ANTITERMITIC ACTIVITIES OF BITTI WOOD EXTRACTIVES (*Vitex cofassus* Reinw.)

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ABSTRACT

Woods with low natural durability are vurnerable to the attack of organisms. Preservation processes increasing wood service life would encounter this problem. However, preservatives that are usually non-biodegradable will lead to some environmental problems. The development of biodegradable and renewable natural preservatives that are environmental friendly is then necessary. This research was aimed at determining bioactivity of bitti wood (*Vitex cofassus* Reinw.) extractives to subterranean termite, *Coptotermes curvignathus* Holmgren. Extraction and successive fractionation of the wood extractive resulted in 2.49% of acetone extract consisting of 1.470%, 0.053%, 0.027%, and 0.940% of n-hexane, ethyl ether, ethyl acetate and residue fractions, respectively. The termite test indicated that ethyl ether fraction was the highest antitermitic activity.

Key word: *Vitex cofassus* Reinw., extractives, *Coptotermes curvignathus* Holmgren.

INTRODUCTION

Most of wood species in Indonesia (about 80-85%) have low natural durability easily being attacked by organisms such as fungi and termites (Syafii, 2000a). From the aspect of forest resources utilization efficiency, these organisms attacks are very harmful because they can shorten the service life of wood and wood products. Therefore, efforts to extend the service life of the wood such as the introduction of chemicals into the wood structure are very important. In fact, however, the preservatives currently used is largely belonging to the non-organic synthetic chemicals, providing environmental impacts due to their non-biodegradable properties. One way to reduce these negative impacts is to find out naturally available preservatives.

Several types of wood extractive has indeed been proven to contain bioactive compounds that could inhibit the growth of organisms. Syafii (2000a) reported that latifolin and new neoflavonoid compounds isolated from sonokeling wood (*Dalbergia latifolia* Roxb.) have bioactive properties inhibiting the growth of *Coptotermes curvignathus* Holmgren. Falah (2001) also proved that the extractives of torem wood (*Manilkara kanosiensis* Lam.) and lara wood (*Mterosideros petiolata* Kds.) were toxic against subterranean termites (*C. curvignathus*) and rot fungus (*Schizophyllum commune* Fries).

Exploration of bioactive components from different wood species are now becoming important for various purposes such as pharmaceutical materials, insecticides, and fungicides. One of wood species potential to be explored for its bioactive components is bitti wood (*Vitex cofassus* Reinw.). The wood species is well known, especially for people in South Sulawesi as it is usually used for traditional boat making as the main raw material. Since the wood shows a good resistant to deteriorating organisms, the current research was conducted to determine antitermitic activity of the wood extractives.

METHODS

Wood sample used in this experiment was obtained from Palopo South Sulawesi with ± 60 years old. For antitermitic properties test used cellulose paper and subterranean termite *Coptotermes curvignathus* Holmgren.

Preparation of Extracts. The heartwood of the sample was converted to woodmeal in a Willey mill to pass 40-60 mesh screen and then air-dried to about 15% moisture content. Two thousand gram samples of air-dried woodmeal was extracted with acetone solvent in a soxleht apparatus for 24 hours. The acetone extract was then concentrated at maximum 30-40^{o}C in a rotary vacuum evaporator. It was then successively fractionated into n-hexane soluble fraction, ethyl ether soluble fraction and ethyl acetate soluble fraction. The general scheme of this successive fractionation is presented diagrammatically in Figure 1.

