ANTIFUNGAL ACTIVITIES OF SELECTED WOOD-DEGRADING FUNGI OF RUBBERWOOD

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TEOH YP, MASHITAH MD & SALMIAH U. 2015. Antifungal activities of selected wood-degrading fungi of rubberwood. The persistent use of boron compounds is of environmental concern and has resulted in the need to search for alternative approach for the preservation of rubberwood, especially using natural resources. Filamentous fungi are major sources of bioactive secondary metabolites that are currently gaining importance for their biochemical application. A total of 12 filamentous fungi from Malaysian forest were evaluated for antifungal activities against selected wood-degrading fungi of rubberwood. Antifungal assays included radial growth inhibition by food poison technique and mycelia growth inhibition by broth dilution assay. Comparison between the efficiency of methanolic and water extract was also studied. Data indicated that the majority of wood-degrading fungi tested were susceptibility to mycelia extract. All species of wood-degrading fungi tested showed inhibition towards methanolic extracts of *Schizophyllum commune* and *Pycnoporus sanguineus*. Results indicated that methanolic extract provided better antifungal activity against wood-degrading fungi as compared with water extract. Bioactive compounds such as glycerine, triacetin, 2,3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-one, 2(3H)-furanone-5-heptyldihydro- and methyl-α-D-galactopyranoside were found in fungal extract.

Keywords: Filamentous fungi, minimum inhibitory concentration, antifungal agent, Malaysian forest, alternative treatment

INTRODUCTION

Rubberwood (*Hevea brasiliensis*) is cultivated in almost 20 countries including Malaysia for natural rubber and production of wood panels (Akhter et al. 1994). Wood-degrading fungi such as *Pycnoporus sanguineus*, *Lenzites palisotii* and *Ganoderma applanatum* rapidly destroy rubberwood (Wong 1993). This has led to the development of economical industrial-scale treatment of rubberwood using boron compounds in the form of disodium octaborate tetrahydrate ($Na_2B_8O_{13}\cdot 4H_2O$), disodium tetraborate decahydrate ($Na_2B_4O_7\cdot 10H_2O$) and boric acid (H_3BO_3), particularly for indoor applications to protect from insects, borers and fungi. Unfortunately, boron compounds are odourless and pose serious health hazards to workers performing treatment and processing of treated timber (Mohd Dahlan et al. 1999).

Filamentous fungi are major sources of bioactive secondary metabolites (Frisvad et al. 2008). There has been much interest in the possible use of wood-degrading fungi as biodegradation agent, particularly in the case of white rot fungi (Reddy & Mathew 2001). White rot fungi belong to the basidiomycete group and exhibit the most efficient and extensive lignin degradation (Pointing 2001). Basidiomycetes exhibit anti-tumour properties by producing lentinan, schizophyllan and meshima during secondary metabolite process. These secondary metabolite products can be used as active ingredients for bioherbicides, bioinsecticides and biofungicides (Song et al. 1998, Bennett et al. 2001).

Biological control can be applied in the rubberwood industry for growth inhibition of wood-degrading fungi. However, there is no report on the use of biofungicides for rubberwood treatment. In this study, antifungal activities of 12 selected filamentous fungi were

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screened using food poison technique. The minimum inhibitory concentrations (MICs) were established for methanol and water extracts from tested filamentous fungi after which the active chemical was detected.

MATERIALS AND METHODS

Fungal strain

Twelve species of fungal strains (*Schizophyllum commune*, *Pycnoporus sanguineus*, *Trametes versicolor*, *Lentinus sajor-caju*, *Lentinus strigosus*, *Trametes feei*, *Trametes menziezi*, *Trametes elegans*, *Gloeophyllum trabeum*, *Lentinus* sp., *Microporus affinis* and *Microporus xanthopus*) were obtained from the Biocomposite and Protection of Timber Forest Products Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Selangor, Malaysia. These 12 species also served as wood-degrading fungi in subsequent testing. Each stock culture was grown on malt extract agar (MEA) containing 30 g L^{-1} malt extract, 3 g L^{-1} peptone and 15 g L^{-1} agar at pH 5.6 at 30 °C and maintained on agar slants prior to use.

