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TOXIC EFFECTS OF THREE SELECTED MALAYSIAN TIMBERS PLANT EXTRACTS AGAINST SUBTERRANEAN TERMITES

Roszaini Kadir [1,*]

ABSTRACT

The toxic effects of selected Malaysian timbers (*Madhuca utilis*, *Anisoptera laevis* and *Endospermum malaccense)* heartwood extracts were studied with the aim to determine and understanding the function of wood extracts as a natural protection against termite. The results show that no-choice experiments revealed toxic properties of all investigated extracts by the contact against *Coptotermes gestroi* and *Coptotermes curvignathus*. However, high termite mortality was only achieved with *Madhuca utilis* extracts and methanol solvents.

Keywords: *Madhuca utilis*, *Anisoptera laevis*, *Endospermum malaccense,* wood extractives, antitermitic activity, *Coptotermes gestroi, Coptotermes curvignathus.*

INTRODUCTION

Extractives are low molecular weight compounds that can be extracted by polar or non-polar solvents and encompass complex, and had diverse physical properties (Fengel and Wegener 1989). Because of this, many different solvents and mixtures have been used for extraction. Solvents such as hexane and acetone/water mixtures have been used with some tropical timbers (Kilic and Niemz 2010); methanol and hexane with *Azadirachta excelsa* (Ahmad Said *et al.* 2006) and ethanol-benzene with *Shorea ovalis* and *Neobalanocarpus heimii* (Ahmad-Said and Mohd-Hamami 1982). In addition, structure, distribution and quantity of secondary metabolites are useful markers for chemotaxonomy (Banthorpe *et al.* 1972).

It is known that wood extracts are a major contributory factor in the natural durability of the wood (Scheffer and Cowling 1966, Hillis 1987). Many studies (Carter *et al.* 1975, Steller and Labosky 1982, Chang *et al.* 2001, Chang and Cheng 2002, Watanabe *et al.* 2005, Elango *et al.* 2012, Roszaini *et al.* 2014, Roszaini *et al.* 2015) show some promising result on wood extractive against termites. They found that bark and Heartwood extractives exhibited antitermitic activity in a certain percentage of concentrations. Meanwhile, Arango *et al.* (1992) reported that several advantages can be obtained from the application of wood extractives as wood preservatives. It is relatively safer than synthetic preservative, but still effective. It is Barnes (1992) also easier to detoxify and dispose off without adverse environmental effects because it's the organic based materials.

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In some instances, it is only present in small amounts (3 to 6% of oven-dry weight) (Rudman 1965, Mori *et al.* 1997, Reyes-Chilpa *et al.* 1998, Celimene *et al.* 1999, Windeisen *et al.* 2002, Haupt *et al.* 2003, Neya *et al.* 2004, Mburu *et al.* 2007). In other cases, Roszaini (2011) found in her study that this value can achieve up to 15% (of oven-dry weight) for heartwood and to 35% (of oven-dry weight) for bark of tropical timbers.

Phenolic compounds, terpenes, carbohydrates, long-chain fatty acids, waxes and other substances, including steryl esters and sterols are among the mainly chemical compounds in heartwood extractives (Fengel and Wegener 1989). Many single or groups of extraneous compounds are known to inhibit the activities of biological agents. For example, sesquiterpenes possess a wide spectrum of biological activity, playing a role in plant defense mechanisms against insects and fungi (Fraga 2003, Wu *et al.* 2005) and, pinosylvin and pinosylvin-monomethyl-ether have been found toxic to fungi (the *Sirex* fungus, presumably *Amylostereum* sp.) (Hillis and Inoue 1968).

Meanwhile, *Madhuca utilis* (Ridley) H.J. Lam ex K. Heyne known as bitis (local name) is one of the large trees which can achieve up to 50 m in height, 1 m in diameter and 2 m high of buttresses (Orwa *et al.* 2009). It belongs to the family of Sapotaceae and classified as heavy hardwood with a density of 820 - 1,120 kg/m^3 air dry (Lim *et al.* 1998).

Anisoptera laevis (Ridl) which is also known as merawan under Malaysian common name is an evergreen tree with a relatively small crown. The tree belongs to the family of Dipterocarpaceae and locally distributed in lowland primary forest of Peninsular Malaysia (Ken 2014). The timber is a medium hardwood with a density of 495–980 kg/m^3 air dry. Its wood is hard and heavy, and particularly used for bridge, rafters, joists, door and window frames, flooring, joinery, furniture manufacture, veneer and plywood manufacture (Wood identification 2010).

Endospermum malaccense Miq. (sesendok) is a timber belonging to the family of Euphorbiaceae. It can be found in lowland to low-Montane forest (up to 1000m altitude) and in all states of Peninsular Malaysia (except Perlis) (Mohd Shukari 1982). It's also one of the timber species that has been proposed for plantation in Peninsular Malaysia as an alternative timber species to rubberwood with an excellent working and nailing properties (Ahmad Zuhaidi *et al.* 2002, Khairul *et al.* 2010).