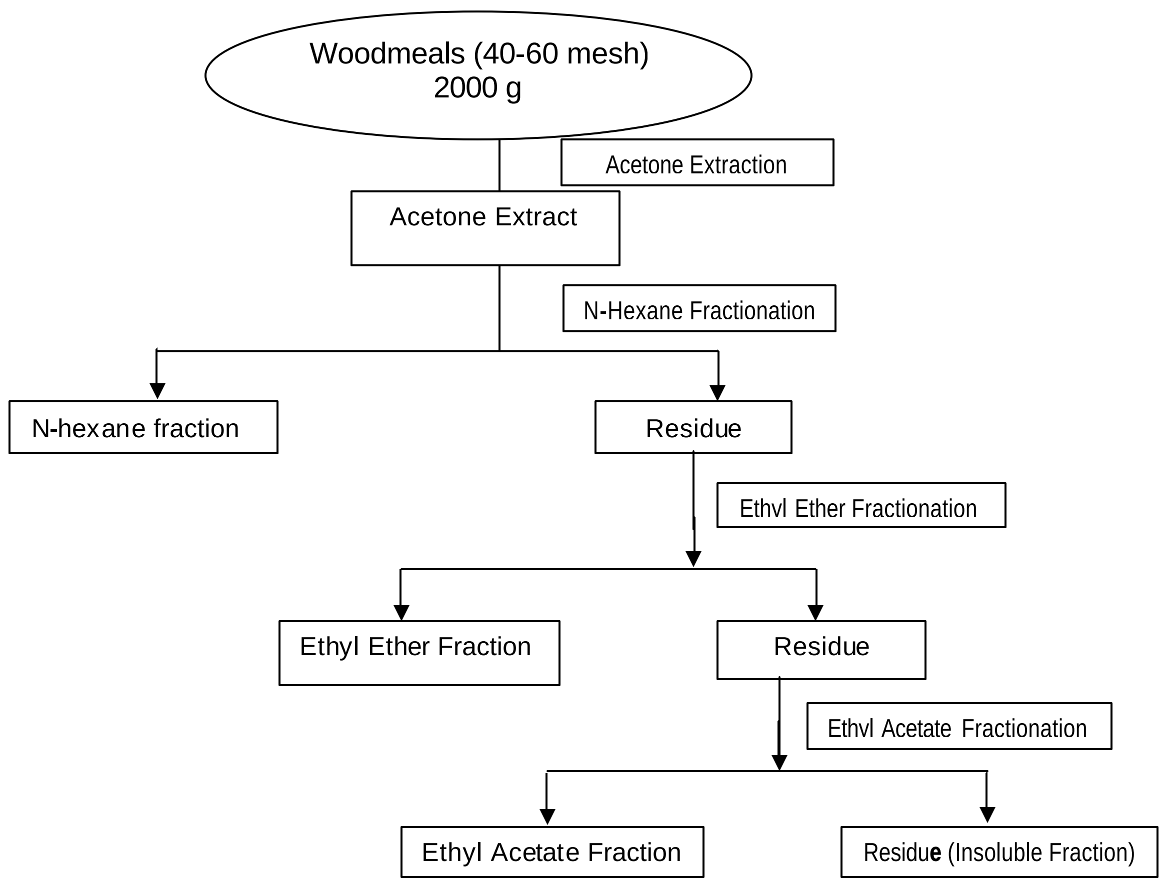

Figure 1. Schematic fractionation of acetone extract

Antitermitic Activity Test. The antitermitic activity test of acetone extract and its fractionations was carried out according to the procedure reported by Ohmura *et al*. (2000) with several modifications. Paper discs (2 cm in diameter, about 0,068-0,078 g in weight, Whatman) were treated with n-hexane soluble fraction, ethyl ether soluble fraction, ethyl acetate soluble fraction and insoluble fraction at five levels of concentration i.e. 2%, 4%, 6%, 8%, and 10% (w/w). The acetone treated paper discs and untreated paper discs were also included in this experiment as a control. After air drying process, the untreated and treated paper discs with different solutions were put on petri dish. This procedure is illustrated in Figure 2. Fourty five of workers and five of soldiers of subterranean termite *C. curvignathus* Holmgren were added to each petri dish and three duplicates were undertaken for each solution. The petri dish were then placed in the dark room for four weeks. Mortality of termite calculated each three days periods. After four weeks, the paper discs were pulled out, dried at 40°C for 24 hours then put in a dessicator for 24 hours. The mortality of termites and weight loss of paper discs were used to determine antitermitic activity of extract.

