Decay Resistance of Wood Treated with Extracts of *Cinnamomum camphora* Xylem

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Four different extracts were obtained by extracting *Cinnamomum camphora* xylem with hot water, methanol, ethyl acetate, and chloroform. Thereafter, wood (Masson pine) was impregnated with these different extracts, and the decay resistance performance of the wood treated with the extracts was studied. The results showed that the mass loss of wood treated with 4% ammoniacal copper quats (ACQ), 4% boric acid, 4% camphor, and extracts made with 10% water, 10% methanol, 10% ethyl acetate, and 10% chloroform were 1.78%, 5.7%, 13.08%, 40.85%, 9.39%, 18.66%, and 21.45%, respectively. The samples impregnated with 4% ACQ, 4% boric acid or 10% methanol extract could meet the demand of degree I (LY/T 1283-2011) for preservation and showed strong resistance to fungal decay. The results from optical microscopy and SEM indicated that treated hyphae (methanol extracts) exhibited an obvious morphological change: the cell wall became rough, and the cell expanded, became twisted, and exhibited uneven growth of hyphae, indicating that the extracts affected the structure and function of the hyphae. The low number of hyphae present within the cell walls revealed that treatment with methanol extracts provided strong resistance to fungal decay.

Keywords: Durable wood extracts; Gloeophyllum trabeum; Impregnation; Decay resistance; Wood preservatives; Scanning electron microscopy

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INTRODUCTION

Brown rot fungi represents an important group of wood decay microorganisms; the group causes billions of dollars of loss each year by destroying wood in forest trees that could be used for timber, attacking urban shade trees, and causing decay in buildings and other wood in service (Wang and Gao 2003). Wood's natural decay resistance varies in different species and parts. In durable species, decay resistance decreases incrementally from the outer heartwood to the pith (Lucchesi *et al.* 2004; Reverchon *et al*. 1994); the durability is thought to be due to the presence of various extracts in the wood (Taylor *et al*. 2008). In recent years, tree extracts as wood preservatives have received increasing attention not only because of the potential utility of decay-resistant wood sources, but also because of the need for decay protection, especially for commercial antifungal products (Qi and Jellison 2004; Wang *et al*. 2005).

Cinnamomum camphora is a type of evergreen tree that is an important non-wood and precious timber tree species in semi-tropical regions (Miyazawa *et al.* 2001); it has been cultivated widely in China. *C. camphora* is very adaptable to different growing conditions. In the wild, it can reach a height of 100 feet, with aromatic, yellowish flowers, oval-shaped red berries, and red leaves that turn dark green as the plant matures.

The leaves and wood stem of *C. camphora* contain a special aroma and volatile oil that has been used as a type of insect repellant and as an antibacterial agent, and it is thought to be ideal for timber construction (Ye and Deng 2008). Throughout history, Chinese doctors have used essential oil extracted from *C. camphora* for treating many diseases according to the principles of traditional Chinese medicine (Gruenwald *et al*. 2000). Camphor is an essential oil by steam distillation from *C. camphora*. It has pungent, hot, and slightly toxic properties, and in traditional Chinese medicine it has been associated with the heart and spleen meridians. Camphor has natural antiseptic and anti-inflammatory effects, and it also contributes to dispelling flatulence, activating blood, and cleansing toxins, *etc*. Camphor oil is usually applied to the skin to treat bruises, sprains, rheumatoid arthritis, and some inflammatory conditions; it has antibacterial effects against *Escherichia coli*, *Proteus vulgaris*, and *Staphylococcus aureus* to some extent. Camphor fumes may treat asthma, bronchitis, emphysema, and other respiratory disorders (Mishra *et al*. 1991). Extensive studies have concluded that extracts of *C. camphora* are renewable bioresources that contain abundant components of rare natural medicinal materials, high-grade spices, and top value-added chemicals that have good potential to offer a high profit margin (Liu *et al*. 2006; Chua *et al*. 2008). There are different inhibiting effects on bacterial strains depending on whether the camphor oil was extracted from the xylem, bark, roots, or leaves of *C. camphora* by different extraction methods, giving different antibacterial activity (Pandey *et al*. 1997). Guo and Yang (2012) found antibacterial activity of extracts from leaves of *C. camphora* to *E. coli*, *Bacillus subtilis*, and *Staphylococcus aureus*.

