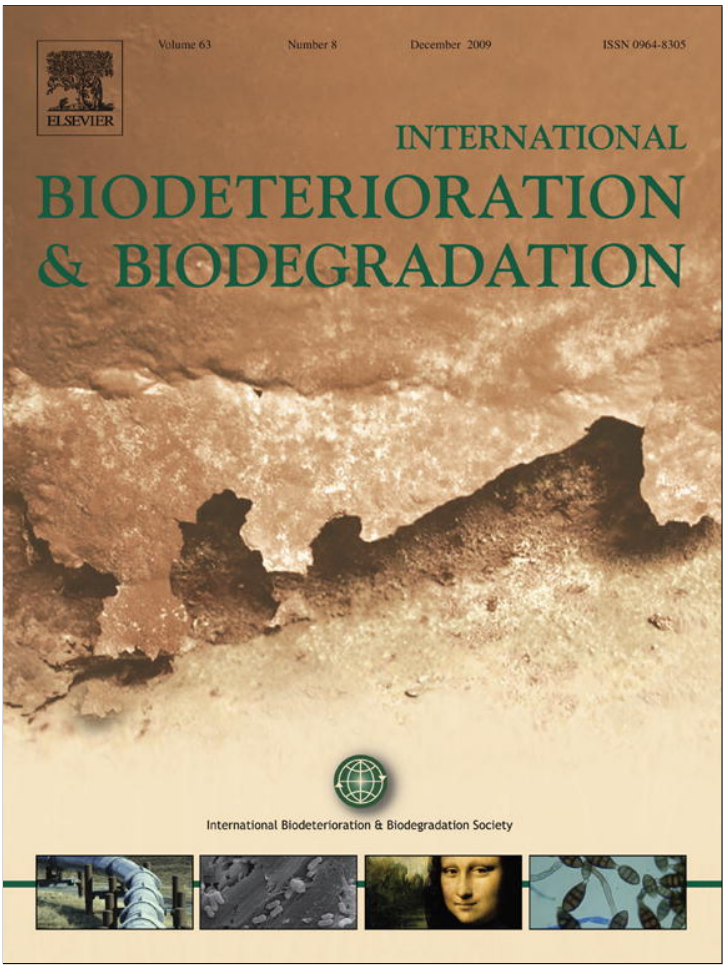

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Evaluation of the decay resistance properties of *Cerbera odollam* extracts and their influence on properties of particleboard

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ABSTRACT

Metallic-based wood preservatives currently face some restrictions over disposal and environmental issues; one possibility to develop new more benign systems is to study extractives in naturally durable woody plants. This study investigated the resistance of extracts from the leaf, fruit, wood, bark, seed and flower of *Cerbera odollam* to deterioration from fungus and termites. Antifungal assays with *n*-hexane, ethyl acetate, ethanol and methanol extracts were evaluated using paper discs. Termite mortality was evaluated with the methanol extract against *Coptotermes gestroi*. Physical and protective properties of particleboard impregnated with *C. odollam* extracts, including thickness swelling, internal bond strength, formaldehyde release, termite-decay and soil burial decay were investigated. Methanol wood extracts from *C. odollam* showed the highest activities against *Trametes versicolor, Pycnoporus sanguineus,* and *Schizophyllum commune* in the paper disc antifungal assay. Methanol flower extracts exhibited high performance in termite mortality, termite-decay and soil burial decay. Thickness swelling, internal bond strength and the formaldehyde emission of particleboard specimens treated with methanol extracts of *C. odollam* were up to the EN Standards.