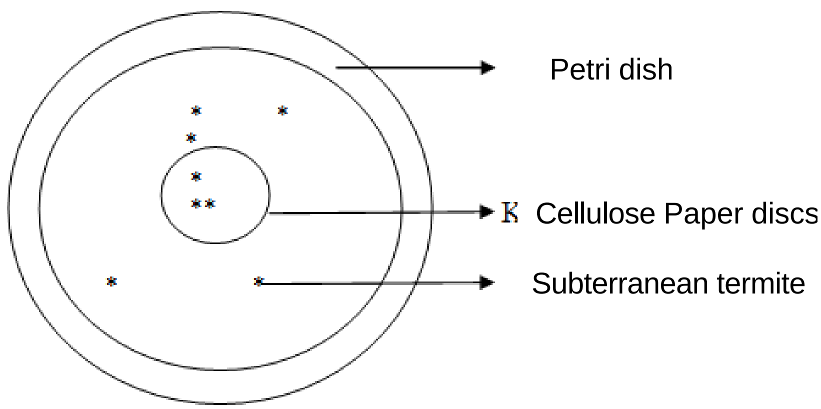

Figure 2. Schematic of antitermite activity test of extractive bitti wood

RESULTS AND DISCUSSION

Acetone Extract Content of Bitti Wood. Extraction with acetone solvent to 2000 g of bitti heartwood mills resulted in 43.3 g or 2.49% acetone extract based on oven dry weight of wood. Acetone extract is then fractionated by non mixed solvent (solvent- solvent extraction) successively with n-hexane, ethyl ether, and ethyl acetate. The results of fractionation is presented in Table 3.

Table 3 Content of Fractionation Extractive of Acetone Extract of Bitti Wood

Soluble Fraction	Weight of Extract (g) *)	Content of Extractive (%)*)
N-hexane	25,56	1,470
Ethyl ether	0,924	0,053
Ethyl acetate	0,472	0,027
Residue (Insoluble Fraction)	16,344	0,940
Acetone Extract	43,3	2,49

Remark : *) based on oven dry weight

The table shows that the residue fraction have a still large value (0.940%) which indicates that there are many kinds of compounds that can not be dissolved in the used solvent. However, the amount of n-hexane fraction has the highest value (1.470%) compared to others. This result indicate that the compounds contained in bitti wood acetone extracts tend to be semi-polar.

Fractionation with n-hexane solvent resulted in fraction which was more difficult to be dried (at temperature 40-60^{o}C) than any other fractions. This was probably caused by fixed oil contained in n-hexane fraction as suggested by Houghton and Raman (1998) that n-hexane can dissolve the fixed and volatile oils.

Bitti wood contained lower acetone extract content compared to sonokeling wood (*Dalbergia latifolia* Roxb.) torem wood (*Manilkara kanoensis* Lam.) and damar laut wood (*Hopea* spp.) with acetone extract contents of 8.23%, 6.05%, 4.53%, respectively, but it higher than ebony wood (*Diospyros polisanthera* Blanco.) with that of only 1.15% (Syafii 2001). Tsoumis (1991) suggested that the content of extractive vary widely from less than 1% to more than 10% (oven dried weight) depending on the species of wood, especially between the sapwood and heartwood. In terms of fractionation, ethyl ether was the dominant fraction in the acetone extracts of sonokeling wood (6.87%) and sonokembang wood (3.77%). Residue was the dominant fraction of johar wood acetone extract (3.01%). N-hexane as the dominant fraction of acetone extract of bitti wood (1.47%) and ebony wood (0.50%) (Syafii 2001). These results indicate that the dominant fraction in the acetone extract of wood species varies depends on the kind of extractives contained in the wood species.

Extractive content variation obtained is influenced by the kinds of compounds contained in samples and the solubility of these compounds in the used solvents. Achmadi (1990) mentioned that the compounds which can be dissolved in ethyl ether are belonging to the group of fatty acids compounds (fats, wax, resin, resin acids, and sterols). On the other hand, Houghton & Raman (1998) suggested that n-hexane contained compounds such as fat, wax, fixed oils and volatile oils. Compounds that can be dissolved in ethyl acetate is a group of alkaloids, aglycones, and the glycoside.

