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Decay Resistance of *Pterocarpus macrocarpus* Kurz Xylem and Toxic Effect of It's Extracts on *Gloeophyllum trabeum*

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Use of botanical preservatives gains attraction due to its environmentally safe, broad spectrum and high efficiency properties in the recent years. Natural decay resistance performance of *Pterocarpus macrocarpus* Kurz Xylem was conducted according to the Chinese standard of GB/T 13942.1-2009. Six different extracts were obtained by extracting *Pterocarpus macrocarpus* Kurz xylem with hot water, methanol, ethyl acetate, acetone, chloroform and mineral ether. Thereafter, Masson pine was impregnated with these different extracts of five different concentration, and the decay resistance performance of the treated wood was studied. The results showed that the natural decay resistance of *Pterocarpus macrocarpus* Kurz xylem meet the demand of degree I and showed strong resistance to fungal decay. The samples impregnated with 10% methanol extracts, 10% ethyl acetate extracts, 10% acetone extracts or 10% chloroform extracts could met the demand of degree I (LY/T 1283-2011) for preservation and showed strong resistance to *Gloeophyllum trabeum* (*G. trabeum*). The results from scanning electron microscopy (SEM) analysis indicated that the tube structure of the decayed sample which treated with methanol extracts was more complete than that treated with hot water extract\s, which revealed that treatment with methanol extracts provided better anti-corrosion effect relatively.

Key words: *Pterocarpus macrocarpus* Kurz*;* Extracts; *Gloeophyllum trabeum*; Decay resistance; Wood preservatives; SEM.

Wood decay is mainly due to the biological attack, especially the fungus invasion is one of the most important corruption. These fungus including wood-decaying fungus(white rot, brown rot and soft rot) and discoloration bacteria[1]. Natural decay resistance performance of wood is connected with wood characteristics, structure and chemical composition[2].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[3]. The main source of the wood's decay resistance is spring from the toxic substances which was engendered in the process of formation of the heartwood[4].

In recent years, botanical 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[5]. Eugene studied the toxic effect of the extracts of the Africa tropical hardwood xylem on wood-decaying fungus in 2002[6]. In Ellera's study, wood blocks were vacuum impregnated with the L-CO_2 and ethanol extracts of *Juniperus virginiana* L and then for insect resistant and corrosion test, and pointed out that extracts from *J. virginianna* may provide a renewable source of safe natural wood preservatives[7]. The poisonous *Sternbergia*

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candidum extracts could be used as wood preservatives when the concentration is proper[8]. Rudman studies have concluded the variation in extractive content and decay resistance in the heartwood of *Tectona grandis*[9]. Philp found that the heartwood extracts of water soluble scats pine and sitka spruce had antifungal activity while the sapwood extracts described as useless[10] Sadhna researched the antifungal activity of ethanol extract of *Lantana camara* Linn to white rot and brown rot fungus[11]. Tor pointed out that extractives in highly durable heartwood may protect wood against fungal colonization and subsequent degradation by dual mechanisms: the extractives have some fungicidal activity and are also free radical scavengers (antioxidants)[12]. Wen-qiang Su found that the methanol extractives of Wild siris had strong anticorrosion activity[13]. Chun-qiu Guo pointed out that the antifungal activity of *P.xerophila* leaves extractives was better than 1%benzoic acid[14].

Pterocarpus macrocarpus Kurz is one of most important exclusive furniture, sculpture and decorative hardwood species that mainly spring from Burma, Thailand and Laos. Presenting fine wood figure, light red to pale purple color, high strength and homogeneous structure[15]. Understanding the natural corrosion resistance and using the extracts of it's small scraps as the wood preservatives is favorable for comprehensive utilization of *Pterocarpus macrocarpus* Kurz. However the report of this part is fewer. This study investigated the natural corrosion resistance of *Pterocarpus macrocarpus* Kurz and the toxic effect of *Pterocarpus macrocarpus* Kurz extracts in different solvents as to provided basic information on the potential of these extracts as natural wood preservatives.

