Bioactive Extracts from Neutrals of Teakwood (*Tectona grandis* L.f.)

Ganis Lukmandaru
Department of Forest Product Technology, Faculty of Forestry, Gadjah Mada University
Email : ganisarema@lycos.com

ABSTRACT

Bioassay-guided investigation by brine shrimp lethality and termite activity tests from the heartwood of teak (*Tectona grandis*) led to the fractionation of *n*-hexane soluble extract. After the washing by alkaline solution and followed by saponification, the unsaponifiable, acidic and insoluble fractions were obtained. The unsaponifiable fraction was the major part and exhibited strong activity both against termites and brine shrimps. Repeated column chromatographic fractionations resulted to the isolation of tectoquinone, and other three compounds that exhibited various levels of activity in brine shrimp and termite tests. The correlation between brine shrimp lethality and termite activity test was also discussed.

Keywords: *Tectona grandis*, extractives, brine shrimp lethality test, anti-termitic test, tectoquinone

INTRODUCTION

Teak is a well-known and very good for general purpose timber. The wood has especially excellent properties in term of durability because of its extract compound. Some bioactive compounds are already isolated, particularly from quinone groups (Rudman and Gay 1961, Sandermann and Simatupang et al. 1966; Khan et al. 1999). However, natural durability of wood includes various factors. Not only quinones but also other compounds may be important in natural conditions. Finding of novel toxicants may be expected by application of more sensitive bioassays and investigations by advanced chemical technology.

In this study, we will report the fractionation and isolation of some compounds from teak extracts with the aim of understanding of its chemical structure and its toxicity by bioassays (termite and brine shrimp). Another purpose was to evaluate the relation between brine shrimp lethality test and anti-termitic test.

Materials and method

Extraction and Isolation

Dried wood meal (1 kg ovendry weight) was refluxed 3 times with *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) successively every 6 hours. Each resulting extract was filtered and concentrated in vacuo at about 45 ^{0}C to obtain dark crude residues.

The *n*-hexane extract of 28 g was dissolved in 250 ml *n*-hexane moved to separatory funnel and partitioned against saturated $NaHCO_3$, followed by 10 % Na_2CO_3, and 1% NaOH, successively (3 times, 250 ml each). As the results, acidic (aqueous layer) and and neutral fractions (*n*-hexane

layer) were obtained, respectively. Saponification was applied to the neutral fraction in order to obtain genuine neutral compounds. The neutral fraction of 25 g was dissolved in 500 ml of 1.3 % ethanolic KOH and refluxed for 90 minutes. After washing with water, acidification and *n*-hexane extraction, the dark substance was obtained as an acidic from neutrals or acidic II fraction. The insoluble part was formed between *n*-hexane – aqueous layer. The dark blue substance as insoluble fraction (acidic III) in considerable amount and the major brown substance as genuine neutral or unsaponifiable fraction were obtained, respectively. The respective yields of acidic II, insoluble, and unsaponifiable parts were 4.6 %, 25.4 %, and 56.9 % on the basis of *n*-hexane extract. Total recovery in this experiment was 25.2 g or 91.1 (%) of the applied sample.

From unsaponifiable fraction, after repeated silica gel column chromatographic separations, four isolated components were obtained using n-hexane, benzene, and EtOAc as eluents. The scheme of separation is summarized in Figure 1.