Mortality of Subterranean Termite *C. curvignathus* Holmgren. Results on termite mortality as one of indicators to determine antitermitic activity of extractive is presented in Fig. 3. As shown in Fig. 3, termite mortality ranging from relatively strong (72,54%) to strong (94,4%). The high value of termite mortality were probably caused by the presence termiticide effects of phenolic compounds dissolved in the ethyl acetate and ethyl ether solvent. Semipolar solvents are an effective solvent for a hydrophylic group of chemical compounds such as phenolic and terpenoids. This result proved that termite mortality was infuenced by the kinds and concentration of fraction (n-hexane, ethyl ether, ethyl acetate, and residue).

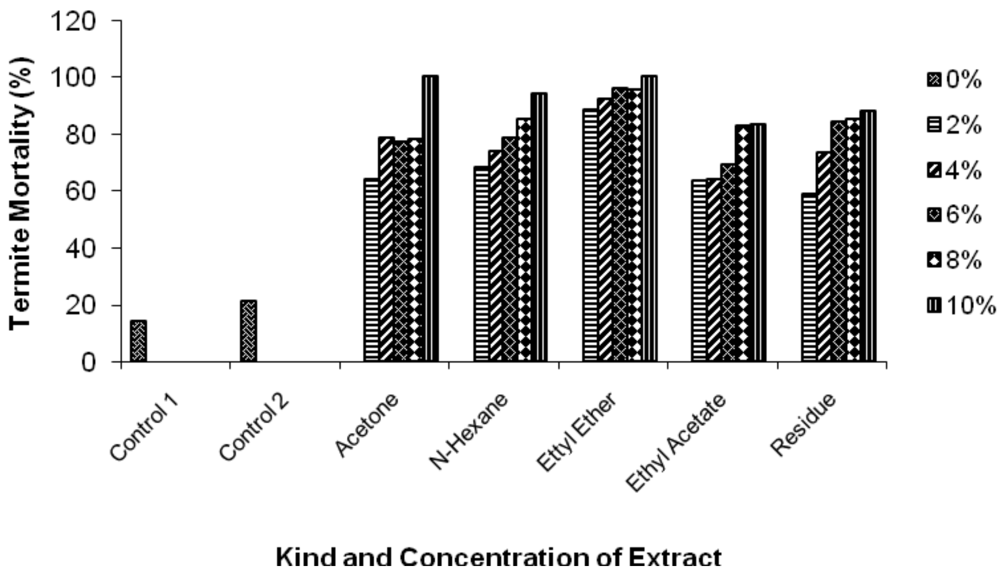

Figure 3. Termite mortality caused by bitti wood acetone extracts and its fractions

N-hexane fraction of lara wood also showed the high toxicity against termites because containing p-hydroxybenzoic acid, vanillic acid, and siringal acid compounds (Syafii 2001). On the other hand, (Syafii 2000[b]) states that chemical compounds which can be identified from n-hexane fraction of sawo kecik wood (*Manilkara kauki* Dubard.) and tanjung wood (*Mimusops elengi* Linn.) by gas chromatography-mass spectroscopy (GC-MS) methods are oleic acid, tetradekana, tridekana, heptadekana 2 methyl, and isoborneol, whereas the main component in n-hexane fraction of damar laut wood i.e., 1-heptadekana, 3-oktadekana, 2-naptalenol, dibutil ptalat, hexadecanoic acid, tarasasterol, and 9-eikosena.

Most active fractions of wood species varies depending on the kind of their extractive. Syafii's research (2001) show that the most active fraction of sonokeling wood acetone extracts is n-hexane fraction followed by ethyl ether fraction, while the most active fraction of torem wood and lara wood are the residues fraction. In the bark of teak

(*Tectona grandis* L.f.), n-hexane fraction showed highest antitermitic activities (Sari & Syafii 2001).

In terms of mortality, the fractions is much greater than control. This is different from the result of Syafii's research (2001), show that the ethyl acetate fraction of ebony and sonokeling wood acetone extract less active against termite *C. curvignathus* Holmgren. The thin layer chromatography (TLC) and gas chromatography mass spectroscophy (GC-MS) methods was indentified that the major compounds contained in the ethyl acetate fraction of damar laut wood are octadecanoic acids, octadecanoic-9-acid, 1,2-benzaldicarboxylate diiso ethyl ester, and eicosadienoic methyl ester acid (Syafii 2000[b]). Fig. 3 shows that all kinds of fractions showed mortality increase with increasing concentrations of extract, but in general the ethyl ether fraction showed a highest mortality at all concentrations of extract.

