PYROLIGNEOUS ACIDS FROM CARBONISATION OF WOOD AND BAMBOO: THEIR COMPONENTS AND ANTIFUNGAL ACTIVITY

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THEAPPARAT Y, CHANDUMPAI A, LEELASUPHAKUL W & LAEMSAK N. 2015. Pyroligneous acids from carbonisation of wood and bamboo: their components and antifungal activity. Pyroligneous acids, i.e. by-products from the manufacture of charcoal by carbonisation of *Eucalyptus camaldulensis*, *Leucaena leucocephala*, *Azadirachta indica*, *Hevea brasiliensis* (rubberwood) and *Dendrocalamus asper* (bamboo) along with two commercial products were studied. Characterisations of their compositions were performed qualitatively by gas chromatography–mass spectroscopy and quantitatively by gas chromatography with flame ionisation detector. Twenty-three components were identified, of which acetic acid was the major component, followed by 2-methoxy-4-propylphenol and 2-methylphenol. Antifungal activity and efficacy of pyroligneous acids as wood preservatives were conducted using Petri dish bioassay and soil block test respectively against two white rot fungi (*Trametes versicolor* and *Rigidoporopsis amylospora*), a brown rot fungus (*Gloeophyllum trabeum*) and a sapstain fungus (*Botryodiplodia theobromae*). All pyroligneous acids exhibited antifungal activity (growth inhibition, minimum inhibitory concentration and minimum fungicidal concentration) especially those from bamboo and rubberwood, which had higher total phenolic concentrations. Soil block experiments for 12 weeks showed that pyroligneous acids from bamboo and rubberwood were more effective as wood preservatives.

Keywords: Wood vinegar, chemical composition, wood rot fungi, wood preservative

INTRODUCTION

Wood products are extensively used in indoor and outdoor applications. However, wood is susceptible to degradation, especially fungi, resulting in tremendous economic and resource losses. South-East Asia is the main area of rubber (*Hevea brasiliensis*) plantations in the world. About 75% of these areas are in Thailand, Indonesia and Malaysia. Natural rubber and rubberwood are important agricultural products. Rubberwood is used mainly for manufacture of indoor furniture. Its potential for exterior use has been hampered by its high susceptibility to biological degradation. To minimise degradation and increase service life of rubberwood, preservative treatments are needed. Synthetic chemicals have long been used to preserve rubberwood. However since 2004, the European Union and the US Environmental Protection Agency no longer allow wood that is pressure treated with synthetic chemicals such as chromated copper arsenate (CCA) for use in residential applications due to public concerns over arsenic exposure (Hingston et al. 2001). Therefore, the development of alternative environmentally-friendly wood preservatives is needed to replace synthetic chemicals. Studies have been conducted on the use of pyrolytic tar from biomass fast pyrolysis, pyrolysis oil and pyroligneous acids from slow pyrolysis as wood preservatives (Suzuki et al. 1997, Mourant et al. 2005, Mohan et al. 2008).

Carbonisation is a slow pyrolysis technique, with similar terms being used for this kind of thermal process such as destructive distillation and dry distillation. The process of wood carbonisation for charcoal production produces liquid by-products and the lighter fraction is pyroligneous acid, also called wood vinegar or locally named as Nam Sam Kwan Mai, which must

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be decanted from sedimented tar (Tiilikkala et al. 2010). Pyroligneous acids have been applied in agricultural and veterinary purposes (Uddin et al. 1995, Mu et al. 2004, Chalermsan & Peerapan 2009, Tiilikkala et al. 2010, Prasertsit et al. 2011). In Asia, wood vinegar products listed on a commercial homepage mostly come from China. The wood vinegar-based pesticide market is very extensive in Japan and other Asian countries such as Thailand, Cambodia and China (Tiilikkala et al. 2010).

The aim of this study was to investigate the chemical composition of carbonising pyroligneous acids from four wood and one bamboo species. Their anti-fungal activity and efficacy as wood preservatives based on growth inhibition, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) as well as soil block tests were determined against two white rot fungi (*Trametes versicolor* and *Rigidoporobsis amylospora*), a brown-rot fungus (*Gloeophyllum trabeum*) and a sapstain fungus (*Botryodiplodia theobromae*).