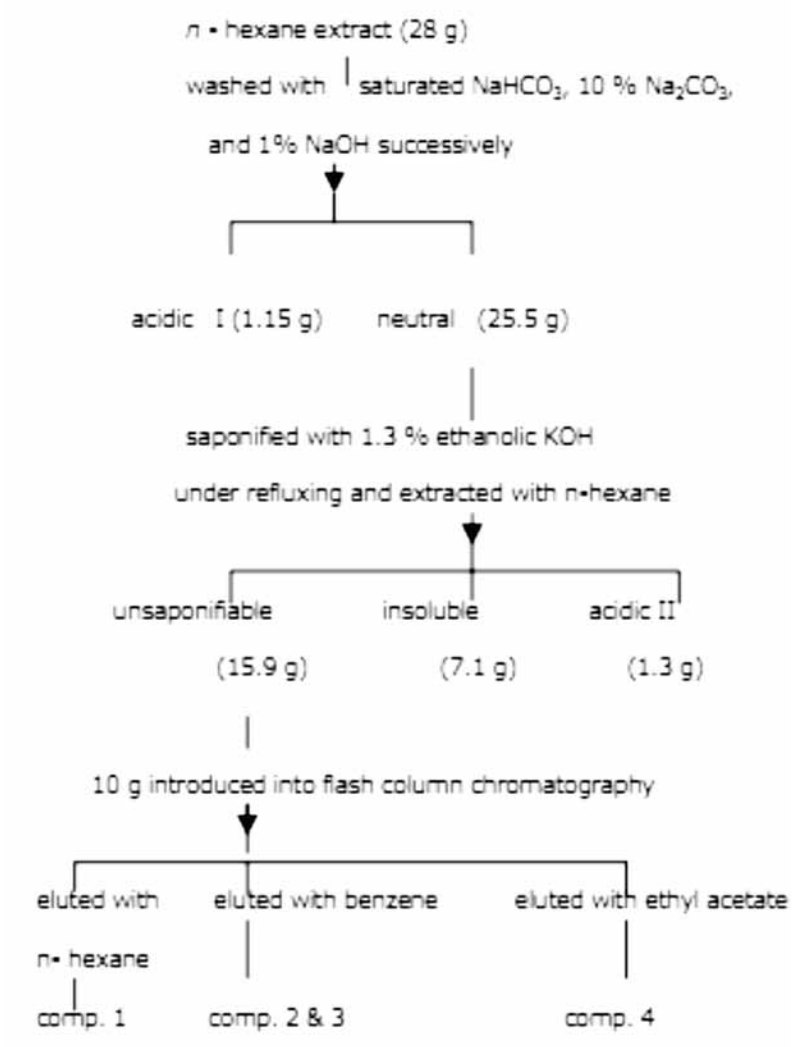

Fig. 1. Separation scheme of *n*- hexane extract

Identification of compounds

Compounds were identified by comparing their mass spectra with literature's data and the injection of standards (tectoquinone, β-sitosterol, fatty acids, squalene). GC-MS (JEOL XS mass spectrometry at 70eV) was used for gas chromatographic separations.

Bioassays

Brine shrimp lethality test (BSLT)

BSLT was conducted according to the method described by Ohira and Yatagai (1984) with some modifications. The concentration of 200, 20 and 2 ppm was applied in 3 replications. Vials added by dimethyl sulfoxide (DMSO) only were used as a control. Data were analyzed by probit analysis with Minitab ver. 13 computer program to calculate the concentration of the extract or fractions that would kill 50% (LC_{50}) at 95 % confidence interval.

Anti-termitic test

No choice antifeedant bioassay test was carried out in this research. A petri dish (diameter 9 cm, height 2 cm) containing 20 g moistened and sterilized sea sand was used as a container test. Paper discs (diameter 8 mm; Whatmann International) were impregnated with chloroform solution containing each of the test fractions. The treatment retention was 5 % (w/w) per disc and 5 duplicates were applied for each sample. Fifty worker *Reticulitermes speratus* Kolbe termites were introduced into the petri dish. After 10 days the disc were taken out, dried and the weight loss was determined. To measure the termiticidal activity, the number of dead termites was also calculated.

RESULTS AND DISCUSSION

To find out bioactive compounds, the extracts obtained from successive extraction were tested by brine shrimp and termite tests as described before. The result is shown in the Table 1.

EtOAc and *n*-hexane extracts exhibited a good level of activity against brine shrimps while MeOH extract did not show some activities in both methods, because its LC_{50} values and weight loss were close to those of the controls. All extracts revealed a low mortality number in termicidal test, and showed little difference of mortality among those extracts. As the *n*-hexane extract exhibited strong activity against brine shrimps and termites, as well as considerable yield, this fraction was used for further experiment.

Table 1. LC_{50} of brine shrimp, weight loss and mortality number due to termite exposure of teak extractives

Tested materials	LC_{50} (ppm)	Weight loss (mg)	Mortality number
n-hexane extract	6.71	8.37	5
Ethyl acetate extract	6.60	5.39	8
Methanol extract	> 200	21.32	6
Control	> 200	20.62	1

The fractionation of *n*-hexane by alkaline solution followed by saponification of neutral part yielded 4 fractions. Unsaponifiable fraction showed the major amount of *n*-hexane extract. The bioactivity of those fractions as shown in the Table 2:

Table 2. LC_{50} of brine shrimp, weight loss and mortality number due to termite exposure

Tested materials	LC_{50} (ppm)	Weight loss (mg)	Mortality number
Unsaponifiable	6.77	8.34	4
Insoluble	5.32	6.51	12
Acidic II	> 200	15.64	6
Control	> 200	21.32	1

Unsaponifiable and insoluble fractions exhibited a good level of activity in BSLT. All fractions showed antifeedant activities. The insoluble fraction among those showed the stronger activity than the others. Unsaponifiable fraction did not show strong toxicity in termicidal test but still gave a strong activity in the antifeedant test. Insoluble fraction was the most active fraction as shown by a weight loss and mortality number. A good correlation between BSLT and antifeedant activity result was found. However, the correlation between weight loss and mortality number of termites is not so clear. The unsaponifiable fraction was used for further investigation because it occupied majority in *n*-hexane extractives and showed good level of activity in both bioassays.

Gas chromatogram of the unsaponifiable fraction is displayed in Fig. 2. The major component of this fraction was squalene (detected at about 40 minutes), which belong to triterpene group. The chromatographic separations of unsaponifiable fraction resulted compound 1 (molecular weight 414), compound 2 or tectoquinone (molecular weight 222), compound 3 (molecular weight 270) and compound 4 (molecular weight 256). On the basis of fragmentation pattern of the mass spectrum as well as TLC analysis, it was indicated that compound 1, 3 and 4 are sterols or terpenes. The results of bioassays of those compounds are given in Table 3.