Based on the antitermitic activity classification of extracts (Prijono 1998), the ethyl ether fraction relatively strong (88.66%) to extremely strong (100%) at all concentrations, while the others tend to be relatively strong to strong. This occurs probably caused by differences in toxic properties of the extracts at different concentrations.

Weight Loss of Cellulose Paper Discs. As one of indicators to determine antitermitic activity of extractive, results on weight loss is presented in Fig. 4. The higher percentage of the paper discs weight loss indicates the lower antitermitic activities of extracts. Weight loss varies greatly depending on the kinds and concentration of fractions.

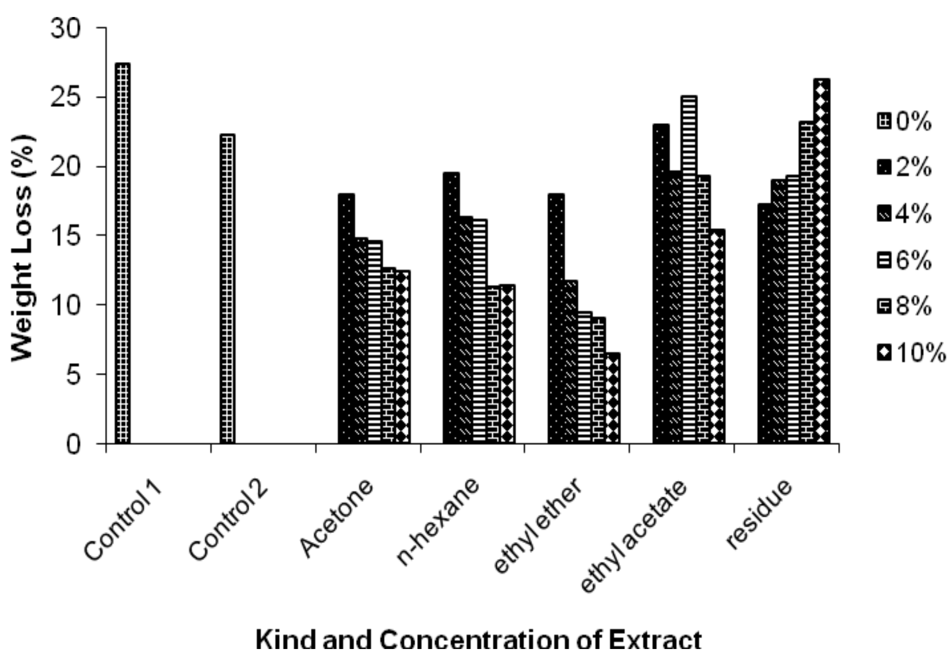

Figure 4. Weight loss caused by bitti wood acetone extracts and its fractions

This is probably caused by these compounds contained in extracts actually become a food additive for termite that makes them better survive, as suggested by Kartal *et al*. (2004) that the filtrate of sugi and *Acacia mangium* wood is not resistant to termite attack because the filtrate contain vanillin the possibility of a food additive that attracts termites.

The ethyl ether fraction showed lower value of weight loss (10,91%) compared to n-hexane fraction (14,90%), ethyl acetate (20,43%) and residue fraction (20,43%). As has been stated that the value of weight loss is inversely to the value of mortality, in the sense that the higher mortality of termites hence smaller the weight loss.

Based on weight loss, the most active fractions in the bitti wood acetone extract different from most active fractions in the acetone extracts from different wood species. Fractions of bitti wood acetone extract which has the lowest value of weight loss is ethyl ether followed by n-hexane, whereas the lowest weight loss of lara wood, for example, the residues fraction (Syafii 2001). However, to determine the most active fraction in the bitti wood extract acetone not only from the value of weight loss, but also the value of termite mortality. The value of termite mortality at all concentrations of ethyl ether fraction showed relatively strong extremely strong, it can be concluded that the most active fraction in bitti wood acetone extracts is ethyl ether fraction.

