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Evaluation of the toxicity and repellency of tropical plant extract against subterranean termites, *Globitermes sulphureus* and *Coptotermes gestroi*

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ABSTRACT

The harmful effects of chemical-based termiticides and the increased incidence of termite resistance have resulted in the need for safer and more effective termiticides. Therefore, the screening of antitermiticidal activity of naturally-occurring products could possibly hamper an alternative means in termite control strategies. The aims of this study were to determine the toxicity and repellency of *L. leucocephala*, *A. paniculata*, *Az. indica* and *P. niruri* crude extracts against two subterranean termites, *G. sulphureus* and *C. gestroi*. Bioassays were conducted by applying varying concentrations of the plant extracts (10,000 ppm, 5000 ppm and 500 ppm) on both termite species under laboratory conditions. All extracts exhibited a significant antitermiticidal activity in time- and concentration-dependent manners after 14 days of exposure. The highest mortality of *G. sulpureus* and *C. gestroi* were noted in all methanolic extracts of *P. niruri*, *L. leucocephala*, *A. paniculata*, *Az. indica* at 10,000 ppm. High repellent activity was also noted in the choice bioassay when both termites were treated with all methanolic extracts at 10,000 ppm.

1. Introduction

Globitermes sulphureus and *C. gestroi* are common subterranean termites that can easily be found in building structures in rural and suburban areas [1–3]. Both species are destructive insect pests, attacking household materials, finished goods and various agricultural crops e.g. sugarcane, millet, barley and paddy [4]. Members of *G. sulphureus* are secondary invaders, of which they infest the treated premises that have been previously infested by *C. gestroi* [5]. Due to their infestations, billions of dollars are spent annually on control and prevention measures worldwide [6]. In 2003, more than USD 10 million were approximately spent for termite control in Malaysia with the cost of total repair was 3–4 times higher [7]. The condition worsens following the overuse of chemical insecticides as termite control tools has resulted in the public's concern on environmental and health issues [8], causing further difficulties in controlling termite infestation. Resistance in the treated pests, residue problem, lethal

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effects on non-target organisms, and increased cost of insecticide application are among disadvantages of using chemical insecticides to control the termites [9].

In the past, synthetic insecticides such as DDT, aldrin, BHC and dieldrin were generally used to provide a longer protection from termite infestation [4]. However, these insecticides have been banned in many countries as their residuals have negatively affected the environment [4]. To date, a myriad of studies are conducted to test the efficacy and safety of plant-based pesticides to develop a novel means to control termites. Plant-based pesticides interfere the feeding, growth, reproduction, and mating behavior [10]. Plant species such as the leaf of *Lantana camara*, the rhizome of *Alpinia galangal*, the seed and leaf of *Azadirachta indica, Jatropha curcas, Maesa laceolata, Chenapodium ambrosoids* and *Vernonia hymenolepis* have been previously reported as promising candidates in termite control studies [11–13].

Furthermore, naturally-occurring pesticides from plants, bacteria, animals and minerals usually degrade in a short period of time [14], hence are safer for environments. Among these, plants are the most common source of biopesticides as the production of secondary metabolites by the plants are not directly involved in physiological or bio-chemical process [15].

Tropical plants are known to have a wide array of chemical compounds that act as natural insecticides. Some plants possess repellent effects by keeping the insects away from the crops due to the smell or taste, while some act on oviposition behavior of insects by preventing them laying their eggs. The plants may also act as antifeedants and inhibitors that promote the suppression of calling behavior and growth [16]. Considering the enormous reports on tropical plants as promising biopesticides, the objectives of this present study were therefore to evaluate the toxicity and repellency of crude extracts of four tropical plants, namely *Leucaena leucocephala, Andrographis paniculata, Azadirachta indica* and *Phyllanthus niruri* on *G. sulphureus* and *C. gestroi* under laboratory conditions.

2. Materials and methods

2.1. Termite collection

Globitermes sulphureus and *C. gestroi* were collected and established in the Universiti Sains Malayisa (USM), Main Campus and Teluk Bahang, Pulau Pinang. The underground monitoring station for both termite species was filled with pine stakes (*Pinus caribaea*) (2.5 cm × 2.5 cm × 15 cm) as their food source. They were placed in hollow plastic containers (16 cm × 18 cm) and were buried in the soil [12].