EXPERIMENTAL

Materials and methods

Small scraps of *Pterocarpus macrocarpus* Kurz xylem were supplied by Hua Ming Hua Ju Home Furnishing Industrial Lt. Company (Putian, China). The *Pterocarpus macrocarpus* Kurz xylem was chopped and milled to 40 ~ 60 mesh particle. *Pinus massoniana* L was about 20 years old and derived from Shunchang(China). Malt agar solid medium (The formula for making Malt agar solid medium is 20 g malt extract, 15 g agar, and 1000 mL distilled water.), *Coriolus versicolor* (*C. versicolor*) and *G. trabeum* were kindly provided by the College of Plant Protection, Fujian Agriculture and Forestry University (Fuzhou, China). Methanol, acetone, ethyl acetate, chloroform and mineral ether were purchased from Shanghai Chemical Reagent Factory (Shanghai, China). Ammoniacal copper quats (ACQ) was purchased from a factory in Fujian province.

Natural corrosion resistance test

Natural decay resistance performance of *Pterocarpus macrocarpus* Kurz Xylem was conducted according to the Chinese standard of GB/T 13942.1-2009.Xylem of *Pterocarpus macrocarpus* Kurz was cut into blocks of 20 mm×20 mm×10 mm ((R×T×L)) and dried at 40 °C to absolute dry weight and weighed, then sterilized in an autoclave for 30 min. Four of the sterilized blocks were placed in a 500-mL flask for the decay test.

The wood-degrading fungus *C. versicolor* and *G. trabeum* were used in the natural decay resistance test .The petri dish containing 25 mL 4% (w/v) malt agar solid medium was inoculated with *C. versicolor* and *G. trabeum*, respectively . Then the tested fungus were cultured at 28 °C ± 2 °C and 75% to 85% relative humidity for seven days. Finally, the rot fungus were transferred to 500-mL sterilized culture flasks, where they were grown to establish active hyphae. 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 formula (1),

$$T(\%)=[(w_1-w_2)/w_1]\times 100 \quad \ldots (1)$$

Where T is Natural corrosion mass loss rate, w_1 is dry mass prior to the test and w_2 is dry mass after the test.

Preservatives preparation

The 100g *Pterocarpus macrocarpus* Kurz powder (40 ~ 60 mesh particle) was placed in a heat reflux extraction. Hot water, methanol, acetone, ethyl acetate, chloroform and mineral ether were used as solvents, respectively. The solvents were heated to their respective boiling point

temperature. The row materials were extracted for two times, first heated for 5 h with a ratio of gardenia to solvent of 1:7(g/mL), then heated for 3 h with a ratio of gardenia to solvent of 1: 5 (g/mL), the extractives of two times were mixed and filtered to obtain the extracted liquid. The next step was to retrieve solvents by reduced pressure distillation, and the *Pterocarpus macrocarpus* Kurz extracts were obtained at the same time. Finally the extracts were diluted with corresponding solvent to obtain 5 different concentrations of 10, 08, 06, 04, 02 (w/w). Meanwhile, ACQ was formulated into 5 different concentrations of 2%01%00.5%00.25%00.125% (w/v) with distilled water..

Preservatives toxic effect test

The wood preservation activities of extracts of *Pterocarpus macrocarpus* Kurz against *G. trabeum* were compared with ACQ under laboratory conditions-according to the "Chinese forest industry standard - Laboratory methods for the toxicity test of wood preservatives on decay fungi (LY/T 1283–2011)", which is referred to the international fungal decay test, JIS K 1571-Qualitative standards and testing methods of wood preservatives (Japanese Industrial Standards (JIS), Japanese Standards Association, Tokyo, JIS, 1998)[16].

Wood Impregnation with extracts and ACQ

The choice wood species for the test was the sapwood of *Pinus massoniana* L. (Masson pine), which is a plantation tree with wide distribution in China[17]. It was found to be very susceptible to biological deterioration by brown rot fungus. Sapwood of Masson pine was cut into blocks of 20 mm×20 mm×10 mm (R×T×L) 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,

$$R = 10(m_2 - m_1)c/V \quad ...(2)$$

where R (kg. m^{-3}) is wood preservative absorption; m_1 (g)and m_2(g) 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. Four of the sterilized blocks were placed in a 500-mL flask for the test.

