# Antifungal Activities of Certain Components of Teak Wood Extractives

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**Abstract**

The present research was aimed to evaluate the antifungal activities of teak (*Tectona grandis*) extracts and their components against *Trametes versicolor, Fomitopsis palustris, Rhizopus oryzae, Cladosporium cladosporioides,* and *Chaetomium globosum*. In this study, wood meal of 72 years old teak heartwood was successively refluxed with *n*-hexane, ethyl acetate and methanol. Bioassay-guided investigation by measuring the growth rate of each fungus on potato dextrose agar (PDA) medium led to the fractionation of *n*-hexane soluble extract. Column chromatographic fractionations resulted in the isolation of tectoquinone, deoxylapachol, squalene and an unknown compound (C1). The *n*-hexane and EtOAc extracts were potent mycelial growth inhibitors for *R. oryzae* (76-78%) and *C. cladosporioides* (65-73%), while MeOH extract had higher antifungal activities against both *T. versicolor* (80.2%) and *C. globosum* (83.3%). In the compound levels, the results were varied, in which deoxylapachol could inhibit all fungi species except for the *C. globosum,* while tecquinone merely deterred the growth of *R. oryzae* (58.9%). Squalene and C1 were growth inhibitors to *C. cladosporioides* (50-63%).

**Key words**: antifungal activities, deoxylapachol, extractive, *Tectona grandis*, tectoquinone

## Introduction

Teak *(Tectona grandis)* is a highly prized commercial timber and amongst the most durable woody angiosperms against wood destroying organisms such as fungi or termites. It has also been understood that wood durability is complex mechanisms determined by various factors (e.g. the presence of heartwood substances toxic to fungi). Wood decaying fungi reduce the mechanical strength of woods, and therefore its prevention with environmentally benign antifungal compounds is paramount (Yen *et al.* 2008).

White- and brown-rot fungi decompose various components of wood cell wall. Brown-rot fungi preference is to depolymerize cellulose resulting in a decrease of fiber strength. On the other hand, white-rot fungi degrade lignin with fewer consequences on the fiber strength. Various works have been done related to the natural durability of woods against brown-rot and white-rot fungi (Bhat *et al.* 2005, Kokutse *et al.* 2006, Da Costa *et al.* 1958 & 1961, Rudman 1967, Narayanamurthi *et al.* 1962). The toxicity of teak wood extracts or its specific bioactive components against wood decaying fungi has also been investigated (Haupt *et al.* 2003, Rudman 1961, Rudman *et al.* 1958, Sumthong *et al.* 2008).

Heartwood extracts contain specific and very active compounds that may block the metabolism of fungi. As certain extractives showed high activity against brown-rot or white-rot fungi, it would be

necessary to assess their activity against soft rot fungi and mold. Although molds do not reduce the mechanical strength of woods, they could discolor any materials including wood. Inhibition of teak extracts against *Aspergillus niger* mold has been reported by Sumthong *et al*. (2006). To provide more information, the toxicity of teak extracts and their components were evaluated against white-rot, brown-rot, and soft-rot fungi and molds. The antifungal activities of teak wood extracts have not been previously tested on these fungi and molds.

## Materials and Methods

### Spectrum determinations

GC analysis was performed with a Hitachi Model G-3 500 equipped with FID detector and 30 m NB-1 bonded capillary column. The analysis was run at column temperature of 120–300 °C (programed for 4 °C $min^{-1}$), detector and injector temperature of 250 °C, and was held at 300 °C for 15 min with helium carrier gas. The direct injection of EI-MS was obtained by using a JEOL XS mass spectrometry at 70eV. FTIR spectra were recorded on HORIBA FT-710. The $^{13}C$ (in 400 MHz), and $^{1}H$ NMR (in 100 MHz) spectra were determined by a JEOL JNX-400 spectrometer.

### Extraction and isolation

Wood samples of the present works were procured from a 72 years old teak wood grown in Randublatung, Central Java. Sufficient amounts of heartwood were collected from the stem base of the felled tree. These heartwood samples were then disintegrated into wood meal. Dried wood meal (equivalent to 1 kg oven dried) was successively refluxed in *n*-$C_6H_{14}$ (*n*-hexane), ethyl acetate (EtOAc), and methanol (MeOH). Each extraction was carried out for 6 hours. Extracts were filtered and vacuumed concentrated at approximately 45 $^{o}C$ to obtained dark crude residues. Total amount of the resulting extracts were 30.24 g, 22.1 g and 2.23 g respectively for n-hexane, EtOAc, and MeOH. These extracts were then tested for their potential to fungal growth inhibitory. Preceding anti-fungal testing, the *n*-hexane soluble fraction was partitioned with saturated $NaHCO_3$, 10% $Na_2CO_3$, and 1% NaOH aqueous solutions in a separatory funnel to obtain neutral and acidic fractions.

