A STUDY ON THE EXPLORATION AND IDENTIFICATION OF BIO-ACTIVE COMPONENTS FROM SOME TROPICAL HARDWOODS AND THE POSSIBILITY OF THEIR USE AS NATURAL PRESERVATIVES

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数種熱帯産樹種の生物活性化物質の検索・同定とその天然防腐剤としての利用可能性に関する研究

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要旨

新しいタイプの防腐剤の開発は重要課題の一つである。新しいタイプの防腐剤の一つは、シロアリと腐朽菌に対して毒性を有する熱帯樹種からの抽出物である。ある種の樹種は、シロアリと腐朽菌に耐性があることはよく知られている。これまでの研究によれば、それら天然耐久性のある木材からの抽出物には、対シロアリ性、耐腐朽性の物質を保有する。そのように、熱帯産樹種のバイオ活性化物質の検索・同定およびその防腐剤としての利用については研究済みである。

本研究の目的は、特定樹種の抽出物の分別及び抽出物（可溶性）そのままのシロアリ（Coptotermes curviganathus）及び腐朽菌（Schizophyllum Commune）に対する毒性調査を行うことにある。

本研究では、4つのインドネシア産樹種がシロアリと腐朽菌に対して強い耐性を示した。その樹種は、インディアンローズウッド（sonokeling wood. Dalbergia latifolia Roxb.）、セランガンバトウ（damar laut wlld, Hopea spp.）、カリン（sonokembang wood, Pterocarpus indicus)、コクタン（ebony wood, Diospyros spp.）である。

材料の心材部分を粉砕機で40－60メッシュに挽き割り、含水量およそ15%まで風乾した。風乾した試料約2000グラムを10リットルのアセトンで室温下48時間抽出した。更に、完全な抽出液を得るため、同じ

操作を4回繰り返した。このアセトン抽出液を30－40℃で真空乾燥機にかけ100mlに濃縮し、nヘキサン、エチルエーテル、エチルアセテートに蒸留分別した。それぞれの分別液をシロアリ防除及び防腐試験に供した。

試験の結果では、抽出物の含有量はインディアンローズウッド、カリン、セランガンバントウは絶乾重量比率で8.06%、6.40%、4.53%と高く、コクタンは1.15%と低かった。

防除効果では、インディアンローズウッド及びカリンのヘキサン分別液は2%溶液でシロアリ100%致死率を示した。インディアンローズウッドのヘキサン分別液、セランガンバトウのアセトン抽出液はシロアリによる食害を低下させる効果があった。

また、カリンのエチルエーテル分別液は腐朽菌の繁殖を抑える効果があった。

これらのことから、インディアンローズウッドのヘキサノン分別液、セランガンバトウのアセトン抽出液は防シロアリ物質が含まれていること、一方、カリンのエチルエーテル分別液には防腐性のある化学複合物が含まれていることが推測された。

今後は、これら防除効果のある化学複合物の種類を決定するための分別物の解析が必要である。

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Summary

The development of the new type of preservatives will be one of the most social demanded subject. One of the new type of preservatives which can be explorated from the tropical forest is wood extractives which has toxicity to the termite and fungal attack. It is well known that certain woods are naturally resistant to the termite and fungal attack. Based on the previous investigations, it is indicated that extract from naturally durable wood are containing substances which have antitermic and antifungal activities. Therefore a study on the exploration and identification of bio-active components from tropical hardwood and their use as a natural preservatives has been carried-out. The objectives of this research are to fractionate the extractives from some selected tropical hardwoods and to test the toxicity of crude extracts (soluble fractions) to the termite of *Coptotermes curviganathus* and to the fungal of *Schizophyllum commune*.

