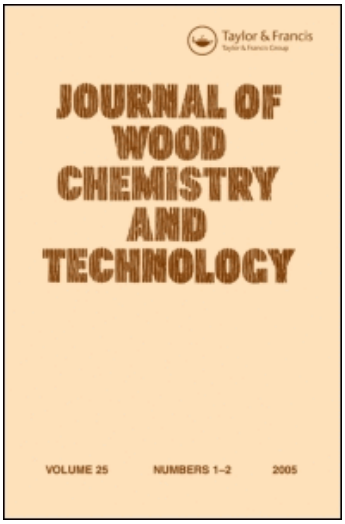

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Identification of Anti-Wood Rot Compounds in Teak (*Tectona grandis* L.f.) Sawdust Extract

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Abstract: The tropical hardwood sawdust of *Tectona grandis* L.f. from the wood processing industry was extracted and tested for anti-wood rot activity. *Tectona grandis* extract inhibited the brown rot fungi *Gloeophyllum sepiarium*, *Gloeophyllum trabeum*, *Piptoporus betulinus* and *Serpula lacrymans,* and the white rot fungi *Bjerkandera adusta, Merulius tremellosus,* and *Phlebia brevispora*. Centrifugal partition chromatography was used to separate these compounds using *n*-hexane-MeOH-H_2O (50:47.5:2.5) as a solvent system. The compounds deoxylapachol, tectoquinone, 2-hydroxymethylanthraquinone, 3′-OH-deoxyisolapachol (2-[(1*E*)-3-hydroxy-3-methylbut-1-enyl]naphthoquinone), hemitectol (2,2-dimethyl-2*H*-benzo[*h*]chromen-6-ol), and tectol were isolated from *Tectona grandis* sawdust $CHCl_3$-MeOH (1:1) extract. Deoxylapachol inhibited the brown rot fungi *Gloeophyllum sepiarium* CBS 353.74 and *Gloeophyllum trabeum* CBS 318.50 and the white rot fungi *Merulius tremellosus* CBS 280.73 and *Phlebia brevispora* CBS 509.92. Hemitectol together with tectol showed a high percentage of cellulase inhibition followed by 3′-OH-deoxyisolapachol and deoxylapachol.

Keywords: *Tectona grandis*, wood rot, brown rot fungi, white rot fungi, cellulase, tropical hard wood, deoxylapachol, tectol

INTRODUCTION

The major wood preservative used for more than 50 years is Chromate Copper Arsenate (CCA). But since 2004 the European Union and the U.S. Environmental Protection Agency (USEPA) no longer allow pressure-treated wood

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containing CCA to be used for residential applications. Pentachlorophenol (PCP) was also used for wood preservation. It was first produced in the 1930s. But since the 1980s the use of PCP is restricted. PCP has been classified as a B2 probable human carcinogen. Nowadays, most of the PCP used is restricted to the treatment of poles and railroads ties.[1–3] Several new chemical formulations such as ACQ, Tanalith-E, Wolmanit CX-8, and copper dimethyldithiocarbamate (CDDC) have been developed and are currently used for building construction.[4] The combination of organic biocides (e.g., propiconazole) with antioxidants and metal chelators was developed for environmentally benign wood preservation.[5] Natural products are a possible approach for developing new anti-wood rot compounds for wood preservation. For example, Nakayama et al.[6] recently reported that the resin from the guayule plant (*Parthenium argentatum* Gray) had insect and microbial resistance properties.

Wood rot fungi is a problem for wood construction such as in residences, museums, and children's playgrounds. The wood rot fungi consist of two major groups: the brown rot and the white rot fungi both belong to the division of Basidiomycetes. Dry rot is a form of brown rot caused by certain fungi that are able to transport water over long distances, thus wood appears dry when it is infected by, for example, *Serpular lacrymans*. The fungi, which grow on the wood in the damp environment, are called soft rot fungi. These fungi are included in the division of Ascomycetes such as *Chaetomium* and *Ceratocystis*.[7]

Brown rot fungi produce endoglucanases and hemicellulases for the degradation of cellulose and hemicellulose, respectively. Ligninolytic enzymes such as lignin peroxidase, manganese peroxidase, H_2O_2-generating enzymes, and laccase are produced by white rot fungi. White rot fungi can degrade cellulose, hemicellulose, and lignin. The lignocellulose-degrading enzymatic system is important for substrate colonization and carbon acquisition by wood rot fungi. Soft rot fungi produce cellulase and it has almost no effect on lignin.[7,8]