Depending on the colony activity, the infested stakes were brought to the Household and Structural Urban Entomology Laboratory, School of Biological Sciences, USM after 7–14 days for further analysis. Termites were transferred into a plastic container covered with black plastic and were kept in the dark along with the soil and wood as foods. They were reared at a room temperature (28 ± 2 °C) with high humidity (RH 70% ± 10) and were subsequently identified based on their morphological characteristics and molecular techniques. Prior to experimental procedures, the termites were separated from debris using the bridging method by allowing them to access five stacks of pre-wetted pine blocks (20 cm × 10 cm) [12,13]. Then, the termites were counted and transferred to a plastic Petri dish (90 mm × 15 mm, Ideal Healthcare, Malaysia) lined with a moistened filter paper (90 mm, Advantec, Japan).

2.2. Plant collection

Four tropical plants, namely *Leucaena leucocephala* (Lam.) de Wit, *Andrographis paniculata* (Burm. f.) Wall. ex Nees, *Azadirachta indica* A. Juss and *Phyllanthus niruri* Linnaeus were acquired in a dried form from Herbagus Sdn. Bhd., MALAYSIA. Only leaf part was used for extraction except for *P. niruri*, of which the whole parts of this plant were used (Table 1).

2.3. Plant extraction

2.3.1. Soxhlet extraction

Soxhlet extraction [17] was employed to obtain crude plant extracts with a slight modification. At first, 30 g of each dried plant were loaded in the thimble. The round bottom flask was then filled with 150 ml of methanol or hexane as a solvent and was heated using an isomantle. The solvent was evaporated through the soxhlet apparatus. This process continued for 20–30 cycles for 4–6 h at 110–130 °C until the solvent completely evaporated and condensed. Plant extracts were continuously evaporated to dryness by using a rotary evaporator. The concentrated extracts were further evaporated in the oven at 80 °C for 48 h and were stored in the freezer at 4 °C for further analysis.

2.3.2. Maceration

The maceration method followed a previous study [18] with a slight modification. Each dried plant sample (30 g) was extracted with 180 ml of distilled water for 24 h. The plant material was mixed with distilled water and frequently shaken using an orbital shaker during the first six hours and was allowed to stand for another 18 h. All extracts were filtered through the Whatmann filter paper and were

Table 1 – Selected plants for crude extraction for developing termite bio-pesticide.

Botanical name	Family	Common name	Plant part
Andrographis paniculata (Burm. f.) Wall. ex Nees	Acanthaceae	Hempedu bumi	Leaves
Azadirachta indica A. Juss	Meliaceae	Mambu/Semambu	Leaves
Leucaena leucocephala (Lam.) de Wit	Fabaceae	Petai belalang	Leaves
Phyllanthus niruri Linnaeus	Phyllanthaceae	Dukung anak	Whole plant

concentrated by using a rotary evaporator. The obtained extracts were kept at 4 °C.

2.3.3. *Plant bio-pesticide solutions*

All extracts were separately re-dissolved using three different solvents i.e. methanol, hexane and water to obtain concentrations of 10,000 ppm, 5000 ppm and 500 ppm based on the equation below,

$$M_1V_1 = M_2V_2$$

where

M_1: the concentration of the stock solution,
V_1: the volume of stock solution,
M_2: the concentration of the dilute solution,
V_2: the volume of dilute solution.

2.4. *Preparation of treated and control filter papers*

Filter papers (90 mm, Advantec, Japan) were soaked with 30 ml of the plant extracts and were left overnight. Filter papers treated with blank solvents were used as controls. All filter papers were air-dried in a fume hood for 1 h.

2.5. *No-choice bioassay*

The no-choice bioassay in this study was a slightly modified method from a previous report [19]. Six Petri dishes (90 × 15 mm, Ideal Healthcare, Malaysia) were previously filled with 25 g of sterile beach sand (moistened with distilled water). Then, the treated filter papers (7 cm in diameter) were placed in the Petri dishes containing the sand. Each plant extraction treatment was prepared in three replicates.

