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The Role of Extractives in Decay Resistance of Ulin Wood (*Eusideroxylon zwageri* T. et B.)

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I. Introduction

Ulin wood (*Eusideroxylon zwageri* T. et B.) is a species of the family Lauraceae. It has long been known as a durable wood, based on its extremely long service life under conditions favorable to decay. The wood has a variety of applications as material for marine construction, bridges, ships, railroad ties and electrical poles.

Decay resistance of wood is its ability to resist the attacks of foreign organisms such as fungi, insects and marine borers. There are many factors which affect the resistance of wood attacked by foreign organisms. There is evidence that the natural durability of wood depends on the concentration of toxic substances from wood. These toxic extractible substances are the principle cause of decay resistance in wood. It has been demonstrated that extracts from durable heartwood are much more toxic than those from sapwood of the same tree, and decay resistance of heartwood is greatly reduced by extraction with hot water and/or organic solvent[1]. Hot water extractives from African mahagoni[2], methanol extractives from tallow wood[3], ether and methanol extractives of teak wood[4] are known to contain substances with antifungal activity.

The purpose of this study is to investigate the relationships between durability of Ulin wood and its extractives caused by wood-rotting fungi.

II. Materials and Methods

A. Preparation of woodmeal and acetone extract

Ulin wood (*Eusideroxylon zwageri* T. et B.) used on this experiment was obtained from Central-Kalimantan, Indonesia. Buna wood (*Fagus crenata)* was also used in this study as a comparative material. The heartwood of the samples were converted to woodmeal in a Willey mill to pass a 40-60 mesh screen and air-dried to about 15% moisture content. A 2100 gram of Ulin air-dried woodmeal was extracted with 10 liters of acetone for 48 hours in a bottle at room temperature. Unextracted and acetone extracted woodmeal were then used for wood decay test.

B. Fungi and medium

The two wood-rotting fungi, kawaratake (*Coriolus versicolor*) and ouzuratake (*Tyromeces polutris*), were used in this experiment for decay test.

The basal medium per liter contained 50 gram glucose, 5 gram polypepton, 0.3 gram K_2HPO_4, 0.3 gram KH_2PO_4, 0.2 gram $MgSO_4 \cdot 7H_2O$, 30 gram agar and 120 gram onion extract, is used to grow the above fungi.

C. Wood decay test

To test the resistance of Ulin wood, the woodmeal dish jar technique of DA COSTA and

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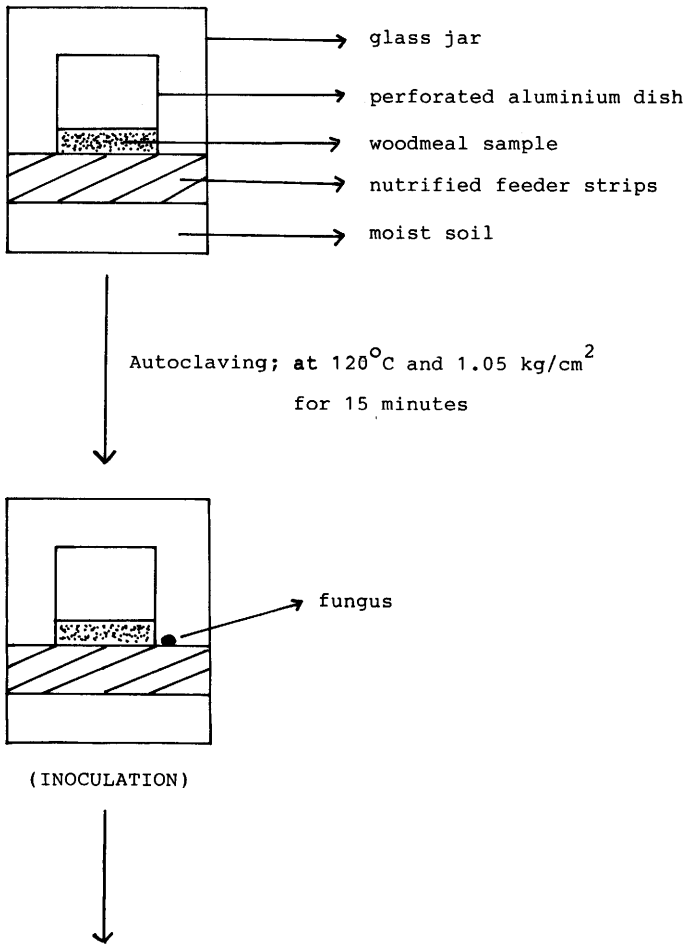

Fig. 1. Chart of wood decay test.

