Antifungal Ability and Decay Resistance of *Fokienia hodginsii* Heartwood Extract and its Inhibitory Effect on *Gloeophyllum trabeum*

Jian-Fei Wu,[a] Xue-Feng Zhuang,[a] Hong-mei Yuan,[a] Quan Li,[a,b] Wei Wang,[a] Ji-Qing Li,[a,*] and Jin-Guo Lin [a]

The inhibitory ability of *Fokienia hodginsii* heartwood (FHH) extracts on *Trametes versicolor* (TV) and *Gloeophyllum trabeum* (GT) as well as the toxic effect of its heartwood extracts on GT were studied. The growth inhibition ability of the samples was analyzed using the growth rate method. The results showed that in the experiment of inhibiting TV, extracts using hot water had little effect, acetone extracts had the best inhibiting effect, and the lowest value of acetone EC_{50} was 0.409 g/L. The parameter EC_{50} is the concentration of the corresponding agent that inhibits the growth of 50% fungi. In the antifungal experiment of GT, methanol extract had the best inhibition effect, and the lowest EC_{50} value was 0.283 g/L. The antifungal effect of five solvent extracts of FHH was good when the concentration was 10% (w/w), and at this time, the mass loss rate of the test pieces was below 11%, all of them were Class I, indicating a strong antifungal level. After observing the samples of GT with SEM, it was found that the structure of methanol extract treatment was more complete and the antiseptic effect was better than that of the hot water extract treatment.

Keywords: Fokienia hodginsii; Extract; Gloeophyllum trabeum; Inhibition; SEM observation; antifungal; Trametes versicolor

Contact information: a: College of Material Engineering, Fujian Agriculture and Forestry University, Fuzhou 35002, Fujian, China; b: Kaili University, Kaili 556011, Guizhou, China;
** Corresponding authors: nfuljq@163.com; fjlinjg@126.com.*

INTRODUCTION

Fungi, such as white-rot fungi and brown-rot fungi, can seriously decompose and destroy wood. The *Trametes versicolor* (TV) studied in this work is a type of white-rot fungus and *Gloeophyllum trabeum* (GT) is a type of brown-rot fungus. Under suitable conditions, they use lignin, cellulose, and hemicellulose as nutrients. They spread and infect in the form of spores, causing wood rot. Antifungal active ingredients are present in various plants in nature, such as trees, and these antifungal active ingredients are plant extracts (Alvarez-Castellanos *et al*. 2001; Barron *et al*. 2003; Bajpai *et al*. 2008; Ahnet *et al*. 2010; Bhardwaj *et al.* 2012). Studies have shown that extracts from more than 40 plants have varying degrees of antifungal effects, including extracts from spruce, larch, peach, juniper, olive, litchi, poplar, African hardwood, toothbrush tree, citrus, rubber wood, *etc.* (Özkan *et al*. 2015; Peng *et al.* 2015; Salem *et al*. 2016; Yang *et al*. 2016; EL-Hefny *et al*. 2017; Onuorah *et al*. 2002; Abhary and Al-Hazmi 2016; Okla *et al*. 2019; Vainio-Kaila *et al*. 2015; Uyup *et al*. 2019). Some of these antifungal substances can be secreted directly on the surface of plants. Nakayama (2005) in the United States reported

that some plants, such as silver geranium, sugar cane, *etc*., secreted resin components that have an antifungal function (Oumzil *et al*. 2002; Tascioglu *et al*. 2013). However, in fact, most of the antifungal components exist in the plant body and need to be extracted with a solvent such as ethanol. Eller *et al*. (2010) treated wood samples with extracts from North American juniper using liquid carbon dioxide and ethanol. The extracts were tested for fungal decay and insect resistance. Nanasombat *et al*. (2018) determined the antifungal activity of methanol extracts from 25 Thailand medicinal plants against 10 strains of fungi. The results showed that ethanol and other organic solvents were suitable for the extraction of antifungal substances in plants. The concentration of FHH extract is relatively low, and some people used to worry that this would affect its antifungal effect. In recent years, scientists have evaluated the antifungal and antimicrobial activities of two types of pyroic acid (PA), and studies have shown that both types of PA inhibit all microorganisms even at a minimum concentration of 20% (De Souza Araujo *et al*. 2017). This suggests that concentration is not the most important determinant of antifungal substances. Because the heartwood has better antifungal properties than sapwood, FH's heartwood was selected in the present work as the material. (Cheng *et al*. 2003; Harju *et al*. 2003; Gierlinger *et al*. 2004; Chua *et al.* 2008).

There are many types of cypress trees, and the effects of cypress extracts have been studied for a long time. For example, Noruzi *et al*. (2012) used cypress extract for the synthesis of gold nanoparticles. Shahali *et al*. (2009) studied the response of immunoglobulin E to cypress pollen extract. Takaiwa and Yang (2014) developed a vaccine against cypress pollen. Many cypress extracts also have antifungal effects, and *Fokienia hodginsii* (FH) is one of them. FH is a kind of cypress tree with straight trunks, solid material, and strong decay resistance. It is often used in architecture and furniture. It is distributed in southern China provinces, such as Fujian, Sichuan, Yunnan, and Guangdong, and belongs to the national 'second-class' of endangered plants. At the same time, the aromatic oil of FH has antifungal and anti-tumor effects, and it can also be used as a spice for making soap. The FH has a strong resistance to fungal decay. At present, people know little about the tree. Therefore, the inhibitory effect of *Fokienia hodginsii* heartwood (FHH) extract on TV and the GT and the toxic effect of the heartwood extract on the GT were studied. It is of great importance for understanding the antifungal and antiseptic mechanism of FHH. This research also has important practical application value for the rational utilization of the wood processing residues, such as scrap, sawdust, and saw powder by FH, in its production and processing.