A 3rd instar of termites were used in the bioassay. A total of 50 workers and 2 soldiers were directly introduced in the middle of the Petri dishes. A few drops of water were periodically added to the bottom edge of each Petri dish to provide moisture. All Petri dishes were placed in an incubator and maintained in darkness at 26 ± 2 °C with 65 ± 5% relative humidity. The mortality of the termites was recorded every 24 h for 14 days. All dead and moribund termites were removed from each Petri dish during the counting. The percentage of termite mortality was corrected using Abbott's formula and arcsine was transformed before the statistical analysis. Correction for control mortality by using the Abbott formula [20].

$$P = \frac{Po - Pc}{100 - Pc} \times 100$$

where

P: corrected mortality (%).
Po: observed mortality (%).
Pc: control mortality (%).

2.6. *Choice bioassay*

The choice bioassay method used in this study was a slightly modified version of the previous study [19]. The experimental procedures was similar to that of no-choice bioassay except the Petri dishes were filled with both treated and untreated (solvent only) filter papers. Each plant extraction treatment was prepared in three replicates.

Repellent assessment [number of termites present in control (N_c) and the treated half (N_t)] was observed for a period of 72 h. The percentage of the repellent was calculated according to the equation [11].

$$\text{Percentage of Repellency} = [(Nc - Nt)/(Nc + Nt)] \times 100$$

2.7. *Statistical analysis*

A factorial analysis of variance (ANOVA) was performed using IBM SPSS Statistics Version 22 to determine the effect of four variables i.e. concentration, species, solvents, and plant on termite mortality. The Kruskal-Wallis H analysis and Man Whitney test at $P < 0.05$ were performed to assess significant differences between the treatment groups. For no-choice bioassay, the mortality and data consumption were analyzed using Two-way ANOVA and Tukey's test at $P < 0.05$.

3. Results

3.1. *Efficacy of plant extracts against* G. Sulphureus *and* C. Gestroi *through no-choice bioassay*

The extracts showed termiticidal activity against *G. sulphureus* and *C. gestroi* under laboratory conditions. High mortality was recorded when methanolic plant extracts were used, followed by hexane and water extracts (Tables 2 and 3). Increased percentage of termite mortality was noted when the concentrations of methanolic extract increased (Fig. 1). A 100% mortality in *C. gestroi* and more than 70% in *G. sulphureus* were observed when the highest concentration (10,000 ppm) of methanolic extract was applied.

Overall, there were significant differences in termiticidal effects between termite species, solvent, concentration and

Table 2 – Mortality activity of different solvents crude extracts of plants against *C. gestroi* after 14days.

Solvent	*P. niruri*	*A. paniculata*	*A. indica*	*L. leucocephala*
Methanol	100.00 ± 0.00[b]	100.00 ± 0.00[b]	100.00 ± 0.00[c]	100.00 ± 0.00[c]
Hexane	80.25 ± 8.25[b]	32.65 ± 9.42[a]	48.20 ± 10.49[b]	44.22 ± 11.04[b]
Water	3.97 ± 2.63[a]	22.32 ± 6.53[a]	18.93 ± 2.86[a]	8.72 ± 5.86[a]

Mean followed by different letters within the same column are significantly different (Tukey HSD; $P < 0.05$).

Table 3 – Mortality activity of different solvents crude extracts of plants against *G. sulphureus* after 14days.

Solvent	*P. niruri*	*A. paniculata*	*A. indica*	*L. leucocephala*
Methanol	89.31 ± 4.33[c]	86.53 ± 5.1[b]	41.67 ± 7.76[a]	40.52 ± 7.48[a]
Hexane	63.43 ± 9.24[b]	55.22 ± 10.92[a]	75.65 ± 9.94[b]	44.71 ± 7.13[a]
Water	16.73 ± 2.67[a]	54.65 ± 6.64[a]	47.49 ± 6.84[a]	13.61 ± 2.82[b]

Mean followed by different letters within the same column are significantly different (Tukey HSD; $P < 0.05$).