However, to the best of our knowledge, a study of the wood extracts of *M. utilis, A. laevis* and *E. malaccense* extracted with different solvent from Malaysia, or any other country, has not been reported to date. One study done by Roszaini *et al.* (2014) shows some promising as antitermitic of *M. utilis* when extract with toluene/EtOH but, they do not include other solvents in their study. In another study, methanol extraction of *E. malaccense* showed highest antifungal activity against a white-rot fungus, *Pycnoporus sanguineus*, at a minimum effective amount of 100 µg (Kawamura *et al.* 2011). No single study was done for *E. malaccense* and *A. laevis* wood extracts against termite. On the other hand, studies (Naczk and Shahidi 2004, Spigno *et al.* 2007, Kajdžanoska *et al.* 2011, Lolita *et al.* 2012) have shown that different solvent extracts different compounds. Based on their studies, it is very important to find the best solvents for extraction of these compounds from plants especially for tropical timbers.

The objective of this paper is to investigate the effects of different solvent extractions on some wood species with respect on the feeding behavior of the two Asian subterranean termites *Coptotermes gestroi* and *C. curvignathus*. Although *M. utilis, A. laevis and E. malaccense* have been reported in durable, moderate and non-durable class timbers respectively, its antitermitic activity has not been tested. So this would be the first study of the antitermitic activity of crude extract of different Malaysian wood species against the two aggressive Asian subterranean termites.

MATERIALS AND METHODS

Plant material

Heartwood of three Malaysian timbers: *Madhuca utilis* (Ridl.) H.J.Lam (bitis), *Anisoptera laevis* (Ridl) (merawan) and *Endospermum malaccense* Miq. (sesendok) were cut from felled trees stored in

the FRIM log yard.

Termite

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

Wood block bioassay against subterranean termites

The un-extracted or had previously been extracted (extracted for 8 hours in an orbital shaker) wood blocks (25 mm x 25 mm x 6 mm) were subjected to no choice feeding tests according to ASTM D3345-08 (ASTM 1988) standard methods with slightly modified. Rubberwood (*Hevea brasiliensis*) were used as controls.

Table 1. Classification of natural durability of wood against termites (ASTM D3345, 1988).

Block aspect after test	Classifications
Sound, surface nibbles permitted	10
Light attack	9
Moderate attack, penetration	7
Heavy	4
Failure	0

Screw-top bottles of 8 cm in diameter by 13 cm high 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 were placed on the surface of the damp sand and 400 termites (360 workers and 40 soldiers) were added to each bottle. All bottles were stored in an incubator maintained at 22±2°C and 65±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. Then the wood blocks were classified according to the standard method used (Table 1).

Extraction and isolation

All the heartwood timber species were ground to fine sawdust powder, passed through a 250 mesh sieve and dried at 60°C (to avoid the possibility of extracts degradation) before extraction. About 50 g of wood sawdust was extracted with four different solvents [absolute methanol (MeOH), absolute ethanol (EtOH), acetone and petroleum ether (PETETHR)] for 8 hours used an orbital shaker (Gallenkamp, UK). The extracts were concentrated under reduced pressure at 45°C, using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan) and stored in a refrigerator (-4°C), until used for analyses. Weight losses of samples were calculated from the oven dry weights at 60°C (48 hours) before and after the extraction. Retention of extractive material was calculated (mg/m^3) as follows:

$$R = \frac{(M_1 - M_0) \times C}{V} \quad (1)$$

where, M_1 is weight after treatment (g), M_0 is a weight before treatment (g), C is concentration levels of solutions and V is volume of filter paper (m^3). The extractive retentions (mg/m^3) in treating filter paper as calculated by solution uptake are presented in Table 2.

Table 2. Mean extractive retentions (mg/m^3) in treated filter paper as calculated by solution uptake.

Solvent	Concentrations	Species/ Retention (mg/m^3)		
		M. utilis	*A. laevis*	*E. malaccense*
Methanol	0,5	0,38 (0,25)	1,78 (0,26)	0,95 (0,25)
	1,0	1,21 (0,10)	2,80 (0,72)	2,19 (0,38)
	2,0	4,38 (1,06)	6,15 (2,49)	3,84 (0,38)
	3,0	8,23 (2,06)	6,26 (3,36)	7,41 (0,99)
	4,0	10,10 (2,12)	9,44 (1,37)	9,44 (1,01)
Ethanol	0,5	1,07 (0,14)	0,93 (0,17)	0,93 (0,26)
	1,0	2,52 (1,25)	1,92 (0,41)	1,87 (0,10)
	2,0	5,93 (0,87)	4,17 (1,48)	3,40 (0,50)
	3,0	7,41 (0,86)	5,27 (0,75)	7,08 (2,06)
	4,0	9,00 (2,66)	7,24 (0,66)	8,56 (0,66)
PETETHR	0,5	0,82 (0,36)	0,38 (0,10)	0,60 (0,41)
	1,0	2,03 (1,19)	0,99 (0,33)	0,99 (0,16)
	2,0	2,85 (0,95)	1,65 (0,00)	1,43 (0,83)
	3,0	4,61 (2,06)	2,30 (0,75)	1,81 (0,57)
	4,0	5,71 (3,04)	3,07 (0,38)	2,85 (0,38)
Acetone	0,5	1,10 (0,05)	1,06 (0,05)	0,96 (0,55)
	1,0	1,98 (0,33)	1,87 (0,41)	1,70 (0,25)
	2,0	3,51 (0,50)	2,41 (2,47)	2,83 (1,87)
	3,0	5,27 (0,29)	3,82 (0,29)	3,27 (1,87)
	4,0	7,24 (0,66)	6,36 (1,52)	5,93 (1,32)

Note: Mean of five replicates, numbers in parentheses are standard deviations.