Preparation of mycelia suspension

Mycelia suspension was prepared by suspending mycelia discs from 7-day-old culture plates in sampling bottles containing sterile distilled water and 0.1% (v/v) Tween 80. Discs of 5-mm diameter were cut on mycelia mats of the agar plate using sterile cork borer. A total of 10 discs for every 100 mL sterile water were vortexed for 5 min in order to homogenise the mycelia suspension.

Preparation of fungal mycelia culture

Ten mL of the mycelia suspension were added to 90 mL of medium containing different compositions of nutrients (mixture of 26.9 g L^{-1} yeast extract, 10.0 g L^{-1} malt extract, 49.2 g L^{-1} glucose, 1.0 g L^{-1} KH_2PO_4, 1.0 g L^{-1} K_2HPO_4, 0.93 g L^{-1} $MgSO_4 \cdot 7H_2O$ and 2.0 g L^{-1} $(NH_4)_2SO_4$) in 250 mL Erlenmeyer flasks. The medium was autoclaved at 121 °C for 15 min before adding the mycelia suspension. The culture was incubated at 30 ± 2 °C and pH 6.5 in an incubator shaker at 200 rpm for 5 days. The culture broth was then harvested and centrifuged at 4000 × g for 15 min. Residues were dried in vacuum oven at 80 °C for 24 hours and homogenised before extraction. Meanwhile, the supernatant was evaporated using rotary evaporator and the residues were maintained in vacuum until the extraction process was carried out.

Preparation of mycelia extract

The conventional Soxhlet extraction apparatus consisting of a condenser, Soxhlet chamber and extraction flask was used. Dry residues (100 g) obtained from the mycelia (biomass) were boiled in methanol for 48 hours in a ratio of 1 g:20 mL. The crude extract obtained was dried and kept at 4 °C for further analysis. The procedure was repeated using water as solvent. All experiments were performed in triplicate.

Test for antifungal activity

Poison food technique

Antifungal activity was determined using poisoned food technique (Das et al. 2010). In this study, methanol was used as negative control in order to confirm that there was no inhibitory effect, while the chemical synthetic fungicide, ketoconazole (> 98%), was used as positive control to make sure that growth of wood-degrading fungi could be inhibited.

A 5-day-old mycelia plate was punched aseptically using sterile 7-mm diameter cork borer. The fungal discs were put in the middle of the agar plate which contained the desired concentration of the biomass extract. The plates were incubated at 30 ± 2 °C. Mycelia growth diameter was recorded by measuring the two opposite circumferences. Percentage inhibition of mycelia growth was evaluated by comparing the mycelia diameter of poisoned plate (with 1.0 µg μL^{-1} biomass extract) with the non-poisoned plate (with distilled water) and calculated using equation (1):

$$MI\ (\%) = \frac{MG_{control} - MG_{treatment}}{MG_{control}} \times 100 \quad (1)$$

where MI = mycelial inhibition, $MG_{control}$ = mycelial growth of control and $MG_{treatment}$ = mycelial growth of treated sample.

Broth microdilution assay

In order to have better understanding of the concentration required for each extract to inhibit growth of wood-degrading fungi, the antifungal activity of each extract was further studied via broth microdilution assay. The procedure for broth microdilution assay followed the protocol of NCCLS (2002) with some modifications. This is a useful technique for determining MICs of large number of samples. MIC is defined as the lowest concentration of antimicrobials that inhibits visible growth of microorganisms after overnight incubation (Ncube et al. 2008, Das et al. 2010). In this study, six concentrations of each biomass extracts were prepared ranging from 0.1–5.0 µg µL. Wood-degrading fungi mycelia suspension was prepared and standardised according to 0.5 McFarland standard turbidity. Biomass extract was dissolved with 5% dimetyl sulfoxide reagent.

Biomass extract (10 µL) was put into a 96-well microtitre followed by 90 µL of malt extract broth (containing 17 g L^{-1} malt extract and 3 g L^{-1} peptone) in each well. Serial dilution technique was carried out in order to obtain the range of desired concentrations, keeping the volume in each well 50 µL. The well was inoculated with 50 µL mycelial suspension of wood-degrading fungi to provide a final volume of 100 µL. For control, 10 µL of malt extract broth, 10 µL of biomass extract and 10 µL of mycelial suspension for each selected wood-degrading fungi were put into separate wells of the microtitre plate. The sample and control were incubated at 30 ± 2 °C for 48 hours. Effectiveness of inhibition was quantified by adding 20 µL of yellow tetrazolium MTT (3-(4,5-dimetylthylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) reagent into each well. Results were obtained after 2 hours of incubation and the formation of blue colour indicated fungal growth while clear solution indicated growth inhibition.