There are many research references on the inhibitory action of volatile oils of natural camphor wood on *Staphylococcus aureus*, *Salmonella*, dysentery, and *Bacillus thuringiensis* for protecting human health and improving quality of life (Yeh *et al*. 2009). However, there has been less research on the antibacterial and antifungal effects of extracts of *C. camphora*, and it is therefore significant to study the relative efficacy of extracts of *C. camphora* as wood preservatives to protect and extend the life of timber. This study investigated the antifungal properties of *C. camphora* extracts in different solvents and provided basic information on the potential of these extracts as natural wood preservatives.

EXPERIMENTAL

Materials and Methods

The experimental tree (*C. camphora*) was 45 years old and grew in the ShangJie area in Fuzhou. The stems were collected in March, 2012. Trees were cut and the zones suitable for samples were cut from the trunk at a height of 1 m to 2 m. Identification of plant material was initially performed using morphological features and then confirmed by Prof. Jin-Guo Lin at the Material Engineering College, Fujian Agriculture and Forestry University. A voucher specimen (No. 978) has been deposited in the herbarium of our laboratory. All samples were taken from three different *C. camphora* trees; the stem of the *C. camphora* tree consists of sapwood and heartwood. Malt agar solid medium was obtained from Plant Protection College, Fujian Agriculture and Forestry University (Fuzhou, China). Methanol, acetone, ethyl acetate, and chloroform were purchased from Shanghai Chemical Reagent Factory (Shanghai, China). Ammoniacal

copper quats (ACQ), boric acid, and camphor powder were purchased from three factories in Fujian province.

Extracts Preparation

The debarked wood (*C. camphora*) was milled to powder having a mesh size within the range 40 to 60. The wood powder was extracted by Soxhlet extractor, the liquor reflux 4 times at 1h. The anhydrous powder was extracted with hot water, methanol, ethyl acetate, or chloroform for 6 h. After the extracted liquor was separated and concentrated, and the concentrated extracts were diluted with extract solvent to obtain a 10% concentration. Meanwhile, ACQ, boric acid, and camphor were kept at concentration of 4%.

Decay Test

Wood Impregnation with extracts

The choice wood species for the test trial was the sapwood of *Pinus massoniana* L. (Masson pine), which is a plantation tree with wide distribution in China (Zhang *et al.* 2010). It was found to be very susceptible to biological deterioration by brown rot fungus. It is usually treated with chromated copper arsenate (CCA), ACQ, *etc*. (Shi *et al.* 2007), but the toxic materials released from wood treated with CCA are dangerous to human health and the environment and have generated a lot of controversy (Weis *et al*. 1998; Ahn *et al.* 2010). We therefore attempted to use plant extracts for wood protection (Schultz and Nicholas 2000, 2002). Sapwood of Masson pine was cut into blocks of 20 mm×20 mm×10 mm (radial×tangential×longitudinal) and dried to absolute dry weight at a temperature of 40 °C before impregnation. Optimal vacuum-pressure preservative impregnation parameters for wood treatment were used. Blocks were impregnated in a small-scale impregnation container by applying pre-vacuum for 30 min, with a relative vacuum of -0.09 MPa. After blocks were sunk into the solvent, the vacuum was released and the samples were removed from the treatment solution, wiped lightly to remove solution from the wood surface, and weighed (to the nearest 0.01 g) to determine the retention of each solution. Wood preservative absorption was calculated using the following formula,

$$R = (m_2 - m_1)c / V \quad (1)$$

where R (kg m^{-3}) is wood preservative absorption; m_1 and m_2 represent the sample weight before and after treatment, respectively, c is the preservative solution concentration, and V is the block volume.

Blocks were dried at 40 °C to absolute dry weight and weighed, then sterilized in an autoclave for 30 min. Three of the sterilized blocks were placed in a 500-mL flask for the decay test.