Four species of Indonesian tropical hardwoods which have been shown to posses strong resistance to the termite and fungal attack have been used in this study. These species are sonokeling wood (*Dalbergia latifolia* Roxb.), damar laut wood (*Hopea* spp.), sonokembang wood (*Pterocarpus indicus*), and ebony wood (*Diospyros spp.*). The heartwood of samples were converted into woodmeals in a Willey mill with 40 - 60 mesh screen, and air-dried to about 15 % of moisture content. About 2,000 gram of air-dried woodmeals were then extracted with 10 liters of acetone at room temperature for about 48 hours. In order to get a complete acetone extraction, the same procedure has been consecutively carried-out four times. The acetone extracts solution obtained from acetone extraction was concentrated to 100 ml by a vacuum rotary evaporator at 3- - 40

C^0, and then successively fractionated into n-hexane, ethyl ether, and ethyl acetate soluble fractions. Each fraction was then subjected to the antitermic and antifungal bioassay tests.

The results of this study showed that amongs four wood species used in this study, sonokeling, sonokembang, and damar laut woods showed a high contain of extractives, those are 8.06 %, 6.40%, and 4.53% (oven-dry wood) respectively, while ebony wood (1.15%) showed a low contain of extractives.

The addition of *n*-hexane soluble fraction from sonokeling and sonokembang woods caused the mortality of termite up to 100% at 2% of concentration level. The addition of n-hexane soluble fraction from sonokeling wood and acetone extract from damar laut wood decreased the weight loss of paper discs against to the termite of *Coptotermes curvignathus*. The ethyl ether fraction from sonokembang wood showed an inhibitory effect to the growth of *Schyzophyllum commune* when this fraction was added to the bioassay medium.

From this experiment, it can be suggested that n-hexane soluble fraction from sonokeling wood and acetone extract from damar laut wood may contain substances having antitermic activities to the *Coptotermes curvignathus*, while the ethyl ether fraction from sonokembang may contain chemical compounds having antifungal fungal activities to the *Schyzophyllum commune*.

Further investigation on those fractions is needed in order to determine what kind of chemical compounds are responsible for those activities.

I. INTRODUCTION

A. Background.

Indonesia is tropical country and has approximately 4.000 species of woods. These species of woods are classified into 5 classes according to their natural durability. About 15-20 % of these species are classified into first and second classes (durable wood classes), and the remaining species are classified as a non-durable species. In addition, the future of wood supply will be coming from plantion forest which dominated by fast-growing species. These woods are mainly undurable woods, therefore it has to be treated with chemical preservatives before using in the field. On the other hand, the using of conventional preservatives would be facing a big problem from the environmental dimension. According to the above condition, the development of the new type of preservatives will be one of the most social demanded subject. One of the new type of preservatives which can be explorated from the tropical forest is wood extractives which has toxicity to the termite and fungal attack.

It is well known that certain woods are naturally resistant to termite and fungal attack. The resistance of wood is primarily caused by the chemical substances in the wood that are toxic to the decaying micro-organisms. Investigators showed that extract from durable heartwood are much toxic than those from sapwood of the same tree, the resistance of durable wood is greatly reduced by extraction with organic solvent (Carter *et.al*, 1975; Nelson 1975; Rust and Reierson, 1977; Steller and Labosky, 1984; Syafii *et. al*, 1985, 1987, 1988, 1991, 1993; Syafii, 1996). Based on the above information, it is indicated that extract from naturally durable wood are containing substances which have antitermic and antifungal activities. Therefore a study on the exploration and identification of bio-active components from tropical hardwood and their use as a natural preservatives would be of great value.

B. Objectives.

The objectives of this research are :

1. Extraction and fractionation of extractives from some selected tropical hardwoods.
2. Toxicity test of crude extracts (soluble fractions) to the termite of *Coptotermes curviganathus* and to the fungal of *Schizophyllum commune*.

II. MATERIAL AND METHOD

A. Material.

Several species of Indonesian tropical hardwoods which have been shown to posses strong resistance to the termite and fungal attack have been used in this study. These species are sonokeling wood (*Dalbergia latifolia* Roxb.), damar laut wood (*Hopea* spp.), sonokembang wood (*Pterocarpus indicus*), and ebony wood (*Diospyros spp.*). The sonokeling wood, sonokembang wood, and ebony wood were obtained from Bogor (West Jawa), while the wood species of damar laut was obtained from Medan (North Sumatera).