1. Introduction

The need for wood and wood products to be chemically treated against biological attack is well known and documented (Hashim et al., 1997; Barnes, 1992; Deppe, 1970). The addition of preservative chemicals is necessary because most wood products are manufactured from non-durable species. However, the addition of preservative chemicals causes safety and the environmental concerns (Yen et al., 2007; Kotan et al., 2008). Because of this, research has been focused on using natural preservatives (Kawamura and Ohara, 2005; Nemli et al., 2006; Cheng et al., 2007; Yen et al., 2007). These natural preservatives include extracts from durable species and natural toxic chemicals from plants that could inhibit the growth of biological degraders such as fungi and termites (Chang et al., 2001; Schultz and Nicholas, 2002; Cheng et al., 2007; Temiz et al., 2008).

Cerbera odollam belongs to the poisonous Apocynaceae family found in coastal swamps, riverbanks and creeks in many Asian countries (Corner, 1952). The tree is also widely grown in parks, gardens and along the roadside as a shade tree. The studies of Laphookhieo et al. (2004) and Gaillard et al. (2004) showed that *C. odollam* contains poisonous compounds used as insecticides against hair mites. Rahman et al. (1993) showed that N-butanol extracts from the *C. odollam* seed caused mortality of *Culex quinquefasciatus* and *Aedes aegypti* larvae. The presence of these chemicals in plants may also influence other properties, such as hygroscopicity, strength and gluing properties (Hse and Kuo, 1988; Hashim et al., 2001). Due to the earlier work on this tree and its wide availability, we carried out a study to investigate the antifungal activities and anti-termite activities of extracts from *C. odollam* and their influence on some properties of particleboard.

2. Materials and methods

2.1. Samples preparation and extraction

Fresh samples of the leaf, fruit, wood, bark, flower and seed of *C. odollam* were collected in July 2007 around Penang, Malaysia. The samples were cut, ground, and then dried in a freeze dryer. Extraction of these samples was performed as in Kawamura et al. (2004) with some modification. Each sample was extracted separately with n-hexane, ethyl acetate, ethanol or methanol for 6 h. The mass of the extracts was completely dried, weighed and the yield determined. The yield of extract was calculated based on oven dry weight of ground sample.

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2.2. Antifungal assay

The antifungal activities of each extract were evaluated with the paper disc assay based on Kawamura et al. (2004) with some modification. The plant extract was diluted with respective solvent. This diluted extract was then pipetted out to 6 mm Whatman Paper AA disc as in the bio-assay. The disc was left uncovered on a clean bench for 3 days to let the solvent evaporate leaving residual pure extract on the paper disc. The amount of this extract in μg on the disc will be used as the weight of the extract. The weight of extract obtained for this assay were 100 μg, 50 μg, 20 μg, or 5 μg. Potato Dextrose Agar (PDA) medium of 3.9% was prepared and autoclaved at 120 °C at 15 psi (0.10 N/mm^2) for 20 min, and approximately 15 ml was dispensed into 9 cm petri dishes to solidify. Each fungus, *Schizophyllum commune* (KUM 790036), *Trametes versicolor* (FFPRI 1030), *Pycnoporus sanguineus* (KUM 70097), *Fomitopsis palustris* (FFPRI 0507) or *Gloeophyllum trabeum* (MI -102) kept in an incubator, was inoculated at the center of the PDA medium. The paper disc treated with the extract was then placed on the dish about 3 cm away from the inoculated fungus. The samples were then incubated in the dark at 25 ± 2 °C. The antifungal activities of each extract were evaluated at 24 h intervals for 7 days. Paper discs permeated with solvent without extract were used as a control. In addition, paper discs were permeated with Antiblu CC, a commercial preservative from Koppers Arch containing chlorothalonil and carbendazim for comparison. The lowest weight of extract in microgram with positive antifungal activities on 7th day was defined as the minimum inhibitory amount.