EXPERIMENTAL

Materials

Test species

The FHH scrap and sawdust were taken from the forest products market of Shunchang County, Nanping City, Fujian Province, China. A small amount of FHH were dried and sawn into 20 mm × 20 mm × 10 mm specimens in the direction of the grain for the determination of natural decay resistance. Most of the cedar heartwood scraps and sawdust were air-dried, crushed, sieved with a pulverizer, and screened to 40- to 60-mesh wood powder as the raw material for the test. Finally, the obtained FHH powder was placed in the freezer and sealed for use. Masson pine, taken from Shunchang County, Fujian Province, had a tree age of 20 to 25 years. Because masson pine is easily degraded

by brown-rot fungus, it is meaningful to study its fungal decay resistance level. The feeding wood for the natural wood test and the preservative toxicity test was 20 mm × 20 mm × 10 mm, and was fed the direction of the grain.

Test strains

The white-rot fungi *Trametes versicolor* was supplied by the China Forestry Microbial Culture Collection Management Center (Beijing, China). The brown-rot fungus *Gloeophyllum trabeum* was supplied by the College of Life Sciences of Fujian Agriculture and Forestry University (Fuzhou, China).

Solvents and other materials

Hot water was made by adding tap water to the distillation flask, then adding some broken porcelain pieces, and then plugging it with a rubber plug inserted with a thermometer. The distillation flask was heated. When the water temperature reached approximately 100 °C, the water was boiled, and the water vapor was collected through the condensation tube after condensation in the bottle. The methanol, chloroform, acetone, petroleum ether, ethyl acetate, all of analytical pure and obtained from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China); the agar, maltose, and potatoes, *etc.* were purchased from the local market.

Preparation of maltose agar medium

After peeling the potatoes, they were chopped and soaked in hot water. Subsequently, 200 g of potatoes, 15 g of agar, 20 g of maltose were weighed, and 1000 mL of hot water was weighed, dissolved by heating, filtered, and then diluted to a volume of 1000 mL. Then, 150 mL of medium was placed in 6 conical flasks. The flasks were placed in the steam sterilizer, where they were subjected to high pressure moist heat sterilization for 30 min at 121 °C.

Methods

Instruments and equipment

The scanning electron microscope (SEM) was mainly used to observe the microstructure of samples (FEI-Quanta-200; FEI Company, Hillsborough, OR, USA). The magnification level was 100 times and 500 times to observe the sample. Samples were dried with a blast dryer at 45 °C (DHG-9246A; Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China). Strains were cultured in a humidified incubator with a temperature of 28 °C and a humidity of 80% (HW-150; Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China).

The virulence regression equation was calculated using Microsoft Office 2010 (Microsoft Corporation, Redmond, WA, USA), the experimental further explains for the specific calculation process. The correlation coefficient R was obtained by the CORREL function, and then R^2 was obtained.

Preparation of extracts

The mixture was subjected to hot reflux extraction using a condenser; approximately 100 g of the 40- to 60-mesh-sized FHH wood powder was placed in a round bottom flask and extracted with one of six different kinds of organic solvents. The extraction was completed in two portions, and the extraction was performed for the first time according to the ratio of the raw material to the solvent of 1:7 (g/mL) for 5 h,

followed by removal of the filtration. The second extraction was performed for 3 h according to the ratio of the raw material to the solvent of 1:5 (g/mL), followed by filtration. Next, the two filtrates were mixed and evaporated under reduced pressure with a rotary evaporator to obtain a paste. Subsequently, the paste was dissolved in 6 different solutions to prepare the samples and then placed in a refrigerator at 4 °C for future use.

Determination of the antifungal activity of extracts

The growth rate of the toxic medium culture method (Huang 1993) was used to determine the inhibition effects of the six chemical solvents of the FHH extract relative to the growth of TV and GT. First, the two wood-decay fungi were cultured on a potato dextrose agar (PDA) plate for 7 days, and the sterilized medium was heated, melted, and cooled to approximately 50 °C. A certain amount of medium was placed in the culture dish, and then pipetted to separately measure the test sample into a sterile Petri dish, where it was shaken. The concentrations of the six medicated media for inhibiting TV are shown in Table 1. The concentrations of the six solvent-containing media for inhibiting brown-rot fungi are shown in Table 2, and blank control group was also set. Then, the inoculation test was performed. A hole cutter was used with a diameter of 7.0 mm to make the mushroom cake at the edge of the colony, and the inoculating ring was used to move fungi to the center of the above six culture dishes (one of them was a blank control). To avoid the inhibition of the two fungi by chemical solvents, 1 mL of each of the different extraction reagents was added to each of the six culture dishes. During the experiment, it was found that a trace amount of methanol solvent highly inhibited the two wood-decaying fungi, so the methanol solvent in the drug-containing medium was volatilized before it was transferred to the mycelium block. The test was repeated three times, and then the culture dish was placed in a constant temperature and humidity incubator at a temperature of 28 °C and humidity of 80%. If the hyphae in the culture dish of the control group grew to approximately two-thirds of the diameter of the culture dish, the growth diameter of the colony was measured by the cross method, and the average value was calculated. The inhibition rate (IR) was calculated according to Eq. 1:

$$\text{Mycelium growth IR (\%)} = \frac{\text{Control colony growth diameter} - \text{Treat growth diameter}}{\text{Control colony growth diameter}} \times 100 \quad (1)$$