MATERIAL AND METHODS

Pyroligneous acid

Five pyroligneous acid by-products from carbonisation of wood (*Eucalyptus camaldulensis*, *Leucaena leucocephala*, *Azadirachta indica*), rubberwood (*Hevea brasiliensis*) and bamboo (*Dendrocalamus asper*) were obtained from a charcoal factory in Nakhon Rachasima Province, Thailand. All pyroligneous acids were prepared under the same conditions. Each species was slowly heated up to 400 °C in a kiln equipped with flame tunnel with chimney closed during carbonisation. The kiln was equipped with temperature gauge with sensors to indicate smoke temperature. The vapours produced were condensed by water-cooled condenser at outlet temperatures of 80–150 °C to give brown bio-oil samples. Each sample was stored at room temperature in a closed container protected from light for at least 6 months which resulted in pyroligneous acid as brown liquid on top of heavy tar. Each pyroligneous acid sample was decanted and used for further studies along with two commercial pyroligneous acids obtained from eastern Asia (Commercial 1) and South-East Asia (Commercial 2).

Characterisation of pyroligneous acid

For qualitative analysis, a gas chromatograph (GC) equipped with mass spectrometer (MS) selective detector was employed. The conditions for GC analysis were as follows: capillary column (60 m × 0.5 mm internal diameter × 0.25 µm film thickness), carrier gas helium 2.0 mL min^{-1} flow rate and splitless injection mode 230 °C. The oven temperature profile was established as follows: initial 62 °C hold time of 6 min, 62–115 °C at 10 °C min^{-1}, 115–215 °C at 3 °C min^{-1}, with final hold time of 15 min resulting in total run time of 55.46 min. The volume of a sample injected was close to 1 µL. Chemstation software was used to control the operation of the GC system as well as data acquisition and analysis of chromatograms. Mass spectra were recorded at ionisation energy of 70 eV. Components were identified by comparing their mass spectra with those in the National Institute of Standards and Technology. The results were accepted when constituents with matched percentage of > 90% were identified. For components having very low response, the co-elution by standards was used to confirm components (Guillén & Ibargoitia 1998).

For quantitative analysis, a GC equipped with flame ionised detector was employed for chemical analysis of pyroligneous acid. The conditions for GC analysis were the same as those mentioned above. The volume of sample injected was 2 µL. Palmitic acid methyl ester was used as internal standard. The Chemstation software was used to control the operation of the GC system as well as data acquisition and analysis of chromatograms. Components were identified by comparing the relative retention time (ratio of component retention time versus that of the internal standard) of their peaks with those of authentic compounds. Quantification of components was based on peak area ratio between the component and internal standard in comparison with the standard curve plotted between peak area ratio versus concentration ratio between the component and internal standard (Achladas 1991, Nilsson et al. 1999).

Bioassays for antifungal activity

Pyroligneous acids were tested for fungicidal effectiveness against two white rot fungi

(*T. versicolor* TISTR 3224 and *R. amylospora* No.05/2550), a brown rot fungus (*G. trabeum* DMST 1398) and a sapstain fungus (*B. theobromae* No.04/2536) obtained from the Royal Forest Department, Ministry of Natural Resource and Environmental as well as Thailand Institute of Scientific and Technological Research, Ministry of Science and Technology, Thailand.

The crude pyroligneous acids were centrifuged at 5000 rpm for 20 min and filtered through 0.45 µm membrane filters. Sterilised distilled water and 3% copper chromium boron (CCB) were used as negative and positive controls respectively. CCB was prepared according to Mazela (2007) by mixing 36% of copper sulphate, 40% of potassium dichromate and 24% of boric acid, then dissolved in sterilised water to 3% w/v.

Fungi were grown on potato dextrose agar (PDA) in Petri dishes until the mycelium covered the surface of the agar plates completely, 12–48 hours, at 25 °C, depending on the fungal species (12 hours for sapstain and 48 hours for white and brown rots). Haematokrit capillary was used to cut out mycelium with diameter about 1 mm which was subsequently pressed on the centre of the test PDA Petri dish. An aliquot of 40 µL of crude pyroligneous acid was added into two agar holes, which were punched 1 cm away from the central fungal mycelium. The Petri dish was sealed with parafilm and incubated at 25 °C until the fungal hyphae in the negative control were 10 mm from the edge of the plate (2 days for sapstain, 3 days for white rot and 5 days for brown rot).