B. Methods.

1. Preparation of woodmeals and acetone extacts.

The heartwood of samples were converted into woodmeals in a Willey mill with 40 - 60 mesh screen, and air-dried to about 15 % of moisture content. About 2,000 gram of air-dried woodmeals were then extracted with 10 liters of acetone at room temperature for about 48 hours. In order to get a complete acetone extraction, the same procedure has been consecutively carried-out four times.

2. Fractionation of acetone extracts.

The acetone extracts solution obtained from acetone extraction was concentrated to 100 ml by a vacuum rotary evaporator at 30 - 40 C^0, and then successively fractionated into n-hexane, ethyl ether, and ethyl acetate soluble fractions. The general scheme of successive is set out diagrammatically in Figure 1.

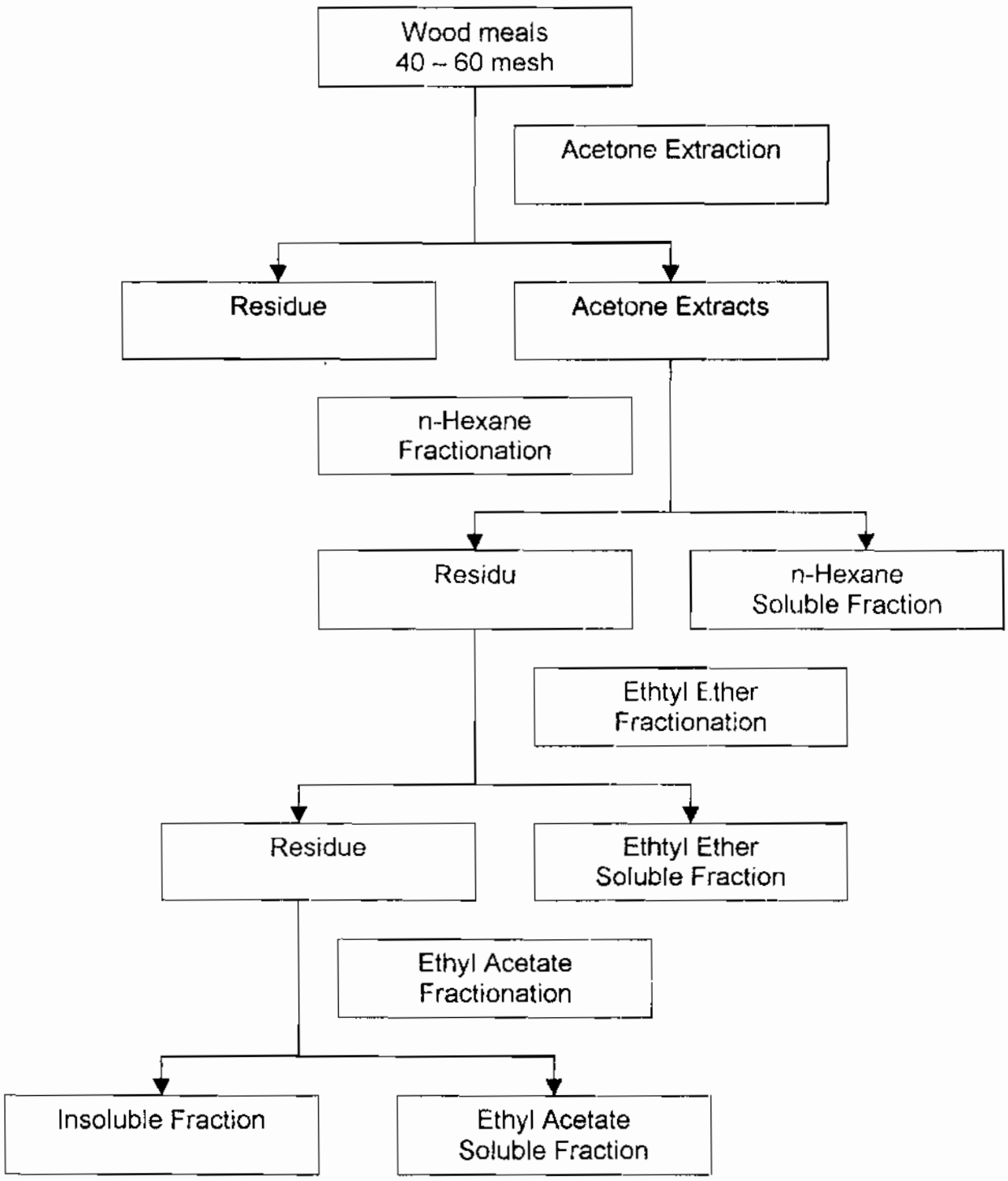

Figure 1 : Schematic fractionation of acetone extracts

3. Preparation of fungal and media.

The fungal of *Schizophyllum commune* was grown on basal media with the following composition : 50 gram/liter glucose, 5 gram/liter polypepton, 0.3 gram/liter K_2HPO_4, 0.3 gram KH_2PO_4, 0,2 gram/liter $MgSO_47H_2O$, 30 gram/liter agar, and 120 gram onion extracts.

4. Antitermic bioassay test.

The antitermic bioassay test carried-out according to the procedure reported by Labosky (1984) and Ohmura *et.al* (1997) with several modifications. Paper discs (about 500 mg in weight, Toyo Seisakusho) were treated with *n*-hexane soluble fraction, ethyl ether soluble fraction, and ethyl acetate fraction at five levels of concentration 2 %, 4 %, 6 %, 8 %, and 10 % (W/W). The untreated paper discs were used in this experiment as a