Fungal strains

The wood-degrading fungus *Gloeophyllum trabeum* (brown rot fungus) was used in the decay resistance test. *G. trabeum* (cfcc isolate 86019) was a generous gift from the Plant Protection College, Fujian Agriculture and Forestry University (Fuzhou, China). The toxicity of wood preservatives to decay fungi was determined by Chinese standard LY/T 1283-2011. The petri dish containing 25 mL 4% (w/v) malt agar solid medium was inoculated with *G. trabeum*, and the fungi were cultured at 28 °C ± 2 °C and 75% to 85%

relative humidity for seven days. Finally, the brown rot fungi were transferred to 500-mL sterilized culture flasks, where they were grown to establish active hyphae.

Exposure conditions

The treated samples were tested for decay resistance according to Chinese standard LY/T 1283-2011. Six replicates for each treatment were used. Untreated wood blocks were included to measure the viability of the fungal strains. The incubation time was 12 weeks at 28 °C ± 2 °C and 75 % to 85 % relative humidity. After incubation, blocks were removed from the culture flask, and fungal hyphae and impurities were removed from the surface of blocks and dried at 40 °C to absolute dry weight and weighed (to the nearest 0.01 g). The mass loss of each block caused by fungi was calculated using the following formula,

$$\text{Mass loss } (\%) = [(m_3 - m_4)/m_3] \times 100 \quad (2)$$

where m_3 is dry mass prior to the test and m_4 is dry mass after the test.

After 12 weeks of decay, the untreated wood was investigated and it was determined that the untreated wood minimum mass loss was not lower than 23% and the average mass loss was 36%; thereafter, the treatment samples were tested.

Microscopic Observation of the Role of Hyphae

Brown rot fungus was cultured in malt agar solid medium for 4 days; then, 1 mL methanol extracts of *C. camphora* were added to a concentration of 8 mg mL^{-1}. After 24 h, a microscopic image analysis system was used to observe the effects of extracts on brown rot fungus hyphal growth.

RESULTS AND DISCUSSION

Decay Resistance

The wood preservation activities of extracts of *C. camphora* against *G. trabeum* were compared with ACQ, boric acid, and camphor under laboratory conditions. ACQ and boric acid were dissolved with hot water, and the camphor was dissolved with chloroform. Following the approach of the Chinese standard, Masson pine sapwood samples were separately impregnated with concentrations of 4% ACQ, 4% boric acid, 4% camphor, or 10% extracts of *C. camphora* made with hot water, methanol, ethyl acetate, or chloroform. The average wood preservative absorption capacity of the blocks treated were 16.37 kg m^{-3}, 13.65 kg m^{-3}, 25.42 kg m^{-3}, 50.39 kg m^{-3}, 50.54 kg m^{-3}, 44.6 kg m^{-3}, and 34.52 kg m^{-3}, respectively. There was no significant difference in wood preservative absorption capacity between the blocks (hot water and methanol) impregnated under the same conditions.

The mass loss test results presented in Table 1 revealed that the range of the mass loss of blocks treated with extracts of *C. camphora* was 9.39% to 40.85% and that the 10% methanol extracts had the greatest resistance to fungal decay of all the extracts. Table 1 shows that the blocks treated with 4% ACQ, 4% boric acid, or 10% methanol extracts could meet the demand of degree I of preservation and showed strong resistance to brown rot fungus attack. The wood treated with 4% camphor, 10% ethyl acetate, or 10% chloroform reached the demand of degree II and showed moderate decay resistance.

The untreated wood and blocks treated with 10% hot water and untreated samples meet the demand of degree III. Sapwood of Masson pine can be considered to be a non-resistant species against *G. trabeum* because the mass loss after 12 weeks of incubation reached 38.16%. The samples treated with hot water extracts had approximately 40.85% mass loss. This is likely due to poor extraction of *C. camphora* by hot water; alternatively, it may be that the extract had some nutrient composition that could promote the growth of brown rot fungus.

