ANTITERMITIC ACTIVITY OF THE BARK EXTRACTS OF TEAK

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Abstract

Teak (*Tectona grandis*) wood is recognized to exhibit antitermitic activities. The antitermitic activities of its bark, however, is still unexplored. This research is aimed to evaluate teak bark extracts and their components against *Reticultermes speratus* Kolbe termites species. Materials used in this study were wood powder from teak trees aged 8 years (6 trees) and 22 years (4 trees). The extraction were performed separately by cold extraction (one week) by three solvents: *n*-hexane, ethyl acetate and methanol. No choice antifeedant bioassay test by paper discs was carried out in this research. By analysis of variance, the results showed that methanol soluble extracts (1.04 %) is significantly higher than those other solvents. The extracts of *n*-hexane exhibited the antifeedant activity (4-5 mg) which is measured by the mass loss due to termites. The similar tendency was also found in the mortality rate of termites (20-38 %) although the magnitude was not so high compared to the controls (17 %). Tree age factor did not affect significantly to both extractive contents and antitermitic properties of the bark. By means of gas chromatography, deoxylapachol detected in the teak wood extracts was also found in the bark extracts. This compound, however, did not significantly correlate to the antitermitic properties.

Keyword : *Tectona grandis,* antitermitic activities, bark extractive, *Reticultermes speratus*, cold extraction

Introduction

The bark is a major by-product of wood processing. It has a formidable barrier providing both constitutive and induced defence mechanisms. Therefore, bark is believed to contain chemical components, which enable the tree to resist those hazards. It is expected that more effective uses of bark will develop from the viewpoint of green environments. Unlike antifungal activities, only few studies have been published on the antitermitic effect of bark extracts of the natural inhabiting wood species. Those species were *Picea glehnii* (Shibutani et al. 2004), *Adina recemosa* (Yaga 1977) and some temperate species (Harun and Labosky 1985).

Tectona grandis is a widely distributed hardwood in Indonesia. The wood is used as raw material for the production of furniture and other wooden structures. Previous works have already reported that extractives from the wood of teak inhibited the wood-destroying temites. Of these extractives, quinones that were present in appreciable levels in the wood of teak showed strong inhibition against the attack of termites (Rudman and Gay, 1961; Sandermann and Simatupang, 1966; Lukmandaru and Ogiyama 2005, Lukmandaru 2012). However, studies about as antitermitic effects as well as chemical constituents of teak bark are still limited.

The aim of this study was to find out the antitermitic effect of bark extracts from teak as well as to detect some quinones and related components in the bark. It is assumed that similar quinones in the wood to also play an important role in the antitermitic activity of the bark. The results are discussed with focus on the bark extracts effect by means of the paper disc tests.and to relate it with the present of quinones. The comparison with the results of our parallel study (Lukmandaru 2013) was also discussed.

Material and methods

Bark extracts

All the plant materials were collected at community forests in Gunungkidul Regency, Jogja. The ages of the test trees were 8 (comprised of 6 samples) and 22 (comprised of 4 samples) years. All the bark samples were collected from the stems of the trees. After drying, these dried bark samples were ground to pass through 40 mesh size. The bark meal (1 g) of each sample was subjected to extraction in *n*-hexane (10 ml) at room temperature for one week. The bark was also separately extracted with ethyl acetate (EtOAc) and methanol (MeOH) in the same manner. The resulting solutions were filtered and the filtrate was evaporated to dryness. The yields of the extractives were calculated on the basis of the weights of the dried extracts to the oven-dried weights of the bark samples.

No-choice feeding test

Subterranean termites were collected from an active wild colony of *Reticulitermes speratus*. The colony was maintained in a dark room at 28°C and 80% relative humidity (RH) until use. A petri dish (diameter 9 cm, height 2 cm) containing 20 g moistened and sterilized sea sand was used as a container test. Paper disc (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 3 duplicates were applied for each sample. After drying at 60 ^{0}C for 2 hours, followed by drying in a vacuum dessicator for 24 hours, they were put on a petri dish. The control discs were impregnated with chloroform only and dried with the same manner. Fifty worker *Reticulitermes speratus* Kolbe termites were introduced into the petri dish. The petri dishes were placed in a dark chamber at 27 ^{0}C and 80 % relative humidity. After 10 days the disc were taken out, dried in the same manner and the weight loss was determined. Mortality was calculated based on the surviving number of termites.