control. After air-dried, the untreated and treated paper discs were dried in a vacuum desicator 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 termite of *Coptotermes curvignathus* 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 4 weeks. After four weeks, the paper discs were pulled out, dried at 40^0 C for 6 hours and dried in a vacuum desicator for 24 hours. The mortality of termite and the weight loss of paper disc after 4 weeks was used to quantify termite attack.

5. Antifungal bio-assay test.

To test the antifungal activity of extractives, the bioassay of fungal growth of Loman (1970) have been used with several modifications. Bioassay was set up on a 2 % malt extract (2 % MEA) medium composed of 600 mg/liter K_2HPO_4, 600 mg/liter KH_2PO_4, 400 mg/liter

$MgSO_4.7H_2O$, 10 gram/liter agar, and 10 gram/liter malt extract. About four ml of bioassay medium were added into atest tube, followed by addition of 1 ml of each soluble fraction at the concentration of 2%, 4%, 6%, 8%, and 10%. A control consisted of all nutrient biassay medium (at 0% of concentration level) was prepared by adding 1 ml of absolute ethanol solution into the test tube. All chemical substances were incorporated by autoclaving at the temperature of 120^0 C and 1,05 kg/cm^2 pressure for about 15 minutes. Lastly, the inoculum plugs obatained from a 5-day-old cultures of the fungi of *Schizophyllum commune* were inoculated into the flat-lying test tube containing a medium and incubated at 26.5^0 C for 4 weeks. The growth of fungus was calculated by measuring length of the grown mycelia at the end of the incubation periode. This procedure is illustrated in Figure 3.