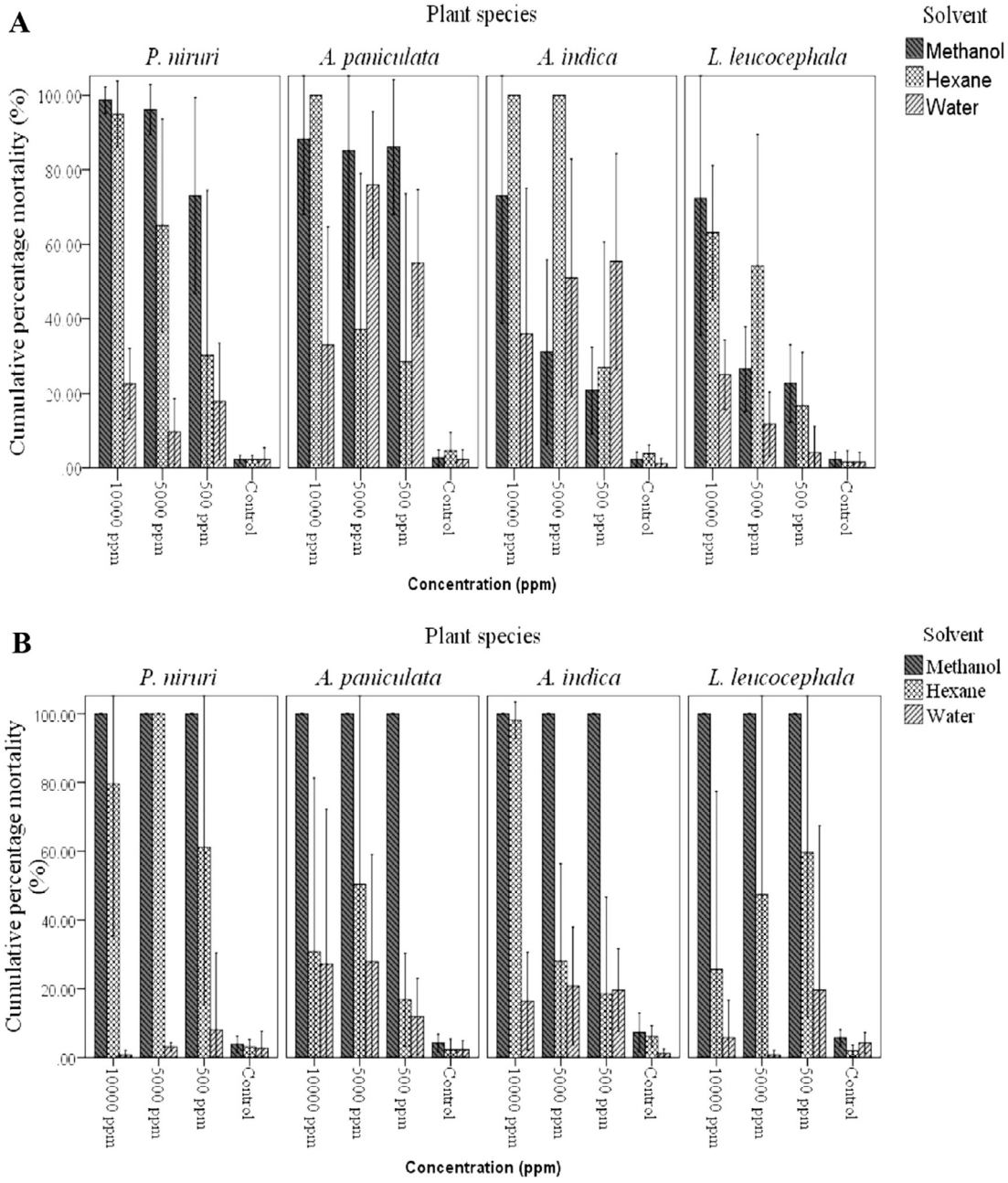

Fig. 1 – Percentage mean mortality of, (A) *G. sulphureus* and; (B) *C. gestroi* in response to different plant extracts after 14days.

plant species ($F (18, 382) = 3.783$, $p < 0.0001$). Termite mortality was significantly high in methanolic extracts of all plant samples ($F (2, 382) = 237.756$, $p < 0.0001$; Table 4). Plant extracts also revealed a significant difference between each other ($F (3, 382) = 13.003$, $p < 0.0001$; Table 4). The plant species, solvent and concentration significantly affected the mortality of

Table 4 – Analysis of variance on termite mortality comparing different concentrations, termite species, solvents and plants used.