RUDMAN[3] was used with several modifications. Unextracted Ulin woodmeals and acetone extracted woodmeals of Ulin were used in this experiment. Unextracted woodmeals of Buna were also used as control. As shown in Fig. 1, the fungi were grown on nutrified feeder strips resting on moist soil in glass jars. The glass jars contained approximately 100 gram of a clay loam soil with a moisture content of about 40–50 percent. The feeder strips used in this experiment were shaving of Buna sapwood, which had been soaked in a nutrient solution. The composition of nutrient solution is as follows: 200 mg/*l* L-Asparagin, 500 mg/*l* $(NH_4)_2HPO_4$, 800 mg/*l* KH_2PO_4, 500 mg/*l* K_2HPO_4, 300 mg/*l* $MgSo_4 \cdot 7H_2O$, 5 mg/*l* $MnSo_4 \cdot 6H_2O$, 4 mg/*l* $ZnSO_4 \cdot 7H_2O$, 5 mg/*l* $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$, 55 mg/*l* $CaCl_2$, 200 mg/*l* yeast extract, and 3 mg/*l* Thyamin hydrochloride. The pH of this nutrient solution was adjusted to 6.0. The dishes containing woodmeal samples were 4 cm in diameter and 3 cm deep of 0.05 cm thick aluminium foil, the bottom being perforated by numerous holes

(0.025–0.25 mm in diameter) to allow the fungus to grow up into the woodmeal samples. Approximately 2 gram of air-dried woodmeal (of known moisture content) was weighed out into each dish. The glass jars containing moist soil, nutrified feeder strips and an aluminium dish with woodmeal samples were then auto-claved for 15 minutes, at 120℃, 1.05 kg/cm^2. Inoculum plugs obtained from a 5-day-old culture of fungi were inoculated to the nutrified feeder strips and incubated at 26.5℃ for two or four months. At the end of the incubation period, the aluminium dishes were taken out from glass jars, oven-dried at 105℃ for 24 hours, weighed. Finally, weight loss of the samples were calculated from the difference of the weight of the samples before and after incubation.

D. Test of antifungal activity of acetone extract

The method of this test was similar to those in wood decay test in II.C. The woodmeal sample used in this experiment was Buna woodmeal which was well known as an undurable species. The acetone extract from the heartwood of Ulin wood was added as acetone solution into the aluminium dishes containing Buna woodmeal at concentration of 2.0%, 4.0%, 6.0%, 8.0% and 10.0%. All percentages are based on oven-dry weight of Buna woodmeal. The control (at concentration of 0.0%) was prepared with aliquots of acetone solvent. Before auto-claving, the aluminium dishes containing Buna woodmeal samples and acetone extracts were air-dried for two days to evaporate the acetone solvent.

E. Effect of chemical substances on the growth of fungi

To test the effect of chemical substances on the growth of fungi, the bioassays of fungal growth by LOMAN[5] was used with several modifications. Bioassay was conducted on a 2% MEA (malt extract agar) medium. The composition of bioassay medium is as follows: 600 mg/*l* K_2HPO_4, 600 mg/*l* KH_2PO_4, 400 mg/*l* $MgSO_4 \cdot 7H_2O$, 10 gram/*l* agar, and 10 gram/*l* malt extract. Four m*l* of bioassay medium was added into the test tube, and followed by addition of 1 m*l* of aliquots of ethanol solution of Ulin acetone extract and its fraction at the concentration of 500 ppm. The concentration of eusiderin solution added into the bioassay medium were 5, 10, 25, 50, and 100 ppm. Control consisted of all nutrient bioassay medium (at 0% concentration of chemical substances) was prepared by addition of 1 m*l* of absolute ethanol solution into the test tubes. All chemical substances were incorporated by auto-claving for 15 minutes at 120℃ and 1.05 kg/cm^2. Inoculum plugs obtained from a 5-day-old culture of fungi were transferred to the test tubes containing flat medium (Fig. 2), and incubated at 26.5℃ for two weeks. The mycelial growth of the test fungi were measured at the end of the incubation period.