To develop cheap natural anti-wood rot compounds sources that are abundant and easy accessible is needed. Therefore, we decided to explore the sawdust from well-known tropical hardwoods as an extractable resource for anti-wood rot preparations. Teak (*Tectona grandis*) wood is commonly used for house construction and furniture in the Indochina region, because this strong hardwood has a beautiful surface and is resistant to termite and fungal damage. For possible new applications, we studied sawdust for the occurrence of interesting phytochemicals. Teak wood contains naphthoquinones (lapachol, deoxylapachol, 5-hydroxylapachol), naphthoquinone derivatives (α-dehydrolapachone, β-dehydrolapachone, tectol, dehydrotectol), anthraquinones (tectoquinone, 1-hydroxy-2-methylanthraquinone, 2-methyl quinizarin, pachybasin) and also obtusifolin, betulinic acid, trichione, β-sitosterol, and squalene.[9–12] Naphthoquinones have been reported to have antimicrobial activity.[13,14] Lapachol has anti-tumor activity.[15,16] Deoxylapachol induced fungal cell wall stress and inhibited *Aspergillus niger* growth.[17]

In this study the inhibition of white and brown rot fungi was studied in order to know that are the compounds from *T. grandis* sawdust extract can be used to protect wood from rotting. The paper-disc diffusion assay and the agar-plate dilution assay were used for screening of anti-wood rot compounds from teak extract. In order to know the mode of action of possible anti-wood rot compounds, cellulase was selected as a target enzyme. Cellulose-azure was used as a substrate for total cellulase assay measuring by UV spectrometry the release of colorant.

EXPERIMENTAL

Plant Extraction and Isolation

The *Tectona grandis* sawdust was appropriately collected from Prasitporn-charoen Company (Bangkhae, Bangkok, Thailand). It was extracted twice with chloroform-methanol ($CHCl_3$-MeOH, 1:1) using a sonicator (Sonicor, New York, USA) for 2 h at 25°C. All organic solvents in this experiment were purchased from Merck (Darmstadt, Germany). The crude extract was dried by an evaporator. Centrifugal partition chromatography (CPC type LLN, Sanki Engineering Limited, Kyoto, Japan) was used to separate the *T. grandis* chloroform-methanol ($CHCl_3$-MeOH, 1:1) extract using *n*-hexane-methanol-water (*n*-hexane-MeOH-H_2O, 50:47.5:2.5) as a solvent system with *n*-hexane as the mobile phase and MeOH-H_2O as the stationary phase. Ascending mode was used with 2.5 mL/min pump flow rate and 800 rpm rotation speed. After 80 fractions were collected (3 mL per fraction) the system was changed to the descending mode and collection of fractions continued (fractions 81–90).

Compounds Identification

All fractions were spotted on TLC plates (aluminum sheets silica gel 60 F_{254}, Merck, Darmstadt, Germany). The TLC plates were developed with $CHCl_3$-MeOH (19:1) or petroleum ether-acetone-acetic acid (75:25:1.5) and observed under UV 366 nm. The TLC plates were sprayed with Anisaldehyde-sulphuric acid reagent or 5% methanolic potassium hydroxide (KOH).[18] The biogram assay was used to test the activity of fractions using *Aspergillus niger* spore suspensions. The active compounds were measured by ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, and HMBC experiments with 300.13 MHz (Bruker DPX-300), 399.68 MHz (Bruker DPX-400), and 600.13 MHz (Bruker DPX-600) spectrometers. LC/MS (Agilent, USA) was used to determine the molecular weight of the active compounds. A C_{18} column (length 70 mm, 5 μM particle diameter, Macherey-Nagel, Germany) was used for the analysis with a gradient elution system of water containing 0.1% formic acid (v/v) (solvent A), and

methanol or ethanol containing 0.1% formic acid (v/v) (solvent B). The gradient range was 50–100% of solvent B for 11 min using a flow rate of 0.5 mL/min with an injection volume of 5 μL. All mass spectrometric analyses were performed in a positive atmospheric pressure chemical ionization (APCI) mode.