Table 1. Inhibiting the Concentration of Drug-containing Medium in Each Solvent of TV

Solvent (g/L)	Methanol	Acetic Acid	Acetone	Chloroform	Petroleum Ether
Concentration a	0.1250	0.1250	0.1250	0.1250	0.5000
Concentration b	0.2500	0.2500	0.2500	0.2500	1.0000
Concentration c	0.5000	0.5000	0.5000	0.5000	2.0000
Concentration d	1.0000	1.0000	1.0000	1.0000	4.0000
Concentration e	2.0000	2.0000	2.0000	2.0000	6.0000

Table 2. Inhibiting the Concentration of Each Solvent-containing Medium in the GT

Solvent (g/L)	Methanol	Acetic Acid	Acetone	Chloroform	Petroleum Ether
Concentration a	0.1250	0.1250	0.0625	0.1250	0.5000
Concentration b	0.2500	0.2500	0.1250	0.2500	1.0000
Concentration c	0.5000	0.5000	0.2500	0.5000	2.0000
Concentration d	1.0000	1.0000	0.5000	1.0000	4.0000
Concentration e	2.0000	2.0000	1.0000	2.0000	6.0000

The EC_{50} value of virulence is the concentration of a poison that causes some harm to half of the test subjects over a certain period of time. In this paper, EC_{50} refers to the concentration of the corresponding agent that inhibits the growth of 50% fungi. If the measured value is smaller than EC_{50}, then the antifungal ability of the agent is stronger. The calculation of virulence EC_{50} value is as follows. Calculate the probability value corresponding to each bacteriostatic rate from the conversion relationship between the bacteriostatic rate and the probability value. Then, use the logarithmic value to indicate the concentration. The logarithmic value (x) at each concentration of each chemical solvent extract is subtracted from the probability value (y). Then, the virulence regression equation is obtained (*i.e.*, $y = a + bx$), and the toxic EC_{50} value that inhibits mycelial growth is calculated (Chattapadhyay *et al.* 2006).

Natural durability test

According to Chinese Standard GB/T 13942.1 (2009), the decay resistance test of FHH was performed. The mass loss rate (T) was calculated after decay of each test piece and expressed as a percentage. The natural decay resistance loss rate percentage T is given by Eq. 2,

$$T = \frac{W_1 - W_2}{W_1} \times 100\% \quad (2)$$

where W_1 is the completely dry mass before the test piece (g), and W_2 is the completely dry mass after the test piece (g).

Preparation of preservatives

The six solvent extracts (methanol extract, ethyl acetate extract, acetone extract, chloroform extract, petroleum ether extract, and distilled water extract) were dissolved in corresponding solvents to prepare test solutions at the concentrations of 10%, 8%, 6%, 4%, and 2% (w/w). Amine copper quaternary (ACQ) was mixed with distilled water to prepare the drug solutions with a concentration of 2%, 1%, 0.5%, 0.25%, and 0.125%.

Preservative toxicity test

The toxicity determination test was performed according to Chinese Standard LY/T 1283 (2011). The drug loading (R) of the preservative for each sample (kg/m^3) was calculated according to Eq. 3,

$$R = \frac{(m_1 - m_2) \times c}{V} \times 10 \quad (3)$$

where m_1 is the constant mass of the test piece before the preservative is absorbed (g), m_2 is the quantity of the specimen immediately weighed after the preservative is absorbed (g), C is the preservative solution concentration (% mass fraction), and V is the sample volume (cm^3).

The percentage of mass loss (L) was calculated after the decay of each test piece using Eq. 4,

$$L = \frac{m_3 - m_4}{m_3} \times 100\% \quad (4)$$

where L is the test piece mass loss rate (%), m_3 is the constant mass of the test piece before decay (g), and m_4 is the constant mass of the test piece after decay (g).

Experimental conditions of SEM

10% methanol extract solution was sprayed on the surface of FHH. The other group was exposed to 10% hot water extract, which was sprayed on the surface of FHH. There was also a blank control group. The sample was placed in a blast oven, the temperature was set to 45 °C, and dried until a constant mass was obtained. Then, the sample was cut into thin slices, the sample was pasted on the sample stage, and the sample was gold sputtered (Spray Konica Minolta MCM-200; Beijing Hanmeng Zixing Instrument Co., Ltd., Beijing, China). Finally, the samples were imaged with different SEM magnifications.

RESULTS AND DISCUSSION

FH Extract Yield and Paste Color

The yields of the six solvent extracts and the color of the paste materials are shown in Table 3. In addition to hot water, the yield of the extract was positively correlated with the polarity of the solvent in the six solvents, namely that methanol was higher than acetone > ethyl acetate > chloroform; the extract obtained from the non-polar solvent, petroleum ether, was the lowest.