Table 1. Mass Loss in Samples Exposed to *G. trabeum* after 12 Weeks

Samples	Wood preservative absorption (kg/m^3)	Mass loss (%)	*Decay resistance level
ACQ	16.37	1.78	I
Boric acid	13.65	5.7	I
Camphor	25.42	13.08	II
Hot water extracts	50.39	40.85	III
Methanol extracts	50.54	9.39	I
Ethyl acetate extracts	44.6	18.66	II
Chloroform extracts	34.52	21.45	II
Untreated	N/A	38.16	III

*Mass loss less than 10% is considered to be degree I, which indicates strong decay resistance; an average mass loss of 11% ~ 24% can be classified as degree II, indicating moderate resistant to decay fungi; an average mass loss of 25% ~ 44% can be classified as degree III, which is considered non-resistant; a mass loss higher than 45% is considered to be degree IV.

The results indicate that the antifungal activity of methanol extracts was stronger than the other three extracts, but the sapwood of Masson pine treated with ACQ or boric acid at concentrations of 4% was significantly more resistant to brown rot fungus than that treated with *C. camphora* extracts at a concentration of 10%. Obviously, none of the extracts' decay-resistant property could reach that of commercial wood preservatives. This finding suggests that to become more resistant to brown rot fungus, the extracts should be mixed with another antifungal material to further improve their fungitoxicity and improve their wood preservative property.

Microscopic Observations

Observed hyphae

G. trabeum was observed microscopically after treatment with methanol extracts at a concentration of 8 mg mL^{-1}. After 24 h of treatment, the morphological characteristics of aerial hyphae appeared uneven, with swollen and excessive branching; part of the swollen hyphal areas continued to expand, which resulted in the formation of extremely swollen bodies of irregular shape; some of the hyphae had apoptosis and condensed endosomes, which leaked out and formed voids. The untreated samples had transparent hyphae with diaphragms and smooth walls and had fewer branches (Wang *et al*. 2011), but the treated hyphae had an obvious change: the cell wall appeared rough; cells expanded and grew twisted, with uneven growth of hyphae, indicating that the extracts affect the structure and function of the hyphae (Fig. 1). After 48 h of treatment, *G. trabeum* hyphae displayed significant morphological changes and lost their distinct structures. The number and size of vesicles increased, its walls thickened, and cytoplasm was seriously degenerated with increasing treatment time. The hyphae were distorted and

adhered together; hyphal surfaces became coarse and sunken, many blank areas appeared, and the hyphal structure appeared destroyed.