Growth inhibition was measured as distance between the centre of the fungal colony and the edge of the growing hyphae by vernier callipers. Percentage of growth inhibition (I) was calculated using the formula

$$\%I = 100 - \left(\frac{R^2}{r^2} \times 100 \right)$$

where R and r = radii (mm) of fungal colony in treated and negative control plates respectively (Gamliel et al. 1989). Five replicates were performed for each pyroligneous acid tested. One-way analysis of variance (ANOVA) was performed on growth inhibition data to determine significant differences within the individual fungus ($p = 0.05$).

Minimum inhibitory concentration and minimum fungicidal concentration

MIC was performed by broth dilution technique using 96-well microlitre plates according to NCCLS (2002). The spore and broken hypha suspension in 0.85% saline buffer was standardised with 0.5% Mc-Farland solution. The final concentration was 1×10^4–10^5 cfu mL^{-1} in final volume of 10 µL well^{-1}.

The stock solution of each pyroligneous acid was dissolved in sterilised water and diluted with sterile RPMI 1640 medium to obtain sample of known concentration. Serial two-fold dilution of each sample needed to be evaluated was made with RPMI 1640 medium to yield final concentrations of 200.00, 100.00, 50.00, 25.00, 12.50, 6.25, 3.13, 1.56, 0.78 and 0.39 mg mL^{-1}. The RPMI 1640 medium was used as negative control.

The 3% CCB was used as positive control. It was prepared by accurately weighing 3 g of CCB and dissolving in Milli-Q water to obtain a concentration of 30 mg mL^{-1}. This was used as stock solution. The two-fold serial dilution was utilised to prepare CCB test solution by mixing an equal volume of CCB stock solution with RPMI 1640 medium. This gave a solution of 15 mg mL^{-1} which was further diluted with RPMI 1640 medium to give a series of CCB solution having concentrations of 15.00, 7.50, 3.75, 1.87, 0.94, 0.47, 0.23, 0.12, 0.06 and 0.03 mg mL^{-1}.

MIC test was done by addition of 200 µL of anti-fungal solution or a positive control with 10 µL of hypha suspension previously prepared into 1800 µL RPMI 1640 media in each well of 96 well micro-plates. The micro-plates were incubated for 7 days at 25 °C. Fungal growth was determined visually, for which MIC was defined as the lowest sample concentration preventing fungal growth.

MFC test or known as the minimal lethal concentration is the most common estimation of anti-fungal activity and is defined as the lowest concentration of antimicrobial agent needed to kill 99.9% of initial inoculum after incubation (Lorian 1996). In this experiment, the MFC was determined by sub-cultivation of 50 µL of suspension from a well of previously incubated micro-plate which showed no visible fungal growth, spread out in a plate containing PDA and further incubated for 72 hours at 25 °C.

The lowest concentration with no visible growth was defined as MFC.

Effectiveness of rubberwood preservative

Soil block test was carried out in accordance with AWPA Standard E10-91 (AWPA 1991) and ASTM D1413-76 (ASTM 1994) in order to evaluate the efficacy of a wood preservative against wood decay fungi. Rubberwood was cut into 20 mm × 20 mm squares, cross-cut into 20 mm thick and sterilised in the autoclave at 125 °C for 30 min. The rubberwood cubes were used in decay testing with two white-rot fungi, a brown rot fungus and a sapstain fungus. Each crude pyroligneous acid was used to perform the rubberwood test by vacuum/immersion (30 min vacuum at 28 inch Hg and 24 hours immersion). Distilled water and 3% CCB were used as negative and positive controls respectively. Six blocks (replicates) were used for each sample. The retention (R) for each treatment solution was calculated using the following equation:

$$R = \frac{G \times 62.4}{V}$$

where $G = (Wt_2 - Wt_1)$ = weight of treating solution absorbed by block (constant weight of block after treatment minus initial weight of block), V = volume of block (cm^3) and 62.4 = factor for converting grams per cubic centimetre to pounds per cubic foot.

After treatment, tested wood block was allowed to air dry in laminar flow until constant weight (24 hours). The treated sterilised block was exposed to fungus in a closed-lid soil bottle for 12 weeks. After that, the block was removed and mycelium was wiped off from the wood surface. The cleaned sample block was dried at 105 °C for 24 hours. The degree of fungal attack was estimated by determining per cent weight loss of the block. One-way ANOVA was performed on weight loss data to determine significant differences within individual fungus ($p = 0.05$).

