradation of lignocellulosic materials because of fungi attack is a complex phenomenon not very well understood. It has been suggested that wood degradation by fungi takes place through oxidative reactions involving metals (Hammel, 1997; Kerem et al., 1999). It has also been postulated that wood extractives can protect heartwood via at least three different mechanisms, namely fungicide action, free radical scavenging/antioxidation and metal chelation (Schultz and Nicholas, 2002). Consequently, antioxidants and metal chelators may be promising additives

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for the development of novel and environmentally benign preservative systems (Suttie et al., 1996; Mabicka et al., 2004; Schultz and Nicholas, 2002).

Decay tests have been used in laboratories to verify the effectiveness of these compounds. Schultz and Nicholas (2000, 2002) found that combining the low-cost antioxidants butylated hydroxytoluene (BHT) or propyl gallate with various organic biocides resulted in enhanced effectiveness against decay caused by fungi compared to using the organic biocide alone. Among all, the best laboratory results were obtained using propyl gallate. It was also reported by Mabicka et al. (2005) that strongly significant synergy was observed using systems of either chelator EDTA and 2-HPNO or antioxidant Irganox 1076 and 2-HPNO. Strippoli et al. (2000) found that the combination of imidazole and propyl gallate gave MIC values 10–150 times lower than those of imidazole alone. However, to the best of our knowledge, antioxidant alone has little or no preservative effect against wood decay fungi as verified using the agar plate test.

Propyl and octyl gallates are currently permitted for use as antioxidant additives in food and cosmetic products (Aruoma et al., 1993) and in general Gram-positive bacteria and fungi are susceptible to the gallates. Besides, propyl gallate has a synergistic effect against wood decay fungi (Schultz and Nicholas, 2000, 2002). However, to our knowledge, there is no report on the antifungal activity against wood decay fungi using octyl gallate. Because propyl and octyl gallates are potential candidates for effective and environmentally benign organic preservatives, it is reasonable to examine their effectiveness in preserving wood. In the present study, the antifungal activity of antioxidants, BHT, propyl gallate and octyl gallate, against four wood decay fungi were evaluated. The synergistic effects of the combination using natural antifungal compound cinnamaldehyde and antioxidants were also studied.

2. Methods

2.1. Fungal strains

The fungal strains used were two white-rot fungi [*Lenzites betulina* (BCRC 35296) and *Trametes versicolor* (BCRC 35253)] and two brown-rot fungi, [*Laetiporus sulphureus* (BCRC 35305) and *Gloeophyllum trabeum* (BCRC 31614)].

2.2. Chemicals

Propyl gallate and octyl gallate were purchased from Tokyo Kasei Kogyo Co. (Japan). Butylated hydroxytoluene (BHT) and 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (America). Cinnamaldehyde was purchased from ACROS (Belgium). Commercial fungicide propiconazole was used as a positive control.

2.3. Free radical scavenging activity

The scavenging activity of DPPH free radical was determined using the method reported by Gyamfi et al. (1999) and Chang et al. (2001). Antioxidants over a range of concentrations dissolved in ethanol (50 μ l) were mixed with 1000 μ l of 0.1 mM DPPH–ethanol solution and 450 μ l of 50 mM Tris–HCl buffer (pH 7.4). Pure ethanol (50 μ l) 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. Ascorbic acid was used as the positive control and the percent inhibition ratio was calculated using the following equation:

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

2.4. Antifungal assays

Antifungal assays were performed based on Chang et al. (1999, 2000) with slight modifications. Propiconazole, cinnamaldehyde, propyl gallate and octyl gallate were dissolved in ethanol; BHT was dissolved in ethanol containing 1% Tween. Solutions of different concentrations of chemicals were added into sterilized potato dextrose agar (PDA) in Petri dish (9 cm dia.) and after transferring the mycelia of fungal strains, 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 antifungal indices were calculated. Each test was repeated three times and the average was calculated. The formula to calculate antifungal index was shown as follows:

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

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

The IC_{50} values (the concentration in that inhibited 50% of the mycelium growth) were calculated by probit analysis.