The amount of neutral and acidic fractions obtained were 25.5 g and 1.3 g, respectively. A portion of the neutral fraction (10 g) was purified by silica-gel 60 N (spherical 63–210 μm, neutral, Kanto Chemical Co., Japan) column chromatography. The extract was eluted successively with *n*-hexane, benzene ($C_6H_6$), and EtOAc. The eluates of EtOAc were dominant with a total weight of 5.09 g followed by these of benzene fractions (3.05 g) and *n*-hexane fractions (1.20 g). The *n*-hexane fractions were then fractionated by Si-gel CC (36 g, 50 x 3.3 cm) eluted with *n*-hexane containing an increasing amount of EtOAc (up to 5%) to yield squalene (220 mg) and deoxylapachol (55 mg). Following a similar procedures, benzene fractions were fractionated to result in tectoquinone (270 mg) and unknown compound, C1 (80 mg). Identification of tectoquinone, squalene, and deoxy-lapachol structures (Figure 1) were carried out based on comparison between the resulting spectral data and published data (Perry *et al*. 1991, Sumthong *et al*. 2008, Windeisen *et al*. 2003, Thomson 1971).

Tectoquinone (yellow crystal), melting point is 175-177 °C. EI-MS: m/z (relative intensity): 222 $[M]^+$ (84), 207

(44) [M-$CH_3$] $^+$, 194 (100) [M-CO]$^+$, 166 (82) [M-2xCO], 165 (94), 164 (26), 163 (34), 139 (30), 76 (20). $^1$H NMR ($CDCl_3$) δ: 2.58 [3H, s, CH3], 7.58 [1H, dd, J= 8.4, 0.8 Hz, H-2], 7.77-7.79 [2H, m, H-6 and H-7], 8.09 [1H, s, H-4], 8.19 [1H, d, J= 8.4 Hz, H-1], 8.28-8.30 [2H, m, H-5 and H-8]. $^{13}$C NMR ($CDCl_3$) δ : 21.5 ($CH_3$), 127.17 (C-5), 127.17 (C-8), 127.46 (C-4), 127.52 (C-1), 131.32 (C-10a), 133.4 (C-3), 133.61 (C-6), 133.64 (C-7), 133.92 (C-9a), 134.03 (C-5a), 134.94(C-1a), 145.29 (C-2), 183.1 (C-9), 183.7 (C-10). IR spectrum υ max KBr cm$^{-1}$: 1718.26 (m), 1671.98 (s), 1590.99 (s), 1326.7 (s), 1295.9 (s), 850 (s), 709 (s).

Deoxylapachol (yellow gum). EI-MS: m/z (relative intensity): 226 [M]$^+$ (60), 211 (100 [M-$CH_3$] $^+$, 197 (6), 183 (15), 165 (14), 155 (8), 152 (6), 128 (6), 115 (6), 105 (8), 104 (6), 76 (6), 51 (2).

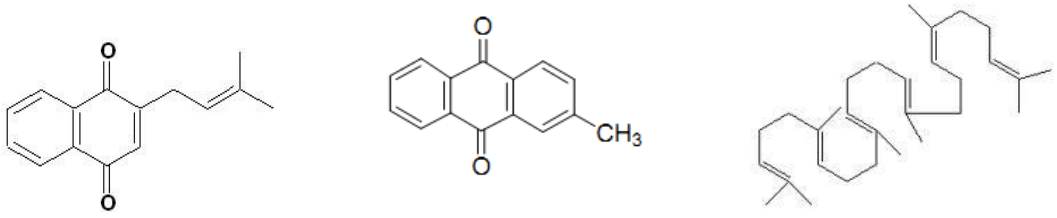
Squalene (colorless oil). EI-MS: m/z (relative intensity): 410 [M]$^+$ (30), 367 (46), 341 (80), 231 (60), 217 (44), 149 (58), 137 (100), 109 (76).

C1 (white powder). EI-MS: m/z (relative intensity ) 414 (98) [M]$^+$, 412 (70), 400 (40) [M-$CH_2$] $^+$, 396 (32), 329 (26), 300 (24), 271 (28), 255 (54), 231 (26), 213 (38), 199 (16), 159 (42), 145 (42), 133 (40), 119 (38), 107 (34), 105 (40), 95 (34), 93 (32), 91 (24), 81 (32), 69 (22), 55 (30), 41 (4). IR spectrum υ max KBr cm-$^1$: 2968.8(m), 2934.1 (m), 1777.2 (w), 1751.0 (w), 1733.6 (m), 1718.2 (m), 1653.6 (m).