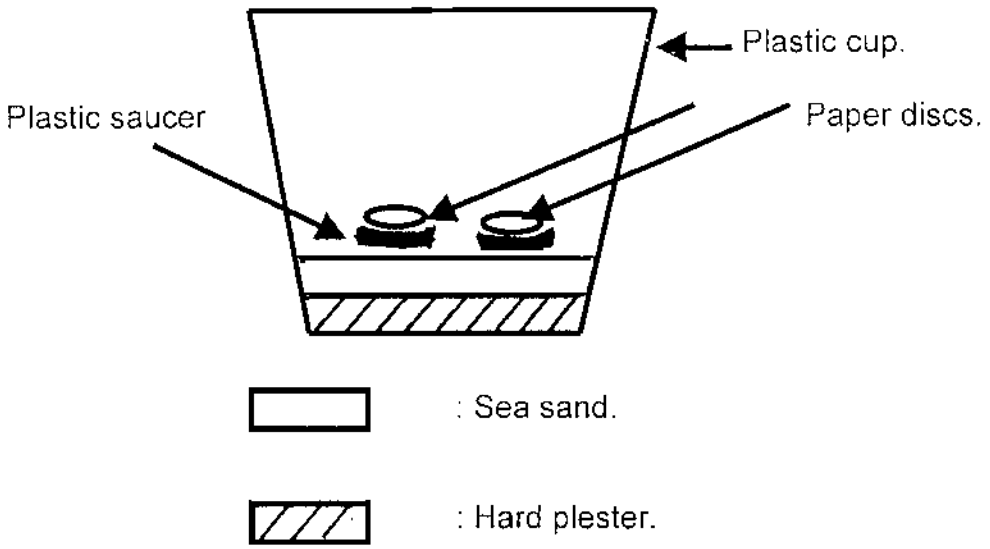

Figure 2 : Schematic of antitermic bioassay test.

