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Antitermite and antifungal activities of *Vitex cofassus* heartwood

Syahidah[*1,*2], Takeshi Katayama[*2], Toshisada Suzuki[*2], Yasuhiko Asada[*2],
Yoshito Ohtani[*3], Wakako Ohmura[*4]

Antitermite activity of *Vitex cofassus* heartwood against a subterranean termite, *Coptotermes formosanus* and its antifungal activity against a white-rot fungus (*Trametes versicolor*) and brown-rot fungus (*Fomitopsis palustris)* were investigated. The heartwood mill was extracted with acetone and methanol to give their extracts, each of which were fractionated successively using *n*-hexane, ethyl acetate, and water to afford their soluble fractions. The yield of the extracts and fractions suggested that the extractives of *V. cofassus* heartwood tend to be polar. It was demonstrated that the methanol extract itself, the ethyl acetate fraction and the *n*-hexane fraction from the methanol extract, and the ethyl acetate fraction from the acetone extract had high antifeedant activity against *C. formosanus*. All the extracts and fractions were shown to have strong-moderate antifungal activity against *T. versicolor*, where the aqueous fraction of the acetone extract had the highest activity, while almost these extracts and fractions were found to have moderate-weak antifungal activity against *F. palustris*.

Key words: *Coptotermes formosanus*, antifeedant activity, wood extractives, white-rot fungus, brown-rot fungus

Vitex cofassus 心材の抗蟻活性と抗真菌活性

Syahidah[*1,*2]，片山健至[*2]，鈴木利貞[*2]，麻田恭彦[*2]，
大谷慶人[*3]，大村和香子[*4]

Vitex cofassus 心材の抗蟻活性を地下シロアリの一種であるイエシロアリ *Coptotermes formosanus* を用いて検討した。また，その心材の抗真菌活性も白色腐朽菌 *Trametes versicolor* と褐色腐朽菌 *Fomitopsis palustris* を用いて検討した。心材粉末は，アセトン及びメタノールを用いて抽出した。それぞれの抽出物は，順次 *n*-ヘキサン，酢酸エチル及び水を用いてそれぞれの可溶部に分画した。得られた抽出物と画分の収率から，*V. cofassus* 心材抽出物には，極性成分が多い傾向にあることが示された。メタノール抽出物，このメタノール抽出物からの *n*-ヘキサン画分と酢酸エチル画分，並びにアセトン抽出物からの酢酸エチル画分が，*C. formosanus* に対して強い摂食阻害作用を有していた。*T. versicolor* に対する抗真菌活性は，すべての抽出物と画分が強～中の程度を示し，特にアセトン抽出物の水溶性画分の活性が最も高かった。一方，*F. palustris* に対する抗真菌活性は，これらの抽出物及び画分のほとんどが，中～弱の程度であった。

キーワード： *Coptotermes formosanus*，摂食阻害活性，木材抽出成分，白色腐朽菌，褐色腐朽菌

[*1]Faculty of Forestry, Hasanuddin University, Makassar 90245, Indonesia
[*2]Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan
[*3]Faculty of Agriculture, Kochi University, Nankoku City, Kochi 783-8502, Japan
[*4]Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan

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1. INTRODUCTION

Wood is widely used for a variety of purpose, but the wood species that have low natural durability are susceptible to degrade by many organisms, such as termites and fungi. The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is currently one of the most destructive pests in the USA[1)]. This termite species can cause damage more rapidly due to the larger amount of colony members. On the other hand, wood deterioration fungi were classified into decaying fungi (brown-rot, white-rot, and soft-rot fungi), staining fungi, and molds. The wood-decaying fungi lead to great economic losses of lignocellulosic materials. From the aspect of the efficiency of forest resource utilization, these attacking organisms are very harmful because they can shorten the service life of wood and wood products. Therefore, the 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 largely belong to the non-organic synthetic chemicals, causing environmental impacts due to their non-biodegradable properties.