RESULTS AND DISCUSSION

The vapour produced from carbonisation in this study was condensed by water-cooled condenser at outlet temperature of 80–150 °C. This is the recommended temperature set by the Japan Pyroligneous Liquor Association, an industrial body for pyroligneous liquor traders (Wada 1997). The smoke with temperature of not more than 80 °C has high water content and does not produce much pyroligneous liquor. Conversely, when smoke temperature exceeds 150 °C, the decomposition of charcoal wood becomes very active, producing more sticky tar and the smoke may also contain substances which are harmful to humans.

All components of pyroligneous acids from five species together with two commercial samples, qualitatively characterised by GC/MS were found to be commercially available. So they were acquired and further used as authentic compounds in quantitative determination by GC.

Twenty-three components of the pyroligneous acids (Table 1) could be categorised into acids, alcohols, furfural and furan derivatives as well as phenol and methoxyphenol derivatives. Many studies have been done on characterisation of pyroligneous acids obtained from slow and fast pyrolysis. The results are highly variable depending on wood species and experimental temperature (Suzuki et al. 1997, Mun et al. 2007, Nakai et al. 2007, Loo et al. 2008, Mohan et al. 2008). Acetic acid, believed to originate from acetyl groups in the hemicellulose (Kartal et al. 2004), was the largest content of pyroligneous acid obtained in this study (Table 1). Their concentrations varied depending on test species. *Hevea brasiliensis* (70.60 mg mL^{-1}) and *D. asper* (69.34 mg mL^{-1}) possessed higher concentrations of acetic acid than the rest of the tested samples (30.45–40.26 mg mL^{-1}). The experiments performed against two white rot fungi, a brown rot fungus and a sapstain fungus showed inhibition of less than 20%. For the test fungus to be considered inhibited, the inhibition must be greater than 20% (Kartal et al. 2004). Hence, we concluded that acetic acid, a major component in pyroligneous acids, did not play a role in antifungal activity. Methanol was identified in all samples (0.69–6.13 mg mL^{-1}) but n-propanol was only found in trace amount in *E. camaldulensis* (Table 1).

Lignocellulosic materials like wood are composed mainly of cellulose, hemicellulose and lignin (Kartal et al. 2004). In this study (Table 1), many phenol derivatives

Table 1 Compositions of pyroligneous acids (mg L^{-1}) from five species and two commercial products