Paper-Disc Diffusion Assay

Seven strains of brown rot fungi, *Gloeophyllum sepiarium* CBS 317.50 and CBS 353.74, *Gloeophyllum trabeum* CBS 318.50 and CBS 335.49, *Piptoporus betulinus* CBS 378.51 and *Serpular lacrymans* CBS 520.91 and CBS 751.79 and six strains of white rot fungi, *Bjerkandera adusta* CBS 595.78 and CBS 230.93, *Merulius tremellosus* CBS 280.73, *Phlebia brevispora* CBS 509.92, and *Trametes versicolor* CBS 410.66 and CBS 114372 were used to test the activity by paper-disc diffusion assay. Fungi were grown on malt extract agar (MEA, Fluka, Spain) plates, which contained 3% malt extract until the mycelium covered the surface of the agar plate completely. Cork borers were used to cut out mycelium with a diameter of 5 mm, which was subsequently pressed on the center of the test Petri dishes containing MEA. The dried plant extract and compounds were dissolved in EtOH or MeOH[19] to a final concentration of 100 and 10 mg/mL, respectively. Either EtOH or MeOH was used as a negative control, and 10 mg/mL of Pentachlorophenol (PCP) 98% (Sigma-Aldrich, Steinheim, Germany) was used as a positive control. The reference compounds, which are 1,4-naphthoquinone, 1,4-naphthohydroquinone, 2-hydroxymethylanthraquinone, and anthraquinone (85–95% purity, Sigma-Aldrich, USA) were used as controls to test anti-wood rot activity from quinones. Sterile filter paper discs (Whatman No .42, Maidstone, England), 5 mm in diameter, were loaded with 10 μL of plant extract or compound solution dried and pressed on a fungus-inoculated plate. The inhibition zones were evaluated after incubating plates in the dark at room temperature (25°C) for 7–18 days (depending on the fungal species). The assays were performed in four replicates.

Agar Plate Dilution Assay

Plant extracts were dissolved in dimethylsulfoxide (DMSO) to obtain a stock solution with a concentration of 400 mg/mL. MEA (3% malt extract) media was autoclaved and cooled to 60–65°C before being divided into 100 mL per treatment, for 14 treatments. Plant extract was added by twofold dilution to the concentrations of 0.97, 1.95, 3.90, 7.80, 15.60, 31.20, 62.50, 125, 250, 5×10^2, 1×10^3, 2×10^3, and $4 \times 10^3 \mu$g/mL. PCP, at the same concentrations, was used as the positive control and DMSO was used as the negative control. Plant extracts were gently mixed with 100 mL of media using a magnetic stirrer and

2 mL media was loaded to the wells of a 12-well plate and left to solidify. Five strains of wood rot—*G. sepiarium* CBS 317.50, *G. sepiarium* CBS 353.74, *G. trabeum* CBS 318.50, *M. tremellosus* CBS 280.73, and *P. brevispora* CBS 509.92, which showed the strong inhibition in paper-disc diffusion assay—were chosen to check the Minimal Inhibitory Concentration (MIC) and the Minimal Fungicidal Concentration (MFC). The wood rot mycelium grown on agar plates was cut using a cork borer (diameter of 5 mm) and subsequently pressed onto the center of the media in a well. The plates were incubated in the dark at room temperature for 7–18 days. The growth of fungi on each plant extract agar well was checked and MIC values were observed. The pieces of fungi that did not grow in the media containing plant extracts or PCP were transferred to MEA media without plant extract or PCP. The MFC value was determined on the basis of no regrowth.