One way to reduce these negative impacts is to find out naturally available preservatives. Plant derivatives have been used for generations in certain parts of the world to enhance appearance of wooden products and to extend the service life of them. Several types of wood extractives have indeed been proved to contain bioactive compounds that could inhibit the growth of organisms[2,3,4)]. Gofasa (*Vitex cofassus* Reinw.) is one of the wood species that is well known as *bitti* wood, especially for people in South Sulawesi Province, Indonesia, and it is usually used as a raw material for housing and traditional boats.

In this study, therefore, the heartwood mill of *V. cofassus* were extracted and fractionated with different solvents, and the antifeedant activity against *C. formosanus* and the antifungal activity against *Trametes versicolor* and *Fomitopsis palustris* of the extracts and the fractions were examined.

2. MATERIALS AND METHODS

2.1 Wood species

Vitex cofassus Reinw. wood was collected from Bulukumba Community Forest, South Sulawesi Province, Indonesia, ca. 70 years of age.

2.2 Extraction and fractionation

Heartwood mill (40-60 mesh) of *V. cofassus* was prepared and extracted according to the procedure reported previously[5)] with slight modification. In this study, the milled wood that was used instead of small wood-pieces was firstly extracted with acetone and then the residue was extracted again with methanol until the extract solution became colorless. These acetone and methanol extracts were then successively fractionated into *n*-hexane, ethyl acetate, and water to give their soluble fractions.

2.3 Termite test

Coptotermes formosanus Shiraki used was collected in black pine forest on the coast of Kochi Prefecture, Japan or bred in Forestry and Forest Products Research Institute, Tsukuba, Japan.

2.4 Fungal strains

The fungal strains used were a white-rot fungus (*Trametes versicolor*, NBRC 4937) and a brown-rot fungus (*Fomitopsis palustris*, NBRC 30339) that were purchased from Biological Resource Center, National Institute of Technology and Evaluation, Tokyo, Japan.

2.5 Termite bioassay

No-choice and two-choice tests were employed to assess the termiticidal activity of *V. cofassus* heartwood[6)]. The weight losses of the paper discs were used to determine the termiticidal properties of the extracts that were obtained by the following equations: in the no-choice bioassay, the absolute coefficient of antifeedancy (A) = $[(KK - EE)/(KK + EE)] \times 100$ (%); while in the two-choice bioassay, the relative coefficient of antifeedancy (R) = $[(K - E)/(K + E)] \times$

100 (%); where KK (K) and EE (E) are the weight losses of the control and treated paper discs, respectively. The total coefficient of antifeedancy (T) is equal to A plus R. All the extracts tested were classified into the following classes according to their T values; feeding preference ($T < 0$), class I ($0 \leq T < 50$), class II ($50 \leq T < 100$), class III ($100 \leq T < 150$), class IV ($150 \leq T < 200$), and 200 for complete antifeedant.

2.6 Fungal bioassay

Fungal bioassay was conducted using the potato dextrose agar (PDA) medium containing 50 and 100 ppm of samples (the extracts and the fractions) in a Petri dish[7]. Triplicate media for each amount of the samples were prepared. The PDA powder in distilled water (39g/L) was autoclaved, and to this warm medium (40-50°C) was added a solution of each of the samples in methanol, and the whole was shaken. Three parts from the resulting mixture were transferred equally into three Petri dishes, respectively, and the mycelium disk was placed at the center of the medium. PDA plates containing methanol without the samples were used as a control. The media were incubated at 23°C. When the mycelium of fungi on the control medium reached the edge of the Petri dish, the antifungal activity (AFA) index (%) was calculated as follows: AFA index (%) = (1－Da/Db) x 100, where Da: diameter of mycelium colony growth with the samples (cm), Db: diameter of mycelium colony growth in the control (cm). Based on the AFA value, the activity of each fraction was classified into the following category levels[8]. AFA ≥ 75% (very strong), 75% ≤ AFA < 50% (strong), 50% ≤ AFA < 25% (moderate), 25% ≤ AFA < 0 (weak), and 0 (not active).