Antitermitic bioassay (Toxicity determination)

The bioassay method used by previous studies (Roszaini *et al.* 2013) with slightly modified was used to evaluate the antitermitic activity of wood extracts against *C. gestroi* and *C. curvignathus*.

Samples of 5,0 mg, 10 mg, 20 mg, 30 mg and 40 mg of wood extract from four different wood species were dissolved in 100 μl of MeOH to obtain solutions (m/v) of 0,5%; 1,0%; 2,0%; 3% and 4%, respectively. Then 20 μl of the solutions were applied to each 30 mg filter paper samples (Advantec, 8 mm diameter and 1,5 mm thickness) and dried in vacuum desiccators for 24 hours. The paper discs were weighed before and after drying. Untreated paper discs were used as a control. 20 active termite workers were introduced into each Petri dish (90 mm diameter and 16 mm height) which contained 3 g of sterile sand. A few drops of water were added periodically to the basal edge of each Petri dish. All the Petri dishes with covers were placed into an incubator (maintained in darkness) at 22 ± 2^{o}C and 65 ± 5% RH and the mortality of the termites was counted and recorded every 24 hours for 10 days. Each test contained 5 replicates including the control. The consumption of the filter papers was calculated from the difference in dry weights before and after the exposure. A dose-mortality line was developed depends on the exposure time(s) and the lethal concentration (LC_{50}) of wood extracts was determined using the probit method (Finney 1971).

Statistical analysis

One way analysis of variance (ANOVA) was performed on all data to determine the significance of variation in extracting compounds and antitermitic between wood species as well as between samples using MINITAB 15 computer programme. The LC_{50} values were determined directly from probit

analysis or calculated by substituting 50% for "y" into the curve equation in the graph.

RESULTS AND DISCUSSION

Extractives yield

The quantities of extractive yield of three tropical timber species are presented in Table 3. Table 3 shows that MeOH yielded greater amounts of extractive than the other three solvents (EtOH, PETETHR and acetone) in all timber species. *M. utilis* were classified as durable timbers under Malaysian grading rules (Lim *et al*. 1998), yielded significantly more extractive (7,00%) than the moderately durable (*A. laevis*) (3,44%) and non-durable (*E. malaccense*) (0,72%) timbers, in MeOH use the shaker method. The same pattern also occurred when EtOH (5,21%; 2,98% and 0,28% respectively) and acetone (3,25%; 1,67% and 0,44% respectively) were used. However, *A. laevis* yielded more (1,29%) compared to *M. utilis* (0,13%) and *E. malaccense* (0,30) when PETETHR was used. As indicated by several authors (Chang *et al*. 2001, Syofuna *et al.* 2012, Ogunwusi *et al*. 2013), extracts which are low molecular weight compounds in the wood can be extracted by many solvents. However, they differ among timber species, between individual tree of the same species and solvents (Scheffer and Cowling 1966, Nacimento *et al.* 2013) due to genetic variation and environmental (Ericsson *et al.* 2001). Furthermore, solvent polarity plays a key role in determining the extract yields to be obtained (Bashash *et al*. 2012). On the other hand, shaker method is not a good method with respect to extraction efficiency for plant materials. As indicated by a few studies before (Park *et al.* 2001, Kothari *et al*. 2012), a heat-employing methods' (soxhlet) proved to be the best option for extraction any plant materials.

In another study, even though MeOH is indeed the most common and effective solvent, it has been reported is an environmental pollutant and more toxic than other alcohols (Kapasakalidi *et al.* 2006, Bridgers *et al.* 2010). Thus EtOH is preferred as solvent extraction (Delgado-Vargas and Paredes-Lopez 2002).

Table 3. Effect of different solvent extraction on extractive yields of three wood species.

Wood species	Extractive yields (%)			
	Methanol	Ethanol	Petroleum ether	Acetone
M. utilis	7,00^{a}	5,21^{b}	0,13^{d}	3,25^{c}
A. laevis	3,44^{a}	2,98^{b}	1,29^{d}	1,67^{cd}
E. malaccense	0,72^{a}	0,28^{c}	0,30^{bc}	0,44^{b}

Note: Means with the same letters are not significantly different at 95% confidence limit.

Bioassay test against termites

Termite mortality

Among the three timber species tested, *M. utilis* heartwood samples were very resistant to attack by both subterranean termite; *C. gestroi* and *C. curvignathus*. Daily assessment shows that majority of the termite in *M. utilis* test bottles died within 15 days compared to 18 days in *A. laevis* and more longer (26 days) in *E. malaccense* test bottles. Result from this study shows that all castes of termite used (workers and soldiers) either in *C. gestroi* or *C. curvignathus* completely died in all *M. utilis* extracts. *A. laevis* extracts using PETETHR showed 1,0% and 0,05% while *E. malaccense* extracts using MeOH showed 0,08% and 0,02% of workers surviving for *C. gestroi* and *C. curvignathus*, respectively. Surprisingly, *E. malaccense* EtOH extracts showed 1,22% of *C. gestroi* workers surviving at the end of the test. All termites also 100% died when tested with unextracted samples of *M. utilis* and *A. laevis* but showed minimal survival (0,01% against *C. gestroi* and 0,005% against *C. curvignathus*, respectively) in unextracted *E. malaccense* samples. Higher survival occurred in *brasiliensis* test bottles (2,1%

against *C. gestroi* and 2,8% against *C. curvignathus*). Even though the mortality percentages due to the used wood of extracts were not significantly different from control (*brasiliensis*), suggesting the toxic effect of these three timber species wood extracts against termites.