Table 3. FH Extract Yield (% of Raw Materials) and Paste Color

Solvent	Methanol	Acetone	Ethyl Acetate	Chloroform	Petroleum Ether	Hot Water
Yield (%)	6.2	4.5	4.3	4.1	2.3	4.2
Paste Color	Orange-red	Dark orange	Orange-yellow	Dark orange-red	Light yellow	Dark red

Determination of the Activity of Two Kinds of Fungi by FHH Extract

From the inhibition rate of the extracts shown in Fig. 1 on white-rot fungi, it can be concluded that in addition to hot water, the five solvent extracts of FHH had different degrees of inhibiting TV. In general, the inhibitory effects of the five solvent extracts increased with the concentration of the drug solution; inhibition was positively correlated with the concentration, because a higher concentration resulted in more antifungal components present in the drug-containing medium. At a concentration of 0.5 g/L, the

inhibition percentages of acetone, ethyl acetate, methanol, petroleum ether, and chloroform extract on the TV were 48.5%, 47.7%, 42.1%, 37.0%, and 35.9%, respectively. Among them, the FHH extract from three solvents, such as acetone, ethyl acetate, and methanol, had an inhibition rate of 50% for TV, and the inhibition rate at the same concentration was appreciably higher than the other two chemical extracts.

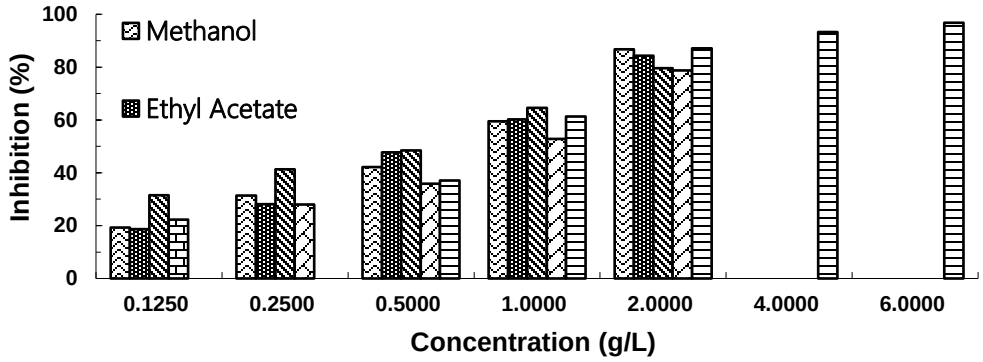

Fig. 1. Antifungal activity of different solvent extracts against TV

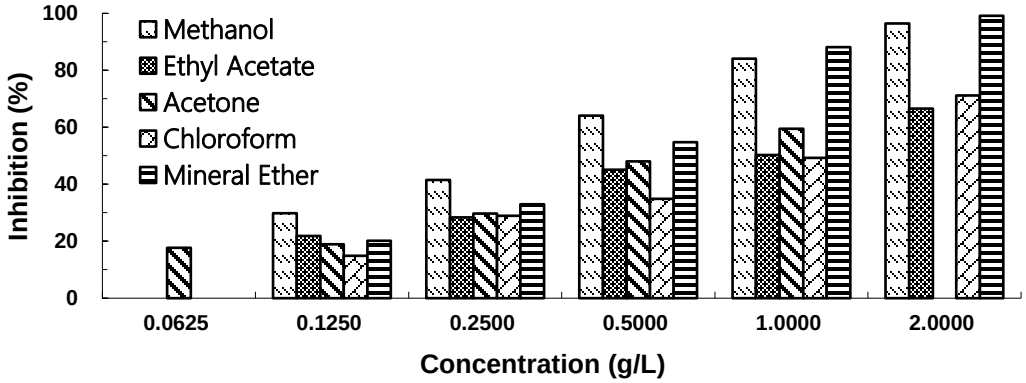

Fig. 2. Antifungal activity of different solvent extracts against *G. trabeum*

Based on the results shown in Fig. 2, the six kinds of reagent extracts of the FH heartwood had inhibitory effects on the viscous fungi, except for the hot water extracts. The inhibition percentages of methanol, petroleum ether, acetone, ethyl acetate, and chloroform extracts against brown-rot fungi at a concentration of 0.5 g/L were 64.0%, 54.8%, 48.0%, 45.1%, and 34.8%, respectively. Overall, the inhibitory effects of the reagent extracts with methanol, petroleum ether, acetone, ethyl acetate, and chloroform increased with increasing concentration.

Determination of the Effects of the Extract of FHH on the TV and the GT

The inhibitory effects of different solvent extracts of FHH to TV (Table 4) showed that the concentration of five extracts of FHH was positively correlated with the antifungal effect. The coefficient of determination, R^2 (Microsoft Office 2010, Microsoft, Office 2010, Redmond, WA, USA), of the virulence regression equation was greater than

0.9. With the increase of extract concentration, the inhibitory effect of the extract on the TV was gradually enhanced.

The EC_{50} values of the five solvent extracts of FH against TV were in the range of 0.409 to 0.690 g/L. According to the EC_{50} value, the order of inhibition (from highest to lowest) of the five solvent extracts of cedar heartwood on *C. versicolor* was acetone extract, methanol extract, ethyl acetate extract, chloroform extract, and petroleum ether extract. The hot water extract had no substantial bacteriostatic action. In the wood antifungal application, for the TV EC_{50} value, the optimum solvent for the extraction of FHH was acetone. However, compared to the traditional wood preservative, ACQ, the EC_{50} value of acetone extract was much greater than that of ACQ (Li and Yan 2005).