III. Results and Discussion

As shown in Table 1, Ulin wood (*Eusideroxylon zwageri* T. et B.) is very resistant to decay. Only 0.54% (on the unextracted woodmeal) lost in four month's incubation with kawaratake (*Coriolus versicolor*) and 1.97% with ouzuratake (*Tyromeces polutris)* respectively. Whereas the percentage of weight loss of Unextracted Buna woodmeal (*Fagus crenata)* was 64.55% and 79.91% caused by fungi of *C. versicolor* and *T. polutris* respectively. The acetone extraction reduced the decay resistance of wood of *E. zwageri.* The weight loss of acetone extracted woodmeals of *E. zwageri* increased 2.30% for fungus of *C. versicolor* and 4.70% for fungus *T. polutris.* Therefore, we expected that acetone extract of this wood may play some role on the activity of the test fungi.

To test the antifungal activity of acetone extract from Ulin wood, the influence of Ulin acetone extract on the weight loss of Buna woodmeal was examined. Buna woodmeal was

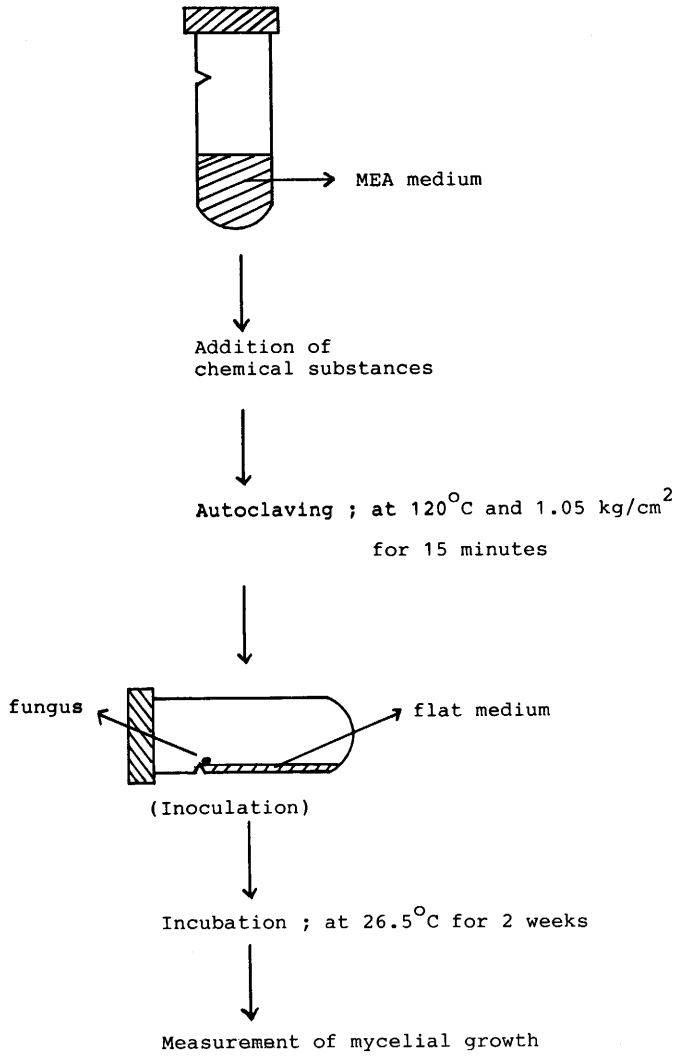

Fig. 2. Flow diagram of toxicity test of chemical substances.

treated with acetone extract from Ulin wood, and then the resistance of this treated Buna woodmeal to fungi was tested. The result is listed in Table 2. It appears that the addition of acetone extract from wood of *E. zwageri* into woodmeal of *F. crenata* showed a significant increase of decay resistance for both two fungi at concentration between 2.0% and 10.0% based on the oven-dried woodmeal of *F. crenata*. The weight loss of woodmeal of *F. crenata* decreased when the concentration of Ulin acetone extract increased. Table 2 also shows that the acetone extract from wood of *E. zwageri* is more toxic to *C. versicolor* than *T. polutris*. From the above result we expected that the acetone extract from wood of *E. zwageri* may contain substances with antifungal activity. The acetone extract of this wood was then successively fractionated into *n*-hexane, ethyl ether, and ethyl acetate soluble fractions. The amount of acetone extract was 8.1% on an oven-dried basis. The *n*-hexane,

Table 1. Percentage weight loss of woodmeal of Ulin (*Eusideroxylon zwageri*) and Buna (*Fagus crenata*) after 4 mounths incubation (% on oven-dried basis).