Extractive analyses

Bark meal samples were separately extracted by *n*-hexane (C_6H_6), EtOAc, and MeOH as mentioned above. Aliquots of each solution (200 µl) were removed, placed in glass tubes and dried. A 20 µl aliquot of C_6H_6 was added to dilute each extract. One µl of this solution was then injected with a micro syringe into a GLC (Hitachi Model G-3000) under following conditions, detector: FID, column: NB-5 bonded capillary 30 m, column temperature: 180 - 280 ^{0}C (programming 4 ^{0}C/min), carrier gas: Helium. For quantification of individual substances, a commercial tectoquinone (2-methyl anthraquinone) was employed as a standard. The amounts of components were expressed as mg per 100 g of oven dry weight. Pure sample of squalene and lapachol purchased from Kanto Chem were also was used for confirmation. The identification of constituent compounds was based on their mass spectra and gas chromatographic retention behavior. GC-MS (JEOL XS mass spectrometry at 70eV) was used for gas chromatographic separations. Deoxylapachol or its isomer was identified by comparison of their mass spectra with those from previous studies by Windeisen et al. (2003) and Perry et al. (1991).

Statistical Analysis

The results were expressed as mean ± standard deviation (S.D.). Statistically significant differences between groups were measured using two-way analysis of variance (ANOVA) with post hoc comparison by Tukey, $^*p < 0.05$ was considered statistically significant. The relationships between the independent variables were studied with a Pearson's correlation analysis. All statistical calculations were conducted using SPSS-Win 10.0.

Results and Discussion

Extractive content

The ANOVA of mean extractive content showed that there was no significant tree age factor (p= 0.72), while the effects of extract (p <0.01) are significant. Comparisons among the extracts revealed that the MeOH soluble extractive content (3.44$\pm$0.70 %) significantly gave the highest values while C_6H_6 soluble extractive content (0.27$\pm$0.07 %) gave the lowest (Fig.1). This result could be explained as methanol dissolves both polar and to some extent non-polar compounds. Theoretically, the polar compounds such as tannins and other polyphenols were abundant in the bark. It was also showed that teak bark contains only small amounts of non-polar compounds. The extractive content levels in this experiment could be higher if the hot-extraction or soxhlett extraction was performed.

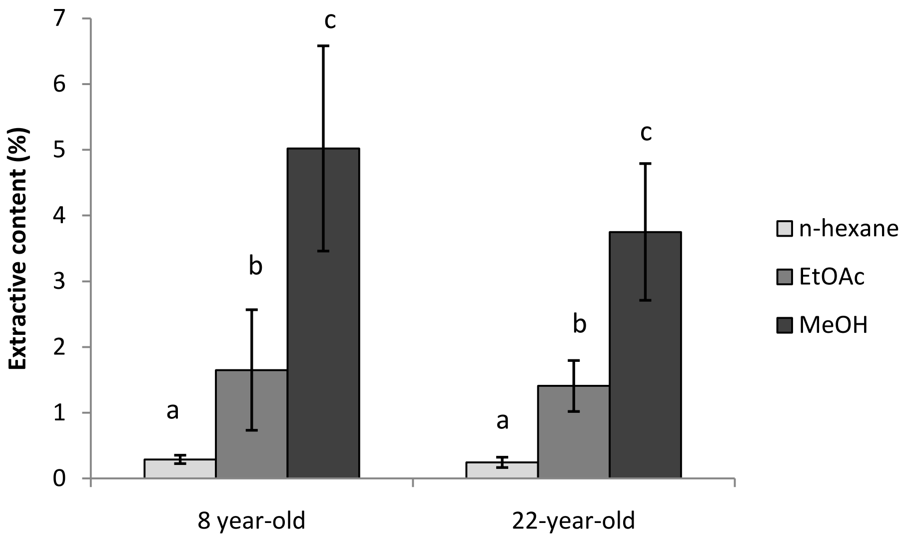

Figure 1. Extractive content (% oven dried mass m/m) of teak bark by tree age and extract. Mean of 6 trees (8- year-old) and 4 trees (22-year-old), with the standard deviation error bar. The same letters on the same graphic are notstatistically different at $P < 0.05$ by Duncan's test.

