JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Antitermitic Activity of Leaf Essential Oils and Components from *Cinnamomum osmophleum*

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The antitermitic activities of the essential oils from the leaves of two *Cinnamomum osmophloeum* clones (A and B) and their chemical ingredients against *Coptotermes formosanus* Shiraki were investigated according to direct contact application. Results from this experiment have demonstrated that the indigenous cinnamon B leaf essential oil has a more effective antitermitic activity than indigenous cinnamon A leaf essential oil. Furthermore, when cinnamaldehyde, eugenol, and α-terpineol are extracted from indigenous cinnamon leaf essential oil and used at the strength of 1 mg/g, their antitermitic effectiveness is much higher than that using indigenous cinnamon leaf essential oil. Among the congeners of cinnamaldehyde examined, cinnamaldehyde has exhibited the strongest termiticidal property.

KEYWORDS: ***Cinnamomum osmophleum*; *Coptotermes formosanus*; cinnamon leaf; essential oils; antitermitic activity; cinnamaldehyde; eugenol; α-terpineol**

INTRODUCTION

Biological deterioration is a main factor that degrades the durability of wooden houses (*1*). Among all factors leading to biodegradation, termites are most damaging to wooden structures worldwide. It is also known that termites cause damage to a variety of materials ranging from paper fabrics to even noncellulosic materials such as asbestos, asphalt bitumen, lead, and metal foils (*2*). Damage to wooden structures and other cellulosic materials attributed to termites has been estimated to exceed $3 billion annually worldwide (*3*). *Coptotermes formosanus* Shiraki is the termite species responsible for most wood destruction in countries such as Taiwan, Japan, and parts of the United States. Thus, many researchers investigating antitermitic compounds have been using *C. formosanus* in their experiments (*4*−*10*).

Indigenous cinnamon (*Cinnamomum osmophloeum* kaneh) (*Cinnamomum*) is an endemic tree that grows in Taiwan's natural hardwood forest at elevations between 400 and 1500 m. Hu and co-workers (*11*) analyzed the composition of the essential oil of *C. osmophloeum* leaves collected from 21 provenances in central, southern, and eastern Taiwan. It was found that cinnamaldehyde was the major ingredient in the leaf essential oil of some *C. osmophloeum* clones, and that eugenol was found in other *C. osmophloeum* clones. On the basis of the chemical composition of different leaf essential oils, *C. osmophloeum* was classified into nine types: cassia type, cinnamaldehyde type, coumarin type, linalool type, eugenol type, camphor type, 4-terpinenol type, linalool−terpinenol type, and mixed type (*11*). *C. osmophloeum* has been of interest to researchers because the chemical constituents of its leaf essential oil are similar to those of *Cinnamomum cassia* bark oil. Cinnamon oil is commonly used in the food industry because of its special aroma. In addition, its antimicrobial and antifungal properties have also drawn great attention from many researchers (*12*−*15*). Chang and co-workers (*16*) found that the leaf essential oils of *C. osmophloeum* have an excellent inhibitory effect against bacteria. To the best of our knowledge, only one preliminary work on the effects of *Cinnamomum* spp. oils on termite control has been reported (*7*). However, there are no prior studies of antitermitic activity of leaf essential oils and their ingredients from *C. osmophloeum*. Huang and Ho (*17*) reported that a methylene chloride extract of cinnamon, *Cinnamomum aromaticum* Nees, was shown to be insecticidal to *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. In this study, the essential oils of leaves collected from two *C. osmophloeum* clones (A and B) were extracted, their chemical compositions were analyzed, and the antitermitic activities were investigated. In addition, the antitermitic activity of cinnamaldehyde congeners was also examined to explain the effects of chemical structure on the antitermitic property.

MATERIALS AND METHODS

Termite. The test termite, *C. formosanus* Shiraki, was collected from Tainan in southern Taiwan. The colony was reared in an incubator at 26.5 °C and 80% relative humidity (RH) for more than 1 year. Water and newspapers were used as food sources.

Essential Oil Distillation. The leaves of two *C. osmophleum* clones (A and B) were collected from the Haw-Lin Experimental Forest located in Taipei. The essential oils of *C. osmophleum* were obtained using water distillation for 6 h. The contents of the essential oils were determined.