Compound	*Leucaena leucocephala*	*Azadirachta indica*	*Eucalyptus camaldulensis*	*Dendrocalamus asper*	*Hevea brasiliensis*	Commercial 1	Commercial 2
Organic acid							
Acetic acid	40.26 ± 2.02	37.38 ± 0.04	32.49 ± 0.08	69.34 ± 0.17	70.60 ± 0.20	39.48 ± 0.16	30.45 ± 0.45
Alcohol derivatives							
Methanol	3.92 ± 0.04	3.85 ± 0.66	6.13 ± 0.14	4.43 ± 0.09	4.52 ± 0.05	3.90 ± 0.04	0.69 ± 0.04
n-propanol	ND	ND	0.02 ± 0.00	ND	ND	ND	ND
∑ Alcohol derivatives	3.92	3.85	6.15	4.43	4.52	3.90	0.69
Furfural and furan derivatives							
2-furfuraldehyde	0.45 ± 0.00	0.60 ± 0.02	5.23 ± 0.01	2.69 ± 0.03	0.67 ± 0.01	6.51 ± 0.05	0.09 ± 0.00
Methyl-2-furoate	0.18 ± 0.01	0.08 ± 0.00	0.87 ± 0.01	0.62 ± 0.02	0.13 ± 0.01	0.49 ± 0.03	0.02 ± 0.00
2-methylfuran	0.23 ± 0.01	0.27 ± 0.00	0.34 ± 0.00	0.79 ± 0.02	0.55 ± 0.03	0.39 ± 0.02	0.16 ± 0.00
∑ Furfural and furan derivatives	0.86	0.95	6.44	4.10	1.35	7.39	0.27
Phenol derivatives							
Phenol	0.94 ± 0.01	0.75 ± 0.01	1.01 ± 0.01	0.67 ± 0.01	0.46 ± 0.02	0.80 ± 0.01	0.91 ± 0.01
2-methylphenol	3.82 ± 0.02	2.76 ± 0.01	3.17 ± 0.01	2.16 ± 0.11	4.06 ± 0.06	2.26 ± 0.02	3.43 ± 0.02
3-methylphenol	1.67 ± 0.01	0.68 ± 0.00	1.28 ± 0.05	0.05 ± 0.00	0.32 ± 0.0	ND	ND
4 methylphenol	0.11 ± 0.00	0.07 ± 0.00	0.16 ± 0.00	0.09 ± 0.00	0.04 ± 0.00	0.11 ± 0.00	0.01 ± 0.00
2-ethylphenol	0.03 ± 0.00	ND	0.03 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	ND
2,6-dimethylphenol	ND	ND	0.05 ± 0.00	0.42 ± 0.00	ND	0.02 ± 0.00	0.03 ± 0.00
2,5-dimethylphenol	0.29 ± 0.01	0.17 ± 0.01	0.24 ± 0.00	0.20 ± 0.00	0.12 ± 0.00	0.46 ± 0.02	0.06 ± 0.00
2,4-dimethylphenol	ND	ND	ND	ND	ND	0.41 ± 0.02	0.08 ± 0.00
2,3-dimethylphenol	0.06 ± 0.00	ND	0.04 ± 0.00	0.05 ± 0.00	ND	0.02 ± 0.00	ND
∑ Phenol derivatives	6.92	4.43	5.98	3.70	5.03	4.12	4.52
Methoxyphenol derivatives							
4-propyl-2-methoxyphenol	6.90 ± 0.05	5.88 ± 0.03	5.54 ± 0.02	11.56 ± 0.04	9.56 ± 0.01	7.29 ± 0.20	8.12 ± 0.01
4-methyl-2-methoxyphenol	1.51 ± 0.01	0.24 ± 0.00	1.27 ± 0.01	2.59 ± 0.06	2.23 ± 0.01	1.12 ± 0.04	1.18 ± 0.01
4-ethyl-2-methoxyphenol	1.46 ± 0.02	0.20 ± 0.01	1.34 ± 0.02	2.81 ± 0.02	2.33 ± 0.01	1.40 ± 0.02	0.82 ± 0.01
Guaiacol	0.40 ± 0.00	0.66 ± 0.01	0.36 ± 0.01	1.33 ± 0.04	4.28 ± 0.02	0.38 ± 0.01	0.24 ± 0.01
Eugenol	0.27 ± 0.01	ND	0.21 ± 0.01	5.23 ± 0.02	2.16 ± 0.02	1.99 ± 0.01	1.38 ± 0.01
Syringol	1.20 ± 0.02	0.93 ± 0.01	3.88 ± 0.01	3.57 ± 0.02	0.06 ± 0.00	2.01 ± 0.01	0.79 ± 0.00
Acetovanillone	ND	ND	ND	0.18 ± 0.01	ND	ND	ND
∑ Methoxyphenol derivatives	11.74	7.91	12.60	27.27	20.62	14.19	12.53
∑ Total phenol concentrations	18.66	12.34	18.58	30.97	25.65	18.31	17.05
1,2 dihydroxybenzene	ND	ND	ND	0.18 ± 0.01	ND	ND	0.08 ± 0.00

ND indicates detected concentration lower than the limit of quantitative detection

(16 compounds) were found and those in higher concentrations were 4-propyl-2-methoxyphenol (5–11 mg mL^{-1}), followed by 2-methylphenol (2–4 mg mL^{-1}). Phenolics basically result from thermal degradation of lignin. Lignin is a high molecular mass randomly cross-linked polymer, consisting of an irregular array of differently bonded hydroxy- and methoxy-substituted phenylpropane units. During pyrolysis, competing thermal degradation reactions take place that generate different bond cleavage according to their bond energies, providing a high number of products due to high structural diversity of lignin (Guillen & Ibargoitia 1998). Only three furfural and furan derivatives were detected in small amounts: 2-furfuraldehyde (0.09–6.51 mg mL^{-1}), methyl 2-furoate (0.02–0.87 mg mL^{-1}) and 2-methylfuran (0.16–0.79 mg mL^{-1}).