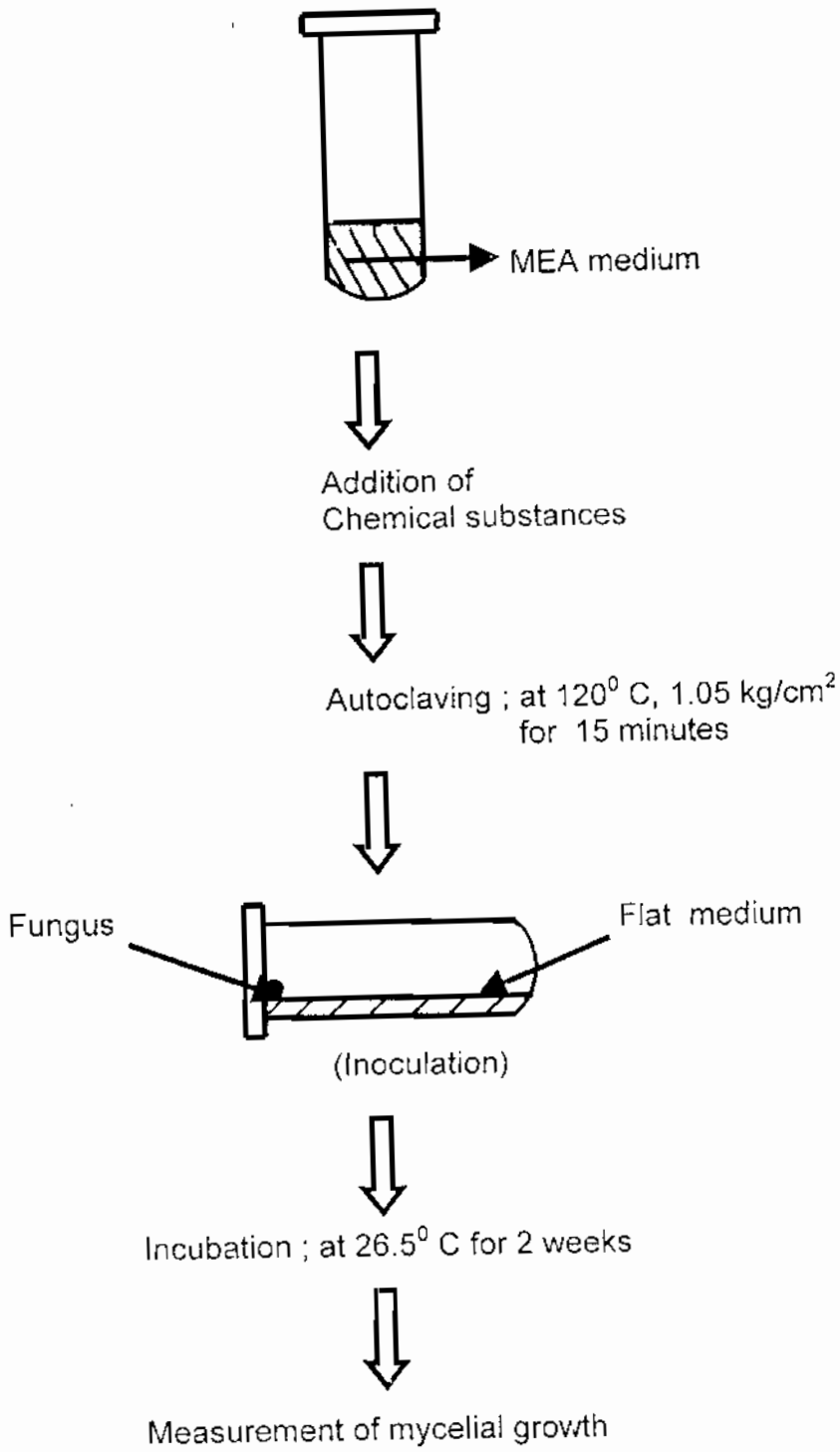

Figure 3 : Schematic of antifungal bioassay test.

III. RESULT AND DISCUSSION

A. Extractives contents.

To assess the normality of extractive content in the wood sample, acetone extractives yield was determined (Table 1). The total amount of acetone extractives obtained from the sample of sonokeling wood, damar laut, wood, sonokembang wood, and ebony wood are 8.06 %, 4.53 %, 6.40 %, and 1.15 % of oven-dry wood respectively.

Tabel 1 :The acetone extracts yield and their fractionation of woodsamples (% of oven-dry wood).

No.	Wood Sample	n-Hexane Fraction	Ethyl Ether Fraction	Ethyl Acetate Fraction	Insoluble Fraction	Total Acetone Extract
1.	Sonokeling	0,32	6.43	0,58	0,73	8,06
3.	Damar Laut	0,27	0,94	2,32	0,99	4,53
4.	Sonokembang	0,46	3,77	0,96	1,21	6,40
6.	Eboni	0,50	0,21	0,26	0,18	1,15

The amount of acetone extract of sonokeling wood is relatively higher than the average of extractive content in the tropical hardwood. On the other hand, the amount of acetone extract of ebony wood is less than those of normal tropical wood. Each acetone extracts were then fractionated into the *n*-hexane soluble fraction, ether soluble fraction, ethyl acetate soluble fraction, and insoluble fraction (residue). Table 1 indicates that each acetone extract shows different characteristic in the results of fractionation. Most of acetone extract from sonokeling wood and sonokembang wood are fractionated into ethyl ether fraction, while acetone extract of damar laut wood and ebony wood are mostly fractionated into ethyl acetate and n-hexane soluble fractions respectively. Each fraction obtained from this successive fractionation was then used in the antitermic and anti fungal bioassay tests.

B. Antitermic properties.

1. Termite mortality.

Termite mortality is one of indicators used to evaluate the antitermic activity of chemical substances. The mortality of *Coptotermes curvignathus* termite exposed to paper discs after treated with extracts are listed in Table 2.

Table 2 : Mortality of *Coptotermes curvignathus* termite after exposed to the treated paper disc for 4 weeks (%).

Wood sample / Soluble fraction	Concentration (%, W/W)					
	0	2	4	6	8	10
Sonokeling						
n-Hexane fraction	18	100	100	100	100	100
Ethyl Ether fraction	18	92	100	100	100	100
Ethyl Acetate fraction	18	68	77	85	90	94
Damar Laut						
Acetone extract	15	90	100	100	100	100
Sonokembang						
n-Hexane fraction	9	100	100	100	100	100
Ethyl Ether fraction	9	85	87	89	93	100
Ethyl Acetate fraction	9	71	77	93	95	99
Eboni						
n-Hexane fraction	14	80	93	100	100	100
Ethyl Ether fraction	17	93	95	98	87	87
Ethyl Acetate fraction	17	49	52	56	59	59

