ANTITERMITIC COMPOUNDS FROM THE HEARTWOOD OF SONOKELING WOOD (*Dalbergia latifolia* Roxb.)

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ABSTRACT

This study was undertaken primarily to isolate and identify antitermitic substances that may be responsible for the natural durability of sonokeling wood (Dalbergia latifolia Roxb.). The woodmeal of the samples were extracted with acetone solvent. The acetone extract was then fractionated into n-hexane soluble fraction, ether soluble fraction, and insoluble fraction. The antifeedant bioassay test was carried-out by treating paper discs with extracts at three level of concentration i.e., 0.1 %, 0.5 %, and 1.0 % (W/W). The antifeedant bioassay test showed that both n-hexane soluble fraction and ether soluble fraction of this wood exhibited high toxicity to subterranean termite Reticulitermes speratus Kolbe. The toxicity of these soluble fractions need further investigation to identify the responsible compounds. Further investigation of the ether soluble fraction led to the isolation and identification of main compound, namely latifolin and new neoflavonoid.

Keywords : sonokeling wood, latifolin, new neoflavonoid, antitermitic activity, *Reticulitermis speratus* Kolbe

Sonokeling (*Dalbergia latifolia* Roxb.) is one species of the family Papilionaceae. It is one of the major commercial wood species of Indonesia and is found throughout Jawa island. In England and the USA, this species is known as Indian rosewood or Bombay blackwood, whereas in Deutch and Germany it is called Indisch palissander and Indisches Rosenholz, respectively. This species is used in Indonesia for furniture, decorative veneer, and wood carving (Martawijaya & Kartasujana, 1977).

According to the wood durable classification, this species is classified as a very durable wood (Martawijaya & Kartasujana, 1977). It means that this species has high ability to resist the attacks of wood destroying organisms, i.e., fungi, insects, and marine borer. The natural durability of wood depends on the concentration of the toxic extractives of wood formed during the formation of heartwood. Pre-investigation using the termite of *Cryptotermes cynopcepahlus* Light indicated that the acetone extractives from the heartwood of this wood play a significant role in its durability against the termite (Syafii & Febrianto, 1995). This study was undertaken primarily to isolate and identify antitermitic compounds that may be responsible for the natural durability of this wood.

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METHODS

The study was conducted at the Laboratory of Forest Products Chemistry, Faculty of Forestry, Bogor Agricultural University and Laboratory of Wood Extractives, Forestry and Forest Products Research Institute, Tsukuba, Japan.

Preparation of Extracts

Wood sample used in this experiment was obtained from West Jawa, Indonesia. The heartwood of the sample was converted to woodmeal in a Willey mill to pass a 40-60 mesh screen and then air-dried to about a 10 % moisture content. One thousand gram sample of air-dried woodmeal (10.55 % moisture content) was extracted with acetone solvent in a soxleht apparatus for 24 hours. The acetone extract was then concentrated at 30-40 ^{0} C in a rotary vacuum evaporator. It was then successively fractionated into *n*-hexane soluble fraction and ether soluble fraction. The general scheme of this successive fractionation is presented diagrammatically in Figure 1.