Inhibition of the Cellulase Enzyme

There are two basic approaches to the assay of inhibition of cellulase activity. The first is based on measuring the individual activities of the cellulase enzyme,[20–23] and the second is based on measuring the activity of the total enzyme complex toward an appropriate substrate, typically filter paper, powdered crystalline cellulose, carboxymethyl cellulose (CMC), trinitrophenyl-carboxymethyl cellulose (TNP-CMC), or cellulose-azure.[22,24–29] Cellulose-azure (product number C1052, Sigma-Aldrich, Steinheim, Germany) was used as the substrate in this study. Before being used in the assay, cellulose-azure was washed by milli-Q water to remove loosely attached dye, and was then dried in a freeze-dryer. A commercially available enzyme from *A. niger* (EC3.2.1.4 Cellulase, product number: C-1184, Sigma-Aldrich, Steinheim, Germany) with an estimated activity of 0.45 units/mg was used. Cellulose-azure solution (1.0 mg/mL) was prepared in acetate buffer at pH 5.0 and gently stirred with a magnetic stirrer. The plant extract and compounds were used as cellulase inhibitors by dissolving in DMSO (20% in acetate buffer) to the concentration of 2 mg/mL. Ammonium-hexachloropalladate (IV) (Sigma-Aldrich, Steinheim, Germany) was used as a positive control,[23] whereas DMSO was used as a negative control. The incubation mixture had the final concentrations of 0.2 mg/mL inhibitor, 0.8 mg/mL cellulose-azure, 0.3 units/mL cellulase, and the total volume was made to 1.5 mL with addition of acetate buffer. The mixtures were incubated in the dark for 2 h at 38°C in a water bath. The sample was filtered using a syringe filter (pore size 0.45 μM, type Spartan RC 30, Sigma-Aldrich, Steinheim, Germany) prior to measurement with spectrophotometer at 595 nm. The percentages of cellulase inhibition were analyzed by SPSS 12.0 statistic analysis software (Chicago, USA) using one way analysis of variance (ANOVA) and least-significance difference (LSD), at the level of significant 0.05.

RESULTS AND DISCUSSION

Compound Isolated from *Tectona grandis* Sawdust

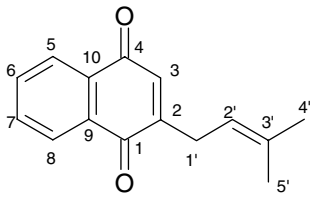
The *T. grandis* crude extract was derived from chloroform-methanol ($CHCl_3$–MeOH, 1:1) extraction. The crude extract was injected in CPC to achieve several fractions. After CPC separation of *T. grandis* sawdust extract using *n*-hexane-MeOH-H_2O as the solvent system, it was found that fractions 19–21 (compound I), 25–29 (compound II), 82 (compound III), 84 (compound IV), and 87 (containing both compound V and VI) inhibited *A. niger* growth in the biogram assay. Inhibition was not found with compound VI, which was present in small amounts (one-fourth of compound V.) in fraction 87. The compound structures are shown in Figure 1. *A. niger* was used as a model to screen for antifungal activity because it can grow fast and can be tested with the biogram assay, which is suitable for the compound isolation methods.[30–32] Another reason to use *A. niger* is for future study on the mode of action in the fungal cell wall.[17]

The ^1H NMR ($CDCl_3$, 300 MHz) of deoxylapachol (compound I) and tectoquinone (compound II) are shown in Table 1. The ^1H and ^{13}C NMR ($CDCl_3$, 600 MH_z) for 2-hydroxymethylanthraquinone (compound III), 3′-OH-deoxyisolapachol (compound IV), and tectol (compound VI) are shown in Tables 1 and 2. The ^1H NMR and ^{13}C NMR ($CDCl_3$, 400 MH_z) of (2,2-dimethyl-2*H*-benzo[*h*]chromen-6-ol) or hemitectol (compound V) are shown in Tables 1 and 2. The data from LC/MS, the UV spectrum absorption at λ_{max}(MeOH + 0.1% formic acid), the color under UV 366 nm, the color after spraying with Anisaldehyde-sulphuric acid reagent (AS) and 5% methanolic KOH and the R_f value in $CHCl_3$-MeOH (19:1) and in petroleum ether-acetone-acetic acid (75:25:1.5) are shown in Table 3.

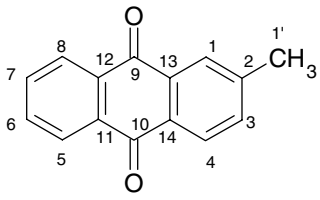
Anti-Wood Rot Growth

Tectona grandis $CHCl_3$-MeOH (1:1) extract inhibited all brown rot fungal strains in this assay, which were *G. sepiarium* CBS 317.50 and CBS 353.74, *G. trabeum* CBS 318.50 and CBS 335.49, *P. betulinus* CBS 378.51, and *S. lacrymans* CBS 520.91 and CBS 751.79. And it inhibited the white rot fungi, which were *B. adusta* CBS 230.93, *M. tremellosus* CBS 280.73, and *P. brevispora* CBS 509.92 (Table 4). The positive control, PCP, at the used concentration, inhibited all wood rot strains in this assay whereas the negative control did not inhibit any strain.