As shown in Fig. 3 and Fig. 4, it can be concluded that all fractions containing bioactive compounds that are toxic against termites. Bioactive compounds that will interfere metabolic food activity in the body of the termite. As already stated Syafii (2000b), termites are one type of insects that can produce cellulase enzymes released by protozoa contained in the stomachs of termites. Cellulase enzymes can break down glucose anhydride units connected by ß types of chemical bonds into simple sugars. Extractive entering into the digestive system of termites will kill protozoa and disrupt the enzymatic process of cellulose into simple sugars so can caused the termites starve and die. Sastrodihardjo (1999) in Sari (2002) suggested that the influence of extractive to termites mortality and other insects are as inhibitors of protein synthesis (extractives from tannins, stilbena, quinon, alcaloids, and resin group). Suparjana (2000) also stressed that the mechanism of toxic effects on the survival of termite probably caused by damage the structural components of cell membranes and disturbing termites transport nutrients necessary for the survival of termites, also caused by inhibiting cell metabolic processes of termites.

Antifeedant Activity. Antifeedant activity as one of indicators to determine antitermitic activity of extractive is presented in Fig. 5.

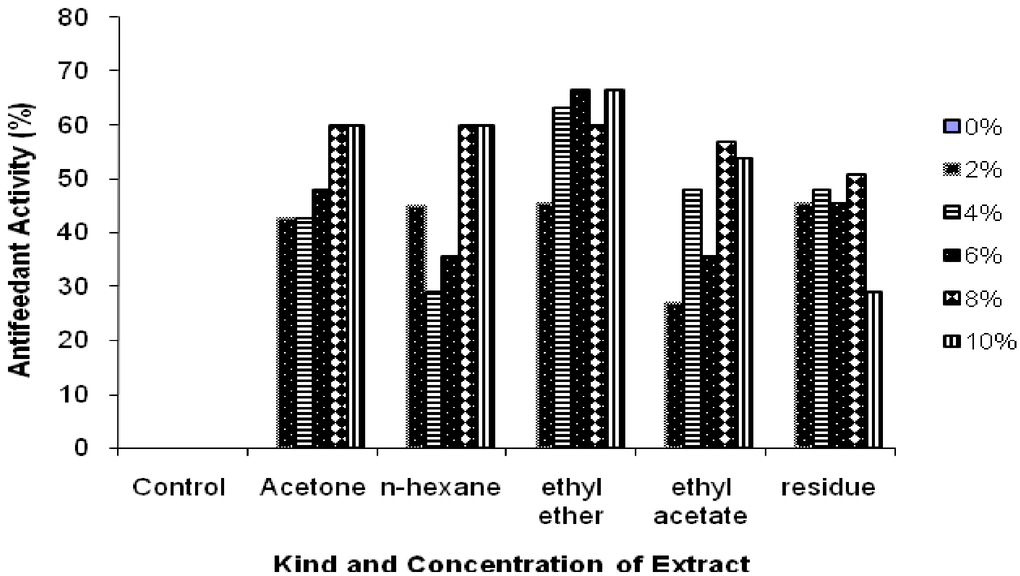

Figure 5. Antifeedant activity caused by bitti wood acetone extracts and its fractions

As shown in Fig. 5, bitti wood acetone extract and it fractions have different value of antifeedant activity. The ethyl ether fraction showed the highest value of antifeedant activity (60,41%), followed by n-hexane fraction (45,93%), ethyl acetate (44.28%), and residual fraction (43.81 %). Based on the classification of antifeedant activity(Ohmura *et.al*., 2000), the ethyl ether fraction has a relatively antifeedant activity strong (class III: A = 50 <75), and the others has a middle value of antifeedant activity (class II: 25 = A <50).

Conclusions and Suggestion

Conclusions

1. Bitti wood containing acetone extract as 43.3 g (2.49%) and resulted n-hexane fraction 25.56 g (1.4707%), ethyl ether 0.924 g (0.053%), ethyl acetate, 0.472 g (0.027%) and residual 16.344 g (0.940%)
2. The most active fraction of bitti wood acetone extract is ethyl ether followed by n-hexane, ethyl acetate, acetone extract, and residue fraction.

Suggestion

Based on the amount of residual have a still large value (0.940%) which indicates that there are many kinds of compounds that can not be dissolved in the used solvent, so that needs to be done fractionation used different solvents.

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