Almost all five pyroligneous acids exhibited inhibition against four fungi tested better than the two commercial products (Table 2): *T. versicolor* (22.41–40.19% compared with 17.24 and 17.93% for Commercial 1 and Commercial 2 respectively), *R. amylospora* (14.41–45.09% compared with 12.10 and 23.79%), *G. trabeum* (33.91–55.39% compared with 35.71 and 56.45%) and *B. theobromae* (58.58–89.70% compared with 62.86 and 74.92%). Pyroligneous acids from *D. asper* and *H. brasiliensis* exhibited per cent inhibition against sapstain fungus almost as high as that of 3% CCB (89.70 and 85.03% respectively vs 89.83%). Phenolics from lignin degradation were critical for antifungal activity against brown rot fungi (Kartal et al. 2004). Our study seemed to support the findings of Kartal et al. (2004) whereby data in Tables 1 and 2 suggested that the synergistic activity of various phenolics in pyroligneous acids, rather than the activity of any single phenolic was responsible for good anti-fungal activity. *Dendrocalamus asper* and *H. brasiliensis* were the two species possessing higher total phenol concentrations (30.97 and 25.65 mg mL^{-1} respectively) compared with *L. leucocephala* (18.66), *E. camaldulensis* (18.58) and *A. indica* (12.34) as well as Commercial 1 (18.31) and Commercial 2 (17.05) (Table 1).

Table 3 reveals that *D. asper* and *H. brasiliensis* exhibit antifungal activities (MIC) against two white rot fungi *T. versicolor* (6.25 mg mL^{-1} each) and *R. amylospora* (6.25 mg mL^{-1} each), a brown rot fungus (3.13 mg mL^{-1} each) and a sapstain fungus (0.78 mg mL^{-1} each), better than the rest of the species. Although the antifungal activities of *D. asper* and *H. brasilensis* were not as good as 3% CCB against *T. versicolor*, *R. amylospora*, *G. trabeum* and *B. theobromae* (1.87, 1.87, 0.12 and 0.23 mg mL^{-1} respectively), they were better than those of Commercial 1 (25.00, 50.00, 12.50 and 3.13 mg mL^{-1} respectively) and Commercial 2 (12.50, 12.50, 6.25 and 1.56 mg mL^{-1} respectively). By comparing *D. asper* and *H. brasiliensis*, the former possessed higher phenol concentrations (30.97 vs 25.65, Table 1). By comparing antifungal activities of all five pyroligneous acids along with two commercials and 3% CCB (Tables 2 and 3), all of them exhibited higher activity against brown rot fungus than the two white rot fungi.

According to ASTM Standard D2017-81 (ASTM 1998), the classification of wood into various resistant classes on the basis of weight loss (%) caused by fungi in wood is as follows: 0–10 very resistant, 11–24 resistant, 25–44 moderately resistant and > 44 non-resistant. However, the durability may vary in terms of climatic and geographic regions (Jusoh & Kamdem 2001). Thus, in our study (Table 4), from weight losses of rubberwood caused by brown rot fungus based on ASTM criteria, all pyroligneous acid were moderately resistant or non-resistant (40–53%). On the other hand, all pyroligneous acids showed resistance to the two white rot fungi *T. versicolor* (12.67–22.79%) and *R. amylospora* (11.36–22.03%). The two pyroligneous acids from *D. asper* and *H. brasiliensis* showed some activities against *T. versicolor* (12.67 and 14.63% respectively compared with 3% CCB, 6.70%) and *R. amylospora* (11.36 and 12.31% respectively compared with 3% CCB, 7.39%), better than the other three pyroligneous acids from *L. leucocephala, A. indica*, *E. camaldulensis* and two commercials. By comparing data in Tables 2 and 3, the antifungal activities of all samples tested were better against brown rot fungus than the two white rot fungi. On the contrary, all samples showed more per cent weight loss (more degradation) with brown rot fungus than with the two white rot fungi (Table 4). Other study showed that fresh rubberwood contained 1.05 to 2.29% of free sugars and 7.53 to 10.17% of starch (Kadir & Sudin 1989). White rot fungi possessed the unique ability of efficiently degrading lignin (found in plant cell wall) to CO_2, whereas brown