2.3. Termite mortality

The extract showing the best antifungal activity was chosen for the termite resistance study. Termite mortality was performed according to Ganapaty et al. (2004) and Cheng et al. (2007) with slight modifications. Termites (*Coptotermes gestroi*) were collected from infested wood in Penang, Malaysia. The extracts were diluted with methanol, evenly permeated into 8.5 cm paper discs at dosage of 10 mg/g, and placed in 9 cm petri dishes. A control test was conducted in parallel with paper disc permeated with methanol alone. The paper discs were left on a bench for 3 days to let the excess methanol evaporate. Termites (*C. gestroi*), 30 healthy mature workers and 3 soldiers, were introduced on each permeated paper disc in the petri dish. Healthy termites refer to termites that are active with live movement and with no physical injuries based on observations. The *C. gestroi* workers and soldiers were identified based on taxonomic key described by Kirton and Brown (2003) and confirmed by molecular techniques by the use of mitochondrial gene (12S, 16S and COII) (Yeap et al., 2007). In general, *C. gestroi* soldiers have a pair of hairs near the rim of the fontanelle, will aggressively bite when challenged and exude a white secretion from the fontanelle. The workers and soldiers were freshly collected from in-ground monitoring stations established earlier according to methods described in Lee (2002) and the insects were used within several days after collection to ensure vigourness. Three replicates of each condition were performed. A few drops of distilled water were dripped on the bottom edge of each petri dish. Mortality counts were performed at 24 h intervals for 14 days, and the number of dead termites was recorded.

2.4. Impregnation, pH of the sawdust and manufacturing of particleboards

Rubberwood samples obtained from a sawmill in Kedah, Malaysia were chipped and ground to form sawdust that could pass through 2.5 mm sieve. To remove possible available methanol extracts in the rubberwood, the sawdust was soaked with technical grade methanol. The methanol extracts were removed from the container and replaced with fresh methanol after 7 days. This process was repeated every 7 days for 28 days. The sawdust were then air dried for 3 days or until all available methanol had evaporated. It was assumed that there was no methanol extract left in the sawdust. The sawdust was then impregnated with methanol extracts from different parts of *C. odollam* diluted with methanol at a 1% concentration based on the over-dried weight of selected saw dust. This sawdust was used to make particleboard. The pH values of the various particles were determined based on the method used by Moore and Johnson (1967). Comparisons were also done using impregnated solid wood. It is generally known that wood-based composites have greater resistance than solid wood but are still susceptible to biological attack (Curling and Murphy, 1999). Solid 2 cm × 1 cm × 0.5 cm rubberwood samples were also immersed into 1% methanol extracts from different parts of *C. odollam* diluted with methanol following the method of Kamdem (1994). Three solid rubberwood replicates were prepared. In addition, the commercial preservative Antiblue CC was used as comparison for solid wood, and samples without treatment were used as controls. After the treatment, all samples were air dried for at least 3 days to remove all available methanol.

Experimental 21.2 cm × 21.2 cm × 0.5 cm particleboards consisting of the sawdust impregnated with *C. odollam* extracts with a target density of 0.60 g/cm^3 were made using a small scale laboratory press based on Hashim et al. (2001). Phenol formaldehyde resin (43% solid) and urea formaldehyde resin (48% solid) obtained from Hexion Specialty Chemicals, Penang, Malaysia were used at 15% adhesives based on the oven-dried weight of the particles. For urea formaldehyde resin, ammonium chloride with 100 parts adhesive and 0.8 part hardener was used. The mat was pre-pressed for 2 min before being hot pressed at 140 °C for 10 min at 500 kg/cm^2. The particleboards were then cut into various test specimens. All test specimens were conditioned at 20 ± 2 °C with 65 ± 5% relative humidity for at least 2 weeks. Particleboards were then evaluated for their physical properties, internal bond strength, formaldehyde release, the termite-resistance decay test and the soil burial decay test.

2.5. Thickness swelling, internal bond strength and the formaldehyde test

Thickness swelling and the internal bond strength of particleboards were determined in accordance with EN 317 (1993) and EN 319 (1993), respectively. Formaldehyde release was conducted according to European Standard EN 717–3 (1996).

