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Chemical compositions and termiticidal activities of the heartwood from *Calophyllum inophyllum* L.

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ABSTRACT

Wood extractives from heartwood of *Callophylum inophyllum* (bintangor) were obtained by shaker method and analyzed for their constituents by gas chromatography-mass spectrometry (GC-MS). Ten compounds were identified by ethanol (EtOH) solvents, fourteen by methanol (MeOH) and only nine by petroleum ether (PETETHR). Major compounds were contributed by monoterpenes (75.11%, 53.75%) when extracted with EtOH and PETETHR solvents. The anti-termitic assay of the wood extracts was also investigated against *Coptotermes curvignathus*. The level of concentration for anti-termite activity may be an indication of the dose application of the wood extracts for new development of termiticide.

Key words: *Calophyllum inophyllum*, chemical compositions, ethanol, methanol, petroleum ether, termiticide.

INTRODUCTION

Calophyllum inophyllum Linnaeus, (Clusiaceae) which is commonly referred to as 'bintangor' (in Malaysia) is a medium to large evergreen tree. It is widely distributed in tropical areas and tolerates varied kinds of soil, coastal sand, clay or even degraded soil (Dweck and Meadowst 2002). The fruits and leaves of *C. inophyllum* are very poisonous (Austin 1998). The extracted oil from the fruit is used as a remedy for sciatica, shingles, neuritis, leprous neuritis and rheumatism, ulcers and skin diseases while and the oil from this tree's seed is reported to have medicinal and healing properties (Cribb and Cribb 1981). Other uses included gum for treatment of wounds and ulcers, bark for vaginal discharge after childbirth, passing of blood and gonorrhea (Burkhil 1994), antiseptic, disinfectant, internal haemorrhages (Nadkarni and Nadkarni 1999), soulattrolide (pyranocoumarins) from latex are active against HIV (Patil et al. 1993) and Calocoumarin-A as an anti-cancer agent (Hathurusingha and Ashwath 2007). The Javanese believed the tree had diuretic properties but in Samoa, the whole tree is considered a virulent poison (Dweck and Meadowst 2002). Recently, *C. inophyllum* has been identified as the most suitable feedstock for future generation biodiesel (Jahirul et al. 2012).

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On the other hand, previous studies (Scheffrahn 1991, Escoubas et al. 1995, Nakayama et al. 2000, Peralta et al. 2004, Ragon et al. 2008) found that wood extractives possessed promising protection/prevention characteristics against termites. They claimed that the protection characteristics came from phenolic compounds like terpenes, flavonoids, quinoids, alkaloids, stilbenes and tannins (Ohmura et al. 2000, Morisawa et al. 2002, Ganapaty et al. 2004, Watanabe et al. 2005, Coelho et al. 2006, Morimoto et al. 2006, Little et al. 2010). In addition, wood extracts which are organic based preservatives are easier to detoxify are considered low hazard and are easier to dispose of without adverse environmental effects (Barnes 1992, Chen et al. 2004).

However, to the best of our knowledge, a study of the wood extracts of *C. inophyllum* species from Malaysia, or any other country, has not been reported to date. A few studies focus on the medicinal properties (Dweck and Meadowst 2002, Misha et al. 2010) rather than on wood preservatives. The aim of this study was to evaluate the *C. inophyllum* heartwood extracts for antitermitic activity against subterranean termite, *Coptotermes curvignathus*.

MATERIALS AND METHODS

Plant Material

A 25 years old disc of *Calophyllum inophyllum* Linnaeus (bintangor) was cut from felled trees stored at FRIM log yard. A disc was cut from the basal portion of one tree.

Termite

Asian subterranean termites, *Coptotermes curvignathus* Holmgren (Isoptera: Rhinotermitidae), was collected from active field colonies at the Forest Research Institute Malaysia (FRIM) campus using a method described before (Roszaini et al. 2009).

Extraction

The heartwood of each timber species was ground to fine sawdust powder, passed through a 250 mesh sieve before extraction. About 5 g of wood powder was extracted with 200 mL of absolute ethanol, absolute methanol and petroleum ether on a shaker for 8h at room temperature. Then the solutions were filtered through Whatman no. 1 filter paper. The filtrate was then evaporated in a rotary vapour at 40°C (Ordoñez et al. 2006) and the extraction yield was determined. The oven-dried cubic samples (25 mm x 25 mm x 6.5 mm) were also prepared from the heartwood of each timber species and were extracted with each of the solvents using the same technique as stated before.