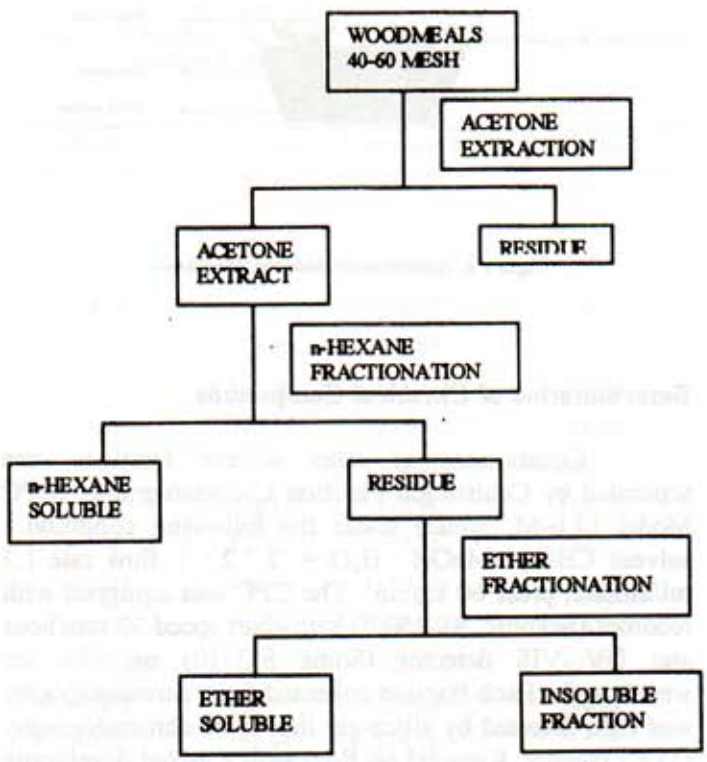

Figure 1. Schematic fractionation of acetone extract

Antifeedant Bioassay Test

The antifeedant bioassay test was carried-out according to the procedure reported by Ohmura *et al.* (1997). Paper discs (8 mm in diameter, about 55 mg in weight, Toyo Seisakusho) were treated with *n*-hexane soluble fraction, ether soluble fraction, and insoluble fraction at three levels of concentration i.e., 0.1 %, 0.5 %, and 1.0 % (W/W). The acetone treated paper discs were also included in this experiment as a control. After air drying process, the untreated and treated paper discs were dried in a vacuum desiccator for 24 hours. Two paper discs treated with different solutions were put on plastic saucers, and then placed diagonally 12 mm away from the center of the test plastic cup. This procedure is illustrated in Figure 2. Fifty workers of the subterranean termite *Reticulitermes speratus* Kolbe were added to each plastic cup and three duplicates were undertaken for each solution. The plastic cups were then placed in the environmental chamber for three days. After three days, the paper discs were pulled out, dried at 40^0 C for six hours and dried in a vacuum desiccator for 24 hours. The weight loss of paper discs was used to determine termite attack.

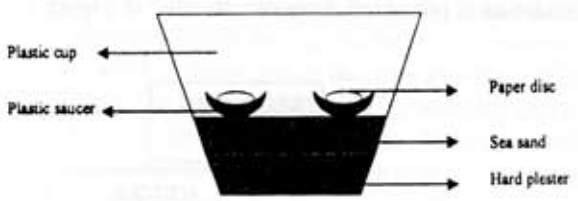

Figure 2. Antitermitic bioassay methods.

Determination of Chemical Compounds

Constituents of ether soluble fraction were separated by Centrifugal Partition Chromatography (CPC Model LLb-M, Sanki) under the following condition : solvent $CHCl_3$: MeOH : H_2O = 2 : 2 : 1, flow rate 1.5 ml/minute, press 60 kg/cm^2. The CPC was equipped with recorder (Sekonic, SS 250 F) with chart speed 30 mm/hour and UV VIS detector (Soma S-3710) on 254 nm wavelength. Each fraction collected from chromatography was then detected by silica-gel thin layer chromatography (TLC) (Merck, Kiesegel 60 F_{254}) with a mixed developing solvent, *n*-hexane : ethyl acetate = 1 : 1 (V/V). Spots on the TLC were sprayed with a sulfuric acid solution, heated on hot plate, and then detected by UV light in the dark room. The chemical structure of compounds isolated from ether soluble fraction was then identified by proton and carbon nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR). Instruments used in this experiment were JEOL GSX-400 and JEOL ALPHA-500 spectrometers.

RESULTS AND DISCUSSION

Acetone Extractive Content

To assess the normality of extractive content in the wood sample, acetone extract yield was determined (Table 1). The total amount of acetone extract obtained from the heartwood of sonokeling wood is 8.23 % of oven-dry wood sample. This amount is relatively higher than the average of extractive content in the tropical hardwood. Tsoumis (1991) stated that the content of extractives varies from less than 1 percent to more than 10 percent depends on the families, species, and tissue. The acetone extract obtained from the heartwood of gonzalo alves *(Astronium fraxinifolium)*, rasamala *(Altingia excelsa)*, and ulin *(Eusideroxylon zwageri)* was 3.34 %, 2.64 %, and 8.18 % respectively (Syafii, 1985 & 1993). The *n*-hexane soluble fraction, ether soluble fraction, and insoluble fraction (residue) obtained from successive fractionation of the acetone extract are 0.74 %, 6.87 %, 0.62 % of oven-dry wood respectively. Each fraction was then used in the antifeedant bioassay test. The ether soluble fraction exhibited the highest extract yield. The TLC spots also resulted only ether soluble fraction showed the major compound. Therefore, this soluble fraction was then separated by CPC for isolating major compound.

Table 1. The acetone extractive yield from sonokeling wood.

Fractions	Content (% of oven-dry wood)
n-hexane soluble fraction	0.74
Ether soluble fraction	6.87
Insoluble fraction (residue)	0.62
Acetone extract	8.23

Antitermitic Activities

As indicated in Figure 3, the addition of each extract on to the paper discs at the concentration level between 0.5 % and 1.0 % significantly decreased the weight loss caused by *R. speratus* Kolbe attack. The weight loss of untreated paper discs was 9.3 %, meanwhile when the concentration of *n*-hexane soluble fraction and ether soluble fraction were increased to 1.0 % (W/W), the weight loss of paper discs decreased to 0.6 % and 3.0 %, respectively. It can be suggested that the *n*-hexane soluble fraction and ether soluble fraction from the heartwood of sonokeling contains antitermitic substances. The toxicity of these fractions warranted further investigation to isolate and identify the responsible compounds.