2.6. Termite deterioration

The termite-decay tests for the particleboard and solid rubberwood were performed according to the No Choice Test Procedure, ASTM D3345-74 (1999) and Ngee et al. (2004) with slight modifications. *C. gestroi* were collected from infested wood in Penang, Malaysia. This species was selected for the termite test due to its high preference for rubberwood (Ngee et al., 2004). The test was performed in a 30 cm × 25 cm × 20 cm polyethylene container with good air circulation. Fine sand was washed with water and sieved to pass a 0.42 mm mesh and oven-dried. The sand was then moistened with drinking water to a ratio of 1 part water to 3 parts sand volume. The moistened sand was placed evenly in the test arena. Treated particleboard and treated solid 2 cm × 1 cm × 0.5 cm rubberwood specimens were oven-dried, weighed and randomly placed in the test arena. A total of 1000 workers and 50 soldiers were introduced into the test arena. Three replicates of each condition were performed. The test arena was protected from light

and left for 30 days. After 30 days, the specimens were taken, washed, and oven-dried, and the weight loss was determined.

2.7. Soil burial

The soil burial test on treated particleboard as well as treated solid rubberwood samples was performed according to the British Standard BS 1982–2 (1990) with modifications. The oven-dried weight of a 10 cm × 1 cm × 0.5 cm specimen was recorded. Samples were randomly buried 8 cm deep in an open-air test arena around Penang, Malaysia for 8 weeks to investigate their natural biodegradation rate by all kinds of natural bio-degraders. The samples were removed from the soil after 8 weeks, carefully cleaned, oven-dried and reweighed to determine their weight loss.

2.8. Statistical analysis

The results were analysed statistically, and the comparison of the means was performed with Tukey HSD (Runyon et al., 2000).

3. Results and discussion

3.1. Paper disc fungal assay

The extract yield is shown in Table 1, indicating that the highest amount of extract was obtained with methanol. The lowest extract yield was from hexane. Fruit gave the highest total yield compared to the others. The fungal assay was evaluated based on the minimum inhibitory amount of extracts as shown in Table 2.

A minimum inhibitory amount was observed for ethyl acetate extracts and methanol extracts from fruit, wood, bark and flower against *T. versicolor*, *P. sanguineus,* and *S. commune*. Methanol wood extracts had the highest antifungal activity against *T. versicolor*, *P. sanguineus,* and *S. commune*, with the lowest dosage compared to the other extracts. There was no antifungal activity of any extract against *G. trabeum* or *F. palustris*. These fungi seem to be tolerant to the active compound found in the extractive. Based on the yield and minimum inhibitory amount, only methanol extracts were selected for further investigation.

3.2. Termite mortality

The termite mortality of *C. gestroi* when exposed to methanol extracts is shown in Table 3. In this mortality test, the termites could only ingest treated paper discs and water during the observation period. All extracts yielded some degree of termite mortality. Flower extracts had the highest activities against termites. Extracts from wood had the lowest termite mortality. All termites in the control survived until the end of the experiment. This indicated that these extracts played an important factor in determining the survival of *C. gestroi*.

After 14 days of exposure with a dosage of 10 mg/g, flower extracts caused total termites mortality. These results show that flower extracts have the highest anti-termite properties compared to other parts of *C. odollam*. The lowest anti-termite properties were observed for wood extract, with only 36% mortality after 14 days of exposure.

Table 1
Yield of extraction from different parts of *C. odollam* with different solvents.

