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NOTE

Antifungal and antitermitic activities of wood vinegar from *Vitex pubescens* Vahl

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Abstract Antifungal and antitermitic activities of wood vinegar produced from *Vitex pubescens* were evaluated. Three kinds of wood vinegar were produced at three different pyrolysis temperatures, i.e. at 350, 400 and 450 °C. A PDA dilution method was employed to assay antifungal activity of the vinegars with a white-rot fungus *Trametes versicolor* and a brown-rot fungus *Fomitopsis palustris*. Termiticidal activity and repellent effect were evaluated by a no-choice test and a choice test with *Reticulitermes speratus* and *Coptotermes formosanus.* All wood vinegars exhibited antifungal activity against both fungi. Wood vinegar of 450 °C had the higher activity than those of 400 and 350 °C. It was assumed that acid component contributed to the increase in controlling the growth of fungal. The wood vinegar exhibited antitermite activity to both *R. speratus* and *C. formosanus* workers in the no-choice experiment. However, it needed relatively high concentration to obtain the perfect mortality. For instance, the wood vinegar of 10 % concentration was needed to achieve 100 % mortality against *C. formosanus*, whereas for *R. speratus* only 3 % of wood vinegar was required. In the direct-choice experiment, wood vinegar had a significantly repellent effect to both termites at the lowest treating concentration of 10 %.

Keywords Wood vinegar · *Vitex pubescens* · Antifungal activity · Antitermite activity

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Introduction

Biodegradation of wood caused by decay fungi and termites are recognized as one of the most serious problems [1, 2]. Chemical control has been a successful method of preventing fungal and termite attack for a long time [2, 3]. The controlling of fungi and termites using synthetic pesticides are considered to have negative effect on human health and the environment due to their residual effects [3, 4].

To avoid environmental pollution and residual problems caused by the synthetic pesticides, there is an increasing interest to naturally producing toxicants from wood hydrolysates, such as wood vinegar. Wood vinegar, which is also called as pyroligneous acid or liquid smoke, is a product by processing of high temperature carbonization of wood with absent of oxygen [4]. As a natural product, it is prospective to be developed as a new environmentally friendly wood preservative because of its fungi toxic and insecticidal properties [5, 6].

Wood vinegars obtained from many different sources of wood are recognized as safe natural inhibitors with various applications, in which they have various bioactivities such as antifungal, termiticidal, and insect-repelling activities [6–11]. For example, the wood vinegars obtained from *Pinus densiflora* Sieb.et. Zucc and *Quercus serrata* Thunb. ex Murray have inhibitory effects against sapstaining fungi [10]. Mazela [9] stated that the extracted tar-product to be an effective wood preservative against brown-rot and white-rot fungi. Yatagai et al. [8] have reported that wood vinegar exhibited high termiticidal activities against *Reticulitermes speratus* (Kolbe). Recently, Kiarie-Makara et al. [11] have shown that the wood vinegar obtained from Konara oak tree (*Q. serrata*) is very effective in repelling *Culex pipiens pallens* Coquillet.

In a previous study, we reported that the effect of the mixed wood vinegar from *Acacia mangium* WILLD and "LABAN" wood (*Vitex pubescens* Vahl) against *Aspergillus flavus* Link [12]. *V. pubescens* is a species that invades grasslands dominated by *Imperata cylindrical* (L.) Raeuschel in West Kalimantan, Indonesia. It fastly grows after burning of *I. cylindrical* [13].

In this study, we provide a follow-up result of the previous study with wood vinegar from "LABAN" wood. Antifungal activities against a brown-rot fungus, *Fomitopsis palustris* (Berk. et Curt.) Gilb. & Ryv. and a white-rot fungus, *Trametes versicolor* (L. ex Fr.) Quel were evaluated as well as termiticidal and termite repellent activities.