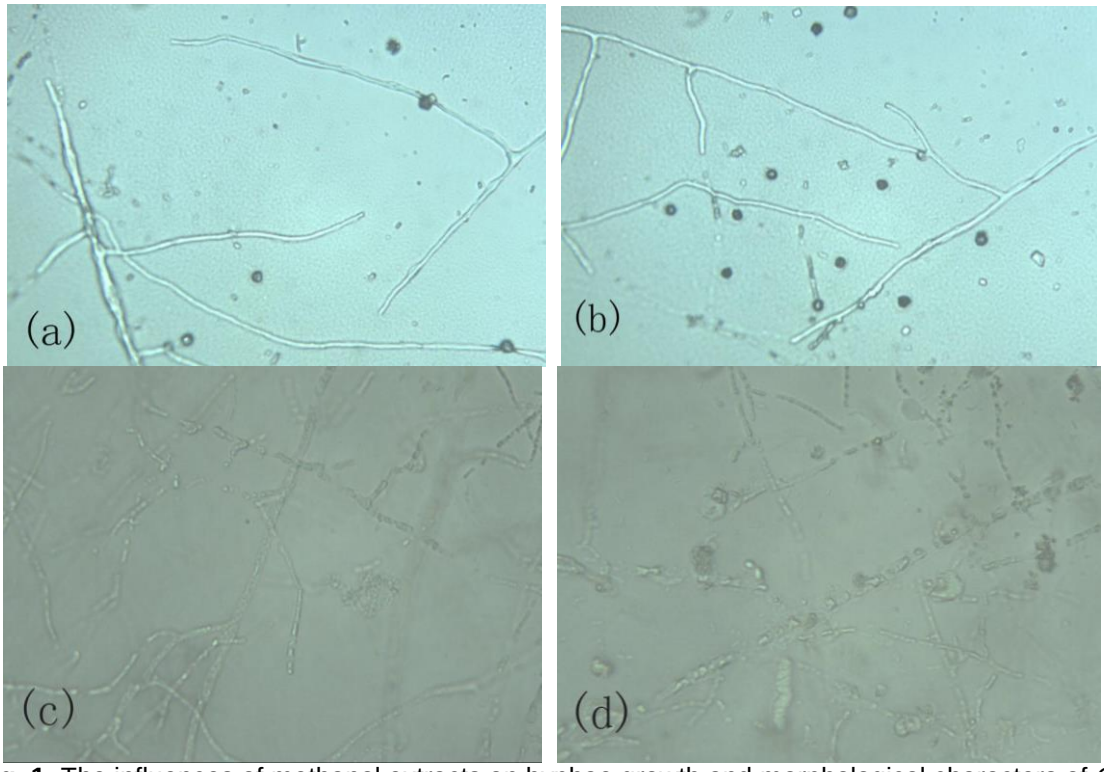

Fig. 1. The influences of methanol extracts on hyphae growth and morphological characters of *G. trabeum*. (a), (b): Natural hyphae (×400). (c): Treatment by methanol extracts (×400) after 24 h. (d): Treatment by methanol extracts (×400) after 48 h

The results from hyphae treated with methanol extracts suggest that *C. camphora* extracts with methanol had good antifungal activity against *G. trabeum*. These figures imply that the antifungal activity of extracts is associated with the chemical composition of *C. camphora*.

Observations of Decayed Wood

The SEM images offered a clear view of the anatomical characteristics of treated and untreated samples (Fig. 2).

Many scholars have discussed the mechanism of wood decay. In the early stages of decay, brown rot fungi use hydroxyl radicals generated by Fenton chemistry. These reactive oxygen species randomly attack compounds within close proximity, causing a rapid depolymerization that alters the chemical composition of wood (Howell *et al*. 2011; Kim *et al*. 2002). The S_2 layer of tracheids is attacked first by the brown rot fungi, whereas the S_3 layer and the middle lamella remain intact, even in the advanced stages of decay (Cho *et al*. 2008; Highley *et al*. 1985). The growth of wood-inhabiting fungus in samples was observed by SEM. As shown in Fig. 2(a), covering the hyphae that were clearly visible in the decayed tracheids, the area of this sample had decay. The brown rot fungus could traverse from one cell to another through natural openings as well as through bore holes.