Table 4. Inhibitory Effects of Various Solvent Extracts of FHH toward TV

Chemical Solvent	Virulence Regression Equation	R^2	[a] EC_{50} (g/L)
ACQ	$y = 1.229x + 7.2931$	0.9612	0.0138
Acetone	$y = 1.064x + 5.4127$	0.9705	0.4094
Methanol	$y = 1.5558x + 5.4287$	0.9482	0.5302
Ethyl acetate	$y = 1.5381x + 5.4108$	0.9758	0.5407
Chloroform	$y = 1.2544x + 5.2104$	0.9170	0.6796
Petroleum ether	$y = 2.0294x + 5.3272$	0.9855	0.6899
Hot water	/	/	/
Note: [a] refers to the average of three replicates; ACQ data from Li and Yan (2005)			

It can be seen from Table 5 that the coefficients of determination (R^2) of the virulence regression equations of the five solvents (excluding hot water) were more than 0.9; the bacteriostatic effect was positively correlated with the extract concentration. The EC_{50} values of the five extracts against GT were 0.283 to 0.856 g/L. According to the EC_{50} value, the order of inhibition (from highest to lowest) of brown-rot fungi by the organic solvent extracts of FHH was methanol extract, petroleum ether extract, acetone extract, ethyl acetate extract, and chloroform extract. According to the measurement results of the GT, it was found that the optimum solvent was methanol. However, the EC_{50} value of methanol extract was greater than ACQ (Li *et al*. 2014) and this extract's antifungal ability was relatively weak when compared to ACQ.

Table 5. Virulence of Various Solvent Extracts of FH

Chemical Solvent	Virulence Regression Equation	R^2	[a] EC_{50} (g/L)
ACQ	$y = 0.7117x + 5.7286$	0.9378	0.095
Acetone	$y = 1.9558\ x + 6.0733$	0.9763	0.283
Methanol	$y = 2.677x + 6.2886$	0.9523	0.330
Ethyl acetate	$y = 1.0505x + 5.2029$	0.9508	0.641
Chloroform	$y = 0.9916x + 5.0913$	0.9768	0.809
Petroleum ether	$y = 1.2411x + 5.0839$	0.9702	0.856
Hot water	/	/	/
Note: [a] refers to the average of three replicates; ACQ data from Li and Yan (2005)			

FH Natural Decay Resistance Test Results

Sample mass loss rate

It can be seen from Table 6 that the quantity loss rate of the FHH sample after rotting from TV exposure was 6.9% (which was less than 11%) and the mass loss rate of the FHH material was 8.2% (which was less than 11%) after GT exposure. According to Chinse Standard GB/T 13942.1 (2009), FHH can be graded as a class I of best decay resistance.