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10.1021/jf010944n CCC: $22.00
Published on Web 02/07/2002

Table 1. Major Components and Their Relative Contents (%) of *C. osmophleum* Leaf Essential Oils

oil	peak	R_t[a] (min)	compound	% of total
A	1	13.8	1,8-cineol	11.32
	2	32.9	benzaldehyde	0.93
	3	34.9	linalool	9.83
	4	43.6	borneol	7.46
	5	44.3	α-terpineol	4.62
	6	47.0	neral	12.82
	7	47.8	geranyl acetate	6.03
	8	53.4	geraniol	4.73
	9	64.6	cinnamaldehyde	8.35
	10	70.1	cinnamyl acetate	9.04
B	1	33.2	benzaldehyde	3.20
	2	34.5	linalool	0.24
	3	49.5	geranyl acetate	3.88
	4	55.8	geraniol	2.96
	5	65.3	cinnamaldehyde	76.00
	6	69.6	eugenol	1.05
	7	71.6	cinnamyl acetate	0.51
	8	84.1	coumarin	0.26

[a] R_t = retention time

Gas Chromatography (GC) Analysis. GC was performed on the oils using a Shimadzu model 14B equipped with an FID and Carbowax capillary column (50 m × 0.22 mm i.d.). The GC settings were as follows: initial column temperature set at 60 °C for 5 min; temperature programmed from 60 to 220 °C with a rate of 2 °C/min; nitrogen flow rate 30 mL/min. Identification of the major components of indigenous cinnamon leaf oils was confirmed by comparison with standards as well as by spiking. The quantity of compounds was obtained by integrating the peak area of the spectrograms.

Essential Oil Constituents. The following compounds of essential oil constituents were purchased from Acros (Belgium): benzaldehyde, linalool, α-terpineol, neral, geraniol, eugenol, cinnamyl alcohol, and cinnamaldehyde. The congeners of cinnamaldehyde, including cinnamic acid, cinnamyl acetate, 4-hydroxybenzaldehyde, and 3-phenylpropionaldehyde, were also obtained from Acros.

Antitermitic Activity. The no-choice bioassay method of Kang et al. (*18*) was used to evaluate the antitermitic activity of the essential oils and their chemical constituents. Samples of 2.5, 5, and 10 mg of two essential oils (A and B) as well as 1 and 5 mg of each compound dissolved in 600 μL of acetone were applied to 1 g filter paper samples (Whatman no. 3, 8.5 cm in diameter), individually. A piece of filter paper treated with solvent only was used as a control. After the solvent was removed from the treated filter papers by air-drying at room temperature, 33 active termites (30 workers and 3 soldiers) above the third instar were put on each filter paper impregnated with the test materials housed in a Petri dish (9 cm in diameter × 1.5 cm in height). The test dishes with covers were then placed in an incubator maintained at 26.5 °C and 80% RH. A few drops of water were periodically dripped to the bottom edge of each Petri dish. Three replicates were made for each test sample, and the mortality of the termites was counted daily for 14 days.

Statistical Analyses. The Scheffe method was used to evaluate differences in percent mortality in termiticidal tests. Results with $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Yields and Chemical Constituents of Essential Oils. In the present study, the yields of leaf oils of indigenous cinnamon A and B on water distillation were 6.02 and 7.93 mL/kg of leaves, respectively. The chemical composition of indigenous cinnamon leaf oils and the relative amount of each component were determined by GC analysis, and the results are shown in **Table 1**. The main component in indigenous cinnamon B leaf essential oil was cinnamaldehyde (76%), whereas there is not a dominant component in the essential oils of indigenous cinnamon A leaves. According to the classification by Hu et al. (*11*), indigenous cinnamon A and B belong to the mixed type and cinnamaldehyde type, respectively.

Table 2. Antitermitic Activity of Indigenous Cinnamon A and B Leaf Oils against *C. formosanus*

	termite mortality[a] (%)	
dosage (mg/g)	7 days	14 days
cinnamon A		
10.0	100 ± 0^{a}	100 ± 0^{a}
5.0	$79 \pm 7^{a,b}$	88 ± 10^{a}
2.5	32 ± 6^{d}	$49 \pm 0^{c,d}$
cinnamon B		
10.0	100 ± 0^{a}	100 ± 0^{a}
5.0	96 ± 6^{a}	100 ± 0^{a}
2.5	56 ± 1^{c}	$66 \pm 1^{b,c}$
control	0 ± 0^{e}	6 ± 1^{e}

[a] Means ($n = 3$) using 33 termites per replicate. Numbers followed by different letters (a–e) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

Antitermitic Activity of Essential Oils. The antitermitic activity tests were carried out by feeding termites with the filter paper impregnated with essential oils, and the results are shown in **Table 2**. The results show that at a dosage of 10 mg/g all termites were killed within 7 days. Similar results were also found in the study by Lin and Yin (*7*) when they compared the antitermitic activity of the *Cinnamomum* spp. leaf oil against *C. formosanus*. Observations of termite activity during the test periods revealed that at a dosage of 10 mg/g all termites were actually killed within 1 day. At a dosage of 2.5 mg/g, cinnamon B samples killed 56% of the termites after 7 days while cinnamon A killed only 32% of the termites, indicating cinnamon B is much more effective in termite control. The antitermitic activity obtained in this study is higher than the results of the Lin and Yin study (*7*) at a dosage of 10 mg/g. Since indigenous cinnamon B leaf oil belongs to the cinnamaldehyde type, the difference in the antitermitic activities between these two indigenous cinnamon leaf oils may be explained by the content of cinnamaldehyde.