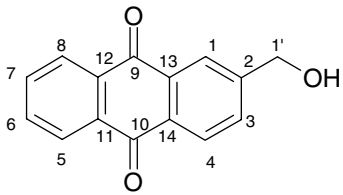
Deoxylapachol from *T. grandis* extract inhibited to *G. sepiarium* CBS 353.74, *G. trabeum* CBS 318.50, *M. tremellosus* CBS 280.73, and *P. brevispora* CBS 509.92 but it did not inhibit to *G. sepiarium* CBS 317.50, *G. trabeum* CBS 335.49, *S. lacrymans* CBS 520.91 and CBS 751.79, and *B. adusta* CBS 230.93. Tectoquinone and fraction 87 (hemitectol + tectol) did



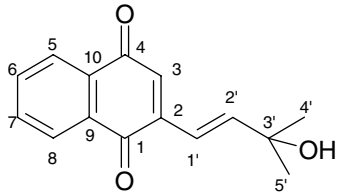
1.1) deoxylapachol (compound I)



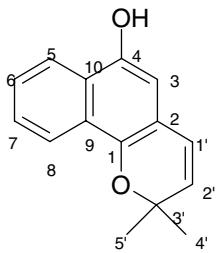
1.2) tectoquinone (compound II)



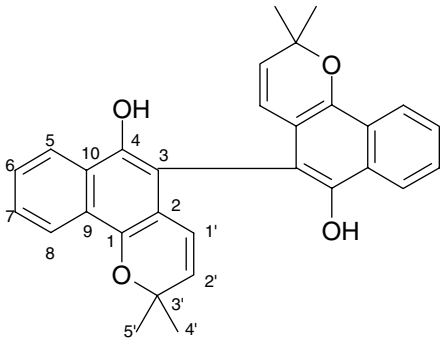
1.3) 2-hydroxymethylanthraquinone (compound III)



1.4) 3'-OH-deoxyisolapachol (compound IV)



1.5) hemitectol (compound V)



1.6) tectol (compound VI)

Figure 1. Compounds isolated from *Tectona grandis* sawdust. 1.1) deoxylapachol (compound I); 1.2) tectoquinone (compound II); 1.3) 2-hydroxymethylanthraquinone (compound III); 1.4) 3′-OH-deoxyisolapachol (compound IV); 1.5) hemitectol (compound V); 1.6) tectol (compound VI). (The numbers presented on the chemical structure are indicated the position of carbon atom which related to the NMR data in Tables 1 and 2.)

not inhibit any of the test organisms whereas 2-hydroxymethylanthraquinone and 3′-OH-deoxyisolapachol were not tested. The reference compounds 1,4-naphthoquinone and 1,4-naphthohydroquinone inhibited *G. sepiarium* CBS 353.74, *G. trabeum* CBS 318.50, *M. tremellosus* CBS 280.73, and *P. brevispora* CBS 509.92, whereas anthraquinone and 2-hydroxymethylanthraquinone did not inhibit any strain.

Table 1. The ^{1}H NMR spectroscopic data (δ in $CDCl_3$) of compound I–VI

	Compounds					
^{1}H	I	II	III	IV	V	VI
1		8.04 *s*	8.29 *s*			
3	6.70 *t*(1.7)	7.53 *d*(7.9)	7.82 *m*	6.98 *s*	6.53 *s*	
4		8.14 *d*(7.9)	8.32 *m*			
5	8.03 *m*	8.23 *m*	8.32 *m*	8.07 *m*	8.04 *m*	8.15 *d*(7.9)
6	7.66 *m*	7.72 *m*	7.81 *m*	7.75 *m*	7.39 *m*	7.46 *m*
7	7.66 *m*	7.72 *m*	7.81 *m*	7.75 *m*	7.39 *m*	7.46 *m*
8	8.03 *m*	8.23 *m*	8.32 *m*	8.12 *m*	8.04 *m*	8.18 *d*(7.7)
1′	3.21 *d*(7.3)	2.47 *s*	4.90 *s*	6.80 *d*(16.3)	6.36 *d*(9.6)	5.86 *d*(9.8)
2′	5.16 *tm*(7.3, 1.4)			6.83 *d*(16.3)	5.68 *d*(9.6)	5.56 *d*(9.8)
4′	1.71 *s*			1.44 *s*	1.45 *s*	1.50 *s*
5′	1.60 *s*			1.44 *s*	1.45 *s*	1.46 *s*