Part	Yield (%)			
	Solvent			
	Hexane	Ethyl acetate	Ethanol	Methanol
Leaf	2.08	5.35	10.53	25.96
Fruit	1.66	1.83	24.27	29.41
Wood	1.04	0.23	4.25	4.62
Bark	2.02	0.88	7.96	18.01
Flower	2.52	1.31	25.78	22.55
Seed	0.45	13.47	4.15	20.51

Table 2
Minimum inhibitory amount of antifungal activities of various extracts of *C. odollam* against *S. commune, T. versicolor* and *P. sanguineus.*

Treatment	Part	Minimum inhibitory amount (μg)		
		Fungal		
		S. commune	*T. versicolor*	*P. sanguineus*
Hexane	Leaf	–	–	–
	Fruit	–	–	–
	Wood	<5	100	<5
	Bark	–	–	–
	Flower	–	–	–
	Seed	–	–	–
Ethyl Acetate	Leaf	–	–	–
	Fruit	10	100	50
	Wood	<5	100	<5
	Bark	<5	100	<5
	Flower	<5	100	<5
	Seed	–	–	–
Ethanol	Leaf	–	–	–
	Fruit	–	–	–
	Wood	–	–	–
	Bark	<5	–	10
	Flower	–	–	−5
	Seed	–	–	–
Methanol	Leaf	–	–	–
	Fruit	10	50	10
	Wood	<5	50	<5
	Bark	10	100	<5
	Flower	10	100	<5
	Seed	–	–	–

Note: There is no minimum inhibitory amount found for *F. palustris* and *G. trabeum*.

3.3. pH of the particles and properties of particleboards

The pH of the particles may influence the curing rate of formaldehyde-based resin (Nemli et al., 2006). The pH values of the particles impregnated with methanol extract from leaf, fruit, wood, bark, flower, seed and control were 6, and 5 from Antiblu. A slight decrease in the pH was seen in the particles impregnated with the Antiblu preservative compared to the control. The presence of extract did not affect the pH of the wood particles compared with the control. The thickness swelling and internal bond of particleboards made from particles impregnated with various extracts of *C. odollam* ranged from 13.75–15.04% for particleboards bonded with urea formaldehyde adhesive and 11.43–12.79% for particleboards bonded with phenol formaldehyde. The maximum 24 h

Table 3
Termite mortality (%) for methanol extracts of *C. odollam* against *C. gestroi* at 10 mg/g after 14 days.

Part	Termite mortality (%)
	Dosage
	10 mg
Leaf	75.76 (±9.09)a
Fruit	60.61 (±6.06)b
Wood	36.36 (±3.03)c
Bark	63.64 (±3.03)bd
Flower	100.00 (±0.00)e
Seed	48.48 (±3.04)f
Blank	0.00 (±0.00)g

Different letter within the same column are statistical significance at $\alpha = 0.05$.

Table 4
Weight loss (%) of particleboards made from particles impregnated with extracts from different parts of *C. odollam* and solid rubberwood exposed to *C. gestroi* and after exposure to soil burial.

Part	Termites, Wt Loss (%)			Soil burial decay, Wt Loss (%)		
	Solid RW	Particleboards		Solid RW	Particleboards	
		UF	PF		UF	PF
Leaf	14.42 (±0.21)a	12.13 (±2.49)a	15.66 (±1.10)a	3.87 (±1.00)a	19.79 (±5.32)ab	27.25 (±7.12)ac
Fruit	32.59 (±3.61)b	29.91 (±1.60)b	33.10 (±0.67)b	3.00 (±1.97)a	15.66 (±5.59)ab	52.38 (±14.52)bd
Wood	41.69 (±2.30)c	34.51 (±1.23)c	37.09 (±1.40)c	4.25 ± 1.87)a	15.29 (±5.73)ab	35.21 (±11.05)abc
Bark	15.84 (±1.66)ad	19.58 (±0.87)d	23.22 (±0.72)d	4.81 (±1.32)a	14.41 (±5.19)a	29.02 (±10.40)ac
Flower	9.53 (±0.37)e	9.35 (±0.47)ae	12.06 (±0.19)e	5.13 (±0.40)a	16.83 (±5.84)ab	15.71 (±6.46)a
Seed	29.61 (±1.13)bf	27.43 (±1.77)bf	31.25 (±0.75)bf	6.54 (±2.02)a	30.47 (±10.16)bc	43.65 (±19.45)bc
Control	68.95 (±0.45)g	45.01 (±4.67)g	54.75 (±4.14)g	13.15 (±4.99)b	45.35 (±15.65)c	64.04 (±11.50)d
Antiblu[a]	7.93 (±0.12)eh	8.71 (±0.31)aeh	10.70 (±0.71)eh	2.85 (±1.88)a	11.88 (±6.09)a	14.50 (±3.24)a