Materials and methods

Preparation and pyrolysis of wood

Wood vinegar was made from burning wood meal from "LABAN" wood (*Vitex pubescens* Vahl). The material was collected from Sekabuk village in Pontianak Regency, West Kalimantan, Indonesia and converted into wood meals by a Willey mill with 40–60 mesh screens, and air-dried to about 15 % of moisture content in Wood Workshop Laboratory, Forestry Faculty, Tanjungpura University, Pontianak, West Kalimantan, Indonesia. This air-dried material (1.000 g) was put into a closed reactor (capacity of reactor is 2.000 g), and was heated up to the desired temperature of 350, 400, 450 °C with the heating rate of 5 °C/min. Smoke was channelled into a cooling column through a pipeline, and then cold water was flowed into the column through a pump to recover the condensed vinegar [14]. All wood vinegars that were produced at temperature of 350, 400 and 450 °C for antifungal test, whereas a wood vinegar which was produced at temperature 450 °C for antitermic test.

Analysis of total phenol

Total phenol in the wood vinegars was measured using the method of Senter et al. [15]. One milliliter wood vinegar was diluted 1,000 times with deionised water (DI). One milliliter of the diluted sample was put into 5 ml 2 % Na_2CO_3 solution. After 10 min, 0.5 ml of Folin–Ciocalteau suspension was added to the solution. The absorbance of the solution at 750 nm was measured by a UV–Vis spectrophotometer (Shimadzu 1601, Shimadzu Manufacturing Co. Ltd, Kyoto, Japan). The total phenol concentration in each wood vinegar was calculated by comparing with that of pure phenol suspension.

Analysis of acid

The acidity of the wood vinegar was analyzed using the method of AOAC [16]. One milliliter of wood vinegar was diluted until 100 ml with DI. The three drops of phenolphthalein (PP) indicator was added into it. The solution was titrated by 0.1 N NaOH.

Antifungal assay

The strains of a brown-rot fungus, *Fomitopsis palustris* (Berk. et Curt.) Gilb. & Ryv. and a white-rot fungus, *Trametes versicolor* (L. ex Fr.) Quel. cultures (7 days old) grown on potato dextrose agar (PDA) plates at 27 °C were used for the preparation of inoculate. The antifungal assay was conducted by the method of Kartal et al. [6]. The wood vinegars were served to determine their ability to inhibit growth of *F. palustris* and *T. versicolor* by PDA dishes in vitro. Growth media containing PDA and the wood vinegar at 0.5, 1.0, 1.5 and 2.0 % (v/v) were autoclaved for 15 min at 121 °C and 103.4 kPa (15 psi), and were poured into Petri dishes (90 mm diameter). The Petri dishes were then centrally inoculated with a single plug (5 mm in diameter) of each fungus.

Controls were set up using Petri dishes with PDA medium only. Four replicates were set up for all the concentrations. The test dishes were incubated in a conditioning room at 27 °C. The colony diameter was measured daily, and the percentage mycelia inhibition rate was calculated by the following equation:

$$I = [(C - T)/C] \times 100\ (\%)$$

where I is the inhibition rate, C, the colony diameter of mycelium from the control Petri dishes (mm), and T is the colony diameter of mycelium from the Petri dishes containing the vinegars (mm).

Antitermite test

Termites

Workers and soldiers of *Reticulitermes speratus* (Kolbe) were collected from three field colonies in the Uji Campus of Kyoto University, Uji City, Kyoto Prefecture, Japan. *Coptotermes formosanus* Shiraki workers and soldiers were obtained from a laboratory colony of the Research Institute for Sustainable Humanosphere (RISH), Kyoto University, which has been maintained at 28 ± 2 °C and more than 85 % RH in the dark.

No-choice test (toxic effect)

The no-choice bioassay method [17, 18] was employed to evaluate the termiticidal activity of the wood vinegar. The 0.3 ml of diluted wood vinegars was pipetted onto filter papers (Whatman No. 1, 55 mm in diameter). The treated filter paper was placed into a Petri dish (60 mm in diameter), and fifty-five active termites (50 workers and 5 soldiers) were put on each the filter paper. A piece of filter papers treated with DI were used as controls. Concentrations of the solutions were 1.0, 3.0, 5.0 % (v/v); and 2.5, 5.0, 7.5, 10.0 % (v/v), respectively, for *R. speratus* and *C. formosanus*. The test dishes with covers were then placed in an incubator maintained at 26.5 °C and 80 % RH. Four replicates were made for each the concentration, and the mortalities of the termites were counted daily for 21 days. Only the wood vinegar produced at 450 °C was use for the test.