Fungal strains

The wood-degrading fungus *G. trabeum* (brown rot fungus) was used in the decay resistance test. 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

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,

$$L(\%) = [(m_3 - m_4)/m_3] \times 100 \quad ...(3)$$

where L(%) is mass loss rate, 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 deter-mined that the untreated wood minimum mass loss was above 23% and the average mass loss was 36%; thereafter, the treatment samples were tested.

SEM observation

Wood before and after decayed was demonstrated by scanning electron microscopy (SEM) Instrument (FEI Quanta 200, FEI Inc., Holland). The sample was cut with a surgical blade and the sample was mounted on sample holders with a double-sided adhesive tape, and then gold sputter-coated.

RESULTS AND DISCUSSION

Natural corrosion resistance test

Natural corrosion mass loss rate

Natural decay resistance performance of *Pterocarpus macrocarpus* Kurz Xylem was presented in the tab.1. It's observed from tab.1 that the mass loss of the *Pterocarpus macrocarpus* Kurz Xylem was 5.51% and 7.49%, both far lower than 10% after *C. versicolor decay* and *G. trabeum* decay respectively. The mass loss rate revealed that the *P. macrocarpus* Kurz Xylem could meet the demand of degree I *a*nd showed strong resistance to fungal decay according to the grade standard of wood natural decay resistance in Chinese standard of GB/T 13942.1-2009 (Table 2).

SEM observation

Fig.1showed *SEM observation of Pterocarpus macrocarpus* Kurz xylem before decayed .The surface was smooth and the organizational structure was complete, wood catheter couldn't be observed at 500 maginification times. *Pterocarpus macrocarpus* Kurz xylem after decayed by *G. trabeum* was presented in the Fig.2(C). The irregular rupture on the surface, the visible hyphae present within the cell walls, and the breaks of the fiber boundaries, revealed the sample was somewhat eroded by *G. trabeum*. Fig.2(D) showed the observation of the sample after decayed by *C. versicolor,* it could be seen that massive hyphae was dietributed on the surface, but the erosion by *C. versicolor* was weak. On the whole, the corrosion of two kinds of wood fungus was slight although the erosion by *G. trabeum* was more destructive than *C. versicolor.* The results of the SEM observation was consistent with the natural corrosion mass loss rate test, which explained that the decay resistance of *Pterocarpus macrocarpus* Kurz xylem is very good.

Wood preservatives absorption

The preservative absorption capacity of masson pine was showed in Fig.3 (different extracts) and Fig.5 (ACQ). It's obvious that the preservative absorption capacity of the samples was increased with the augment of the concentration of the preservatives, which indicated that masson pine has good permeability to water-borne and oil-borne preservatives.

Preservatives toxic effect

The results of mass loss rate trend in the Fig.4 and Fig.5 indicated that the mass loss of samples which treated with extracts and ACQ was descended with the increase of the concentration. In other words, the inhibition capability to *G..Trabeum* of the tested preservatives and the concentration of the tested preservatives were directly related. Corrosion resistance of the samples treated with extracts and ACQ was presented in Tab.1. The corrosion resistance performance of the different extracts and ACQ was varied. The blocks treated with 10% methanol extracts, 10% acetone extracts, 10% ethyl acetate extracts or 10% chloroform extracts could meet the demand of degree I of preservation and showed strong resistance to *G..Trabeum;* and the order of the corrosion resistance is : 10% methanol extracts>10% ethyl acetate extracts>10% acetone extracts> 10% chloroform extracts. The change of the mass loss of the blocks which treated with ACQ of five different concentrations was not obvious. All blocks treated ACQ could meet the demand of degree I of preservation. However, all blocks treated with hot water met the demand of degree IV of preservation; the samples treated with hot water extracts had approximately 42.44% mass loss. This is likely due to poor extraction of *Pterocarpus macrocarpus* Kurz xylem by hot water; alternatively, it might be that the extract had some nutrient composition that could promote the growth of *G .trabeum*.