Table 6. Mass Loss Rate of FHH Samples After Decay by TV and GT

Wood Rot Fungus Type	*C. versicolor*	*G. trabeum*
Mass Loss Rate (%)	6.89	8.19

SEM observation before and after the fungal decay of the sample

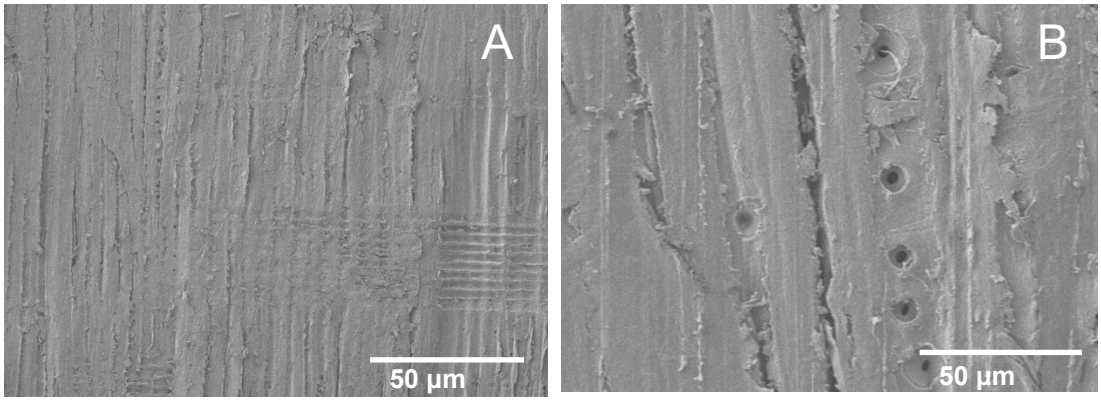

Fig. 3. SEM images of FHH: A: 100× magnification; B: 500× magnification

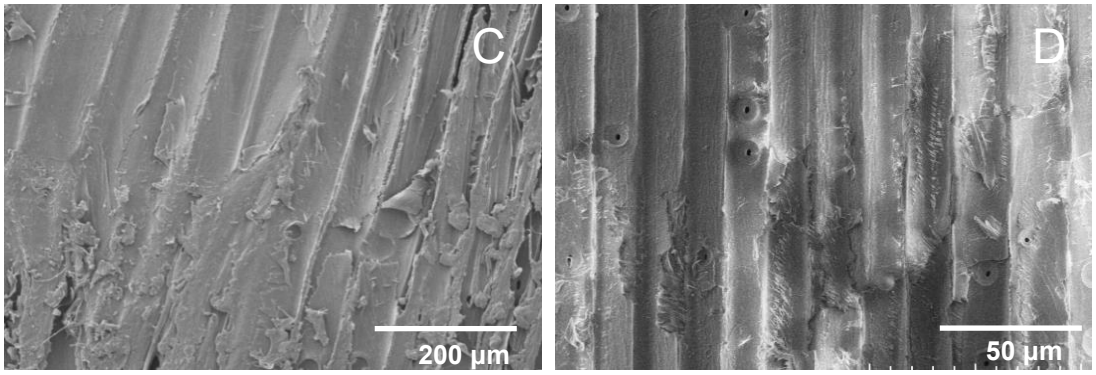

Fig. 4. SEM images of FHH after fungal decay: C: GT, 500× magnification; D: TV, 500× magnification

The samples before and after brown rot of FHH were observed. Figure 3 shows that the surfaces of the FH sample were flat, and its various structures were observed to be complete and compact at 100× and 500× magnification, respectively. Figure 4 (C) shows the FH specimens were eroded by the TV. Mycelia were visible on the surface, especially at the rupture. It can be observed that the boundary between the rupture and the fibers was destroyed. The sample was attacked to some extent. Figure 4 (D) shows the FH specimens were eroded by TV. The surface was also covered with some hyphae, but the internal hyphae were few, the structure was relatively intact, and the original shape

was well maintained. The results showed that the destructive ability of TV to FHH was weaker than that of GT. In general, both of these fungi were less damaging to FHH. These observation results were consistent with the previous mass loss rate test results, which indicated that FH extract imparts good rot resistance.

Preservative Toxicity Test Results

Sample preservative retention

According to the method of a preservative-loaded drug in Chinese Standard LY/T 1283 (2011), the drug loading of FH extract and ACQ in the sample was calculated. The results are shown in Figs. 5 and 7. It can be seen from the figures that the drug loading of the test piece increased with the increasing concentration of the water-based preservative and the organic solvent preservative; these observations indicated that Masson pine wood has good permeability.