Minimal inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) were also examined using the methods reported by Kubo and Lee (1998) with slight modifications. Solutions of different concentrations of the test compounds were added to sterilized PDA media. After transferring the mycelium of four fungi strains, the testing dishes were then incubated similar to above. When the mycelium of fungi reached the edges of the control dishes, the lowest concentration at which no visible growth was observed was defined as MIC. After the MIC was determined, a plug cut from the colony of the MIC plate was taken and was inoculated on a drug-free PDA medium. After a period of time, MFC was determined by the incubation of 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 $\pm$ SD ($n = 3$). The significance of difference was calculated using SAS Scheffe's test and results with $P < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Free radical scavenging activity of antioxidants

The free radical scavenging activities of antioxidants were assessed by DPPH assay. As shown in Table 1, both propyl gallate and octyl gallate showed stronger free radical scavenging activities than the control using (–)-ascorbic acid. The EC_{50} values were 0.0075, 0.0074 and 0.0159 μ M, respectively. The effectiveness of both compounds was much stronger than that of BHT, which had an EC_{50} value of 0.27 μ M. From the molecular viewpoint, both propyl gallate and octyl gallate are gallate esters and the only difference in their molecular structures is the alkyl chain length. Comparisons of the free radical scavenging activities of these two compounds showed that the length of the alkyl chain did not have much effect on their free radical scavenging effectiveness. It was the hydrophilic pyrogallol moiety of gallates that endowed them with the free radical scavenging activity.

Table 1
Free radical scavenging activity of propyl gallate, octyl gallate and BHT by the DPPH assay

Compounds	EC_{50}	
	(μ g/ml)	(μ M)
Propyl gallate	1.6	0.0075
Octyl gallate	2.1	0.0074
BHT	59.6	0.2704
(–)-Ascorbic acid	2.8	0.0159

Results are mean $\pm$ SD ($n = 3$).

Table 2
Antifungal index (%) of test compounds against four wood decay fungi at the concentration of 100 μ g/ml

Compounds	Fungi			
	<i>L. betulina</i>	<i>T. versicolor</i>	<i>G. trabeum</i>	<i>L. sulphureus</i>
Propiconazole ^a	100 $\pm$ 0	92 $\pm$ 0.6	52 $\pm$ 3.7	100 $\pm$ 0
Cinnamaldehyde	100 $\pm$ 0	34 $\pm$ 2.2	31 $\pm$ 0.7	100 $\pm$ 0
Octyl gallate	43 $\pm$ 2.5	69 $\pm$ 9.1	85 $\pm$ 26.2	100 $\pm$ 0
Propyl gallate	1 $\pm$ 1.9	0 $\pm$ 0	2 $\pm$ 0.6	0 $\pm$ 0
BHT	16 $\pm$ 3.4	23 $\pm$ 2.6	0 $\pm$ 0	21 $\pm$ 5.6

^a The concentration of propiconazole was 1 μ g/ml.

3.2. Antifungal activity

The antifungal activities of samples are shown in Table 2. It was noticed that *L. sulphureus* was the most susceptible one. Among all samples tested, the commercial fungicide, propiconazole, was the most effective with an antifungal index of 100% against *L. betulina* and *L. sulphureus* at the concentration of 1 μ g/ml. At the concentration of 100 μ g/ml, octyl gallate exhibited stronger antifungal activity against all fungi than other two antioxidants, propyl gallate and BHT. Octyl gallate also exhibited stronger antifungal activity against *T. versicolor*, *G. trabeum* and *L. sulphureus* than cinnamaldehyde.

At 100 μ g/ml, the antifungal indices of octyl gallate were 43%, 69%, 85% and 100% against *L. betulina*, *T. versicolor*, *G. trabeum* and *L. sulphureus*, respectively. Whereas the antifungal indices of propyl gallate against these fungi were 1%, 0%, 2% and 0%, respectively. Although propyl gallate was also a strong free radical scavenger like octyl gallate, it showed no antifungal activity at 100 μ g/ml and only limited antifungal activity even at the concentration up to 500 μ g/ml (41%, 27%, 47% and 15% against *L. betulina*, *T. versicolor*, *G. trabeum* and *L. sulphureus*, respectively). It, therefore, was very clear that there was a huge gap between propyl gallate and octyl gallate in antifungal properties.

The effect on the antifungal activity against food related fungus *Zygosaccharomyces bailii* by the chain length of gallates has been reported by Kubo et al. (2001) and Fujita and Kubo (2002). They proposed a mechanism that the head and tail structure of hydrophilic pyrogallol moiety of gallates bound with an intermolecular hydrogen bond like a “hook” and attaches itself to the hydrophilic portion of the membrane surface; meanwhile the alkyl length moiety interfered with the hydrophobic interior surfaces of the membrane. As a result, the increment of the chain length increased the lipophilicity and the movement into the mem-

Fig. 1. IC_{50} and IC_{90} values of cinnamaldehyde and octyl gallate against four wood decay fungi (■: cinnamaldehyde; □: octyl gallate).