Table1. Mass loss of *Pterocarpus macrocarpus* Kurz xylem after the decay of *C. versicolor* and *G. trabeum*

wood-decaying fungus species	*C. versicolor*	*G. trabeum*
mass loss rate (%)	5.51	7.49

Table 2. Grade standards of wood natural decay resistance

Grade	Mass loss for softwood (%)	Mass loss for hardwood (%)
I Best	0--10	0--10
II Better	11--20	11--30
III Good	21--30	31--50
IV Not good	>31	>51

SEM observation of the decayed masson pine blocks

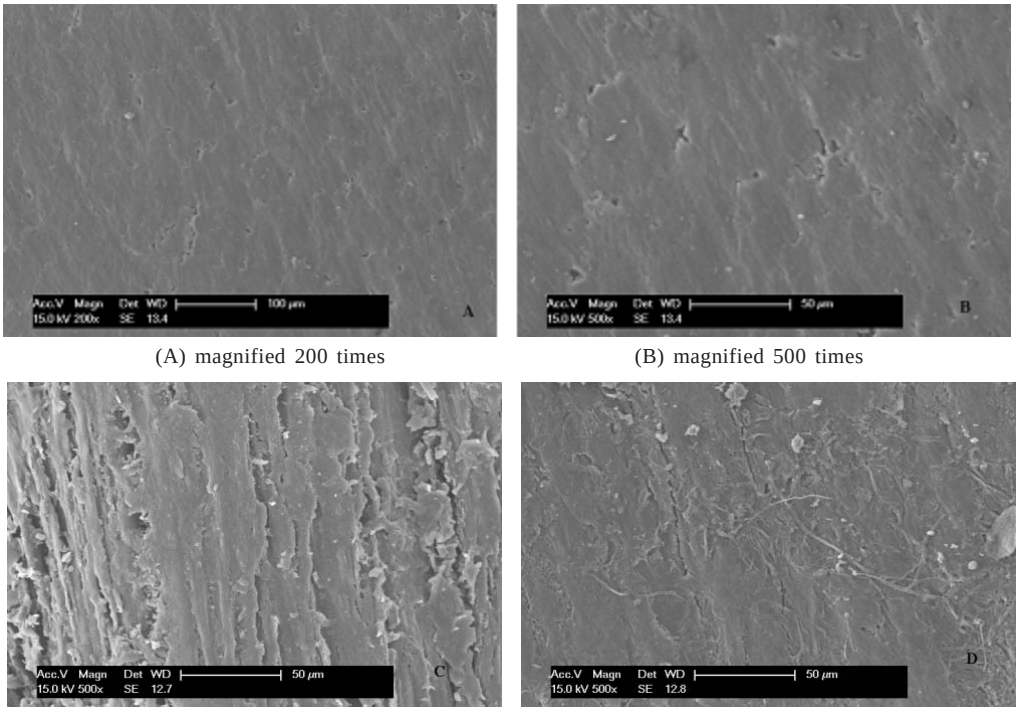
Many scholars have researched the mechanism of wood decay. Reactive oxygen species randomly attack compounds within close proximity, causing a rapid depolymerization that alters the chemical composition of wood[18] . The S_2 layer of tracheids was attacked first by the brown rot fungi, whereas the S_3 layer and the middle lamella remained intact, even in the advanced stages of decay[19].

The SEM images offered a clear view of the anatomical characteristics of decayed samples (Fig.6 and Fig.7). From Fig.6, an SEM micrograph of a tangential section of the sample treated with hot water extracts showed a tracheid plugged with large amounts of hyphae, the main cell boundaries were broken, big hole was existed in tracheids. The SEM micrograph in Fig.7 clearly showed that slight decay can be seen within the cell walls, the number of hyphae in the cell lumens was less. The Brown rot fungi hyphae entered the xylem through tracheids or pits[20]. Brown rot fungi degrade wood and produce extracellular enzymes that break down the woody cell wall[21].