Table 2 Average per cent inhibition of pyroligneous acids from five species, two commercial products and synthetic chemical (3% CCB) against wood destroying fungi

Sample	Average per cent inhibition (SD, n = 4)			
	White-rot		Brown-rot	Sapstain
	Trametes versicolor	*Rigidoporopsis amylospora*	*Gloeaphyllum trabeum*	*Botryodiplodia theobromae*
Leucaena leucocephala	22.41 d, e (2.98)	14.41 d (2.11)	36.23 c (5.93)	62.33 d (2.22)
Azadirachta indica	35.61 b, c (4.68)	27.16 c (1.71)	33.91 c (5.93)	68.22 c (1.06)
Eucalyptus camaldulensis	29.10 c, d (3.01)	26.31 c (2.74)	48.56 b (4.86)	58.58 d (1.26)
Dendrocalamus asper	40.19 a, b (4.44)	23.77 c (4.67)	55.39 b (5.15)	89.70 a (0.18)
Hevea brasiliensis	37.20 b (2.07)	45.09 b (4.86)	50.47 b (5.53)	85.03 a, b (3.13)
Commercial 1	17.24 e (1.72)	12.10 d (0.69)	35.71 c (3.75)	62.86 d (1.32)
Commercial 2	17.93 e (0.39)	23.79 c (3.72)	56.45 b (3.34)	74.92 b (1.20)
3% CCB	45.20 a (4.34)	86.70 a (0.66)	94.55 a (0.54)	89.83 a (0.90)

Different letters within a column mean statistically different at $p \geq 0.05$; SD = standard deviation; CCB = copper chromium boron

Table 3 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) (mg mL^{-1}) of pyroligneous acids from five species, two commercial products and synthetic chemical (3% CCB) against wood destroying fungi

Sample	Fungus							
	White-rot				Brown-rot		Sapstain	
	Trametes versicolor		*Rigidoporopsis amylospora*		*Gloeaphyllum trabeum*		*Botryodiplodia theobromae*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Leucaena leucocephala	12.50	100.00	12.50	100.00	12.50	100.00	1.56	6.25
Azadirachta indica	12.00	100.00	12.50	100.00	12.50	100.00	1.56	6.25
Eucalyptus camaldulensis	12.50	100.00	12.50	100.00	6.25	100.00	1.56	6.25
Dendrocalamus asper	6.25	12.50	6.25	12.50	3.13	12.50	0.78	1.56
Hevea brasiliensis	6.25	50.00	6.25	50.00	3.13	25.00	0.78	1.56
Commercial 1	25.00	100.00	50.00	100.00	12.50	100.00	3.13	12.50
Commercial 2	12.50	100.00	12.50	50.00	6.25	100.00	1.56	3.13
3% CCB	1.87	3.75	1.87	3.75	0.12	1.87	0.23	0.47

CCB = copper chromium boron

rot fungi rapidly depolymerised carbohydrates, preferentially the amorphous components, with the rate of removal of hemicelluloses greater than cellulose during the early stages while only modifying lignin (Pandy & Nagveni 2007, Sánchez 2009). Pandy and Nagveni (2007) used FTIR spectroscopy to study modification in the chemical structure of degraded chir pine and rubberwood. They reported significant increase in lignin/carbohydrate ratio indicative of selective removal of carbohydrates during decay by brown rot fungi. In contrast, in the treatment with white rot fungi, lignin/carbohydrate ratio decreased with increase in degree of decay, suggesting that the white rot preferentially decayed lignin. Xylan, a component of plant cell wall showed degradation as shown by the decrease in the intensity. It could be suggested that since rubberwood contained high amounts of carbohydrates, the per cent weight loss by brown rot fungi was more than that by white rot fungi.