Determination of chemical compounds

Analysis using gas chromatography–mass spectrometry

Crude extract was dissolved in 70% (v/v) ethanol before gas chromatography–mass spectrometry (GC–MS) analysis. GC–MS was used to analyse samples quantitatively by referring to the molecular weights of compounds in the NIST library. Chromatography analyses were performed using gas chromatograph equipped with an ELITE-5 column (length 30 m, internal diameter 0.25 mm). Gas chromatography was coupled to the mass spectrometer. Oven temperature was programmed at 65 °C for 4 min increasing to 280 °C at 8 °C min^{-1}.

Analysis using high performance liquid chromatograph

Concentration of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-one (DDMP) was measured by high performance liquid chromatography (HPLC) system equipped with a 150 mm (outer diameter) × 4.0 mm (inner diameter) column. The mobile phase was a mixture of deionised water and acetonitrile in a ratio of 95:5 at flow rate of 1 mL min^{-1}. The analysis was conducted at 30 °C. Twenty µL of sample were diluted with ethanol and injected into the HPLC column. The DDMP peak was detected within retention times of 3.5 to 4.5 min.

Biomass extract samples that contained DDMP were collected and concentrated to approximately 20% of its initial volume under nitrogen purge at 60 °C. The concentrated extract was injected several times into the HPLC column using similar conditions as described above. Eluate at each injection was collected into a vial from retention times of 3.5 to 4.5 min. Injection and collection of eluate were repeated several times. Content in the vial was evaporated to dryness under nitrogen purge at 60 °C and then injected back to the HPLC so as to confirm the presence of DDMP in the tested sample.

RESULTS AND DISCUSSION

Antifungal activity using food poison technique

Antifungal activities of the selected fungal strains were assayed against 12 selected species of wood-degrading fungi of rubberwood. The growth inhibitory activities of the methanolic extracts (1.0 µg $µL^{-1}$) of the tested fungi are summarised in Table 1. Most of the wood-degrading fungi tested showed inhibition towards the biomass extract. All species of wood-degrading fungi tested showed inhibition towards the methanolic

Table 1 Radial growth inhibition of methanolic extracts of selected filamentous fungi against wood-degrading fungi

Methanolic extract	Wood–degrading fungus											
	1	2	3	4	5	6	7	8	9	10	11	12
Schizophyllum commune	ND	–	+++	++	+	++	+++	++	+	+++	+++	+++
Pycnoporus sanguineus	–	ND	–	–	–	–	+	++	–	+++	+++	+++
Trametes versicolor	×	×	ND	++	±	±	–	±	×	+++	+++	+++
Lentinus sajor–caju	×	×	++	ND	+	++	++	+	×	+++	+++	+++
Lentinus strigosus	×	×	+	+	ND	–	++	++	×	+++	+++	+++
Trametes feei	×	×	+++	++	+	ND	+	+	×	++	+	+
Trametes menziezi	×	×	++	±	+	×	ND	++	×	++	++	–
Trametes elegans	×	×	++	–	×	±	±	ND	×	+++	+++	++
Gloeophyllum trabeum	×	×	++	–	×	–	–	±	ND	+++	+++	++
Lentinus	×	×	+++	+	+	×	×	±	×	ND	+	+
Microporus affinis	×	×	+	+	±	±	×	×	×	+	ND	–
Microporus xanthopus	×	×	+	+	±	±	–	×	×	+	–	ND
Positive control (ketoconazole)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Negative control (methanol)	×	×	×	×	×	×	×	×	×	×	×	×