**Assay for antifungal activity**

*Trametes versicolor* (white-rot) (NBRC: 30340), *Fomitopsis palustris* (brown-rot) (NBRC: 30339), *Chaetomium globosum* (soft rot) (NBRC: 6347), *Rhizopus oryzae (mold)* (NBRC: 31005), and *Cladosporium cladosporioides* (mold) (NBRC: 6348) were provided by the Natural Institute of Technology and Evaluation Biological Resource Center (NBRC; Tokyo, Japan). Some of these fungi are chosen routinely for antifungal tests according to Japan Industrial Standard (JIS) K1571. Culture of each fungus was maintained on potato dextrose agar (PDA) medium (Eiken Chemical Co., Japan) and stored at 4±1 °C. Before antifungal tests, each strain was incubated on PDA medium in a Petri dish at 26±1 °C until the fungus covered most of the surface of the plate.

Antifungal assays (Figure 2) were conducted referring to Sekine *et al*. (2009). Before fungal inoculation, 1 mg of sample was dissolved in 1 ml of respective solvents (*n*-hexane, EtOAc, and MeOH), and 300 µl of each solution was then applied to the surface of each 15 ml PDA medium in a Petri dish (88 mm of diameter) with the final concentration of 5 µg cm$^{-2}$ of solid agar.




Deoxylapachol Tectoquinone Squalene

Figure 1 Chemical structures of isolated compound from *n*-$C_6H_{14}$ extract of teak heartwood.

After the application, each plate was air-dried on a clean bench for 1 h. An equal amount of the solvent was spread on the control medium. An inoculum of each strain was obtained by using a 5.5 mm in diameter of cork-borer, and was placed on the center of the test medium. The dishes were cultured in the dark at 26±1 °C and 70% relative humidity in an incubator. When mycelia reached the edge of the control petri dish, the average mycelium diameter per treatment was calculated from four radial directions measurement.

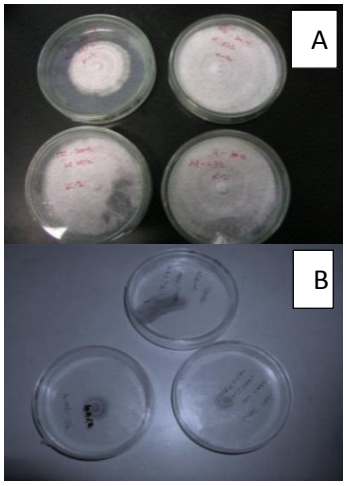



Figure 2 Antifungal assays in PDA medium of *T. versicolor* (A) and *C. globosum* (B).

All results are expressed as means ± SE. The antifungal assay was carried out in three replicates for each sample. The antifungal activity was expressed as the percentage of mycelium diameter growth that was calculated in accordance with the following formula:

Average growth rate (%) = 100 x Da/Db

Da: Average of the sample mycelium diameter

Db: Average of the control mycelium diameter.

## Results and Discussion

### Yields and anti-fungal properties of extracts

The weight and yield of n-hexane, EtOAc, and MeOH extract on the basis of oven dry weight of wood meal (1000 g) was 30.24 g (3.93%), 22.1 g (2.87%) and 2.23 g (2.20%), respectively. The *n*-hexane was comparatively potent mycelia growth inhibitors for *R. oryzae* (76%), C. *cladosporioides* (73%) and *C. globosum* (77%), whereas EtOAc extract was active against *F. palustris* (81 %), *R. oryzae* (78%), and *C. cladosporioides* (65%) (Figure 3).

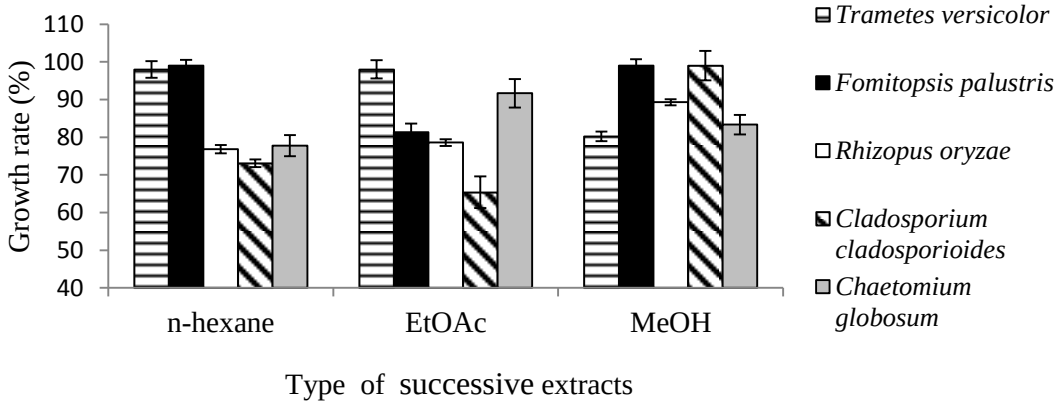



Figure 3 Antifungal activities of successive extracts from teak heartwood against five fungi species.