Choice test (repellent effect)

The choice bioassay method [18, 19] was carried out to evaluate repellent effect of the wood vinegars using Petri dishes of 60 mm diameter containing a pair of paper discs (8 mm in diameter, Advantec Co. Ltd., Tokyo, Japan): a wood vinegar-treated disc (treated) and a DI-treated disc (untreated). The treated and untreated paper discs were placed into a Petri dish by approximately 25 mm apart from each other.

The tested wood vinegar concentrations were 100, 50 and 10 % (v/v), and the paper discs were saturated by 0.1 ml wood vinegar solutions or DI. Thirty workers were put into the Petri dish at the center of the paired disc. Ten replicates for each concentration were performed. The number of workers in contact with each disc was counted every 5 min for 60 min ($n = 12$ counts). Only the wood vinegar produced at 450 °C was use for the test.

Data analysis

The analysis of antifungal activity data was done using factorial 3×5 in a completely randomized design: the first factor was pyrolysis temperature of wood vinegar (350, 400, and 450 °C), and the second factor was concentration of wood vinegar (0, 0.5, 1.0, 1.5, and 2.0 %, v/v). The means were separated using Tukey's highly significant difference (HSD) test at $P = 0.05$.

The Tukey's comparison was employed to evaluate the differences in percent mortality and mass loss in the termiticidal activity test (no-choice test). The results for $P < 0.05$ were considered statistically significant. The mean numbers of termites on the wood vinegar-treated and DI-treated discs for each 60-min period were compared by a paired comparisons *t* test ($P \geq 0.05$) for the direct-choice test. All data were analyzed using Statistical Package for Social Science software (SPSS version 17.0).

Results and discussion

Wood vinegar properties

Wood vinegar was collected at three temperatures of pyrolysis, i.e. 350, 400 and 450 °C. Table 1 shows the basic properties of wood vinegars for the different temperatures. The acid content of the wood vinegars ranged from 2.10 to 3.27 %, and the phenol content ranged from 5.15 to 5.89 %. These vinegars ranged from light yellow to yellowish-brown in color and the darkness of the vinegar increased with the increase in temperature. It thus was assumed that total acid content contributed to the color difference of wood vinegar. Hwang et al. [20] reported that the result of determination of wood vinegar from sapwood of *Cryptomeria japonica* (Thunb. ex L.f.) D. Don contained methanol, acetone, carboxylic acids, furans, phenols, quaiacols, cyclotene, and maltol were 15.34, 0.54, 43.77, 2.34, 8.38, 7.59, 2.66, and 0.38 mg/ml, respectively. The differences in the results obtained from present study and previous study may be due the mainly differences in species of wood and pyrolysis technique.

In this study, only total acid was analyzed. Therefore, in the future, it is worthwhile to analyze acid derivatives.

Antifungal activity

The effect of increasing concentration of the wood vinegar on the growth of *F. palustris* and *T. versicolor* are summarized in Table 2. Overall, wood vinegar exhibited a significant inhibition to the fungal growth, and the effectiveness of the wood vinegar against fungi increased as pyrolysis temperature increased. The wood vinegar from 450 °C showed significantly higher inhibition rate against *T. versicolor* than those of wood vinegars from 350 and 400 °C at 0.5 %. As shown in Table 1, the acid content of wood vinegar from 450 °C was significantly higher in

Table 1 Total acid and total phenol concentrations of the wood vinegars

Pyrolysis temperature (°C)	Total acid (%)[a]	Total phenol (%)[a]
350	2.13 ± 0.01 A	5.22 ± 0.19 A
400	2.10 ± 0.05 A	5.89 ± 0.13 B
450	3.27 ± 0.01 B	5.15 ± 0.19 A