Species of fungi	Unextracted Ulin	Acetone extracted Ulin	Unextracted Buna
Kawaratake (*Coriolus versicolor*)	0.22	2.79	63.42
	0.86	2.35	64.59
	0.66	1.75	68.22
	0.43	2.34	61.96
Average	0.54	2.30	64.55
Ouzuratake (*Tyromeces polutris*)	1.30	8.42	75.09
	2.15	3.59	78.60
	2.00	3.02	83.14
	2.44	4.10	82.81
Average	1.97	4.78	79.91

Table 2. Percentage weight loss of Buna woodmeal (*Fagus crenata*) with addition of acetone extract from Ulin wood (*Eusideroxylon zwageri*) after 2 months incubation (% of oven-dry Buna woodmeal).

Species of fungi	Concentration of acetone extract (%)*					
	0.0	2.0	4.0	6.0	8.0	10.0
Kawaratake (*Coriolus versicolor*)	26.92	25.48	24.05	25.32	22.78	17.09
	28.89	24.34	22.78	20.73	19.00	17.19
	22.15	26.32	24.28	20.86	18.35	18.47
	28.30	26.11	26.58	24.05	20.37	15.92
Average	26.56	25.56	24.42	22.74	20.13	17.16
Ouzuratake (*Tyromeces polutris*)	35.03	33.54	32.90	25.64	25.32	22.64
	33.75	33.96	29.38	25.79	23.57	24.05
	36.77	33.76	29.38	28.48	23.13	22.50
	35.89	34.59	31.88	26.58	24.05	22.44
Average	35.36	33.96	30.88	26.62	24.02	22.91

* Based on an oven-dried basis of Buna woodmeal.

ethyl ether, and ethyl acetate soluble fractions of the acetone extracts amounted to 1.2%, 0.8%, and 3.0% on an oven-dried basis respectively. Each fraction was then subjected to the fungal bioassay. As shown in Fig. 3, with the exception of ethyl acetate soluble fraction, all of the fractions inhibited mycelial growth of both *C. versicolor* and *T. polutris*. Furthermore, the highest toxicity was found in the *n*-hexane soluble fraction to both of fungi tested.

In order to determine what chemical compound is responsible for the toxicity of *n*-hexane soluble fraction, further purification of this fraction was carried out by column chromatography. In our previous report[6], we demonstrated that *n*-hexane soluble fraction was composed of neolignans; eusiderin, eusiderin A, and eusiderin B. Eusiderin is a major compound among these and amounted to 0.80% on an oven-dried basis.

Eusiderin isolated from wood of *E. zwageri* was then subjected to the fungal bioassay.

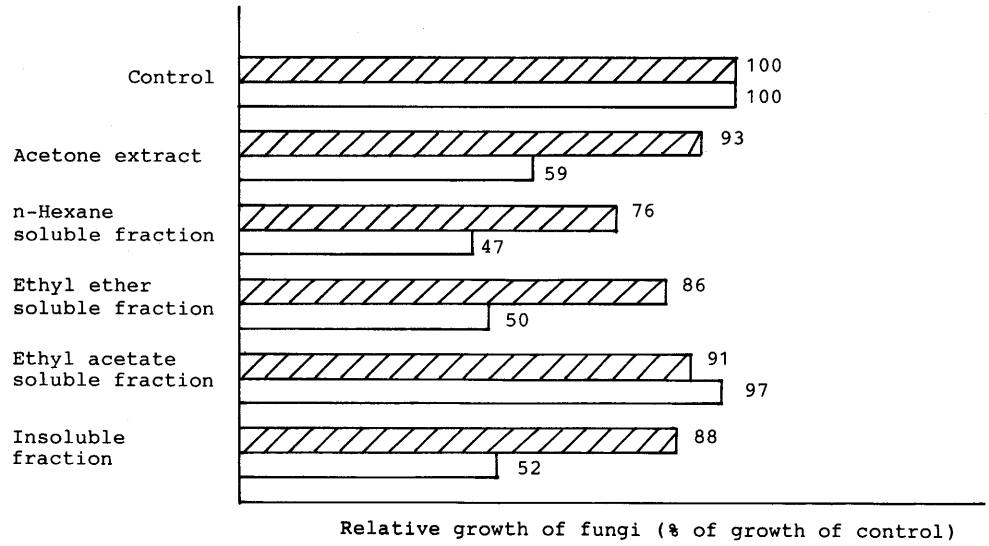

Fig. 3. Relative growth of fungi with addition of extracts from Ulin wood (*Eusideroxylon zwageri*) at concentration of 500 ppm.
////// Kawarataka (*Coriolus versicolor*), ☐ Ouzuratake (*Tyromeces polutris*).

Table 3. Relative growth of fungi with addition of eusiderin after incubation for 2 weeks (% of growth of control).