Table 2 shows that amongs the fractions tested, the n-hexane soluble fraction of sonokeling wood, acetone extract of damar laut wood, and n-hexane soluble fraction of sonokembang wood are very toxic to the termite of *Coptotermes curvignathus*. At the concentration of 2 %, the n-hexane soluble fraction of sonokeling wood and sonokembang wood caused the termite mortality up to 100 %. The results also indicated that the acetone extract of damar laut wood showed antitermic properties, although the toxicity of this extract is less than those of n-

hexane soluble fractions of sonokeling and sonokembang woods. At the concentration of 4 % of extract of damar laut wood, the mortality of termite is 100 %. From this experiment, it can be suggested that the n-hexane soluble fractions from sonokeling and sonokembang woods as well as the acetone extract of damar lat wood contains substances having antitermic activities.

2. Weight loss of paper discs.

An experiment of antitermic activity of extracts (*n*-hexane soluble fraction, ether soluble fraction, and residue or insoluble fraction) from wood samples have been carried out. In this experiment, the influence of each soluble fraction on the weight loss of paper discs was examined. The weight loss of paper discs after exposed to the termite of *Coptotermes curvignathus* for about 4 weeks is listed in Table 3.

Table 3 : The weight loss of treated paper discs after exposed to the termite of *Coptotermes curvignathus* for about 4 weeks (% of oven-dry paper discs).

Wood sample / Soluble fraction	Concentration (%, W/W)					
	0	2	4	6	8	10
Sonokeling						
n-Hexane fraction	19	4	4	2	2	1
Ethyl Ether fraction	19	18	16	16	11	6
Ethyl Acetate fraction	19	19	16	15	10	5
Damar Laut						
Aceton extract	18	3	3	3	1	1
Sonokembang						
n-Hexane fraction	23	11	10	10	7	7
Ethyl Ether fraction	23	13	11	8	7	7
Ethyl Acetate fraction	23	21	20	16	10	9
Eboni						
n-Hexane fraction	22	14	11	7	7	7
Ethyl Ether fraction	22	5	5	7	13	14
Ethyl Acetate fraction	22	10	16	13	12	11

As indicated by Table 3, the addition of n-hexane soluble fraction of sonokeling wood and acetone extract of damar laut wood showed a significant decrease of paper disc weight loss against termite of *Coptotermes curvignathus* at the concentration level between 2.0 %. The weight loss of untreated paper discs (concentration level of 0.0 %) was 18 – 23 %. When the concentration of *n*-hexane soluble fraction of sonokeling wood and acetone extract of damar laut wood were increased to 2.0 % (W/W), the weight loss of paper discs decreased to 4 % and 3 %, respectively. From this experiment, it can also be suggested that the *n*-hexane soluble fraction of sonokeling wood and acetone extract of damar laut wood contains substances having antitermic activities. The toxicity of these fractions warranted further investigation to isolate and identify the responsible compounds.

C. Antifungal properties.

Table 4 : Relative growth of fungi (*Schizophyllum commune)* in 2 % MEA (malt extract agar) with addition of soluble fraction after incubation for 4 weeks (% of growth of control).

Wood sample / Soluble fraction	Konsentrasi (%, W/W)					
	0	2	4	6	8	10
Sonokembang						
n-Hexane fraction	100	67	54	47	46	42
Ethyl Ether fraction	100	0	0	0	0	0
Ethyl Acetate fraction	100	100	100	100	100	100
Eboni						
n-Hexane fraction	100	91	79	37	25	4
Ethyl Ether fraction	100	91	94	74	62	19
Ethyl Acetate fraction	100	100	100	95	85	76

As shown in Table 4, among the fractions tested, ethyl ether fraction from sonokembang wood showed the highest toxic activity to the mycelial growth of *Schizophyllum commune.* Compare to the control, the mycelial growth of *Schizophyllum commune* decreased to 0 % when the

concentration of ethyl ether fraction from sonokembang wood was added into bioassay medium. In addition, the n-hexane fraction from ebony wood also showed the toxic activity to the fungi tested but at the higher concentration. The mycelial growth of fungi tested decreased to 37 % when the concentration of n-hexane fraction from ebony wood was increased to 6 %. In general, no significant increase in inhibition was found when the concentration of other fractions were added into bioassay medium.