Bioassay Test Against Subterranean Termite

The un-extracted and extracted samples were subjected to no choice feeding tests according to ASTM D3345-74 (ASTM 1988) standard methods slightly modified. Screw-top bottles of 8 cm in diameter by 13 cm height were filled with 200 g of sterilized sand and 30 ml distilled water. The bottles were left overnight to equilibrate to laboratory conditions before test initiation. One block of each timber species was placed on the surface of the damp sand and 400 termites (360 workers and 40 soldiers) were added to each bottle. Each tests contained 5 replicates. All bottles were stored in an incubator maintained at 22°C and 70 ± 5% relative humidity for 28 days. Within this period, if it was found that all termites appeared dead, the bottle would be taken out and the number of days until 100% mortality would be recorded. At the end of the fourth week the blocks were removed, cleaned, dried overnight and reweighed. The remaining live termites were weighed and recorded for each of the bottles. As detailed in the standard, the condition of the test blocks were rated visually using a 0-10 scale where 0 was total failure and 10 was sound.

Termite Assays

The toxicity of *C. inophyllym* wood extract was determined according to previous methods (Sharma and Raina 1998) with slight modification. Samples of 5 mL, 10 mL, 20 mL, 40 mL and 80 mL of wood extracts were dissolved in analytical grade acetone separately, to obtain solutions of 5%, 10%, 20%, 40% and 80%, respectively. Then the solutions were applied to a 9.0 cm in diameter of filter paper samples (Advantec and 1.5 mm thickness) and dried in a laminar flow for 1h. The filter paper weights were measured before and after treatment process. Untreated filter paper and treated filter paper with acetone were use as a controls and each of the tests contained 3 replicates. Fifty active termite (3rd instar) (45 workers and 5 soldiers) of *C. curvignathus* were introduced onto each Petri dish (9 cm diameter and 1.6 cm height). A few drops of water were added periodically to the bottom of each Petri dish. All the Petri dishes with covers were placed into an incubator which was maintained in darkness at 26°C ± 2°C and 65% ± 5% relative humidity and the mortality of the termites was counted and recorded every 24h for 25 days.

Chemical Analysis

The extracts were analyzed by GC-MS (Siti Humeirah et al. 2010) [Agilent Technologies GC-MS 7890A/5975C Series; mass selective detector (MSD), EI mode (70eV direct inlet). GC: Hewlett-Packard 5MS; silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness]. Temperature program: 75°C → 230°C at 3°C min^{-1} and finally held at 230°C for 5 min. Injector temperature: 270°C. Carrier gas: He 1 ml min^{-1}. Injected volume: 1.0 μl. Each extract was analyzed by GC twice. The GC peak areas were integrated and the component identification was done by comparing the MS with standards and with a library search (National Institute of Standards and Technology, NIST).

Statistical Analysis

One way analysis of variance (ANOVA) was performed on all data to determine the significance of variation in extractive compounds and antitermitic between wood species as well as between samples using MINITAB 15 computer programme. Values of $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Extractvie Yield

Extraction of the air dry heartwood of *C. inophyllum* gave dark brown extracts in 3.89% (EtOH), 4.29% (MeOH) and 3.31% (PETETHR) (Fig. 1). In the same way, MeOH gave a higher yield of extracts in camparison to the other two solvents which implies that MeOH was the best extraction proficiency while petroleum was the least proficient. Our findings are in agreement with previous study by Mahamadi et al. (2011), who reported that maximum extract yield from *Temnocalyx obovatus* was obtained with absolute methanol. Analysis of variance (ANOVA) also demonstrated that there was a significance variance between the yield extracts.

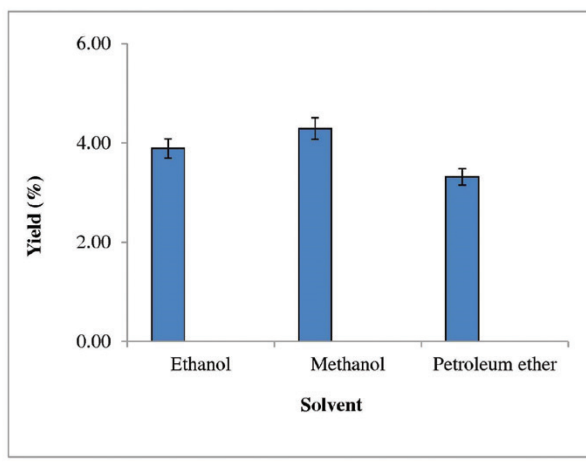

Fig. 1 - Yield of wood extractives from *C. inophyllym* extracted with different solvents (shake for 8 hour).