This determination of the efficacy of preservatives against wood rot fungi by soil block test was not applied to sapstain fungus

Table 4 Average retention contents of pyroligneous acids in rubberwood and per cent weight losses of rubberwood treated with pyroligneous acids from five wood species, two commercial products and a synthetic chemical (3% CCB)

Sample	Average retention content (pcf) (n = 20)	Average percentage weight loss (SD, n = 6)			
		White-rot		Brown-rot	Sapstain
		T. versicolor	*R. amylospora*	*G. trabeum*	*B. theobromae*
Leucaena leucocephala	1.47 b, c (0.14)	22.79 d (4.38)	22.03 e (3.13)	48.09 c, e (2.90)	8.12 c (1.36)
Azadirachta indica	1.22 a, b (0.24)	19.53 d (4.40)	21.68 e (4.65)	40.40 d (5.53)	7.26 c (1.67)
Eucalyptus camaldulensis	1.49 b, c (0.20)	18.42 d, e (2.06)	16.11 c, e (3.35)	53.72 c (4.02)	10.20 c (1.09)
Dendrocalamus asper	2.24 c (0.21)	12.67 c (1.97)	11.36 b (2.57)	41.63 d (6.78)	3.80 b (1.59)
Hevea brasiliensis	1.98 c (0.29)	14.63 c, e (2.31)	12.31 b, c (4.32)	44.31 d, e (5.31)	4.30 b (1.12)
Commercial 1	1.10 a, b (0.20)	20.79 d (3.38)	24.03 e (3.54)	50.19 c (2.60)	7.62 c (1.06)
Commercial 2	1.24 a, b (0.19)	21.79 d (3.01)	25.30 e (3.13)	51.09 c (1.90)	7.85 c (1.26)
3% CCB	1.58 b, c (0.22)	6.70 b (1.28)	7.39 d (1.85)	3.79 b (0.61)	4.88 b (0.78)
Water	0.94 a (0.20)	53.59 a (1.05)	55.71 a (3.54)	65.87 a (5.49)	18.00 a (2.41)

pcf = Pound per cubic foot; different letters within a column are statistically different at $p \geq 0.05$; CCB = copper chromium boron; SD = standard deviation

because it predominantly caused sapstain on rubberwood due to the production of melanin in ray parenchyma tissues and cell lumens of fungal hyphae (Geo & Breuil 1998, Velmurugan et al. 2009). A soil block experiment exposing rubberwood to *B. theobromae* reported weight loss of 8.5% after 12 weeks and 12.2% by the end of 16 weeks (Florence et al. 2002). In our study, after 12 weeks, the weight losses of negative control (water) and positive control (3% CCB) were 18.00 and 4.88% respectively. Blocks treated with five pyroligneous acids along with two commercial samples showed some anti-fungal activities against sapstain fungus with per cent weight losses of 3.80, 4.30, 7.26, 7.62, 7.85, 8.12 and 10.20%. The first two weight losses of 3.80 and 4.30 which belonged to *D. asper* and *H. brasiliensis* respectively were less than those of 3% CCB (4.88), Commercial 1 (7.62) and Commercial 2 (7.85). This implied that *D. asper* and *H. brasiliensis* showed better resistance to sapstain fungus than the rest of the species. It is generally accepted that the sapstain fungi do not adversely affect the strength of the material they infect. Weight loss is attributable to utilisation of sugars and nitrogeneous materials present in parenchyma cells. Florence et al. (2002) who used transmission electron microscope to examine morphological changes in cell wall caused by penetration of fungal hyphae revealed that *B. theobromae* might cause degradation of lignified cell walls of rubberwood in the form of erosion of walls exposed to the cell lumen. This caused additional weight losses in the rubberwood exposed to *B. theobromae* longer than 12 weeks. Data in Table 4 also revealed that pyroligneous acids showed better retention in terms of pcf in *H. brasiliensis* (1.98) and *D. asper* (2.24) compared with 3% CCB (1.58), *A. indica* (1.22), *E. camaldulensis* (1.49), *L. leucocephala* (1.47), Commercial 1 (1.10) and Commercial 2 (1.24). Comparing per cent inhibition MIC, per cent weight loss and per cent retention values (Tables 2, 3 and 4), it was found that those of bamboo were better than those of rubberwood except in one case, in which per cent inhibition against a white rot fungus (*R. amylospora*) of rubberwood was better than that of bamboo (45.09 vs 23.77).

In conclusion, all five pyroligneous acids by-products from carbonisation for charcoal production along with two commercial products showed good potential in growth inhibition of white rot fungi, brown rot fungus and sapstain fungus. The studies indicated that pyroligneous acids from *H. brasiliensis* and *D. asper* showed better antifungal activity. Hence, they could be used as environmentally-friendly rubberwood preservatives. Total phenolic concentration seemed to play a role in antifungal activity.

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