Numbers followed by different letters (A, B) are significantly different at the level of $P < 0.05$ according to Tukey's test

[a] Means ($n = 3$) ± SD

Table 2 The effect of wood vinegars the growth of *Trametes versicolor* and *Fomitopsis palustris*

Treatment		Inhibition (%)[a]	
Wood vinegar	Concentration (%)	*T. versicolor*	*F. palustris*
Control	0	0.0 ± 0.00 A	0.0 ± 0.00 A
W1	0.5	24.72 ± 3.67 C	0.00 ± 0.00 A
	1.0	86.11 ± 4.11 E	6.11 ± 2.13 B
	1.5	100 ± 0.00 F	18.34 ± 2.30 D
	2.0	100 ± 0.00 F	45.28 ± 9.49 G
W2	0.5	14.17 ± 4.39 B	0.00 ± 0.00 A
	1.0	100 ± 0.00 F	9.45 ± 2.13 BC
	1.5	100 ± 0.00 F	34.44 ± 0.91 F
	2.0	100 ± 0.00 F	54.46 ± 2.02 H
W3	0.5	76.39 ± 7.11 D	0.00 ± 0.00 A
	1.0	100 ± 0.00 F	13.89 ± 1.44 CD
	1.5	100 ± 0.00 F	29.72 ± 3.67 E
	2.0	100 ± 0.00 F	64.45 ± 1.29 I

Numbers followed by different letters (A–I) are significantly different at the level of $P < 0.05$ according to Tukey's test

W1 wood vinegar produced at 350 °C, *W2* wood vinegar produced at 400 °C, *W3* wood vinegar produced at 450 °C

[a] Means ($n = 4$) ± SD

comparison with those of wood vinegar from 350 °C and wood vinegar from 400 °C, whereas the total phenol content was the highest in the wood vinegar from 400 °C. It thus was assumed that total acid content contributed to inhibit the growth of fungal. These results are consistent with the results of the previous report showing that acetic acid which is the largest component of wood vinegar from wood wastes (mixed chips of *A. mangium* and *V. pubescens*) at 450 °C exhibited high antifungal activity against *A. flavus* [12].

Besides acid content, phenolic compounds of wood vinegar have also been reported to play a main role in the antimicrobial activity [20–24]. The variation in chemical composition of wood vinegar is influenced by temperature and several parameters, such as species of wood, moisture content of wood, and time of combustion [25, 26]. However, for the practical point of view, we only focussed on the temperature in this study.

The present results show that the antifungal activity of wood vinegar is likely to be higher against white-rot fungi than brown-rot fungi (Table 2). It is well known that some brown rot fungi, including *F. palustris* secrete oxalic acid and have the higher resistance against Cu-based wood preservatives [27, 28]. This leads us to speculate that *F. palustris* has the higher resistance against wood vinegars with more than 2 % acid in this study.

White-rot fungi are also more sensitive to chemicals, not only synthetic chemicals but also natural chemicals, than brown-rot fungi. Celimene et al. [29] stated that three stilbenes, pinosylvin (PS), pinosylvin monomethyl ether (PSM) and pinosylvin dimethyl ether (PSD), were extracted from white spruce (*Picea glauca*), jack pine (*Pinus banksiana*), and red pine (*Pinus resinosa*) pine cones inhibited growth of white-rot fungi, but slightly stimulated growth of brown-rot fungi.

Effects of the wood vinegar against termites

Tables 3 and 4 show the results of termite mortalities and mass losses of filter papers in the no-choice termites test with the wood vinegar from 450 °C.