The MeOH extract revealed mycelium growth inhibition for *T. versicolor* (80%) and *C. globosum* (83%), but not for *T. versicolor*, *R. oryzae*, and *C. cladosporioides*. It was obvious that n-hexane extracts retained higher antifungal activities against soft rot fungi and molds than those of MeOH and EtOAc extracts. In general, due to apparently a very low extract concentration (5 µg $cm^{-2}$) in solid agar, the inhibition seems very low and could not generate a strong antifungal activity (below 50% of the mycelial growth rate).

The *n*-hexane extract was further investigated due to its higher activity compared to those of EtOAc and MeOH extracts. After successively washing the extract with saturated $NaHCO_3$, 10% $Na_2CO$, and 1% NaOH aqueous solutions in a separatory funnel, neutral fraction (25.5 g) and acidic fraction (1.3 g) were obtained. The major portion of n-hexane (neutral fraction) was then specifically characterized.

**Chemical compositions of *n*-hexane extract**

It has been reported previously that GC-FID analysis of *n*-hexane neutral fraction of teak wood indicated the presence of quinones (deoxylapachol or its isomer, tectol, lapachol, and tectoquinone), palmitic acid, myristic acid, squalene, and some steroids (Lukmandaru & Takahashi 2009, Weindesen *et al*. 2003). Squalene, a triterpene, was the major component. Deoxylapachol (1), tectoquinone (2), squalene (3), and an unknown compound, C1 (4) were isolated by column chromatography separation (Figure 4). C1 was thought to be a steroid on the basis of fragmentation pattern of its mass spectra and positive under Liebermann-burchard staining test. This compound showed a parent ion at m/z 414 and other ions were at 271, 255, 133, 107, 105, 95, 81, 69, 55, and 41 which similar to *β*-sitosterol (Yahya *et al*. 2011). In order to evaluate the antifungal properties of teak heartwood extract, it was necessary to confirm the activities of isolated compounds.

**Antifungal properties of isolated compounds**

In order to evaluate the antifungal activities of isolated compounds 1 to 4, a 5 µg $cm^{-2}$ of every compound were tested against 5 species of fungi (Figure 5). Tectoquinone was comparatively strong mycelium growth inhibitor for *R. oryzae* (58%). Deoxylapachol retained a medium antifungal activity against *T. versicolor* and *F. palustris* (64-75%) nevertheless was strong against *R. oryzae* and *C. cladosporioides* (57-59%). Squalene revealed stronger mycelium growth inhibitions (50%) than these of C1 (63%) and deoxylapachol (57%) when tested against *C. cladosporioides.* No compound is considered inhibitory to soft rot *C. globosum.*

The present study also indicated that deoxylapachol reduced growth rates of molds (*R. oryzae* and *C. cladosporioides*), while tectoquinone reduced that of *R. oryzae* only. This is in line with previous finding of Sumthong *et al*. (2006) who demonstrated deoxylapachol and tectoquinone inhibited *Aspergillus niger* growth. The triterpene structure compounds such as squalene and C1, as expected, showed weak antifungal properties to brown-rot and white-rot fungi, however, both of them were growth inhibitors to *C. cladosporioides*. This suggests that certain mold could be inhibited by quinones, triterpenes, or sterol compounds.