Species of fungi	Concentration of eusiderin (ppm)					
	0.0	5.0	10.0	25.0	50.0	100.0
Kawaratake (*Coriolus versicolor*)	100	100	95	72	57	48
	100	100	99	74	60	48
	100	93	96	76	54	51
	100	93	93	74	56	45
Average	100	97	96	74	57	48
Ouzuratake (*Tyromeces polutris*)	100	97	80	86	47	35
	100	94	78	84	43	32
	100	94	78	83	43	36
	100	93	75	68	44	34
Average	100	95	78	80	44	34

The result is listed in Table 3. The addition of eusiderin at concentration of 5 ppm and 10 ppm did not inhibit the mycelial growth of *C. versicolor*, but, when the concentration of eusiderin was increased at concentration between 25 ppm and 100 ppm the growth of *C. versicolor* was greatly inhibited. The addition of eusiderin also showed significant inhibition on the mycelial growth of *T. polutris* at concentration higher than 10 ppm. The degree of inhibition varied with the concentration of eusiderin. The addition of eusiderin into bioassay medium at concentration of 100 ppm produced a greatly significant inhibition of *T. polutris*. At this level of concentration the mycelial growth of *T. polutris* was only about

34% compared with the growth of the control.

Based on the results of the present investigation, the conclusion can be stated that eusiderin, a main compound of *n*-hexane soluble fraction of the acetone extract, is responsible for the natural decay of Ulin wood. As mentioned before, eusiderin is a chemical compound of neolignan which dominated acetone extractives of Ulin wood. Evidence has been presented that lignans as well as neolignans are produced in wood in response to fungal attack and that they play some role in preventing the degradation of wood. Spruce wood (*Picea abies*) produces a number of lignans with fungistatic activity[7], this species is also known to contain a high level of matairesinol, conidenrin, hydroxymatairesinol and liovil, but only hydroxymatairesinol and matairesinol however were inhibitory to the growth of the fungus of *Fomes annosus*[8]. Some of the fungistatic activity of lignans is attributable to their inhibition of the extracellular fungal enzymes, cellulase, polygalacturonase, aryl-β-glucosidase and laccase[9].

Summary

The role of extractives from heartwood of Ulin (*Eusideroxylon zwageri* T. et B.) on durability of wood was investigated. Decay resistance of Ulin heartwood is reduced by extraction with acetone. The addition of acetone extract from Ulin heartwood into woodmeal of Buna (*Fagus crenata*) showed a significant increase of decay resistance of this wood. From these observations, it can be stated that the Ulin acetone extract may contain fungal toxic substances. Acetone extract from the heartwood of Ulin was then successively fractionated into *n*-hexane, ethyl ether, and ethyl acetate soluble fractions, and each fraction then subjected to the fungal bioassay. The highest toxicity on mycelial growth of kawaratake (*Coriolus versicolor*) and ouzuratake (*Tyromeces polutris*) was found in the *n*-hexane soluble fraction. Therefore, the chemical components of *n*-hexane soluble fraction were investigated, and eusiderin, a neolignan, was found as a major compound in *n*-hexane soluble fraction. Eusiderin showed a toxic activity on mycelial growth of wood-rotting fungi. From these results, eusiderin is considered to be responsible for durability of Ulin wood.

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Ulin 材 (*Eusideroxylon zwageri* T. et B.) の耐朽性と抽出成分との関係について

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摘　要

Ulin 材の耐朽性と抽出成分との関係について調べた。Ulin 材をアセトン抽出するとカワラタケ (*Coriolus versicolor*) およびオオウズラタケ (*Tyromeces polutris*) に対する耐朽性が低下した。一方，Ulin 材アセトン抽出をブナ木粉に添加すると木材腐朽菌に対するブナ木紛の耐朽性は高くなった。このことからアセトン抽出物が Ulin 材の耐朽性に関与することを示した。そこで，アセトン抽出物中の抗菌活性成について検索した。アセトン抽出物を *n*-ヘキサン，エチルエーテル，酢酸エチルで逐次抽出し，各画分を MEA 培地に添加し，木材腐朽菌の菌糸生長抑制効果について調べた。その結果，*n*-ヘキサン可溶部を添加したときに，腐朽菌の生長が最も阻害された。*n*-ヘキサン可溶部をさらにカラムクロマトグラフィーで分画し，Eusiderin を単離した。Eusiderin は *n*-ヘキサン可溶部の主要成分であった。Ulin 材抽出物による腐朽菌生育抑制活性の一因は Eusiderin によるものと推定された。