2.7 Statistical analyses

The SPSS software (IBM SPSS Statistics Version 21) was utilized as a statistical tool. As a result of multiple analysis of variation (ANOVA) test, the type of all the samples (the extracts and fractions) were evaluated their antitermite and antifungal activities. For post hoc analysis, Scheffe's test was used to compare the values at a level of significance of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Extract yield

Extraction of *Vitex cofassus* heartwood and subsequent fractionation of the extracts showed that the methanol extract (10.4%) had higher percentage than the acetone extract (2.7%) did, and the successive fractionation of the acetone extract gave the following percentages of the fractions: *n*-hexane (13%), ethyl acetate (19%), and aqueous (65%). Moreover, that of the methanol extract afforded the following percentages of the fractions: *n*-hexane (1%), ethyl acetate (7.5%), and aqueous (71%). These data indicated that the *V. cofassus* heartwood extractives tend to be polar.

The amount and composition of wood extractives depend on several factors such as wood species, age, and growth location[9,10]. Wood contains a large variety of aromatic extractives from simple phenols to complex polyphenols and their related compounds. Those are often colored compounds which are accumulated abundantly in the heartwood of many species. This kind of extractives also has antifungal activity and thus protects the tree against microbiological attack.

3.2 Antitermite activity

3.2.1 Weight loss

Weight losses of the paper discs are displayed on Figures 1 and 2, representing the ability of the extracts and their fractions to protect against *C. formosanus*. The no-choice bioassay was used for testing termiticidal activity, while the two-choice bioassay for testing antifeedant activity[6].

Figure 1 (no-choice bioassay) shows that the weight losses for all the extracts and their fractions were lower than that of the control, indicating that *C. formosanus* disliked to consume the treated discs, although there were significant differences of the protection ability against the termites in the extracts and their fractions. These results confirmed that the extracts and fractions had termiticidal activity, which indicated that they were able to provide reliable protection against the termites. The aqueous fraction of the methanol extract gave the lowest weight loss (1.01%) of paper discs compared to the other fractions.

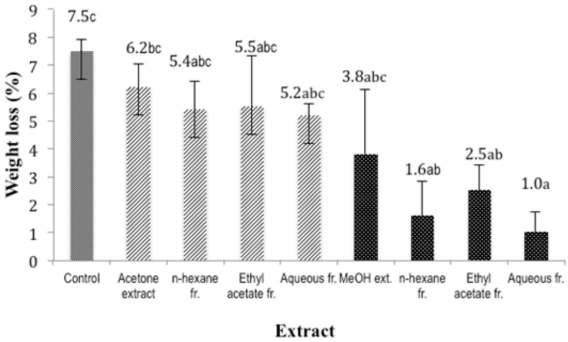

Figure 1. Weight loss of paper discs in the no-choice bioassay.
Notes: The numbers in the blanket represent the percentage of the paper discs. Different letters at the top of the bars indicate the significant difference of the weight loss at the level of $P < 0.05$ according to the Scheffe's test.
a: The lowest weight loss and significantly different from b and c.
b: The weight loss and significantly different from a and c, but not significantly different from ab and abc.
c: The highest weight loss and significantly different from a and b.
ab: The weight loss not significantly different from a or b.
abc: The weight loss not significantly different from ab or c.