Antitermitic Activity of the Main Constituents. To understand the relationship between the constituents of indigenous cinnamon leaf oil and antitermitic activity, seven constituents of indigenous cinnamon leaf oil were tested for antitermitic activity against *C. formosanus*. At a dose of 5 mg/g, benzaldehyde, α-terpineol, neral, geraniol, eugenol, cinnamyl alcohol, and cinnamaldehyde all showed 100% mortality after 1 day posttreatment. The only exception is linalool, with only 61% mortality at 5 mg/g after 1 day. **Figure 1** shows the antitermitic activity of the same seven constituents at a dose of 1 mg/g. Among the seven constituents, tests using cinnamaldehyde, eugenol, and α-terpineol exhibit significantly greater termite mortality, followed by cinnamyl alcohol (78%), geraniol (66%), benzaldehyde (48%), and neral (28%) after 7 days. When the test was extended to 14 days, the termite mortalities of those constituents were increased. The effectiveness of all these ingredients in terms of termite mortality at 1 mg/g is ranked as cinnamyl alcohol (100%) > geraniol (83%) > benzaldehyde (70%) > neral (40%). These results are in agreement with those of Cornelius et al. (*19*), who reported that the toxicity of monoterpenoids, especially eugenol, exhibited significant mortality against *C. formosanus*. In another investigation, Ohtani et al. (*6*) also found that the termite mortality of α-terpineol was 100% at a 4 mg/g dosage for 7 days.

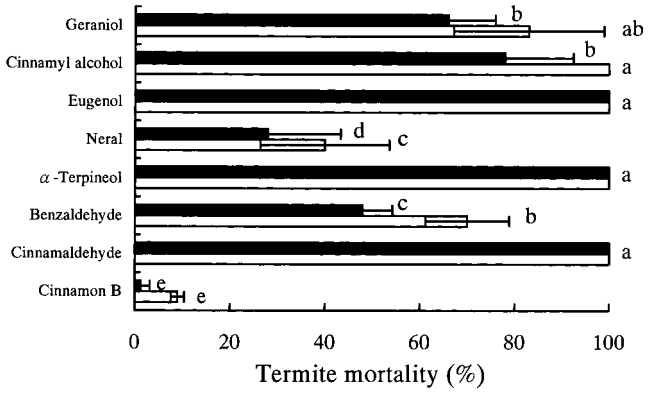

Figure 1. Antitermitic activity of seven constituents at a 1 mg/g dosage (black bar, after 7 days; white bar, after 14 days). Means ($n = 3$) using 33 termites per replicate. Numbers followed by different letters (a–e) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

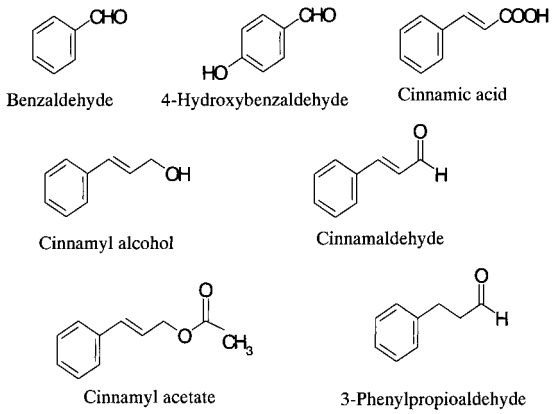

Figure 2. Chemical structures of cinnamadehyde congeners.

Antitermitic Activity of Cinnamaldehyde's Congeners. On the basis of the above results, it is found that cinnamaldehyde, eugenol, and α-terpineol have the strongest antitermitic activity, and that cinnamaldehyde is the main component in the indigenous cinnamon B leaf essential oil. Meanwhile, comparison of the antitermitic activity of cinnamaldehyde with that for indigenous cinnamon B leaf oil reveals that cinnamaldehyde is more effective (**Figure 1**). To ascertain the chemical groups and structures that confer cinnamaldehyde with such strong antitermitic activity, four compounds whose chemical structures are similar to cinnamaldehyde were selected for the study. These four compounds were 4-hydroxybenzaldehyde, cinnamic acid, cinnamyl acetate, and 3-phenylpropionaldehyde, and their chemical structures are shown in **Figure 2**. The results of antitermitic activity of these four congeners of cinnamaldehyde are presented in **Table 3**. The table shows that, at a dosage of 5 mg/g, cinnamaldehyde and cinnamyl alcohol killed all the termites within 1 day. However, the termite mortalities of cinnamaldehyde and cinnamyl alcohol decreased to 96% and 6% when the dosage was decreased to 1 mg/g. The results suggest that cinnamaldehyde has the strongest antitermitic activity. When the antitermitic activity of these four cinnamaldehyde congeners having both a benzyl ring and a conjugated double bond was ranked, the result was cinnamaldehyde > cinnamyl alcohol > cinnamyl acetate > cinnamic acid.