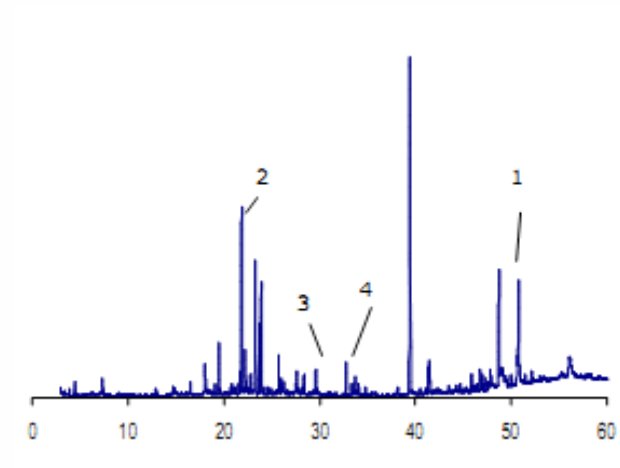

Fig. 2. Gas chromatogram of unsaponifiable fraction of *n*-hexane soluble extract from teak heartwood. The number of the peaks referred to the isolated compounds in Fig. 1.

Table 3. LC_{50} of brine shrimp, weight loss and mortality number due to termite exposure

Tested materials	LC_{50} (ppm)	Weight loss (mg)	Mortality number
Compound 1	> 200	14.02	8
Tectoquinone	11.8	0.72	36
Compound 3	> 200	21.32	6
Compound 4	> 200	14.24	16
Control	> 200	21.59	9

Tectoquinone was the most active in both antifeedancy and termiticidal tests. This finding confirms the activity of quinone derivatives of which concluded by Sandermann and Simatupang (1966). Tectoquinone, showed a high activity to the brine shrimps. Despite the fact that squalene is the most abundant compound in unsaponifiables, the role of this compound in mediation of natural durability remains poorly understood. It is generally known that squalene is a natural anti-oxidant. Yamamoto et al. (1998) suggested that anti-oxidants are necessary to provide long-life durability in the teak sample. One of the possibilities is synergistic mechanism as proposed by Schultz and

Nicholas (2000) who found the combination of a commercial antioxidant and biocide showed an increase in efficacy compared to the organic biocide alone. Therefore, further studies should be undertaken to explore the role of squalene in mediation of termite resistance.

As inexpensive, easy, and time-saving method, the use of brine shrimp in this study deserved an attention. BSLT have been introduced in various bioassay systems as the analysis of allelophaty or medicinal purposes (Ohira and Yatagai 1984). The present finding showed a good correlation between termite antifeedant activity and BSLT in the fraction stage. With regard to the isolated compounds, theoretically, the result trend of BSLT is expected to show a good correlation to those of termiticidal activities. However, the lower toxicity of the isolated compounds in BSLT was found as mentioned before. It may due to insufficient solubility in artificial sea water and/or DMSO solution. Thus, it can be suggested that BSLT can be used to monitor the toxicity of fractions in teak wood extractives, but it may be more preferable in more polar fractions which dissolved in water.

CONCLUSIONS

1. Less polar fractions of teak heartwood extractives exhibited strong activities against brine shrimps and termites.
2. Genuine neutral or unsaponifiable fraction was the major part of n-hexane soluble extract.
3. Tectoquinone showed a strong activity level against termites and brine shrimps.
4. BSLT showed a good correlation with termite antifeedant and termiticidal test in a screening test and maybe useful as monitors of bioactivity.

References

Khan, R.M., Mlungwana, S.M. 1999. 5-hydroxylapachol : A Cytotoxic Agent from *Tectona grandis.* Phytochemistry 50 : 439-442.

Ohira, T., Yatagai, M. 1984. Allelophatic Compounds Produced by Forest Plants II. Mokuzai gakkaishi 40 : 541-548.

Rudman, P., Gay, F.J. 1961. The Causes Natural Durability in Timber part VI. Measurement of Anti-termite Properties of Anthraquinones from *Tectona grandis* L.f. by Rapid Semi-micro Method. Holzforschung 15 : 117-120.

Sandermann, W., Simatupang, M.H. 1966. On the Chemistry and Biochemistry of Teakwood (*Tectona grandis* L. fil). Holz Roh-Werkst. 24: 190-204.

Schultz, T.P., Nicholas, D.D. 2000. Naturally Durable Heartwood : Evidence for a Proposed Dual Defensive Function of the Extractives. Phytochemistry 54 : 44-52.

Yamamoto, K., Simatupang, M.H., Hashim, R. 1998. Caoutchouc in teak wood (*Tectona grandis* L f): formation, location, influence on sunlight irradiation, hydrophobicity and decay resistance. Holz Roh-Werkst. 56:201-209.