Inhibition is reported as × no inhibition, – < 10% growth inhibition, ± between 10 and 20%, + between 20 and 40%, ++ between 40 and 80%, +++ > 80% and ND = not done; all experiments were run in triplicates; 1 = *Schizophyllum commune*, 2 = *Pycnoporus sanguineus*, 3 = *Trametes versicolor*, 4 = *Lentinus sajor-caju*, 5 = *Lentinus strigosus*, 6 = *Trametes feei*, 7 = *Trametes menziezi*, 8 = *Trametes elegans*, 9 = *Gloeophyllum trabeum*, 10 = *Lentinus*, 11 = *Microporus affinis* and 12 = *Microporus xanthopus*

extracts of *S. commune* and *P. sanguineus*. The other 10 extracts (1 µg µL^{-1}) did not inhibit growth of *S. commune*, *P. sanguineus* and *G. trabeum*. On the other hand, *T. versicolor*, *L. sajor-caju*, *Lentinus*, *M. affinis* and *M. xanthopus* were susceptible to all methanolic extract assayed. According to Anke (1989), most basidiomycetes were capable of producing varieties of antimicrobial activities.

The methanolic extract from *T. versicolor* biomass showed inhibition on the tested wood-degrading fungi, particularly against *Lentinus*, *M. affinis* and *M. xanthopus* with mycelia growth inhibition greater than 80% (Table 1). On the contrary, biomass extract produced from *T. versicolor* showed no inhibition against *S. commune*, *P. sanguineus* and *G. trabeum*. This phenomenon might be due to the fact that some antifungal proteins had specificity of action against only certain fungal species (Pushpa & Purushothama 2010). For example, ethanolic extract of *T. versicolor* provided no inhibition towards *Eschrichia coli*, *Flavobacterium* sp. and *Bacillus cereus* (Fagade & Oyelade 2009). However, several researchers found that *T. versicolor* was among the most versatile white-rotters with ongoing intensive research into bioremediation applications such as antibacterial, antifungal, antioxidant, antitumor and antiviral activities (Farghali & Masek 1998, Hsieh & Wu 2001, Nyanhongo et al. 2007, Getha et al. 2009). Meanwhile, growth inhibition of *L. sajor-caju* was between 40 and 80% when using 1.0 µg µL^{-1} of *S. commune* biomass extract (Table 1).

Antifungal activity using broth microdilution assay

MIC values mainly depended on the types of solvent used and fungal strains assayed (Table 2). Water and methanol extracts from *G. trabeum* biomass showed antifungal activities against six wood-degrading fungi tested; MIC values for *S. commune*, *P. sanguineus*, *L. sajor-caju*, *T. feei* and *T. menziezi* were greater than 5 µg µL^{-1}. Among the wood-degrading fungi tested, *M. affinis* showed growth inhibition to water extract of *G. trabeum* in which the MIC value recorded was 0.63 µg µL^{-1}. *Trametes versicolor*, *L. strigosus*, *T. elegans*, *Lentinus* and *M. xanthopus* had MIC values of 5.0, 5.0, 5.0, 1.25 and 2.5 µg µL^{-1} respectively. MIC values obtained from methanol extracts of *T. versicolor*, *L. strigosus*, *T. elegans*, *Lentinus*, *M. affinis* and *M. xanthopus* were 0.63, 5.00, 5.00, 0.31, 0.31 and 0.63 µg µL^{-1} respectively. This phenomenon might be due to the fact that *G. trabeum* produced several aromatic metabolites that could be used as antimicrobial agent (Paszczynski et al. 1999, Newcombe et al. 2002).

Methanolic extract of *P. sanguineus* exhibited considerable in-vitro activities against all wood-degrading fungi tested, particularly against *Lentinus* with MIC 0.1 µg µL^{-1} (Table 2). Methanol extracts of *P. sanguineus* showed considerable antifungal activities against human fungal pathogens such as *Candida albicans*, *C. krusei*, *Trichophyton mentagrophytes* and *Aspergillus fumigatus* (Al-Fatimi et al. 2013). The fraction obtained from the culture fluid of *P. sanguineus* fungus contained a compound with biological activity against strains of *E. coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and members of the genus *Streptococcus* (Smania et al. 1995).