Antitermitic test

The mass loss due to termites was displayed in Figure 2. By ANOVA, it was calculated that tree age factor was not significant (p= 0.88) but the extract factor was significant (p= 0.01). The mass loss of C_6H_6 extracts levels were significantly lower (5.01±3.34 mg) compared to those of methanol (8.59±4.63 mg) or EtOAc extracts (10.35±2.63 mg). Compared to the control samples (11.35±3.60 mg), the C_6H_6 extracts exhibit a higher antifeedant activity although it was not a huge difference. This finding also indicated the existence of some active components in that extract. The similar tendency was also found with regard to mortality rate of termites. The results showed that tree age (p= 0.28) did not show significant effects, on the other hand, the extract factor (p= 0.04) had significant effects. As shown in Fig. 3, the highest mortality rate levels were obtained in the C_6H_6 and MeOH soluble extracts (24-29 %). The ANOVA of mortality rate between the C_6H_6 and MeOH soluble extracts did not vary significantly. The mortality rate value of the control was about 17 %. Based on these results, the toxicity of teak bark is thought to be weak. In general, by the same samples and method (Lukmandaru 2013), compared to the outer heartwood extracts (mortality rate of 27-65 %, mass loss of 1-3 mg), those of teak barks exhibit lower degree of antitermitic activity. No significant effect of tree age factor to the termite resistance properties is likely due to the low variation in chemical properties between the 8- and 22-year-old barks.

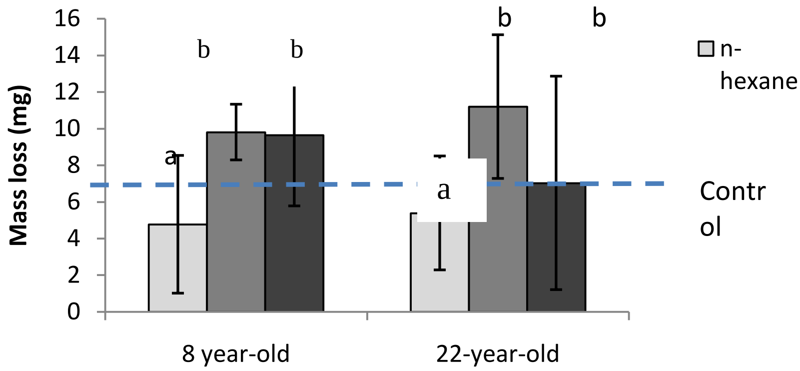

Figure 2. Mass loss due to *Reticulitermes speratus* on 10-day observation of teak bark by tree age and extracts. Mean of 6 trees (8- year-old) and 4 trees (22-year-old), with the standard deviation error bar. The same letters are not statistically different at $P < 0.05$ by Duncan's test.

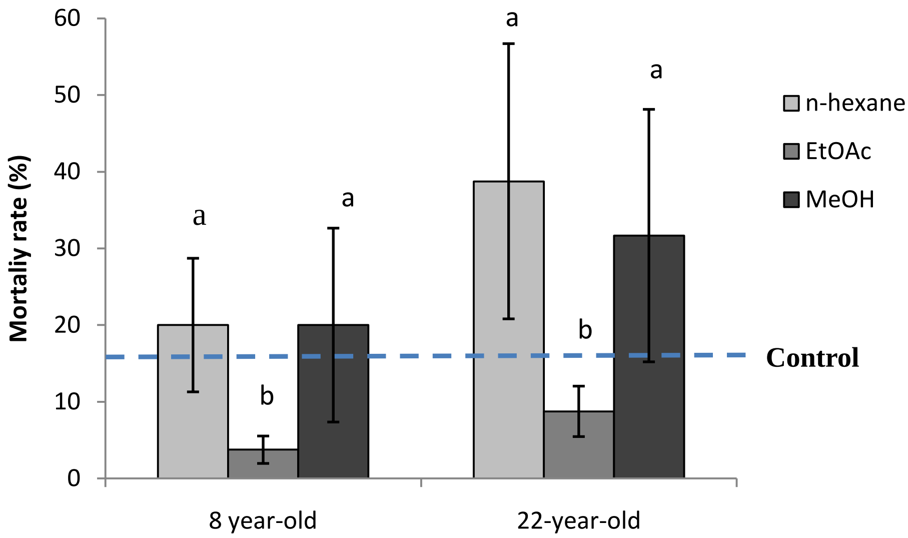

Figure 3. Mortality rate against *Reticulitermes speratus* on 10-day observation of teak bark by tree age and extracts. Mean of 6 trees (8- year-old) and 4 trees (22-year-old), with the standard deviation error bar. The same letters are not statistically different at $P < 0.05$ by Duncan's test.