Determination of Chemical Compounds

As it was described previously that the acetone extract fractionation produced *n*-hexane soluble fraction, ether soluble fraction, and insoluble fraction (residue). Among three fractions, only ether soluble fraction was subjected to further separation and identification. It is due to the fact that only ether soluble fraction showed a remarkable spots on thin layer chromatography (TLC), indicating the presence of a main compound. On the other hand, the two other fractions showed no distinctive spots, and so it seemed to be very difficult to isolate any compound from these fractions. The ether soluble fraction was then separated by Centrifugal Partition Chromatography and yielded two main compounds, namely compound 54 and compound 20. By silica-gel TLC with a mixed developing solvent, *n*-hexane : ethyl acetate = 1 : 1, spots on the TLC showed that the Rf of compound 54 and compound 20 were 0.62 and 0.40 respectively.

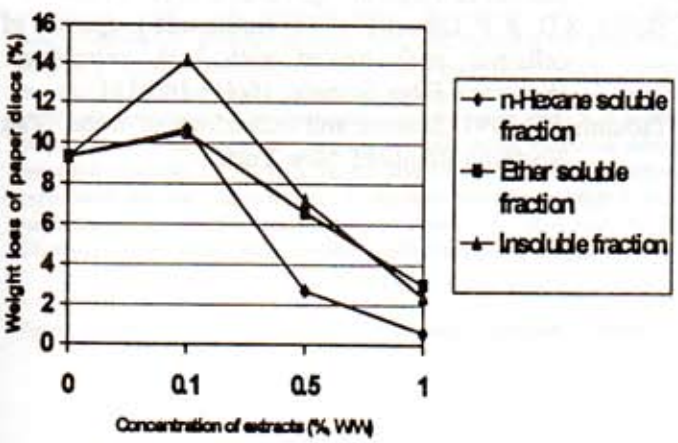

Figure 3. **The wieght loss of paper discs after three days biossay test against *R. speratus* Kolbe.**

The chemical structures of the compounds isolated were then determined by proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR) spectroscopies. The ^{13}C-NMR spectral data of compound 54 and compound 20 are listed in Table 2. Based on the ^{1}H-NMR spectral data obtained from this investigation, it was concluded that compound 54 is a neoflavonoid that seemed to be the latifolin reported by previous researcher (Muangnoicharoen & Frahm, 1982). The chemical structure of latifolin is presented in Figure 4. This conclusion was supported by an informative ^{13}C-NMR spectral data of compound 54 which was very similar to that of the spectral data of latifolin.

Figure 4. **Chemical structure of latifolin and new neoflavonoid.**

The ^{1}H-NMR spectral data of compound 20 was very similar to that of compound 54 with the exception of a double doublet at δ 6.50 and a doublet at δ 6.54 which represented a hydroxyl substitution at C-5' in ring B. The chemical shift of C-4' and C-6' in ring B of compound 20 are of comparative size for the corresponding carbon atoms in compound 54 and compound 20 due to the hydroxyl substitution at C-5'. On the basis of the above spectral data, compound 20 was deduced as a new neoflavonoid, and its chemical structure is proposed by the formula shown herein (Figure 4). The ^{13}C-NMR spectral data of this compound (Table 2) is very informative in supporting this conclusion.

Table 2. **Chemical shift of ^{13}C-NMR spectra of latifolin, compound 54, and compound 20.**

Carbon No.	Latifolin	Compound 54	Compound 20
C-1	122.61	124.37	124.37
C-2	149.41	151.25	151.27
C-3	97.13	99.24	99.32
C-4	145.43	146.79	146.83
C-5	139.99	140.86	140.90
C-6	115.10	116.82	116.89
C-1'	129.24	130.48	131.30
C-2'	153.58	155.51	148.45
C-3'	116.16	115.91	116.52
C-4'	128.35	127.81	114.05
C-5'	120.46	119.94	150.93
C-6'	127.51	130.02	116.79
C-HA	40.01	40.97	41.03
C-HX	138.91	141.43	141.47
=CH_2	116.47	115.12	115.12
2-OCH_3	55.98	56.47	56.51
4-OCH_3	57.01	57.05	57.08

CONCLUSIONS

1. The addition of *n*-hexane soluble fraction and ether soluble fraction from the heartwood of sonokeling decreased the weight loss of paper discs caused by *Reticulitermes speratus* Kolbe attack. Therefore it can be suggested that these extracts contain atitermitic substances.
2. Further investigation on the ether soluble fraction from this wood led to the isolation and identification of two main compounds, namely latifolin and new neoflavonoid.

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