For *R. speratus*, termite mortalities were increased in accordance with the increase in concentration from 1.0 to 5.0 % after 7, 14 and 21 days (Table 3). Forced contact to the filter paper treated with the wood vinegar with concentrations of 5.0 % showed high mortality from 7 day and achieved 100 % termite mortalities. On the other hand, the mass losses of the filter papers generally were decreased as concentration was increased with exception for the concentration 3.0 %. For *C. formosanus,* the characteristics of antitermite activities were generally the same as those of *R. speratus.* However, we needed 10.0 % concentration to achieve 100 % mortality of *C. formosanus* (Table 4), whereas for *R. speratus* only 5.0 % of wood vinegar was required as stated above. This might be partly because of the difference in size of the body of termites and in feeding activities between these two species (Tables 3, 4). *C. formosanus* has a bigger size than *R. speratus,* and has higher feeding activities as well. Yoshimura et al. [30] reported the wood consumption rates of *C. formosanus* and *R. speratus* as 0.0961 mg/termite/day and 0.0737 mg/termite/day for sapwood of *P. densiflora*, respectively. For *C. formosanus*, the feeding activity was increased in accordance with the decreased mortalities in the same concentration, exception for the concentration 7.5 %.

In our work, in which the wood vinegar made from LABAN was used, the vinegar showed resistant against both fungi and termites, but it needed the relatively high concentrations to perform as an antitermite agent. The wood vinegar of 10 % concentration was needed to achieve 100 % mortality against *C. formosanus*, whereas for *R. speratus* only 5 % of wood vinegar was required. Whereas wood vinegar from coconut shell exhibited high termicidal activity against termite workers, *Odontotermes* sp. at a dilution of 1:50, wood vinegar: sterile water (v/v) [31]. Yatagai et al. [8] investigated the termiticidal activity of three wood vinegars against *R. speratus.* They were made from the mixed wooden chips of *Cryptomeria japonica* (Thunb. ex L.f.) D. Don and *Pseudotsuga menziesii* (Mirb.)

Table 3 Toxic effect of the wood vinegar produced at 450 °C and mass losses of the filter papers in a no-choice test against *Reticulitermes speratus*

Treatment (%)	Termite mortality (%)[a]			Mass loss (%), after 21 days[a]
	7 days	14 days	21 days	
Untreated	1.82 ± 1.49 A	2.73 ± 3.15 A	3.64 ± 3.64 A	23.74 ± 5.56 A
1.0	6.82 ± 4.75 B	6.82 ± 3.75 A	7.27 ± 2.97 A	17.63 ± 1.63 A
3.0	13.64 ± 1.82 C	19.09 ± 4.57 B	64.09 ± 4.78 B	23.28 ± 5.58 A
5.0	100 ± 0.00 D	100 ± 0.00 C	100 ± 0.00 C	6.69 ± 1.87 B

Numbers followed by different letters (A–D) are significantly different at the level of $P < 0.05$ according to Tukey's test

[a] Means ($n = 4$) ± SD using 55 termites per replicate

Table 4 Toxic effect of the wood vinegar produced at 450 °C and mass losses of the filter papers in a no-choice test against *Coptotermes formosanus*

Treatment (%)	Termite mortality (%)[a]			Mass loss (%), after 21 days[a]
	7 days	14 days	21 days	
Untreated	0.46 ± 0.91 A	0.46 ± 0.91 A	0.91 ± 1.05 A	72.54 ± 5.01 A
2.5	2.27 ± 2.73 A	8.18 ± 11.16 AB	32.73 ± 17.18 B	65.65 ± 2.81 A
5.0	11.82 ± 3.15 A	21.82 ± 7.86 B	38.19 ± 6.47 B	34.98 ± 4.63 C
7.5	72.27 ± 23.35 B	87.73 ± 9.66 C	94.09 ± 11.82 C	48.80 ± 2.10 B
10.0	86.36 ± 1.05 B	100 ± 0.00 C	100 ± 0.00 C	15.65 ± 2.98 D

Numbers followed by different letters (A–D) are significantly different at the level of $P < 0.05$ according to Tukey's test

[a] Means ($n = 4$) ± SD using 55 termites per replicate

Table 5 Repellency of the filter papers treated with the wood vinegar produced at 450 °C against *Reticulitermes speratus* workers in a choice test