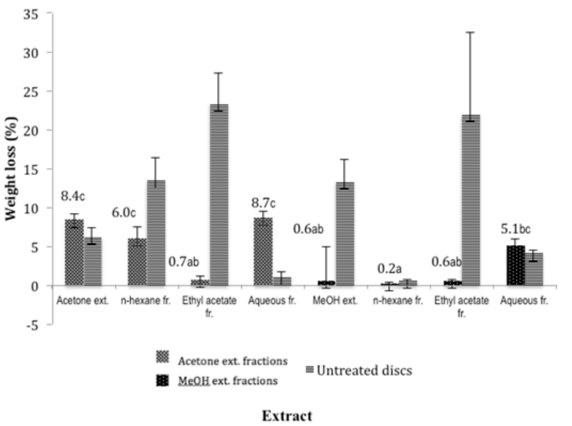

Figure 2. Weight loss of paper discs in the two-choice bioassay.
Notes: The numbers in the blanket represent percentage of the weight loss of the treated discs only. Different letters at the top of the bars indicate the significant difference of the weight loss at the level of $P < 0.05$ according to the Scheffe's test.
a: The lowest weight loss and significantly different from b and c, but not significantly different from ab.
ab: Not significantly different from a or b.
bc: Not significantly different from ab or c.
c: The highest weight loss and significantly different from the a and ab, but not significantly different from bc.

Furthermore, Figure 2 (two-choice bioassay) shows that weight losses of most of the treated discs were lower than that of the control. This indicated that the extracts and their fractions had antifeedant activity.

3.2.2 Antifeedant activity

Table 1 shows antifeedant activity of *V. cofassus* heartwood extracts and their fractions. The methanol extract had higher activity than the acetone extract. The ethyl acetate fraction of the methanol extract had the highest activity, followed by the *n*-hexane fraction. The activity of the acetone extract might be enriched in the ethyl acetate fraction. These active extracts and fractions were classified into class III of antifeedancy. On the contrary, the aqueous fraction of the acetone extract revealed feeding preference activity. Feeding preference activity means that the Formosan termites preferred to consume the filter paper containing those extracts. The residue fraction could contain substances, such as sugar, vanillin and something else that are digestible and preferable by termites[11,12].

Table 1. Antifeedant activity of *V. cofassus* heartwood extracts and their fractions.

Extracts/ fractions	T value	Antifeedant classes
Acetone ext.	4.9 ± 6^{d}	I
n-Hexane fr.	59.9 ± 8^{c}	II
EtoAc fr.	110.8 ± 7^{bc}	III
Aqueous fr.	-60.2 ± 4^{e}	Feeding Pref.
MeOH ext.	125.3 ± 4^{ab}	III
n-Hexane fr.	115.9 ± 4^{b}	III
EtoAc fr.	145.2 ± 5^{a}	III
Aqueous fr.	66.9 ± 9^{c}	II

Notes: The letters after the numbers indicates the significant differences of the activity at the level of $P < 0.05$ according to the Scheffe's test.
a: The highest antifeedant activity and significantly different from b, c, and d, but not significantly different from ab.
b: The antifeedant activity significantly different from a, c, and d, but not significantly different from ab or bc.
c: The antifeedant activity significantly different from a, b, and d, but not significantly different from bc.
d: The antifeedant activity significantly different from a, b, and c.
e: The lowest antifeedant activity and significantly different from a, b, c, and d.
ab: Not significantly different from a or b.
bc: Not significantly different from b or c.

We have also studying antifeedant activity of gofasa fruit against another subterranean termite, *Coptotermes curvignathus* and reported that the activity of the acetone extract was classified into class II of antifeedant classification[13]. On the other hand, we had studied antifeedant activity of *V. cofassus* heartwood against *C. curvignathus* and reported that the ethyl ether fraction of the acetone extract had higher antifeedant activity which was classified into the class III of antifeedant classification[14].

Extractives present in these active extracts and fractions could be toxic or unfavorable for the termites, because the ethyl acetate extract of teak heartwood was responsible for the greater resistance of the teak heartwood[15]. Isolation of antitermite compounds have not been reported from any genus of *Vitex* yet. Moreover, some flavonoids, such as quercetin and taxifolin might be useful for termite control agents, because they are abundant in plants[6]. The resistance to termite attack is due to the presence of some active components of wood as part of their natural defense, such as flavonoids that possess both toxicity and antioxidant properties as natural repellents [16].

Our recent work is purification of the active fractions to isolate and identify the antitermite compounds. Separation of the active fractions showed that the ethyl acetate fraction of the acetone extract had two active sub-fractions, while that of the methanol extract had three active sub-fractions.