From the present investigation, it can be suspected that the ethyl ether fraction from sonokembang and the n-hexane fraction from ebony woods may contain chemical compounds with antifungal activity. Further investigation is needed in order to determine what kind of chemical compounds is responsible for the inhibition of this fungi.

It was shown by previous investigators that lignans are produced in wood in response to fungal attack and that they play some role in preventing the subsequent degradation of wood. Spruce wood (*Picea abies)* produces a high content of extractives with fungistatic activity (Alcubilla et.al., 1974 *in* Donald and Towers, 1984). This species is known to contain a high level of matairesinol, conidendrin, hydroxymatairesinol, and liovil, but only matairesinol and hydroxymatairesinol, however, were inhibitory to the growth of *Fomes annosus* (Shain, 1971). Some of the fungistatic activity of extractives is attributable to their inhibition of the extracellular fungal enzymes, cellulose, polygalacturonase, aryl-β-glucosidase, and laccase (Johansson, *et. al.,* 1976).

The durability of wood or its natural resistance to decay is variable. The factors responsible for such variation are, however, numerous. Syafii *et. al.* (1993) reported that no inhibition in growth of *Coriolus versicolor* and *Tyromeces polutris* was apparent as the concentration of catechin and

gallic acid in medium was increased from 5 ppm to 100 ppm. By using other fungus, Alfenas *et. al.* (1982) showed that gallic acid enhanched the mycelial growth of *Cryphonectria cubensis* at concentration between 10^{-3} M to 10^{-2} M. On the contrary, gallic acid seems to be main inhibitor to growth of *Collybia velutipes* (Minami, 1975).

In this investigation, the n-hexane fraction from sonokembang showed high toxicity to the mortality of *Coptotermes curvignathus*, but it relatively has no inhibitory effect to the growth of *Schyzophyllum commune*. On the contrary, the ethyl ether fraction of sonokembang showed a high toxicity to the growth of *Schyzophyllum commune*, but this fraction showed no inhibition to, both the paper pads weight loss as well as the mortality of *Coptotermes curvignathus*. From this experiment, it seems that the toxicity of extractives to be linked to the kind of the test microorganisms.

IV. CONCLUSION

1. Amongs four wood species used in this study, sonokeling, sonokembang, and damar laut woods showed a high contain of extractives, those are 8.06 %, 6.40%, and 4.53% (oven-dry wood) respectively, while ebony wood (1.15%) showed a low contain of extractives.

2. The addition of *n*-hexane soluble fraction from sonokeling and sonokembang woods caused the mortality of termite up to 100% at 2% of concentration level.

3. The addition of n-hexane soluble fraction from sonokeling wood and acetone extract from damar laut wood decreased the weight loss of paper discs against to the termite of *Coptotermes curvignathus*.

4. The ethyl ether fraction from sonokembang wood showed an inhibitory effect to the growth of *Schyzophyllum commune* when this fraction was added to the bioassay medium.

5. From this experiment, it can be suggested that n-hexane soluble fraction from sonokeling wood and acetone extract from damar laut wood may contain substances having antitermic activities to the *Coptotermes curvignathus*, while the ethyl ether fraction from sonokembang may contain chemical compounds having antifungal fungal activities to the *Schyzophyllum commune*.

6. Further investigation on those fractions is needed in order to determine what kind of chemical compounds are responsible for those activities.

Acknowledgements

- The author wishes to express his cordial gratitude to the Tanabe Southeast Asia Nations Friendship Foundation for granting the financial assistance in conducting this research.

- My thanks goes also to Mr. Tata Brata Suparjana and Mr. Eko Kuswanto, graduate students of Bogor Agricultural University, for their excellent work in conducting this research.

- I also take this opportunity to thanks to Inter University Center for Life Sciences Studies, Bogor Agrcultural Uiversity, for permitting us to use the facilites of the Center in carrying-out the antitermic bioassay test.

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