Experiment	Number of termites in contact with the filter paper discs for 60 min[a]	Difference in mortality
Control[b]	4.53 ± 2.40	No ($P > 0.05$, $df = 9$)
Control	5.64 ± 1.53	
Control	5.02 ± 1.93	Yes ($P < 0.05$, $df = 9$)
Wood vinegar (10.0 %)	0.12 ± 0.18	
Control	5.69 ± 2.41	Yes ($P < 0.05$, $df = 9$)
Wood vinegar (50.0 %)	0.29 ± 0.59	
Control	4.41 ± 1.52	Yes ($P < 0.05$, $df = 9$)
Wood vinegar (100 %)	0.13 ± 0.18	

Paired comparison t test was used for each experiment, $df = 9$

[a] Mean ± SD. No. of counts in a 60-min period ($n = 12$). Each mean is based on 300 termites (ten replicates × 30 termite workers per replicate

[b] Control: the paper discs were saturated with the deionised (DI) water

Franco, *Q. serrata* and *P. densiflora* and the largest component of the three vinegars that exhibited high termiticidal activity was acetic acid. They stated that all three wood vinegar exhibited high termiticidal activities at against *R. speratus*. The content of organic fraction of wood vinegar and acetic acid might be responsible for the differences in termicidal activities [8].

A 60-min repellent effect in the choice test was employed in this study. During the test period, *C. formosanus* and *R. speratus* workers made the equal contact to both the water treated filter papers (control) with no significant difference ($P > 0.05$, $df = 9$, paired comparisons t test, Tables 5, 6). Similar results were obtained by Blaske and Hertel [32] and Ganapaty et al. [18].

When exposed to paper discs treated with the wood vinegar solution at 100, 50, and 10 % concentrations, the termite workers exhibited significant avoidance toward the treated paper discs in all concentrations. These results reinforced the results of the previously reports, showing that wood vinegar had a repellent effect to mosquitoes, snails and slugs [11, 33]. Kiarie-Makara et al. [11] stated that wood vinegar was very effective in repelling *Culex pipiens pallens* Coquillet. The characteristics of mosquito repellence were varying depending on the concentration of wood vinegar used and the species of mosquitoes.

Table 6 Repellency of the filter papers treated with the wood vinegar produced at 450 °C against *Coptotermes formosanus* workers in a choice test

Experiment	Number of termites in contact with the filter paper discs for 60 min[a]	Difference in mortality
Control[b]	2.54 ± 1.49	No ($P > 0.05$, $df = 9$)
Control	3.03 ± 1.10	
Control	2.89 ± 2.17	Yes ($P < 0.05$, $df = 9$)
Wood vinegar (10.0 %)	0.09 ± 0.22	
Control	2.67 ± 1.42	Yes ($P < 0.05$, $df = 9$)
Wood vinegar (50.0 %)	0.00 ± 0.00	
Control	3.03 ± 1.10	Yes ($P < 0.05$, $df = 9$)
Wood vinegar (100.0 %)	0.00 ± 0.00	

Paired comparison t test was used for each experiment, $df = 9$

[a] Mean ± SD. No. of counts in a 60-min period ($n = 12$). Each mean is based on 300 termites (ten replicates × 30 termite workers per replicate

[b] Control: the paper discs were saturated with the deionized (DI) water

Lindqvist et al. [33] found that birch (*Betula* sp.) tar oil is effective in repelling both on a snail *Arianta arbustorum* L. and a slug *Arion lusitanicus* Mabille.

Conclusions

Our result shows that the wood vinegar from *V. pubescens* can act as an antifungal and antitermite agent. This result can be a good preliminary indication for future application of wood vinegar made from *V. pubescens* to wood protection, although in the current work only two fungi and two termites were used as test organisms. Therefore, in the future, it is worthwhile to investigate the effectiveness of the wood vinegar to a wide variety of wood deteriorating organisms. Furthermore, we also need to conduct the detailed analysis of the acid and phenol derivatives in the wood vinegar from *Vitex pubescens* to know the chemical characteristics of the vinegar.

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