The results of termite mortality could be the reaction of termites to the toxic, anti-feeding and / or repellent effects (Yuan and Hu 2011). In all cases, higher termite mortality exists when exposed to un-extracted samples compared to extracted indicates the function of heartwood extractives as a natural wood preservative against termites (Syofuna *et al.* 2012, Tascioglu *et al.* 2012, Kirker *et al.* 2013). In other words, wood extracts are one of the factors that increase the termite mortality.

On the other hand, the results, evidently indicate that *M. utilis* heartwood extractives contain biologically active compounds that were potent to *C. gestroi* and *C. curvignathus*. MeOH apparently was a better solvent in extracting the toxic chemical compounds followed by EtOH, acetone and PETETHR. *M. utilis* extract completed the mortality of *C. gestroi* and *C. curvignathus* at the concentration of 4% compared to *A. laevis* and *E. malaccense* even though there was not much difference between *C. curvignathus* and *C. gestroi* mortality in every concentration. MeOH extracts that killed both termite species tested may have reacted and made a food substrate to be toxic to both termite species. Golpayegani *et al.* (2014) in their study on mulberry wood (*Morus alba*) extractives against *Reticulitermes flavipes* also found that MeOH is the second best solvent besides acetone that give a low termite survival. On the other hand, Syofuna *et al.* (2012) reported that different compounds obtained from different solvents will show a different effect towards termite resistance. In conclusion of Taylor *et al.* (2006) study, to understand the natural durability of wood against termites, we can't just focus on a single compound alone, but this resistance is a combination of several compounds that are present in the wood.

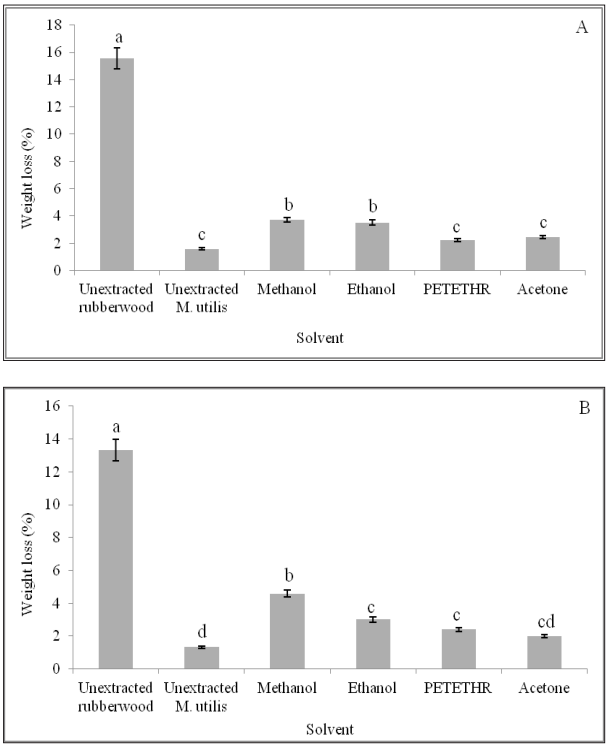

Figure 1. Weight loss of *M. utilis* extracted in selective solvents and exposed for 4 weeks to A: *C. gestroi* and B: *C. curvignathus* blocks that were subjected to various extraction procedures. Columns with the same letter are not significantly different at $\alpha = 0,05$.

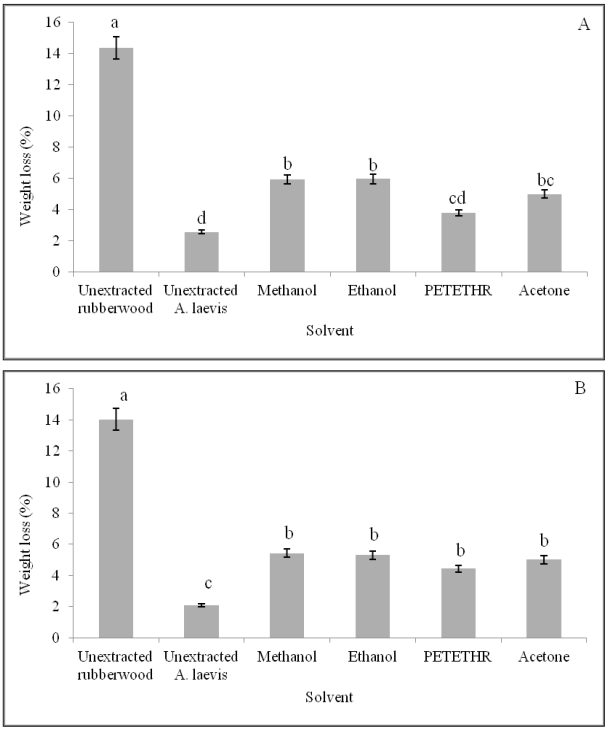

Figure 2. Weight loss of *A. laevis* extracted in selective solvents and exposed for 4 weeks to A: *C. gestroi* and B: *C. curvignathus* of blocks that were subjected to various extraction procedures. Columns with the same letter are not significantly different at α = 0,05.