Chemical Compositions

A total of ten, seventeen and nine compounds comprising 99.46%, 99.99% and 99.28%, respectively, of the total constituents of the wood extracts (Table I) were identified using different solvents of EtOH, MeOH and PETETHR.

TABLE I
Chemical constituents of ethanol, methanol and petroleum ether of *C. inophyllum* wood extracts.

Compound	RI	RA		
		EtOH	MeOH	PETETHR
Abietadiene	3	-	2.95	-
β- Funebrene	409	2.96	-	-
Coniferyl alcohol <E->	464	2.63	-	-
Agarospirol	544	-	1.15	-
Presilphiperfolan-8-ol	647	-	-	1.83
Incensole acetate	722	2.08	-	-
Hexenyl cinnamate <3Z	810	-	-	2.64
Hydroxy Citronellol	825	-	1.63	-
Indipone	839	15.16	38.46	39.32
Larixol	982	-	12.38	-
Tetradectene <1->	1215	0.55	-	-
Stilbene	1437	-	1.47	-
Isobornyl isobutanoate <5-oxy->	1447	43.27	-	46.60
Occidenol	1481	-	1.38	-
Thujaplicinol	1495	-	8.02	-
Carvacrol, methyl ether	1503	29.91	19.12	2.30
Thymol	1506	-	-	2.70
Rosifoliol	1595	-	10.56	0.27
Isoprenyl cinnamate-(E)-methyl	1671	-	1.25	-
Methyl p-tert butylphenyl acetate	1697	1.93	2.45	-
Hexadecanoic acid	1742	0.56	1.53	-
Thujic acid	1850	-	-	2.15
Allo-hedycaryol	1856	-	-	1.47
Isovalencenol<(E)-	2173	0.41	-	-
Identified compounds (%)		99.46	99.47	99.28
Group components				
Monoterpenes		75.11	28.27	53.75
Sesquiterpenes		0.41	14.96	3.57
Diterpenes		2.08	15.33	-
Phenylpropanoids		3.52	2.45	2.64
Alkaloids		18.34	38.46	39.32

RI = retention index as determined on Db-5 using the homologous series of n-hydrocarbons; RA = relative area (peak area relative to total peak area); - = not found, EtOH = Ethanol; MeOH = Methanol; PETETHR = Petroleum ether.

The major compound in each of these wood extracts was isobornyl isobutanoate (43.27 – 46.60%) followed by indipone (15.16 – 39.32%) and carvacrol (2.30 – 29.91%) in EtOH, MeOH and PETETHR fractions, respectively. Other compounds that were significantly present were larixol (12.38%), rosifoliol (10.56%) and thujaplicinol (8.02%) in MeOH fractions. Quite a few of the compounds were only present in low concentrations (below 3%). It is also clear that monoterpenes (53.75-75.11%) were the main group of compounds in the *C. inophyllum*, except when extracted with MeOH (28.34%). Previous studies have shown that monoterpenes had significant insecticidal effects (Kordali et al. 2007, Abdelgaleil et al. 2009, Abdelgaleil 2010, Santos et al. 2011, Zahran and Abdelgaleil 2011, Xie et al. 2014). Alkaloids were the second most important compound group in *C. inophyllum* (15.16-39.32%).

Mao and Henderson (2007) reported that alkaloids had a strong antifeedant effect against Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Sesquiterpenes, diterpenes (except with PETETHR) and phenylpropanoids were also present in all fractions. Furthermore, an aromatic compound, abietadiene was only detected in the MeOH extract.

Out of twenty four different compounds identified in three solvents, indipone and carvacrol were the two compounds detected in each of solvents used. The toxicity of MeOH solvent extracts against *C. curvignathus* may be attributed to the presence of carvacrol (Seo et al. 2009). Morales-Ramos et al. (2003) reported that the absence of carvacrol is one of the factors that made the infected wood susceptible to Formosan subterranean termite attack while Ahn et al. (1998) found that carvacrol had broad insecticidal and acaricidal activity against agricultural, stored-product, and medical arthropod pests including termite. Methyl p-tert butyphenyl acetate and hexadecanoic acid were detected in EtOH and MeOH fractions, only isobornyl isobutanoate in EtOH and PETETHR; and rosifoliol in MeOH and PETERHR, respectively. On the other hand, β- funebrene, coniferyl alcohol <E>, incensole acetate, tetradectene <1-> and isovelencenol <E> were detected only in EtOH fracion while abietadiene, agarospirol, hydroxyl citronellol, larixol, stilbene, occidenol, thujaplicinol and soprenyl cinnamate-(E)-methyl were only found in MeOH fraction. (+)-Citronellol has been reported as a mosquito repellent chemical (Taylor and Schreck 1985) and as being toxic against *C. curvignathus* (Roszaini et al. 2013). According to Shibutani et al. (2004), stilbenes which are well known as biologically active compounds are very effective against *Reticulitermes speratus* (Kolbe) while methylated compounds increased termiticidal activity. Meanwhile, only five other compounds; presilphiperfolan-8-ol, hexenyl cinnamate <3Z, thymol, thujic acid and allo-hedycarvol were detected from PETETHR fraction. Thymol was reported give a good result of termite mortality (88.27%) when tested against harvest termites *Anacanthotermes ochraceus* (Moawad et al. 2012). Pandey et al. (2012) also found that thymol, eugenol and carvacrol exhibited 100% termite mortality of *Odontotermes assamensis.*