Table 2. The ^{13}C NMR spectroscopic data (δ in $CDCl_3$) of compound III–VI

	Compounds			
^{13}C	III	IV	V	VI
1	124.91	185.40	142.13	143.20
2	147.58	143.81	116.74	117.40
3	131.97	131.28	106.99	114.00
4	127.73	184.90	147.80	145.30
5	127.27	125.93	123.13	123.57
6	134.19	133.90	126.49	126.46
7	134.11	133.77	125.81	126.78
8	127.25	126.77	122.45	122.68
9	183.17	132.30	126.95	127.17
10	182.93	132.40	127.14	127.27
11	133.53			
12	133.54			
13	133.64			
14	132.72			
1′	64.40	118.52	123.97	122.37
2′		147.44	131.27	130.92
3′		71.61	77.07	76.73
4′		29.55	27.69	27.84
5′		29.55	27.84	27.67

Table 3. The chemical characteristic of compound I–VI

Compound	LC/MS data	λ_{max} (nm)	Under UV 366	Spraying reagent		R_f value in A*	R_f value in B**
				AS	5% methanolic KOH		
I	227 $[M+H]^+$	205, 250, 335	orange	green-gray	violet	0.72	0.44
II	223 $[M+H]^+$	205, 255, 330	red-orange	purple	red-purple	0.72	0.37
III	240 $[M+H]^+$	205, 257, 330	bright orange	purple	red	0.29	—
IV	225 $[M+H-18]^+$	208, 252, 300, 345	dark orange	blue-gray	dark red	0.31	—
V	227 $[M+H]^+$	222, 273, 360	violet	blue-green	red-purple	0.47	—
VI	451 $[M+H]^+$	230, 275, 362	violet	gray	violet	0.77	—

*TLC plate were developed with $CHCl_3$-MeOH (19:1)

**TLC plate were developed with petroleum ether-acetone-acetic acid (75:25:1.5); —, data not shown

Table 4. The inhibition of wood rot fungi by teak sawdust extract and the MIC values of teak sawdust extract compared to pentachlorophenol (PCP)

Wood rot fungal strains	Inhibition from teak extract (by paper disc diffusion assay)	MICs (μg/mL)	
		Teak extract	PCP
Brown rot fungi			
Gloeophyllum sepiarium CBS 317.50	++	2×10^3	3.90
Gloeophyllum sepiarium CBS 353.74	++	4×10^3	3.90
Gloeophyllum trabeum CBS 318.50	++	1×10^3	1.95
Gloeophyllum trabeum CBS 335.49	+	—	
Piptoporus betulinus CBS 378.51	+	—	—
Serpular lacrymans CBS 520.91	+	—	—
Serpular lacrymans CBS 751.79	+	—	—
White rot fungi			
Bjerkandera adusta CBS 230.93	+	—	—
Bjerkandera adusta CBS 595.78	n	—	—
Merulius tremellosus CBS 280.73	++	2×10^3	7.80
Phlebia brevispora CBS 509.92	++	1×10^3	7.80
Trametes versicolor CBS 410.66	n	—	—
Trametes versicolor CBS 114372	n	—	—

+, inhibited; ++, strong inhibited; n, not inhibited; —, data not shown.

MIC and MFC values from the agar plate dilution assay were used to evaluate plant extracts and compounds that showed strong inhibition on the wood rot fungi. These might be suitable for the development of anti-wood rot compounds for wood preservation. The results show that *T. grandis* sawdust extract inhibited *G. sepiarium* CBS 317.50 and CBS 353.74, *G. trabeum* CBS 318.50, *M. tremellosus* CBS 280.73, and *P. brevispora* CBS 509.92 at the MIC value of 2×10^3, 4×10^3, 1×10^3, 2×10^3, and 1×10^3 μg/mL, respectively, whereas other strains were inhibited by *T. grandis* sawdust extract at higher than the tested concentration. A fungicidal effect was not found at the test concentrations, while the positive control, PCP, inhibited those fungi at the lower MIC values (Table 4).