Methanolic extract of *S. commune* biomass possessed antifungal activity with MIC values between 0.16 and 5.00 µg µL^{-1} against all tested wood-degrading fungi (Table 2). In this study, methanolic extract from *S. commune* biomass effectively inhibited the growth of *P. sanguineus* with MIC value of 5 µg µL^{-1}. Oxidised schizophyllan produced from *S. commune* secondary metabolite possessed antimicrobial activity against a broad range of microorganisms (Hao et al. 2010, Jayakumar et al. 2010). Contrary to results found in this study, Fagade and Oyelade (2009) found that extracts of *S. commune* had the poorest antimicrobial activity (no inhibition) against all of the tested microorganisms including *Bacillus cereus*, *E. coli* and *S. aureus*. This suggested that different proteins from different fungi exerted different antimicrobial activities with different mechanisms such as inhibiting alternate microorganism-secreted enzymes or they could interfere directly with the carrier proteins (Ordonez et al. 2006).

Not all selected wood-degrading fungi was inhibited when using extract concentration range within 0–5 µg µL^{-1} (Table 2). For example, in the case of water extract from *S. commune* biomass, concentration set below 5 µg µL^{-1} did not effectively inhibit mycelia growth of *P. sanguineus*, *T. versicolor*, *L. sajor-caju* and *L. strigosus*. On the

Table 2 Antifungal activity from crude extract of selected filamentous fungi towards wood-degrading fungi

Crude extract	Minimum inhibitory concentration (MIC) (μg μL^{-1})											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Schizophyllum commune (water)	ND	> 5.00	> 5.00	> 5.00	> 5.00	5.00	5.00	2.50	5.00	0.31	0.63	0.63
Schizophyllum commune (methanol)	ND	5.00	0.63	1.25	1.25	0.63	0.31	0.63	2.50	0.16	0.31	0.31
Pycnoporus sanguineus (water)	> 5.00	ND	5.00	5.00	5.00	>5	2.50	2.50	> 5	2.50	1.25	1.25
Pycnoporus sanguineus (methanol)	5.00	ND	5.00	5.00	5.00	5.00	1.25	1.25	5.00	0.16	0.31	0.31
Trametes versicolor (water)	> 5.00	> 5.00	ND	1.25	5.00	5.00	> 5.00	5.00	> 5.00	0.63	0.63	1.25
Trametes versicolor (methanol)	> 5.00	> 5.00	ND	1.25	1.25	5.00	> 5.00	1.25	> 5.00	0.31	0.31	0.31
Lentinus sajor-caju (water)	> 5.00	> 5.00	5.00	ND	1.25	5.00	5.00	5.00	> 5.00	0.63	1.25	1.25
Lentinus sajor-caju (methanol)	> 5.00	> 5.00	1.25	ND	1.25	0.63	0.63	1.25	> 5.00	0.63	0.63	0.63
Lentinus strigosus (water)	> 5.00	> 5.00	5.00	1.25	ND	> 5.00	0.63	0.63	> 5.00	0.63	0.63	1.25
Lentinus strigosus(methanol)	> 5.00	> 5.00	2.50	1.25	ND	> 5.00	0.63	0.63	> 5.00	0.63	0.63	0.63
Trametes feei (water)	> 5.00	> 5.00	1.25	5.00	2.50	ND	5.00	5.00	> 5.00	5.00	> 5.00	> 5.00
Trametes feei (methanol)	> 5.00	> 5.00	0.63	1.25	1.25	ND	2.50	2.50	> 5.00	1.25	2.50	5.00
Trametes menziezi(water)	> 5.00	> 5.00	0.31	> 5.00	> 5.00	> 5.00	ND	2.50	> 5.00	2.50	2.50	> 5.00
Trametes menziezi (methanol)	> 5.00	> 5.00	1.25	1.25	2.50	> 5.00	ND	0.63	> 5.00	1.25	0.63	> 5.00
Trametes elegans (water)	> 5.00	> 5.00	2.50	> 5.00	> 5.00	5.00	5.00	ND	> 5.00	> 5.00	0.63	> 5.00
Trametes elegans (methanol)	> 5.00	> 5.00	1.25	> 5.00	> 5.00	2.50	2.50	ND	> 5.00	0.31	0.31	0.63
Gloeophyllum trabeum (water)	> 5.00	> 5.00	5.00	> 5.00	5.00	> 5.00	> 5.00	5.00	ND	1.25	0.63	2.50
Gloeophyllum trabeum (methanol)	> 5.00	> 5.00	0.63	> 5.00	5.00	> 5.00	> 5.00	5.00	ND	0.31	0.31	0.63
Lentinus (water)	> 5.00	> 5.00	2.50	2.50	> 5.00	> 5.00	> 5.00	5.00	> 5.00	ND	2.50	> 5.00
Lentinus (methanol)	> 5.00	> 5.00	0.63	1.25	> 5.00	> 5.00	> 5.00	5.00	> 5.00	ND	1.25	2.50
Microporus affinis (water)	> 5.00	> 5.00	5.00	5.00	> 5.00	> 5.00	> 5.00	> 5	> 5.00	> 5.00	ND	> 5.00
Microporus affinis (methanol)	> 5.00	> 5.00	1.25	1.25	2.50	> 5.00	> 5.00	> 5	> 5.00	2.50	ND	> 5.00
Microporus xanthopus(water)	> 5.00	> 5.00	2.50	1.25	> 5.00	> 5.00	5.00	> 5	> 5.00	5.00	> 5.00	ND
Microporus xanthopus (methanol)	> 5.00	> 5.00	1.25	2.50	2.50	5.00	5.00	> 5	> 5.00	1.25	> 5.00	ND