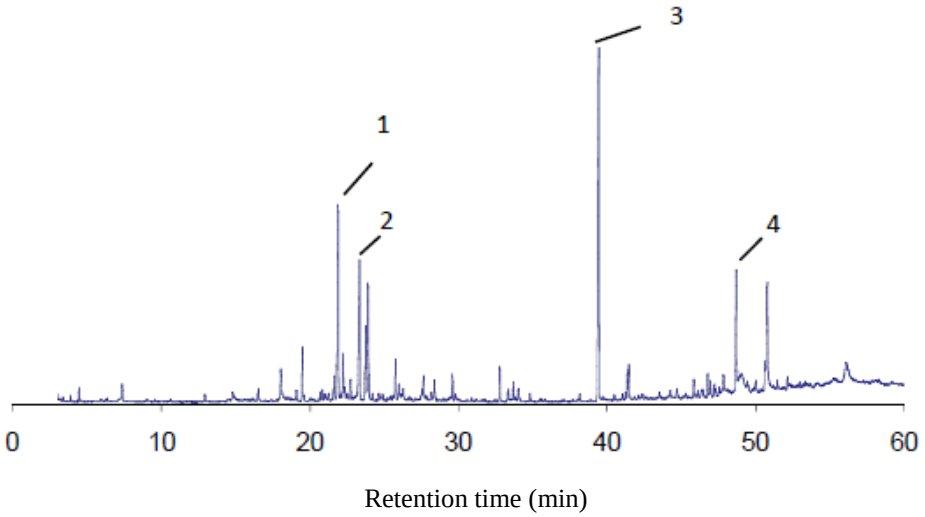



Figure 4 GC chromatogram of neutral fraction in n-hexane extract of teak heartwood. 1= Deoxylapachol, 2= tectoquinone, 3 = squalene, and 4= C1.

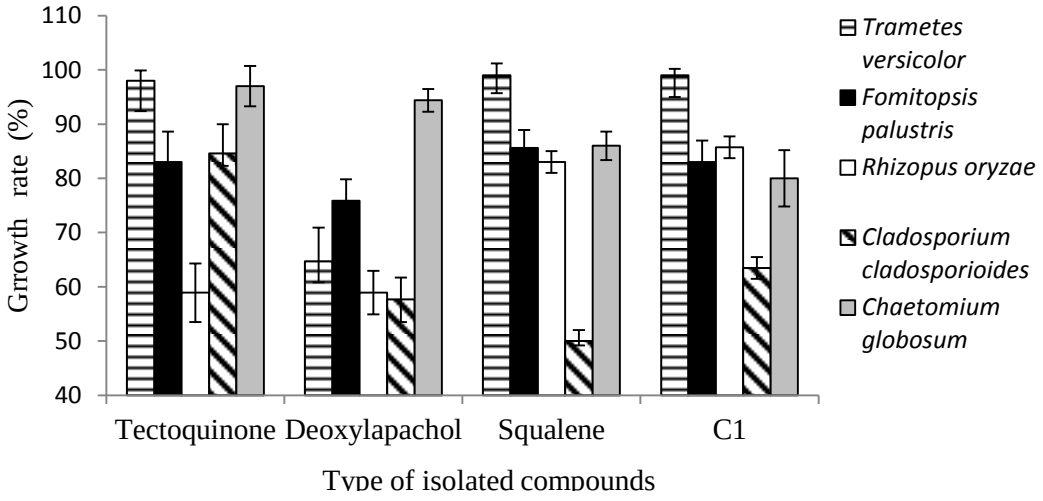



Figure 5 Antifungal activities of isolated compounds against five fungi species.

It is necessary to find out the mechanism of inhibition and bioassay of teak active components to more species of fungi. The relationship between antifungal activity and chemical structure was then discussed. Deoxylapachol, which showed strong antifungal properties to both brown-rot and white-rot, is a naphtaquinone. Previous finding by Sumthong *et al*. (2008) showed that this compound has antifungal properties against brown-rot fungi (*G. sepiarium* and *G. trabeum*) and the white-rot fungi (*Merulius tremellosus* and *Phlebia brevispora*). Tectoquinone did not reduce growth rates of both *T. versicolor* and *F. palustris*. This compound did not have antifungal properties in previous reports (Rudman 1958, Rudman 1961, Sumthong *et al*. 2008) against various brown-rot or white-rot fungi. These results suggest that, in teak heartwood extract, the napthaquinone structure retained stronger influence than

anthraquinone structure on the antifungal activities especially against brown-rot or white-rot fungi.

## Conclusion

In the present study, agar plate method has been used to test the antifungal activity of teak wood extracts. The n-hexane showed higher activities against *R. oryzae*, C. *cladosporioides* and *C. globosum*. The EtOAc extract possessed notable inhibition to *F. palustris*), *R. oryzae*, and *C. cladosporioides*, while the MeOH extract had moderate effect to *T. versicolor* and *C. globosum*. From the neutral fraction of *n*-hexane extract, four compounds were isolated (deoxylapachol, tectoquinone, squalene, and an unknown compound, C1). Tectoquinone was active against *R. oryzae*, whereas deoxylapachol was potent mycelial growth inhibitors for *R. oryzae T. versicolor*, *F. palustris*, R. *oryzae* and *C. cladosporioides*. Both squalene and C1 just inhibited mycelium growth of *C. cladosporioides*.

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