Source of variation	df	Mean Square	F-value	P-value
Termite species	1	556.925	1.671	0.197
Plant species	3	4334.673	13.003	**0.000**
Concentration	3	83234.545	249.693	**0.000**
Solvent	2	79255.293	237.756	**0.000**
Termite species * Plant species	3	3036.370	9.109	**0.000**
Termite species * Concentration	3	3135.133	9.405	**0.000**
Termite species * Solvent	2	19327.139	57.979	**0.000**
Plant species * Concentration	9	955.976	2.868	**0.003**
Plant species * Solvent	6	4342.088	13.026	**0.000**
Concentration * Solvent	6	11848.937	35.545	**0.000**
Termite species * Plant species * Concentration	9	1163.677	3.491	**0.000**
Termite species * Plant species * Solvent	6	2275.211	6.825	**0.000**
Termite species * Concentration * Solvent	6	2995.444	8.986	**0.000**
Plant species * Concentration * Solvent	18	1404.750	4.214	**0.000**
Termite species * Plant species * Concentration * Solvent	18	1261.079	3.783	**0.000**

df, degree of freedom; MS, mean-squared value. Significant values are given in bold.
* Interaction between the parameters measured.

G. sulphureus (F (18, 192) = 5.090, p < 0.0001). Statistical analysis revealed a significant difference between the solvent used on *G. sulphureus* mortality (F (2, 192) = 44.547, p < 0.0001), where all methanolic plant extracts were highly effective compared to other solvents. However, the effect of the plant extracts on *G. sulphureus* was not significantly different when used against *C. gestroi* (F (1, 382) = 1.671, p = 0.197; Table 4). A similar result was obtained in *C. gestroi* where the plant species, solvent and concentration also significantly affected their mortality (F (18, 192) = 3.177, p < 0.0001). Plants extracted with methanol significantly caused 100% mortality at all concentrations (F (2, 192) = 234.331, p < 0.0001) and were also highly effective than the other solvents.

3.2. *Repellency test of different crude plant extracts through choice bioassay*

Repellency of the different plant extracts on *G. sulphureus* and *C. gestroi* are presented in Tables 5 and 6. All methanolic plant extracts were strongly repellent toward both termites at all concentrations during three days of observation. Hexane and water extracts appeared to be repellent at least after one dose, irrespective of the concentrations.

Repellent activities of all plant extracts did not show any significant difference on *C. gestroi* after three days of exposure (F (6, 168) = 1.027, p = 0.054). In contrast, the repellent activities of plants extracts displayed a significant difference on *G. sulphureus* after three days of exposure (F (6, 168) = 2.806, p = 0.013). One-way ANOVA indicated that the repellent activity of *G. sulphureus* from methanolic plant extracts was significantly higher than water and hexane plant extracts, F (2, 177) = 7.163, p = 0.001. In spite of that, there was no significant different on the repellent activity among methanolic plant extracts, F (3, 56) = 0.900, p = 0.447.

Plant species, solvent and concentration significantly affected the mortality of *G. sulphureus*, F (12, 144) = 3.554, p = 0.000. However, all plant extracts exhibited low mortality rates (<30%). The highest mortality was 21.53% when *G. sulphureus* was treated with *A. paniculata* extracted with water at 10,000 ppm. Similar observations were noted in *C. gestroi*, of which the plant species, solvent and concentration significantly affected the mortality rate of this termite species, F (12, 144) = 3.649, p < 0.0001 (Figs. 2 and 3). Among the hexane plant extracts, *Az. indica* showed higher termite mortality and repellency at all concentrations compared to other plants. Methanolic extracts of *P. niruri* and *A. paniculata* also showed high termite mortality and repellency.

4. Discussion

This study demonstrated the biocontrol potential of four crude plant extracts i.e. *P. niruri*. *A. paniculata*, *Az. indica* and *L. leucocephala* against *G. sulphureus* and *C. gestroi* under laboratory conditions. Numerous plants are reported to possess a biological activity against different insects and other organisms [21]. For instance, an evaluation of the toxicity of polar and non-polar extracts of *Milletia ferruginea* seed at different concentration levels has been previously done on different castes of adult *Macrotermes* sp. and *Pachnoda interrupta*. The extracts were also compared with other plant extracts and carbaryl, a standard insecticide used in pest control [22]. Another study demonstrated eight plant species extracted with four different solvents (hexane, ethyl acetate, acetone and methanol) has revealed a promising significant antitermiticidal activity against *Coptotermes formosanus* after 24 h and 48 h of exposure [4].