Activity concentration: weak activity = MIC > 5.0 μg μL^{-1}, moderate activity = 1.0 μg μL^{-1} < MIC ≤ 5.0 μg μL^{-1}, strong activity = MIC ≤ 1.0 μg μL^{-1}, ND = not done; F1 = *Schizophyllum commune*, F2 = *Pycnoporus sanguineus*, F3 = *Trametes versicolor*, F4 = *Lentinus sajor-caju*, F5 = *Lentinus strigosus*, F6 = *Trametes feei*, F7 = *Trametes menziezi*, F8 = *Trametes elegans*, F9 = *Gloephyllum trabeum*, F10 = *Lentinus*, F11 = *Microporus affinis* and F12 = *Microporus xanthopus*

other hand, methanolic extract from *S. commune* biomass inhibited all tested wood-degrading fungi within the range of concentration used. Water extract of *S. commune* biomass showed growth inhibition of *M. affinis* and *M. xanthopus* with MIC value of 0.63 µg µL^{-1}. With methanolic extract of *S. commune* biomass, the MIC value was lower at 0.31 µg µL^{-1} and lower concentration of MIC was achieved in later inhibition process. Thus, it could be summarised that methanolic extract for each fungal strain provided better antifungal activity compared with water extract. Methanol extract has wider spectrum of activity compared with water extract (Parthasarathy et al. 2009). Furthermore, the methanolic extraction provided more complete reaction compared with water extraction and produced less polar compounds in the crude extract. Saponins may be produced during methanol extraction which can exhibit high toxicity against fungi and, thus, better antifungal activity (Webster et al. 2008, Parthasarathy et al. 2009).

From this study, it was observed that the filamentous fungus *S. commune* effectively exhibited antifungal activity towards all tested wood-degrading fungi. Thus, *S. commune* was used as antifungal producing fungus for further study.

Analysis of chemical compounds

Analysis using gas chromatography mass spectrometry

DDMP was one of the active components present in the fungal extract. DDMP provided pathway for saponins to scavenge superoxides by forming hydroperoxide intermediates which prevented biomolecular damage to free radicals (Teoh et al. 2012). DDMP consisted of flavonoid fractions which had been proposed for use against fungal pathogens of man due to their widespread ability to inhibit spore germination of plant pathogens (Harborne & William 2000). This analysis proved that methanolic extract from *S. commune* mycelia could provide good antifungal activity towards selected wood-degrading fungi since it contained the highest amount of bioactive compounds (Table 3). Thus, the presence of DDMP produced was quantified using HPLC.

Analysis using high performance liquid chromatograph

In order to precisely determine the presence of DDMP in the methanol extract of *S. commune*, the purification process was carried out

Table 3 Bioactive compounds in the methanol extract of fungi species studied

Chemical compound	Percentage of chemical composition (%)											
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Glycerin	0.540	0.538	NF	0.112	0.184	NF	NF	NF	NF	0.129	NF	NF
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	7.799	7.431	7.23	5.442	5.518	7.17	7.011	7.082	0.912	5.198	2.355	2.391
2(3H)-Furanone, 5-heptyldihydro-	1.920	1.606	1.587	1.329	1.021	1.582	1.509	1.437	0.228	1.218	NF	NF
Triacetin	0.726	0.711	NF	0.281	NF	NF	NF	NF	NF	NF	NF	NF
Methyl-α-D-galactopyranoside	NF	NF	NF	NF	NF	NF	NF	NF	21.114	NF	0.781	0.653