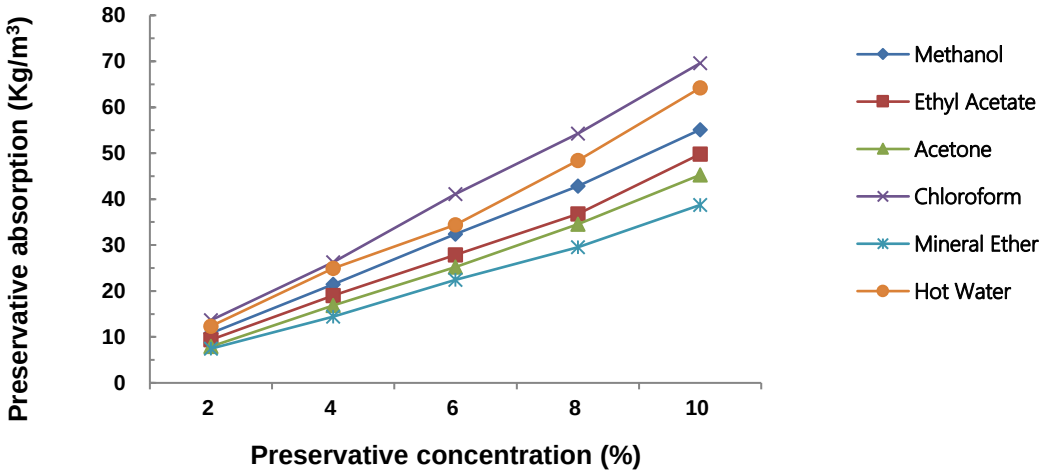

Fig. 5. Drug loading of *Pinus massoniana* wood-impregnated FH extract

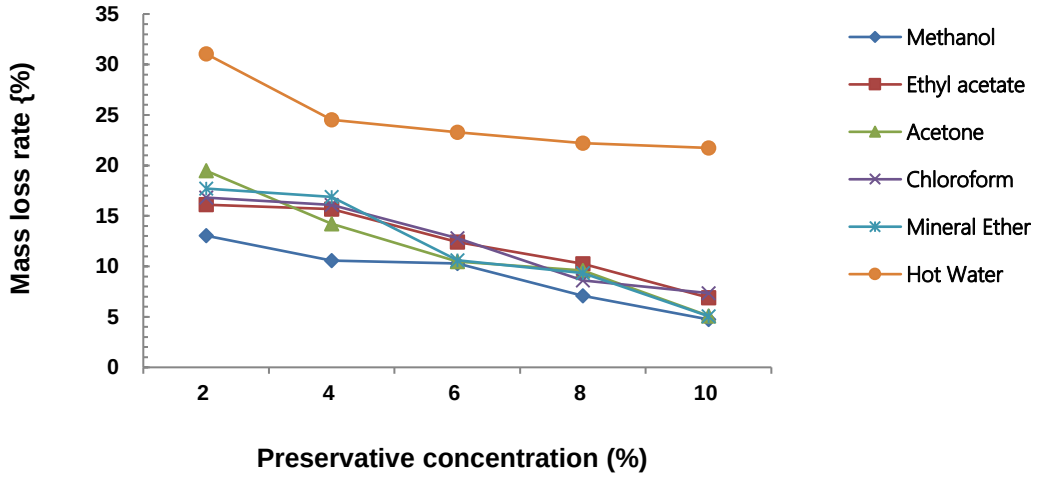

Fig. 6. Mass loss rate after the treatment of the test piece of FH extract

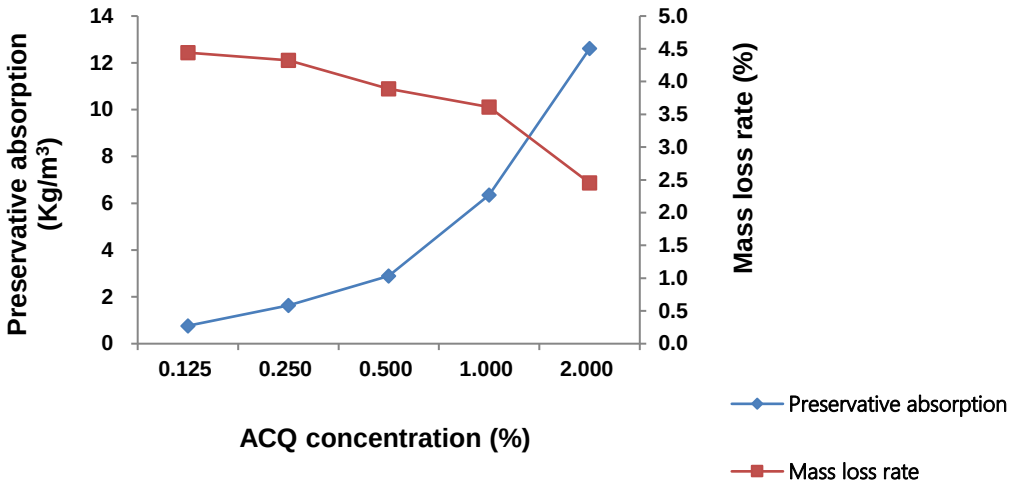

Fig. 7. ACQ-treated specimens exposed to dense viscous fungi

Virulence effects of preservatives

It can be seen from Figs. 6 and 7 that with increased concentration of the liquid preservative of various drugs, the mass loss rate of the test piece after the FHH extract and the ACQ-treated Masson pine test piece was reduced. Results indicated that the six chemical preservatives were enhanced with increased concentration at inhibiting GT. It is shown in Table 7 that there were some differences in the preservative ability of different concentrations of FHH extract and ACQ treatments. The antifungal effect of the FH extracts of methanol, ethyl acetate, acetone, chloroform, and petroleum ether at a 10% (w/w) concentration was good, and the mass-loss rate was below 11%, which is a level I for best resistance. The mass-loss rate of the hot water extract at a concentration of 2% (w/w) was greater than 31%, which is a non-resistance (level IV of not good); the mass loss rate of the sample at a concentration of 10% was greater than 21%, which is a grade III of slightly antifungal. However, the mass-loss rate of the samples treated with the five concentrations of ACQ did not change much, and all had a rating of best resistance (level I). In terms of inhibiting the GT, the FHH extracts of the other five solvents (except for hot water) had antiseptic effects, and the antiseptic effect was closely related to the concentration. However, there was still a certain gap when compared with the resistance of ACQ.

SEM observation of the of the treated material of FHH extract

The SEM images of the brown-rot samples of 10% (w/w) concentration of cedar heartwood hot water extract-treated material and 10% (w/w) concentration of cedar heartwood methanol extract-treated material were observed. It can be seen from Fig. 8 that the sample treated by the hot water extract contained more rot, and a large amount of hyphae of the bacterium was densely formed inside the sample. The boundary between the cells was broken, and a large cavity was also formed. This indicated that the hyphae of GT critically invaded the inside of the sample of *Pinus massoniana* through the tissues, such as through ducts and pits.

Table 7. Antifungal Performance of FHH Extracts Preservative and ACQ Preservative Treated Material

Preservative	Concentration (%)	Mass Loss Rate (%)	Resistance Grade
ACQ	0.125	4.44	I
	0.25	4.32	I
	0.5	3.89	I
	1	3.61	I
	2	2.45	I
Methanol extract	2	13.04	II
	4	10.58	I
	6	10.28	I
	8	7.08	I
	10	4.75	I
Ethyl acetate extract	2	16.11	II
	4	15.69	II
	6	12.42	II
	8	10.26	I
	10	6.91	I
Acetone extract	2	19.48	II
	4	14.21	II
	6	10.46	I
	8	9.60	I
	10	5.10	I
Chloroform extract	2	16.81	II
	4	16.08	II
	6	12.81	II
	8	8.61	I
	10	7.35	I
Petroleum ether extract	2	17.71	II
	4	16.89	II
	6	10.60	I
	8	9.33	I
	10	4.69	I
Hot water extract	2	31.06	IV
	4	24.53	I
	6	23.27	III
	8	22.20	III
	10	21.74	III

Figure 9 shows a sample of the methanol extract-treated material after brown-rot exposure. When compared with Fig. 8, there were not as many hyphae in Fig. 9; the cell boundary damage was not obvious, and the tracheid and other structures were more complete with less damage caused by GT (*i.e.*, better antiseptic effect). This observation was consistent with the test results of the mass-loss rate in the antifungal experiments.