Bioassays

Results of the antitermitic activity tests of *C. inophyllum* wood extracts were examined by termite mortality (Fig. 2) and percentage of wood consumption (Fig. 3). The results of analysis showed that the strong antitermitic activity of *C. inophyllum* was extracted using MeOH followed by EtOH and lastly PETETHR. MeOH are commonly used to get the higher yield of extracts (Ali et al. 2010) and this solvent is also a good solvent system for the extraction of polar and non polar compounds (Richard et al. 2012). The strong antitermitic activity by MeOH solvent is likely from the presence of hydroxy citronellol and stilbene (Taylor and Schreck 1985, Shibutani et al. 2004, Roszaini et al. 2013) which can't be extracted by other solvents.

By all assessment methods, extracted wood species were particularly susceptible to *C. curvignathus* (Fig. 3). Results show that MeOH solvents resulted in a higher (45.49%) impact on the durability of *C. inophyllum* followed by EtOH (21.96%). Only 6.64% of *C. inophyllum* durability affected by PETETHR. This means that, when a high extractive is removed, it will cause a lack of wood resistance against *C. curvignathus* and this is in line with the results obtained for wood extracts yield (Fig. 1). The result from this study is in accordance with the previous study by Taylor et al. (2006). The samples of *C. nootkatensis* extracted using MeOH solvent were significantly increased the susceptibility of this timber species against *C. formosanus*.

This shows that the extract yields and resulted of antitermitic activities of the wood species are strongly dependent on the nature of extracting

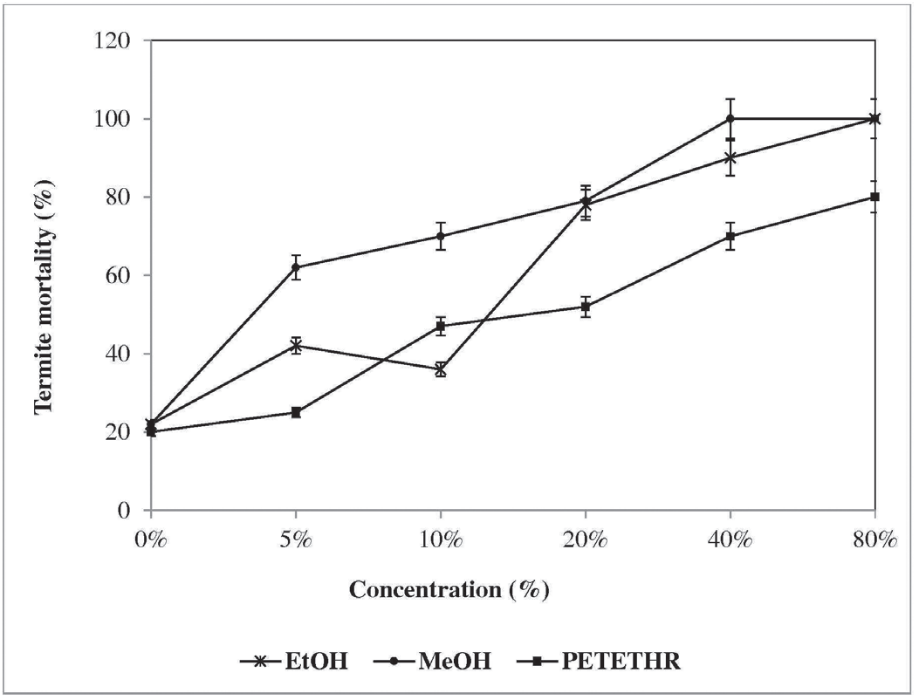

Fig. 2 - Effect of different level of concentration on termite mortality of *C. curvignathus*.