In a word, the more hyphae that penetrate

Table 3. Corrosion resistance of the blocks treated with extracts and ACQ

Preservatives	Concentration(%)	Mass loss rate(%)	Corrosion grade
ACQ	0.125	4.43	I
	0.25	4.35	I
	0.5	3.38	I
	1	3.03	I
	2	2.69	I
methanol extracts	2	32.20	IV
	4	28.36	III
	6	26.64	III
	8	18.20	II
	10	8.39	I
ethyl acetate extracts	2	33.85	IV
	4	31.38	IV
	6	20.70	III
	8	14.49	II
	10	9.92	I
acetone extracts	2	36.49	IV
	4	17.2	II
	6	13.28	II
	8	11.47	II
	10	10.31	I
Chloroform extracts	2	26.37	III
	4	25.59	III
	6	17.68	II
	8	11.58	II
	10	10.43	I
mineral ether extracts	2	35.91	IV
	4	30.38	III
	6	18.94	II
	8	14.77	II
	10	14.66	II
hot water extracts	2	42.44	IV
	4	41.85	IV
	6	38.81	IV
	8	37.19	IV
	10	35.28	IV



(A) magnified 200 times (B) magnified 500 times

(C) *G. trabeum* (D) *C. versicolor*

Fig.1. Scanning electron micrographs of *Pterocarpus Macrocarpus* Kurz xylem after decayed