Table 3

Minimum inhibitory concentration and minimum fungicidal concentration ($\mu\text{g/ml}$) of cinnamaldehyde and octyl gallate against four wood decay fungi

Compounds	<i>L. betulina</i>		<i>T. versicolor</i>		<i>G. trabeum</i>		<i>L. sulphureus</i>	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Cinnamaldehyde	100	100	300	300	300	300	100	100
Octyl gallate	>500	–	>500	–	>500	–	50	–

– Not tested.

brane lipid bilayer portions and subsequently disturbed the fluid bilayer of the membrane. The alkyl chain length of propyl gallate was too short to influence the physical composition of the membrane and, therefore, it showed no antifungal property against *S. cerevisiae* and *Z. bailii*. This hypothesis explained the observations in this test well and warranted further study and confirmation.

BHT alone also showed little antifungal property and this observation was in agreement with results obtained by Schultz and Nicholas (2002). Among these antioxidants, only octyl gallate showed potency in inhibiting wood decay fungi. The IC_{50} and IC_{90} values obtained for octyl gallate and cinnamaldehyde against four decay fungi are shown in Fig. 1. The IC_{50} values of cinnamaldehyde were 0.65, 1.11, 1.05 and 0.17 mM against *L. betulina*, *T. versicolor*, *G. trabeum* and *L. sulphureus*, respectively, while the IC_{50} values of octyl gallate against these four fungi was 0.47, 0.16, 0.24 and 0.04 mM, respectively. This result clearly showed that octyl gallate had better antifungal property than cinnamaldehyde. As for IC_{90} , octyl gallate was also more effective than cinnamaldehyde for growth inhibition of *T. versicolor*, *G. trabeum* and *L. sulphureus*, but not for *L. betulina*.

The results of the MIC and MFC tests using cinnamaldehyde and octyl gallate are shown in Table 3. Cinnamaldehyde was more effective with a MIC of 100, 300, 300 and 100 $\mu\text{g/ml}$ against *L. betulina*, *T. versicolor*, *G. trabeum* and *L. sulphureus*, respectively, while octyl gallate showed no fungicidal activity against *L. betulina*, *T. versicolor* and *G. trabeum* up to 500 $\mu\text{g/ml}$. It was interesting to see that if the antifungal activity were measured by antifungal index, IC_{50} or IC_{90} , octyl gallate showed better performance than cinnamaldehyde; while if MIC or MFC was used, cinnamaldehyde was better. A reasonable explanation for this observation could be that octyl gallate had a stronger fungistatic property and a weaker fungicidal property than cinnamaldehyde.

3.3. Synergistic effects of the combination of octyl gallate with cinnamaldehyde

Antioxidants combined with cinnamaldehyde were studied to determine whether the combination has interactions and enhanced actions against the fungi. The antifungal index of cinnamaldehyde against *L. betulina* at the concentration of 50 $\mu\text{g/ml}$ was 6% and that of octyl gallate against *L. betulina* at the concentration of 25 and 100 $\mu\text{g/ml}$ was 16% and 43% (Fig. 2A), respectively. The antifungal index

Fig. 2. Effects of cinnamaldehyde on the antifungal activity against *L. betulina* (A) and *G. trabeum* (B) in the presence of octyl gallate. Cin: cinnamaldehyde, OG: octyl gallate. Cin₅₀: cinnamaldehyde at the concentration of 50 $\mu\text{g/ml}$. OG₂₅: octyl gallate at the concentration of 25 $\mu\text{g/ml}$.

for the treatment using the combination of cinnamaldehyde with octyl gallate was greatly increased to 64% and 100%, respectively, indicating that the cinnamaldehyde/octyl gallate combination had significant synergism against *L. betulina*. The same synergistic effect was also observed for *G. trabeum* (Fig. 2B). However, the synergistic effect was not observed for the combination of cinnamaldehyde with propyl gallate and the combination of cinnamaldehyde with BHT.

4. Conclusions

From the results it could be concluded that among the three antioxidants, octyl gallate showed good antifungal properties even better than cinnamaldehyde in terms of antifungal index, IC_{50} or IC_{90} . The antifungal action could be attributed to its pyrogallol group functioning as an attached moiety to the hydrophilic portion of the membrane surface and the octyl moiety interfering with the hydrophobic interior surfaces of the membrane. Apparently a synergistic effect existed when octyl gallate was combined with cinnamaldehyde.

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