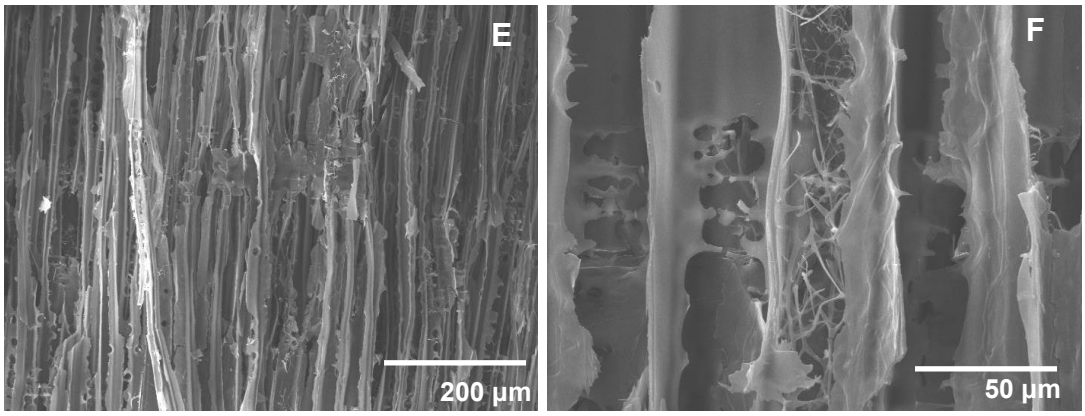

Fig. 8. SEM images of the FHH hot water extract treated material after brown-rot exposure (E: 100×magnification; F: 500× magnification)

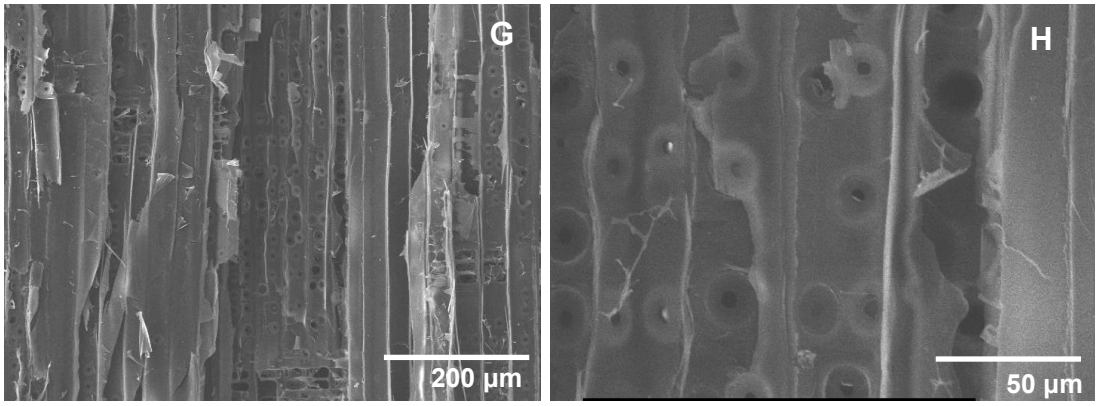

Fig. 9. SEM images of the chlorinated extract of FH after brown-rot exposure (G: 100× magnification; H: 500× magnification)

CONCLUSIONS

1. Hot water extract of *Fokienia hodginsii* heartwood (FHH) had no inhibitory effect on *Trametes versicolor*. The inhibitory effects of the other five solvent extracts on *T. versicolor* were positively correlated as concentrations increased. The acetone extract had the highest effect. The order observed (from highest to lowest effect) was acetone extract, methanol extract, ethyl acetate extract, chloroform extract, and petroleum ether extract.
2. The extract from hot water from FHH had no effect on *Gloeophyllum trabeum*. The inhibitory effects of the other five solvent extracts on *G. trabeum* were positively correlated as concentrations increased. The methanol extract had the highest resistance effect. The order observed (from highest to lowest effect) was methanol extract, petroleum ether extract, acetone extract, ethyl acetate extract, and chloroform extract.
3. The natural resistance grade of FHH belongs to grade I (best resistance).
4. The test results of 6 kinds of liquid preservatives on *Gloeophyllum trabeum* showed that the preservatives increased with increasing concentration in inhibiting *Gloeophyllum trabeum*. The antifungal effect of the five kinds of FHH extract at 10%

(w/w) concentration was good. At this time, the mass-loss rate of the specimens was below 11%, which implies that all the specimens qualified as grade I (best resistance). The order observed (from highest to lowest) for resistance at this time was methanol extract, petroleum ether extract, acetone extract, ethyl acetate extract, and chloroform extract.

5. SEM was used to inspect the samples of the *Gloeophyllum trabeum* treated with the methanol extract, and the findings were compared the samples of the *Gloeophyllum trabeum* treated with the hot water extract. It was found that the structure treated with methanol extract was more complete and the antifungal effect was relatively better. This observation was consistent with the experimental results of the mass-loss rate of the antifungal tests.
6. This research demonstrated the antifungal and antifungal mechanism of FHH and provided a scientific basis for the rational use of residue extracted from FH in the production process.

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