3.3 Antifungal activity

Table 2 shows that against *T. versicolor* the aqueous fraction of the acetone extract of *V. cofassus* heartwood possessed strong antifungal activity, and all the other fractions belong to moderate activity.

On the other hand, against *F. palustris* the ethyl acetate fraction of the methanol extract revealed the highest but moderate activity, and all the other fractions belong to moderate or weak activity.

The strong activity means that the extract or the fraction strongly inhibits the fungal growth. Although the antifungal compounds have not reported from *V. cofassus* until now, from the ethanolic extract of leaves of *Vitex negundo*, vitegnoside and negundoside which had significant antifungal activity against *Trichophyton mentagrophytes* and *Cryptococcus neoformans* were isolated[17]. On the other hand, many antifungal compounds came from the other plants have reported so far. A recent example was liriodenine, an alkaloid, that was isolated and identified from the *n*-hexane fraction of *Michelia formosana* and could effectively inhibit the growth of wood-rotting fungi[18].

On the other hand, the weak activity means that the extracts or the fractions weakly inhibit the fungal growth. Such fractions might contain the substances that actually support the life of the fungi or that are digestible by and preferable to the fungi[19].

Table 2. Antifungal Index of the extracts of *V. cofassus* heartwood and their fractions against wood-rotting fungi.

Extracts/ fractions	Antifungal Index (%)	
	Trametes versicolor	*Fomitopsis palustris*
Acetone ext.	40.6 ± 7^{ab}	22.7 ± 5^{ab}
n-Hexane fr.	26.3 ± 4^{b}	5.6 ± 5^{c}
Ethyl acetate fr.	30.3 ± 7^{b}	11.1 ± 5^{bc}
Aqueous fr.	54.4 ± 3^{a}	27.2 ± 4^{ab}
Methanol extract	46.6 ± 5^{ab}	11.1 ± 5^{bc}
n-Hexane fr.	31.9 ± 6^{b}	17.4 ± 4^{abc}
Ethyl acetate fr.	36.1 ± 8^{ab}	30.3 ± 4^{a}
Aqueous fr.	32.8 ± 8^{ab}	11.1 ± 5^{bc}

Notes: The letters after the numbers indicates the significant differences of the activity at the level of $P < 0.05$ according to the Scheffe's test.
a: The highest activity and significantly different from b and c, but not significantly different from ab or abc.
b: Moderate activity and significantly different from a and c, but not significantly different from ab, bc, or abc.
c: The lowest activity, but not significantly different from bc or abc.
ab: Not significantly different from a or b.
bc: Not significantly different from b or c.
abc: Not significantly different from ab, bc, or c.

Another active compounds reported as antifungal activity was cinnamaldehyde, a major constituent of cinnamon essential oil, which occurs naturally in the bark and leaves of cinnamon trees of the genus Cinnamomum. It has been proven to have strong antifungal activities against a wide variety of wood decay fungi[7,20], and is a potential candidate for effective and environmentally-benign wood preservatives. Eugenol has been demonstrated as an excellent fungicide against wood decay fungi[7]. High antifungal activity of mimosa and quebracho extracts are believed to be related their high tannin contents[21]. T-muurolol and α-cadinol possess antifungal activities against a broad spectrum of tested plant pathogenic fungi and could be used as potential antifungal agents[22].

4. CONCLUSIONS

The yield of the extracts and fractions suggested that the extractives of *V. cofassus* heartwood tend to be polar. The methanol extract itself, the ethyl acetate fraction and the *n*-hexane fraction from the methanol extract, and the ethyl acetate fraction from the acetone extract had high antifeedant activity against *C. formosanus*. Furthermore, all the extracts and fractions had strong-moderate antifungal activity against *T. versicolor*, where the aqueous fraction of the acetone extract had the highest activity, while almost these extracts and fractions had moderate-weak activity against *F. palustris*.

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