Inhibition of Cellulase

The inhibition of cellulase by *Tectona grandis* extracts and compounds was evaluated using cellulose-azure (0.8 mg/mL) as a substrate. The crude extract at the concentration of 0.2 mg/mL showed 68% of cellulase inhibition, whereas the positive control, ammonium-hexachloropalladate (IV), showed

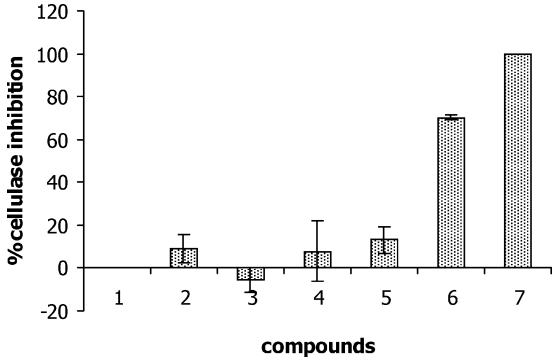


Figure 2. Inhibition of cellulase by compounds isolated from *Tectona grandis* sawdust compared with ammonium-hexachloropalladate IV (0.2 mg/mL); (n = 4, $p < .05$). 1, negative control (DMSO), 0% cellulase inhibition; 2, deoxylapachol; 3, tectoquinone; 4,2-hydroxymethylanthraquinone; 5,3′-OH-deoxyisolapachol; 6, hemitectol+tectol; 7, positive control (ammonium-hexachloropalladate IV).

99% of cellulose inhibition at the same concentration. The concentration of 0.2 mg/mL deoxylapachol, tectoquinone, 2-hydroxymethylanthraquinone, 3′-OH-deoxyisolapachol, and fraction 87 (hemitectol + tectol) from *T. grandis* extract were also tested for inhibition of cellulase. Fraction 87 (hemitectol + tectol) showed the highest percentage of inhibition, followed by 3′-OH-deoxyisolapachol and deoxylapachol (70.3, 13.1, and 9.1%, respectively). The results are shown in Figure 2.

Concerning the possible mode of action in wood rot fungi, cellulase inhibition might be involved as a target, but not for all compounds. It is possible that the other compounds inhibit through other fungal mechanisms. Most antimicrobial agents used for the treatment of microbial infections may be categorized according to their principle mode of action or mechanism of action. The most common modes of action are interference with the cell membrane and cell wall, interference with nucleic acids, and enzyme interactions.[33–36]

Apparently the broad activity of the total extract is due to a combination of activities of the compounds present, which is generally called synergistic effect or synergy.[37,38] The results indicate similar activities for isolated compounds as for the crude plant extracts. For application in wood protection we can use either active crude extracts or isolated compounds, but as the activities are similar for extracts and pure compounds it may be more cost-effective to use the extract. The extracts also contain many compounds that may inhibit wood rot fungi through different modes of action. For example, the *T. grandis* extract has approximately 0.8% of deoxylapachol. In the paper-disc diffusion assay we used a 10% less concentrated extract, and the results were the same.

The percentage of cellulase inhibition by *T. grandis* extract and fraction 87 (hemitectol + tectol) at the same concentration (0.2 mg/mL) are not different. This means that either pure compound or *T. grandis* extract can be used to inhibit wood rot fungi. The tropical hardwood sawdust such as *T. grandis* is thus a good source to further explore as raw materials for composite woods, due to its anti-wood rot properties.

CONCLUSIONS

Tectona grandis sawdust extract inhibited all seven strains of brown rot fungi and three strains of white rot fungi. The compounds deoxylapachol, tectoquinone, 2-hydroxymethylanthraquinone, 3′-OH-deoxyisolapachol, hemitectol, and tectol were isolated from *Tectona grandis* sawdust $CHCl_3$-MeOH (1:1) extract. After isolation of the active compounds from *T. grandis*, it was found that deoxylapachol inhibited two brown rot fungi, *G. sepiarium* CBS 353.74 and *G. trabeum* CBS 318.50, and two white rot fungi, *M. tremellosus* CBS 280.73 and *P. brevispora* CBS 509.92. The reference quinone compounds were also tested for the inhibition of wood rot fungi. 1,4-naphthoquinone and 1,4-naphthohydroquinone inhibited the same wood rot fungi as deoxylapachol. The isolated anthraquinone and the reference anthraquinone did not inhibit any wood rot strains in this study. The result from cellulase inhibition showed that fraction 87 (hemitectol + tectol), which was isolated from *T. grandis*, showed the highest inhibition of cellulase activity compared to other isolated compounds.

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