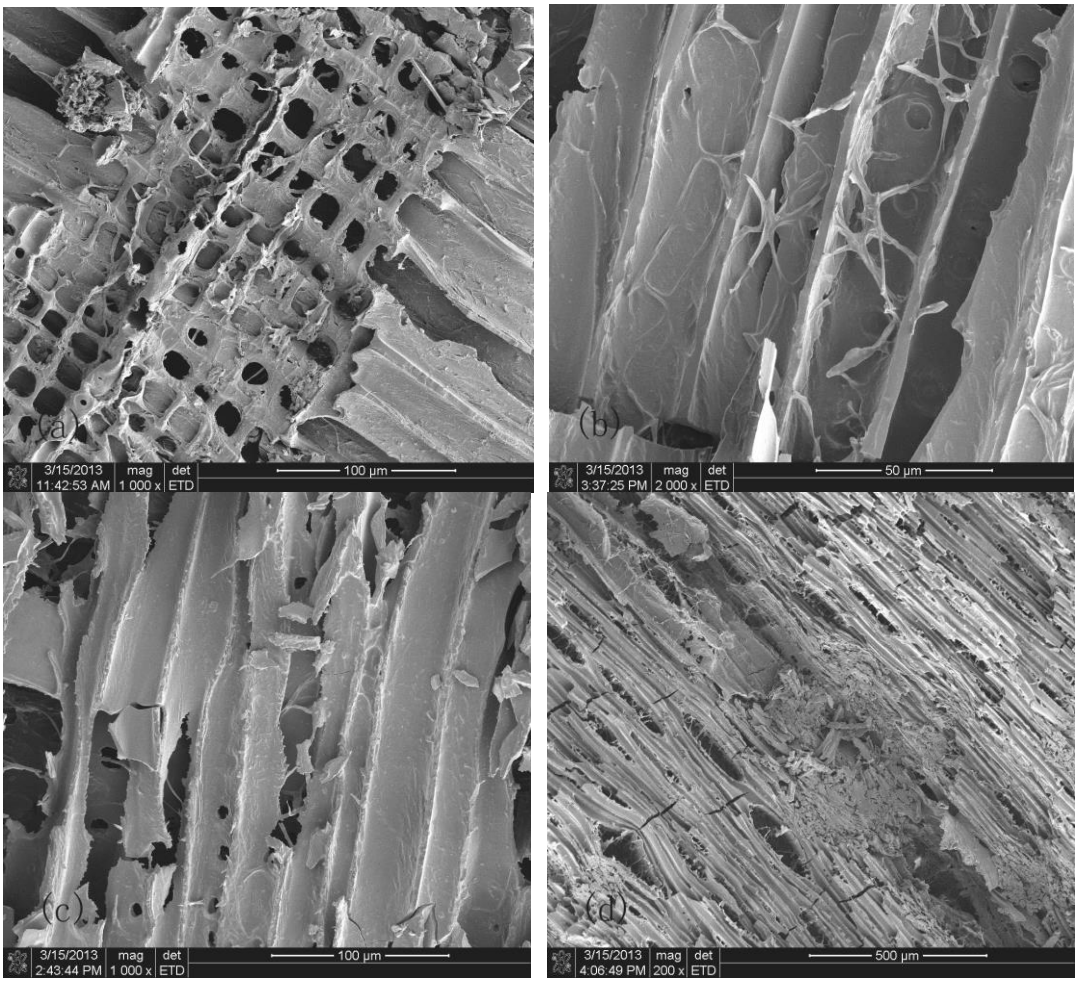

Fig. 2. Scanning electron micrographs of the samples decayed for 12 weeks. (a): Treatment with 10% hot water extracts. (b): Treatment with 10% chloroform extracts. (c): Treatment with 10% methanol extracts. (d): Treatment with 10% ethyl acetate extracts.

From Fig. 2(b), an SEM micrograph of a tangential section of the sample showing a tracheid plugged with hyphae, the right side of the hyphae gradually penetrated to the left. The SEM micrograph in Fig. 2(c) clearly shows that slight decay can be seen within the cell walls, but the left of the cell walls appear to be ruptured. The wood blocks treated with ethyl acetate extracts are shown in Fig. 2(d), and a large number of hyphae were found in the cell lumens. Brown rot fungi degrade wood and produce extracellular enzymes that break down the woody cell wall (Highley *et al*. 1983). The hyphae not only grew along the longitudinal axis of the cells, but also traversed cells perpendicularly and made bore holes (Patel and Rao 1993).

The observation from scanning electron microscopy indicated that the more hyphae that penetrate holes in the cell walls, the higher the amount of decay. In all samples, treatment with methanol extracts showed strong resistance to fungal decay, whereas treatment with hot water extracts showed the worst. The decay-resistant property of the ethyl acetate extract was better than that of the chloroform extract.

CONCLUSIONS

1. The range of the mass loss of blocks treated with extracts of *C. camphora* was 9.39% to 40.85%, and the antifungal activity of methanol extracts was stronger than that of the other three extracts.
2. The blocks treated with 4% ACQ, 4% boric acid, or 10% methanol extracts could meet the demand of degree I of preservation and showed strong resistance to brown rot fungus attack.
3. Microscopy revealed that hyphae treated by methanol extracts of *C. camphora* had an obvious morphologic change: the cell wall became rough, and the cell expanded, became twisted, and exhibited uneven growth of hyphae.
4. The observations from SEM indicated that treatment with methanol extracts of *C. camphora* conferred strong resistance to fungal decay because relatively little hyphae were observed within the cell walls.

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