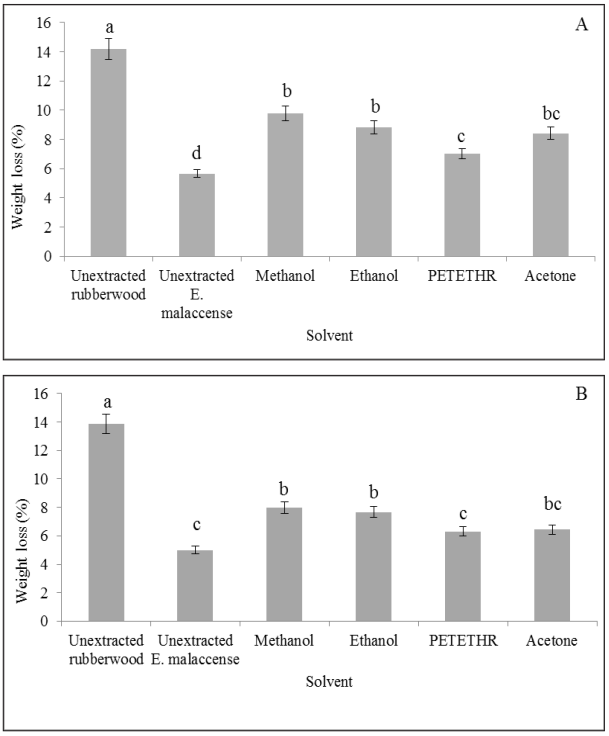

Figure 3. Weight loss of *E. malaccensis* extracted in selective solvents and exposed for 4 weeks to A: *C. gestroi* and B: *C. curvignathus* of blocks that were subjected to various extraction procedures. Columns with the same letter are not significantly different at α = 0,05.

Weight loss

Figure 1, Figure 2 and Figure 3 reports the effects of the different extracts on the feeding behavior of two different subterranean termite species. The result of no-choice termite bioassays also shows that extracted blocks gave a higher wood consumption compared with un-extracted blocks in all wood species tested. Both termite species consumed more wood on un-extracted *E. malaccense* samples (5,66% against *C. gestroi* and 5,01% against *C. curvignathus*, respectively) than the other two timber species; *M. utilis* (1,58% against *C. gestroi* and 1,33% against *C. curvignathus*, respectively) and *A. laevis* (2,57% against *C. gestroi* and 2,09% against *C. curvignathus*, respectively). Indirectly, these results suggest that *M. utilis* heartwood is more resistant to both termites than the other two timber species. *E. malaccense* extracted blocks had the highest mass loss at all solvent used [MeOH – 9,78%, EtOH – 8,84% PETETHR – 7,01% and acetone – 8,41% against *C. gestroi* and MeOH – 8,00%, EtOH – 7,68% PETETHR – 6,31% and acetone – 6,44% against *C. curvignathus* (Figure 3)] whereas *M. utilis* extracted blocks had the lowest weight loss [MeOH – 3,71%, EtOH – 3,52% PETETHR – 2,22% and acetone – 2,44% against *C. gestroi* and MeOH – 4,59% EtOH – 3,01% PETETHR – 2,40% and acetone – 1,98% against *C. curvignathus* (Figure 1).

In all cases, a significant effect was observed on the weight loss of wood blocks against *C. gestroi.* All solvents (MeOH, EtOH, acetone and PETETHR) lead to similar trends on the two termites tested (except *M. utilis* against *C. gestroi*), probably indicating the presence of the same molecules. Both species of termites also show a similar trend in wood weight loss. However, the performance of wood durability for each timber species depends on the type of solvent used. As reported by González-Laredo *et al.* (2015), both quantity and particularly the quality of extractives have a key role, but their relative contribution varies considerably from substrate to substrate. On the other hand, studies have shown that extracts of wood is the important factor that determine the durability of wood. This agreed with the study done by Roszaini and Hale (2012) on twelve species of tropical timbers against *C. curvignathus* and *C. gestroi*. They found that timbers with high extractive content had high termite resistance and species with lower extractive content showed poor performance. In addition, Lapornik *et al.* (2005) reported that different solvent extracts different chemical compound. The differences also could be due to the properties of the phenolic components of the plants concerned.

Antitermitic bioassay

The result of antitermitic activities of wood extract is depicted in Table 4, Table 5 and Table 6. The antitermite functions were dependent on the chemical composition of the wood extracts. Previous study indicates that *M. utilis* extracts under varied concentrations inhibit termite feeding. Similarly, *M. utilis* extracts showed the strongest anti-termitic activities against *C. gestroi* and *C. curvignathus* (Roszaini *et al.* 2014). It is evident from an earlier study that constituents of wood extracts could affect their anti-termite activity; some influenced greater potency while some others lower, e. g. Monoterpene hydrocarbon possessed lower anti-termite activity as compared with oxygenated constituents (Watanabe *et al*. 2005, Roszaini *et al.* 2014).

Table 4, Table 5 and Table 6 indicated that heartwood extracts of *M. utilis* had more anti-termitic activities against both subterranean termites; *C. gestroi and C. curvignathus* than *A. laevis* and *E. malaccensis*. The percentage of paper consumption was 1,01% (MeOH extracts), 1,37% (EtOH extracts), 1,92% (PETETHR extracts) and 1,68% (acetone extracts) at the concentration of 4% against *C. gestroi* while it was 1,15%; 2,24%; 2,11% and 1,72% respectively, for *A. laevis* and 1,66%; 2,34%; 2,38% and 2,27%; respectively, for *E. malaccensis.* The same trend (*M. utilis* extracts) also occurs on tests against *C. curvignathus*.