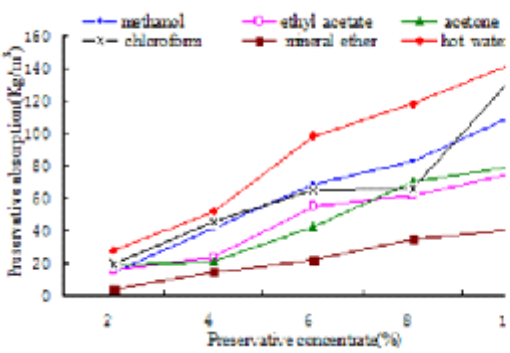


Fig. 3. Preservatives absorption of Masson pine

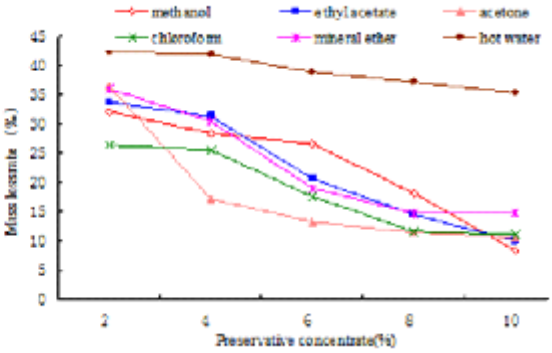


Fig. 4. Mass loss rate of Masson pine treated preservatives

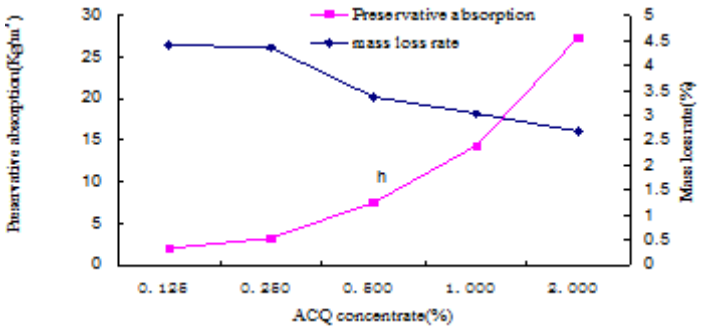
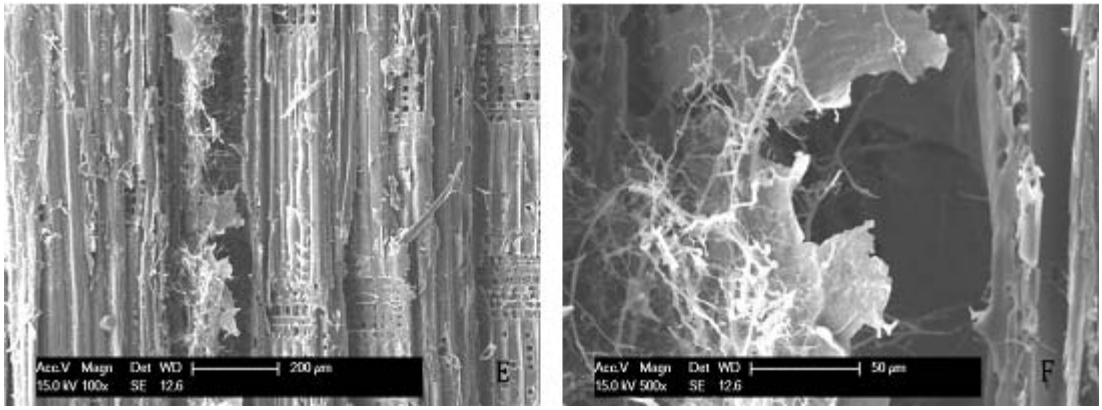
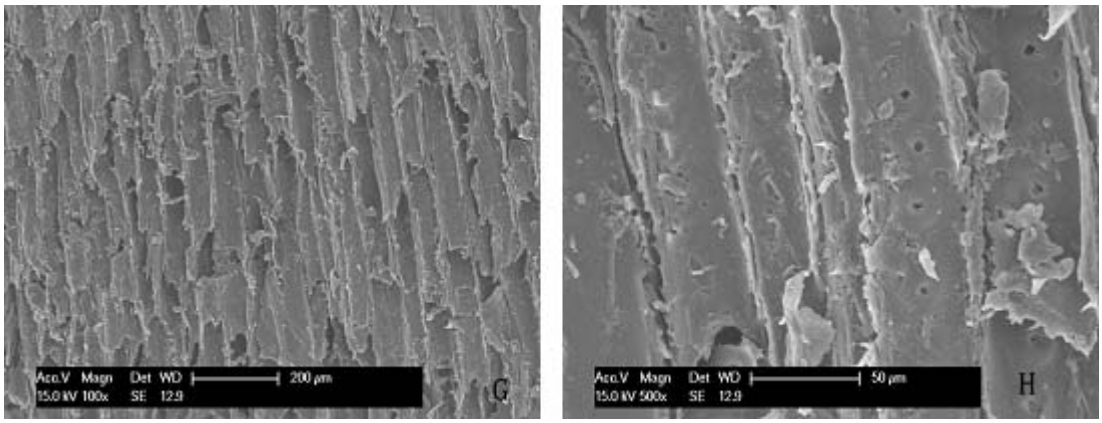


Fig. 5. Resistance performance to *G. Trabeum* of masson pine treated with ACQ



(E) magnified 100 times (F) magnified 500 times)

Fig. 6. SEM observation of the sample treated with hot water extracts



(G) magnified *100* times (H) magnified *500* times)

Fig. 7. SEM observation of the sample treated with methanol extracts

holes in the cell walls, the higher the amount of decay. The decay-resistant property of the methanol extracts was better than that of hot water extracts.

CONCLUSION

The *Pterocarpus macrocarpus* Kurz Xylem could meet the demand of degree I *a*nd showed strong resistance to fungal decay, the decay resistance is very good. The inhibition capability to *G. trabeum* of the tested preservatives and the concentration of the tested preservatives were directly related. All extracts of *Pterocarpus macrocarpus* Kurz Xylem had corrosion resistance effect except the hot water extracts, but with ACQ's corrosion resistance performance was still a certain gap. The observations from SEM indicated that treatment with methanol extracts of *Pterocarpus macrocarpus* Kurz Xylem conferred stronger resistance to fungal decay compared with hot water extracts because relatively little hyphae were observed within the cell walls, which was consistent with the results of mass loss rate. On the whole, biomass resource of *P. macrocarpus* Kurz could provide a renewable source for wood preservatives.

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