Table 1. Pearson correlation coefficients (r) for the termite resistance parameters and extractive contents.

Extractive content	Mass loss	Mortality rate
n-hexane	-0.76**	-0.24
EtOAc	-0.31	0.05
MeOH	0.02	-0.02

Note : ** Significant at the 1% level; * significant at the 5% level.

Correlation analysis (Tab.1) confirmed a highly significantly negative correlation between the mass loss and C_6H_6 soluble extracts content ($r = -0.76$). This meant that teak bark was more resistant when it contained higher amounts of these extracts.This indicated that non-polar extractives in the bark contained compounds that were distasteful or repellent to termites. However, the relationship between mortality rate and any extractive content levels was not clear in this study. This outcome was reasonable since the bark extracts do not exhibit a strong toxicity. It was observed in our previous findings (Lukmandaru 2013) that lower degree correlations were measured between mortality rate or mass loss levels and extractive content levels in the teak wood extracts.

Analysis of n-hexane extract

As C_6H_6 soluble extracts levels were related to the termite antifeedancy property (Table 1), GC and GC-MS investigation was further performed. This analysis refered to previous work in the teak wood extract (Lukmandaru and Takahashi 2009) which some bioactive quinones (tectoquinone, lapachol, deoxylapachol or its isomer) as well as non-quinone constituents (squalene) were detected. The quantification of those compounds in the C_6H_6 soluble extracts was presented in Table 2.

Table 2. Contents of some components (mg/100 g of oven-dry wood) in the *n*-hexane soluble extract of teak bark aged 8 and 22.

Teak samples	Compounds of *n*-hexane extracts			
8-year-old trees	Deoxylapachol	Lapachol	Tectoquinone	Squalene
1	10.17	0	0	14.37
2	9.95	0	0	74.04
3	168.11	0	0	37.43
4	13.18	0	0	15.43
5	15.25	0	0	17.72
6	49.22	0	0	30.40
Average	44.31	0	0	31.55
22-year old -trees				
1	8.54	0	0	14.14
2	0	0	0	4.53
3	0	0	0	16.27
4	0	0	0	16.52
Average	2.13	0	0	12.86

In a literature review, Sandermann and Simatupang (1966) mentioned that tectoquinone is the principal component responsible for natural durability against termites in the wood. Furthermore, desoxylapachol has been found to exhibit strong antitermitic activity, but lapachol has only weak antitermitic activity. Squalene have never been mentioned as factors in natural durability. The presence of deoxylapachol and squalene in the bark was confirmed, although at very low levels compared to that in the heartwood (Lukmandaru and Takahashi 2009). Both lapachol and tectoquinone were not detected. Therefore, the lower activity against termites in the bark compared to its wood was probably due to the absence of tectoquinone or comparatively low amounts of quinones.

Previous work reported that some of the polyphenols in the bark were tested to have antitermitic properties (Harun and Labosky 1985). Stilbenes from bark of *Picea glehnii* acted as toxicants against termites rather than as mere feeding deterrents (Shibutani et al. 2004). Yaga (1977), found that scopoletin and oily scopolin were the main termicidal substances from the *Adina recemosa* bark. The Pearson's correlation coefficients between total deoxylapachol content and mass loss levels was only 0.32. This weak link means that this compound is not the sole cause of termite resistance in the bark. Thus, further investigation with regard to non-polar compounds (fats, resin, oils, terpenes etc.) in the teak bark would be useful in determining the relationships between termite resistance and extractives of the bark.

Conclusions

Methanol soluble extractive content significantly gave the highest values in the teak bark extracts. No significant tree age factor affected the variation in the extractive content as well as termite resistance properties. As *n*-hexane soluble extracts exhibited the largest antifeedant activity (low mass loss values), it seemed that the repellent or distasteful substances existed extensively in non-polar constituents of bark materials. Further, correlation analysis showed significantly negative correlation (r=-0.76) between the mass loss due to termite attacks and *n*-hexane soluble extract contents. There was no strong antitermicidal activity was observed in all extracts. Deoxlylapachol was detected in the *n*-hexane soluble extracts, however, it explained relatively little the variation in termite antifeedancy of teak bark.

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