Table 4, Table 5 and Table 6 reveal that there was a significant increase in the number of termites in contact with the solvent control disc in comparison to the number of termites on the corresponding extract-treated disc ($P<0,05$; DF =5) for all wood extracts tested. The current study demonstrated that all four concentrations of the heartwood extract from three different timber species were less preferred and avoided by the both subterranean termite, *C. gestroi* and *C. curvignathus.* Lower percentage of paper consumption was obtained at the highest concentration (4%) of every solvent extracts compared to 0,5% of the minimum concentration, respectively. This trend is the same for both termite species

tested. According to the statistical analyses, lower concentration levels (0,5%) of all timber extracts resulted in significant reductions in weight loss when compared to the untreated controls.

Extracts from all timber tested strongly inhibited termite feeding against *C. gestroi* and *C. curvignathus* although *E. malaccensis* heartwood extracts were much less than *M. utilis* and *A. laevis* extracts. *M. utilis* heartwood extracts with MeOH solvents is the most active against both termite species at any level of concentration. At 0,5% level of concentration of any solvent used, *M. utilis* extracts inhibited ~ 1,5-fold (against *C. gestroi*) and ~ 1,3-fold (against *C. curvignathus*) than *A. laevis* the heartwood extracts and ~ 2,0-fold and ~ 2,3-fold than *E. malaccensis,* respectively. At the highest level of concentration (4,0%) also in any solvent used, *M. utilis* extracts inhibited ~ 1,3-fold (against *C. gestroi*) and ~ 1,5-fold (against *C. curvignathus*) than *A. laevis* heartwood extracts and ~ 1,8-fold and ~ 2,0-fold than *E. malaccensis,* respectively. The strong feeding inhibition from *M. utilis* heartwood extractives could be due to the higher value of monoterpenes and sesquiterpenes groups where both are interfering with basic behavioural functions of insects (Werner and Illmann 1994, Roszaini *et al.* 2014). As indicated by other studies, the presence of flavonoids (Ohmura *et al.* 2000, Wang *et al.* 2004) and quinones (Nacimento *et al.* 2013) which possess natural repellent and toxic properties will also increase the durability of the timber against termites.

Findings suggested that all wood extracts may produce larvicidal effects (behaving like general toxicants) against both termites; *C. gestroi* and *C. curvignathus* but depends on the solvents used. Laboratory bioassay of *M. utilis* against both subterranean workers showed that the LC_{50} value of *C. gestroi* was higher than *C. curvignathus* in all solvents used. Numerical LC_{50} values differed based on solvent used (MeOH>EtOH>Acetone>PETETHR). The LC_{50} of *M. utilis* heartwood MeOH extracts was 8,86% for *C. gestroi* and 8,51% for *C. curvignathus*, 9,17% and 8,98% for EtOH extracts 10,24% and 10,01% for PETETHR and 9,79% and 9,50% for acetone extracts, respectively (Table 4). Same trend also exists in *E. malaccensis* except with PETETHR solvents. The LC_{50} for *E. malaccensis* was 10,35% for *C. gestroi* and 9,88% for *C. curvignathus* when using MeOH as a solvent 11,42% and 10,85% for EtOH, 11,14% and 11,66 for PETETHR and 13,31% and 12,67% for acetone, respectively (Table 6). Contrarily, with *M. utilis and E. malaccensis*, the LC_{50} result of *A. leavis* heartwood extract against *C. curvignathus* was higher than *C. gestroi* (except when using MeOH solvents). The LC_{50} values of MeOH solvents were 9,79% for *C. gestroi* and 9,15% for *C. curvignathus*, 10,34% and 11,98% for EtOH, 11,52% and 11,88% for PETETHR and 9,82% and 10,11% for acetone, respectively (Table 5). The lowest of LC_{50} values of MeOH extracts followed by EtOH and acetone compared to PETETHR solvents could be to the phenolic content that they extracted. Phenolic content is one of the chemical constituents that influenced the rate of degradation. The higher the total phenolic content, the higher resistivity of the wood species. This means that only low concentrations necessary to turn off at least 50% of the number of termites (Shanbhag and Sundararaj 2013).

Table 4. Effect of *M. utilis* wood extracts on feeding and mortality of *C. gestroi* and *C. curvignathus.*