M = methanolic extract, NF = not found; 1 = *Schizophyllum commune*, 2 = *Pycnoporus sanguineus*, 3 = *Trametes versicolor*, 4 = *Lentinus sajor-caju*, 5 = *Lentinus strigosus*, 6 = *Trametes feei*, 7 = *Trametes menziezi*, 8 = *Trametes elegans*, 9 = *Gloeophyllum trabeum*, 10 = *Lentinus*, 11 = *Microporus affinis* and 12 = *Microporus xanthopus*

using HPLC assay. After several trial and error analyses, it was found that the column 150 mm × 4.0 mm, incorporated with mobile phase 0.02 M monopotassium phosphate and acetonitrile was able to separate DDMP from its matrix. Figure 1 describes the HPLC chromatogram for a purified DDMP from a sample and a standard DDMP. Based on the analysis, the DDMP content was more than 100 ppm for 10 mg μL^{-1} *S. commune* methanol extract.

CONCLUSIONS

This study provided new scientific information about filamentous fungi based on its antifungal activity. Antifungal activity was different for different fungal genera assayed. The antifungal activity might be attributed to various phytochemical constituents present in the crude extract. Bioactive compounds from each extract revealed the exact potential and mechanism to inhibit growth of wood-degrading fungi of rubberwood.

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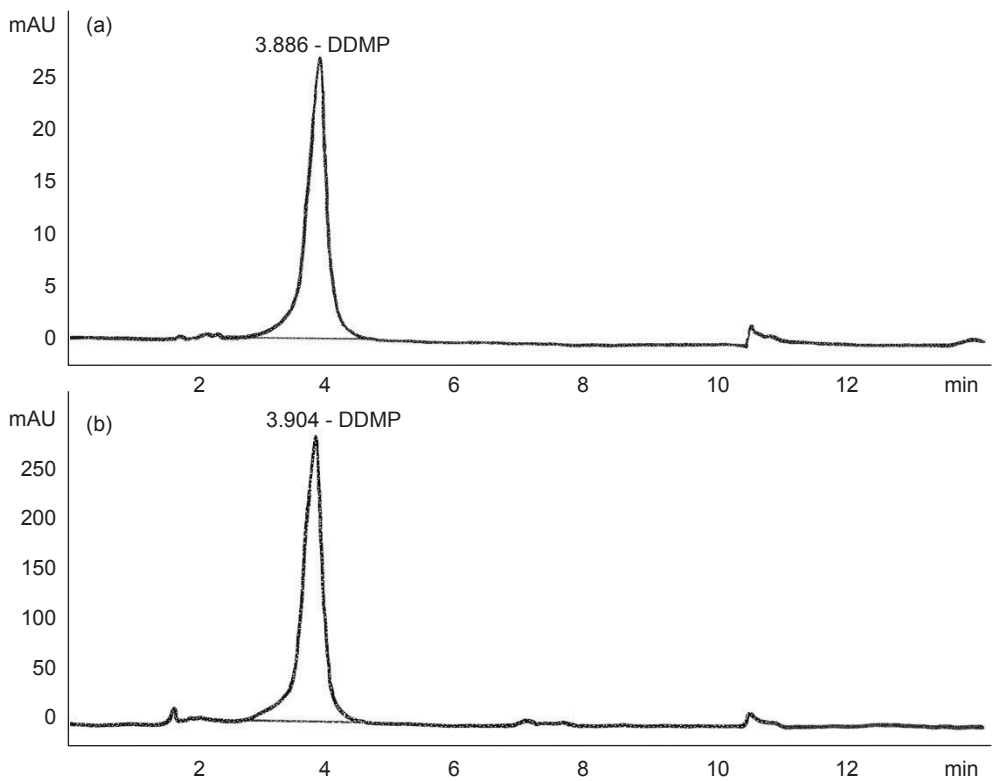

Figure 1 HPLC chromatogram for (a) purified 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-one (DDMP) from *S. commune* extract (retention time = 3.886 min) and (b) standard DDMP (retention time = 3.904 min)

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