RW, Rubberwood; UF, Urea formaldehyde; PF, Phenol formaldehyde; Figures in parentheses are standard error. Different letter within the same column are statistical significance at $\alpha = 0.05$.
[a] Commercial wood preservative.

thickness swelling requirements are 16% for non-load-bearing boards used in humid conditions (Type P3) and 19% for load-bearing board for use in dry conditions (Type P4), as specified by EN 312 (2003). The internal bond strength values obtained ranged from 0.51 N/mm^2 to 0.57 N/mm^2 for particleboards bonded with urea formaldehyde and 0.66 N/mm^2 to 0.78 N/mm^2 for particleboards bonded with phenol formaldehyde. The internal bond strength requirements are 0.5 N/mm^2 for non-load-bearing boards used in humid conditions (Type P3) and 0.45 N/mm^2 for load-bearing for use in dry conditions (Type P4), as specified by EN 312 (2003). There were no significant effects of the extracts on the thickness swelling rate or on the internal bond strength.

The formaldehyde release of the particleboards made from particles impregnated with extracts from different parts of *C. odollam* passed the requirements for formaldehyde emissions as stated in EN 312 (2003). According to the standard, the formaldehyde value for type E1 boards bonded with urea formaldehyde adhesive and phenol formaldehyde adhesive should be ≤80 mg/kg based on oven dry weight of the board. The formaldehyde values obtained ranged from 18.0 mg/kg to 19.2 mg/kg for urea formaldehyde bonded boards and less than 1 mg/kg for phenol formaldehyde bonded boards. The presence of extracts did not significantly affect the formaldehyde emission of treated boards compared to the control.

Table 4 shows the termite–decay resistance of various extracts from various parts of *C. odollam* compared to particleboard and wood. The termites decay test is a direct choice test, with termites showing avoidance behaviour. The condition with the lowest weight loss in the termites decay test has the best repellent effect among the samples. Flower extracts exhibited the best effects against termite–decay and were comparable to commercial preservatives. This was followed by leaf, bark, seed, fruit and wood.

The soil burial decay test results are also shown in Table 4. These results showed that there is an improvement in the durability of the test sample after treatment with extract from *C. odollam*. This was especially so for samples treated with flower and bark extracts. The weight loss of the samples treated with flower extract was as low as those treated with Antiblu CC, a commercial preservative, with only 5.13% weight loss for solid wood and 16.8% and 15.7% for urea formaldehyde and phenol formaldehyde particleboard, respectively. It seems that there is a small difference between commercially treated boards and boards treated with extract from *C. odollam*. Not much difference could be seen in boards treated with flower extract compared to boards treated with the commercial preservative. Treatment with leaf extract also showed promising results, indicating that leaf extract was able to reduce weight loss in the soil burial test.

4. Conclusions

Methanol wood extracts showed strong antifungal activities against *T. versicolor*, *P. sanguineus,* and *S. commune*, while methanol flower extracts had excellent performance against *C. gestroi*. Thickness swelling, internal bond, and formaldehyde release of particleboard were not negatively affected by the extracts. *C. odollam* extract has the potential to preserve wood and/or particleboard from fungus and termite attack and achieve the required EN standards. Based on the outcomes of the study we found out that extract of *C. odollom* could inhibit *S. commune*, *T. versicolor* and *P. sanguineus*. However, the fungi *F. palustris* and *G. trabeum* were tolerant to this extractive. Further study is also required to compare the properties with a wider range of commercial synthetic preservatives.

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