Treatment	Con. (%)	% paper consumption		% Feeding-Inhibition (FI%)		LC50 (%)	
		CG	*CC*	*CG*	*CC*	*CG*	*CC*
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Methanol		4,891 (0,66)[b]	4,282 (0,80)[b]				
	0,5	2,444 (0,38)[c]	2,533 (0,52)[c]	55,21 (2,39)[d]	59,88 (1,85)[d]	8,86[b]	8,51[b]
	1	1,992 (0,84)[d]	1,844 (0,22)[c]	68,44 (0,12)[c]	70,44 (0,88)[c]		
	2	1,787 (0,56)[d]	1,109 (0,19)[d]	76,94 (2,69)[b]	78,36 (0,11)[b]		
	3	1,554 (0,22)[de]	0,927 (0,22)[d]	79,52 (1,44)[ab]	85,64 (1,35)[ab]		
	4	1,012 (0,11)[e]	0,772 (0,45)[d]	82,38 (0,09)[a]	89,37 (1,35)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Ethanol		5,173 (0,27)[b]	5,007 (0,51)[b]				
	0,5	3,267 (1,17)[c]	2,863 (1,43)[c]	50,44 (0,22)[c]	52,22 (1,22)[c]	9,17[b]	8,98[b]
	1	3,014 (0,66)[e]	2,222 (0,05)[cd]	55,38 (0,08)[c]	57,37 (0,36)[c]		
	2	2,447 (1,37)[d]	1,970 (1,11)[d]	62,10 (2,28)[b]	70,18 (0,64)[b]		
	3	1,633 (2,21)[e]	1,818 (0,44)[de]	69,99 (0,08)[ab]	74,49 (1,22)[ab]		
	4	1,379 (0,07)[e]	1,220 (1,52)[e]	76,43 (0,09)[a]	78,27 (1,35)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Petroleum ether		5,487 (0,11)[b]	5,334 (1,24)[b]				
	0,5	4,111 (1,13)[c]	3,697 (0,07)[c]	46,62 (2,65)[c]	47,34 (0,08)[c]	10,24[a]	10,01[a]
	1	3.339 (0.59)[d]	3,018 (0,27)[cd]	50,11 (1,12)[bc]	50,98 (0,65)[bc]		
	2	2,445 (0,91)[e]	2,625 (0,18)[de]	55,33 (2,66)[b]	54,57 (0,22)[b]		
	3	2,008 (1,00)[e]	1,872 (0,66)[e]	68,21 (0,44)[a]	69,69 (2,64)[a]		
	4	1,927 (0,06)[e]	1,671 (0,83)[e]	70,58 (0,58)[a]	73,35 (3,22)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Acetone		5,551 (0,11)[b]	5,221 (0,03)[b]				
	0,5	3,512 (1,11)[c]	3,421 (0,22)[c]	48,33 (1,66)[c]	49,11 (1,46)[c]	9,79[ab]	9,50[a]
	1	3,017 (1,09)[cd]	2,990 (0,33)[cd]	50,22 (0,88)[c]	51,52 (0,06)[c]		
	2	2,455 (0,77)[d]	2,510 (0,52)[de]	54,87 (0,08)[c]	58,88 (1,22)[b]		
	3	2,383 (0,06)[d]	2,077 (0,47)[e]	62,01 (1,35)[b]	63,31 (1,64)[b]		
	4	1,682 (0,05)[e]	1,456 (0,01)[e]	75,37 (0,98)[a]	77,51 (1,64)[a]		

CG = *C. gestroi*, CC = *C. curvignathus*. Con. = Concentration. 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. LC_{50} = Lethal Concentration which causes a 50% reduction in feeding as compared to the non-treated control.

Table 5. Effect of *A. laevis* wood extracts on feeding and mortality of *C. gestroi* and *C. curvignathus.*

Treatment	Con. (%)	% paper consumption		% Feeding-Inhibition (FI%)		LC50 (%)	
		CG	*CC*	*CG*	*CC*	*CG*	*CC*
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Methanol		4891 (0,66)[b]	4,284 (0,80)[b]				
	0,5	4,050 (0,23)[b]	3,893 (0,59)[bc]	40,39 (2,56)[d]	45,33 (1,12)[c]		
	1	3,344 (0,08)[c]	3,342 (1,26)[c]	45,22 (1,13)[c]	50,01 (2,69)[b]		
	2	2,593 (0,22)[d]	3,017 (0,85)[c]	50,01 (1,14)[c]	52,33 (1,16)[b]	9,79[b]	9,15[c]
	3	1,875 (0,44)[d]	2,540 (2,36)[d]	57,35 (8,55)[b]	56,33 (2,25)[ab]		
	4	1,156 (0,22)[e]	1,245 (0,22)[e]	65,71 (3,69)[a]	60,38 (7,14)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Ethanol		5,174 (0,27)[b]	5,000 (0,51)[b]				
	0,5	4,508 (0,11)[b]	4,144 (0,03)[c]	35,62 (1,12)[c]	40,61 (2,44)[b]	10,34[ab]	11,98 [a]
	1	3,775 (0,67)[c]	3,69 (2,21)[cd]	48,33 (0,82)[b]	46,46 (0,97)[b]		
	2	2,924 (0,43)[cd]	3,110 (1,92)[d]	48,59 (0,07)[b]	52,38 (0,02)[a]		
	3	2,416 (0,52)[d]	2,598 (0,08)[d]	51,68 (0,65)[b]	56,66 (1,17)[a]		
	4	2,248 (0,09)[d]	1,875 (1,47)[e]	60,44 (2,87)[a]	58,53 (3,44)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Petroleum ether		5,484 (0,11)[b]	5,332 (1,24)[b]				
	0,5	4,965 (2,25)[b]	4,627 (0,81)[b]	32,28 (3,34)[d]	35,51 (1,80)[c]	11,52[a]	11,88[a]
	1	4,036 (1,28)[c]	3,882 (0,65)[c]	38,80 (0,44)[d]	40,01 (1,64)[bc]		
	2	3,773 (0,67)[c]	3,267 (2,22)[c]	43,45 (0,65)[c]	42,57 (1,22)[b]		
	3	2,995 (0,09)[cd]	2,393 (0,98)[cd]	47,31 (2,29)[b]	48,88 (0,88)[ab]		
	4	2,115 (0,88)[d]	1,821 (0,14)[d]	51,11 (3,12)[a]	52,21 (4,12)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Acetone		5,551 (0,11)[b]	5,228 (0,03)[b]				
	0,5	4,330 (2,27)[b]	3,982 (0,18)[b]	39,92 (0,47)[c]	37,74 (0,87)[c]	9,82[b]	10,11[b]
	1	4,010 (0,12)[c]	3,332 (0,53)[c]	42,22 (1,88)[bc]	46,22 (1,62)[b]		
	2	3,427 (0,04)[c]	2,763 (0,64)[c]	46,36 (1,01)[b]	48,88 (1,25)[b]		
	3	2,186 (0,66)[cd]	2,344 (0,15)[cd]	48,00 (2,22)[b]	52,22 (1,11)[ab]		
	4	1,722 (0,72)[d]	1,082 (0,22)[d]	56,81 (0,87)[a]	59,22 (3,39)[a]		

CG = *C. gestroi*, CC = *C. curvignathus*. Con. = Concentration. 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. LC_{50} = Lethal Concentration which causes a 50% reduction in feeding as compared to the non-treated control.