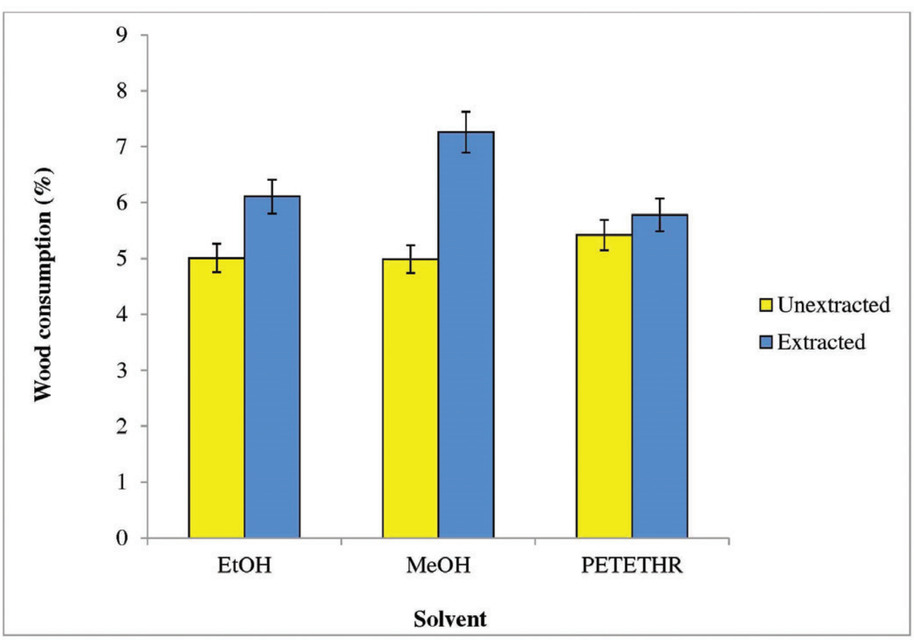

Fig. 3 - Wood consumption in bio-assay tests of *C. inophyllum* against subterranean termites.

solvent, due to the presence of different antitermitic compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent.

The two reported reasons why termites respond differently in wood in bioassay tests are the amount and type of extractives and the wood density. Generally, extractives have a bigger impact on durability than density (Sen-Sarma 1963). This is clearly shown in the data presented here (Table II and Fig. 3). Samples with less amount of extractive extracted, had higher termite resistance while sample with higher extractive content extracted showed poor performance. This result corroborated the results of earlier works by Hashimoto et al. (1997). They claims that lower extractive content has been correlated with reduced termite and fungal resistance. Significant variation in termite resistance properties of *C. nootkatensis* wood also were also observed by Grace and Yamamoto (1994) and they suggested that this was a function of heartwood extractive variability. However, samples extracted with PETETHR (of lower density and lower extractive content) showed poorer performance; much better performance was achieved with samples extracted with ethanol, with a little bit higher extractive content but had similar density. The effect of this wood density on the natural durability was still unclear.

TABLE II
Density of unextracted and extracted samples of *C. inophyllum* using different solvents.

Solvent	Unextracted	Extracted
Ethanol	0.68 (0.03)[a]	0.65 (0.03)[a]
Methanol	0.69 (0.02)[a]	0.66 (0.02)[a]
Petroleum ether	0.66 (0.02)[a]	0.65 (0.02)[b]

Mean (± SD) of 5 replicates for each species. Percentage values followed by the same letter are not significantly different in the same group at the 0.05 level of probability.

CONCLUSIONS

In conclusion, analysis of chemical compounds and antitermitic activity of *C. inoplyllum* wood extracts showed differences depending on solvents and level of concentration used. The results show that methanol had the greatest extraction yields whereas petroleum ether had the least.

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RESUMO

Extratos de madeira de cerne de *Calophyllum inophyllum* (bintangor) foram obtidos pelo método de agitação e os seus componentes analisados por cromatografia em fase gasosa-espectrometria de massa (GC-MS). Dez compostos foram identificados pelo emprego de solvente etanol (EtOH), e catorze por metanol (MeOH) e apenas nove por éter de petróleo (PETETHR). Os principais compostos dessas frações foram identificados como monoterpenos (75,11%, 53,75%), quando extraídos com os solventes EtOH e PETETHR. O ensaio anti-cupins dos extratos da madeira também foram investigados contra *Coptotermes curvignathus*. O nível de concentração necessário para a atividade anti-fungica pode ser uma indicação da dose de aplicação dos extratos de madeira para o novo experimento da ação termiticida.

Palavras-chave: *Calophyllum inophyllum*, composições químicas, etanol, metanol, éter de petróleo, termiticidas.

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