It is clear that compounds with an aldehyde group have the best antitermitic activity. Thus, we selected four compounds containing an aldehyde group to examine their antitermitic activities. These four compounds were cinnamaldehyde, 4-hydroxybenzaldehyde, 3-phenylpropionaldehyde, and benzaldehyde (**Figure 2**). **Table 3** shows the antitermitic activity of the four selected compounds. The table reveals that cinnamaldehyde, benzaldehyde, and 3-phenylpropionaldehyde killed 100% of the termites within 1 day at a dosage of 5 mg/g. The antitermitic activities of these three compounds were virtually the same at 5 mg/g, but they decreased and a big difference surfaced when the dosage was decreased to 1 mg/g. The ranking of antitermitic activity of these three compounds at 1 mg/g is cinnamaldehyde (96%) > benzaldehyde (3%) ≈ 3-phenylpropionaldehyde (1%) for 1 day. The Scheffe test showed no significant differences in termite mortality between benzaldehyde and 3-phenylpropionaldehyde at the level of $P < 0.05$. These results suggest that a compound having a conjugated double bond and a long CH chain outside the ring, such as cinnamaldehyde, possesses much stronger antitermitic activity.

Table 3. Antitermitic Activity of Cinnamaldehyde's Congeners against *C. formosanus*

compound	dosage (mg/g)	termite mortality[a] (%) 1 day	4 days	7 days
cinnamyl alcohol	5	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
	1	6 ± 1^{e}	$45 \pm 11^{b,c}$	$78 \pm 14^{a,b}$
cinnamic acid	5	0 ± 0^{e}	$80 \pm 12^{a,b}$	100 ± 0^{a}
	1			
cinnamyl acetate	5	94 ± 5^{a}	100 ± 0^{a}	100 ± 0^{a}
	1			
cinnamaldehyde	5	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
	1	96 ± 6^{a}	100 ± 0^{a}	100 ± 0^{a}
benzaldehyde	5	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
	1	3 ± 2^{e}	$32 \pm 9^{c,d}$	$48 \pm 7^{b,c}$
4-hydroxybenzaldehyde	5	0 ± 0^{e}	96 ± 4^{a}	100 ± 0^{a}
	1			
3-phenylpropionaldehyde	5	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
	1	1 ± 2^{e}	$31 \pm 12^{c,d}$	$48 \pm 5^{b,c}$
control	0	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{e}

[a] Means ($n = 3$) using 33 termites per replicate. Numbers followed by different letters (a–e) are significantly different at the level of $P < 0.05$ according to the Scheffe test.

In conclusion, the results of antitermitic tests demonstrated that *C. osmophloeum* B leaf oil (cinnamaldehyde type) exhibited the strongest antitermitic activity. All termites were killed at a level of 5 mg/g after 14 days. The *C. osmophloeum* A leaf oil (mixed type) killed all termites at a higher level of 10 mg/g after 14 days of test. Cinnamaldehyde, eugenol, and α-terpineol in indigenous cinnamon leaf essential oil exhibited significant antitermitic activities which were higher than that of indigenous cinnamon leaf essential oil alone at a level of 1 mg/g. In addition, comparisons of the antitermitic activity of cinnamaldehyde's congeners revealed that cinnamaldehyde exhibited the strongest termiticidal activity, followed by cinnamyl alcohol, benzaldehyde, 3-phenylpropionaldehyde, cinnamyl acetate, 4-hydroxybenzaldehyde, and cinnamic acid.

ACKNOWLEDGMENT

We thank Prof. His-Tai Chen (The Haw-Lin Experimental Forest) for supplying *C. osmophleum* leaves and Mr. Chien-Yung Hung and Mr. Wen-Ching Cheng (Jong Shing Pest Control Co., Ltd.) for providing the termites. We also thank Dr. Sheng-Yang Wang (Institute of BioAgricultural Sciences, Academia Sinica) for his suggestions.

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Received for review July 20, 2001. Revised manuscript received November 20, 2001. Accepted November 25, 2001.

JF010944N