Table 6. Effect of *E. malaccensis* wood extracts on feeding and mortality of *C. gestroi* and *C. curvignathus.*

Treatment	Con. (%)	% paper consumption		% Feeding-Inhibition (FI%)		LC50 (%)	
		CG	*CC*	*CG*	*CC*	*CG*	*CC*
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Methanol		4,891 (0,66)[b]	4,288 (0,80)[b]				
	0,5	4,390 (0,64)[b]	4,641 (0,59)[b]	28,56 (3,36)[b]	29,33 (3,38)[b]	10,35[b]	9,88[b]
	1	3,664 (0,14)[b]	4,017 (1,14)[b]	30,00 (2,33)[b]	32,45 (1,14)[b]		
	2	3,033 (0,64)[bc]	3,010 (1,11)[bc]	36,84 (0,02)[ab]	35,64 (7,25)[ab]		
	3	2,672 (0,65)[bc]	2,883 (0,99)[bc]	42,11 (5,52)[a]	43,29 (2,26)[a]		
	4	1,667 (0,25)[c]	2,089 (0,06)[c]	48,88 (0,08)[a]	45,31 (1,17)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Ethanol		5,170 (0,27)[b]	5,009 (0,51)[b]				
	0,5	4,502 (0,11)[b]	4,144 (0,03)[b]	25,17 (3,25)[b]	23,48 (2,82)[b]	11,42[b]	10,85[b]
	1	3,773 (0,67)[b]	3,680 (2,21)[b]	29,99 (1,42)[b]	27,55 (2,02)[b]		
	2	2,929 (0,43)[bc]	3,110 (1,92)[bc]	30,14 (0,56)[ab]	29,65 (0,64)[ab]		
	3	2,592 (0,08)[bc]	2,598 (0,08)[bc]	40,01 (0,08)[a]	30,63 (0,11)[a]		
	4	2,343 (1,47)[c]	2,016 (1,47)[c]	42,44 (1,22)[a]	41,77 (0,35)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Petroleum ether		5,487 (0,11)[b]	5,330 (1,24)[b]				
	0,5	4,482 (0,12)[b]	4,732 (2,98)[b]	23,67 (1,28)[b]	24,99 (1,69)[b]	11,14[b]	11,66[a]
	1	3,471 (2,64)[b]	4,014 (3,56)[b]	27,27 (3,44)[b]	29,25 (2,78)[b]		
	2	3,013 (1,89)[bc]	3,472 (0,44)[bc]	31,02 (1,36)[ab]	31,04 (0,66)[ab]		
	3	2,668 (0,06)[bc]	3,111 (0,63)[bc]	35,64 (1,78)[a]	31,98 (0,08)[a]		
	4	2,381 (1,45)[c]	2,521 (1,17)[c]	38,20 (0,88)[a]	32,11 (1,22)[a]		
Control		6,382 (0,35)[a]	6,015 (0,46)[a]				
Acetone		5,555 (0,11)[b]	5,222 (0,03)[b]				
	0,5	4,423 (0,03)[b]	4,670 (0,11)[b]	28,44 (1,26)[b]	26,62 (0,66)[b]	13,31[a]	12,67[a]
	1	3,212 (1,38)[b]	3,880 (0,07)[b]	29,98 (3,24)[b]	29,65 (0,98)[b]		
	2	2,885 (2,20)[bc]	3,111 (1,25)[bc]	33,19 (2,64)[ab]	32,46 (0,44)[ab]		
	3	2,452 (1,21)[bc]	2,623 (1,48)[bc]	38,57 (0,02)[a]	37,23 (0,22)[a]		
	4	2,278 (0,08)[c]	2,484 (1,14)[c]	46,33 (0,84)[a]	40,04 (0,74)[a]		

CG = *C. gestroi*, CC = *C. curvignathus*. Con. = Concentration. 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. LC_{50} = Lethal Concentration which causes a 50% reduction in feeding as compared to the non-treated control.

On the other hand, the LC_{50} values which is an appropriate measure for determining the toxicity of a chemical has always been questioned before. However, it is a good basis for a preliminary assessment to determine the potential risk of a compound under conditions specified and also it gives an idea to generate on the order of magnitude of the lethal concentration (Duffus 1980).

CONCLUSIONS

This study shows that the solvents influence the yield and wood extracts properties against subterranean termites; *C. curvignathus* and *C. gestroi*. MeOH solvent had higher extraction yields in every timber species tested. The MeOH extraction increases the anti-termite activity than EtOH, PETETHR and acetone extraction. A comparative study on field trials is recommended to ascertain the respective needed dose of the extracts. Studies should also be conducted to characterize the chemical compound that causes the durability of a timber against subterranean termites.

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