The effectiveness of any plant extract mainly depends on concentrations and types of extraction solvents [23]. In this study, methanol, hexane and water were used as solvents as they had different polarity indexes of 5.1, 0.1 and 10.2, respectively. In general, the solvents diffuse into solid plant materials and solubilize compounds of similar polarity during the extraction [24]. Adding to this, polar solvents would extract polar molecules while non-polar solvents extract non-polar molecules [25]. In the present study, the bioactivity of the plant extracts varied significantly according to the

Table 5 – Mean percentage repellency of termites by different plant extracted with different solvent in *C. gestroi*.

Solvent	Plant	Concentration (ppm)	Time exposure		
			24 h	48 h	72 h
Methanol	*P. niruri*	500 ppm	60.00 ± 24.49[a]	80.00 ± 20.00[a]	40.00 ± 24.49[ab]
		5000 ppm	40.00 ± 24.49[a]	0.00 ± 0.00[a]	40.00 ± 24.49[ab]
		10,000 ppm	38.67 ± 19.02[a]	80.00 ± 20.00[a]	80.00 ± 6.32[a]
		Control	18.52 ± 36.48[a]	26.67 ± 37.12[a]	−50.00 ± 28.87[b]
	A. paniculata	500 ppm	60.00 ± 24.49[a]	80.00 ± 20.00[a]	80.00 ± 20.00[a]
		5000 ppm	80.00 ± 20.00[a]	53.33 ± 29.06[a]	49.52 ± 25.98[a]
		10,000 ppm	60.00 ± 24.49[a]	22.22 ± 27.44[a]	32.38 ± 32.38[a]
		Control	0.00 ± 0.00[a]	−22.22 ± 40.06[a]	−3.70 ± 57.85[a]
	A. indica	500 ppm	95.56 ± 4.44[a]	58.79 ± 24.03[a]	80.00 ± 20.00[a]
		5000 ppm	46.67 ± 22.61[a]	20.00 ± 48.99[a]	60.00 ± 40.00[a]
		10,000 ppm	86.67 ± 13.33[a]	80.00 ± 20.00[a]	74.00 ± 16.61[a]
		Control	−22.22 ± 22.22[b]	0.00 ± 0.00[a]	77.78 ± 22.22[a]
	L. leucocephala	500 ppm	60.00 ± 24.49[a]	40.00 ± 24.49[a]	60.00 ± 24.49[a]
		5000 ppm	34.29 ± 38.23[a]	40.00 ± 24.49[a]	24.00 ± 37.09[a]
		10,000 ppm	72.86 ± 19.48[a]	81.67 ± 13.02[a]	15.38 ± 38.31[a]
		Control	25.93 ± 37.59[a]	33.33 ± 33.33[a]	0.00 ± 57.74[a]
Hexane	*P. niruri*	500 ppm	−30.00 ± 22.61[a]	33.33 ± 42.16[a]	46.67 ± 22.61[a]
		5000 ppm	−9.94 ± 39.41[a]	13.36 ± 40.07[a]	60.00 ± 40.00[a]
		10,000 ppm	3.23 ± 33.66[a]	43.24 ± 29.33[a]	65.00 ± 35.00[a]
		Control	33.33 ± 33.33[a]	53.33 ± 29.06[a]	−25.00 ± 62.92[a]
	A. paniculata	500 ppm	−4.00 ± 44.90[a]	−15.26 ± 44.82[a]	33.33 ± 42.16[a]
		5000 ppm	90.00 ± 10.00[a]	−23.33 ± 39.30[a]	32.57 ± 36.70[a]
		10,000 ppm	−46.67 ± 38.87[a]	−81.14 ± 15.53[a]	36.67 ± 36.67[a]
		Control	0.00 ± 57.74[a]	−8.10 ± 54.94[a]	−25.19 ± 62.60[a]
	A. indica	500 ppm	44.00 ± 39.19[a]	52.00 ± 38.78[a]	20.95 ± 34.65[a]
		5000 ppm	14.67 ± 31.65[a]	70.00 ± 20.00[a]	63.09 ± 13.81[a]
		10,000 ppm	100.00 ± 0.00[a]	85.09 ± 10.64[a]	85.65 ± 7.44[a]
		Control	0.00 ± 57.74[a]	31.48 ± 57.76[a]	−2.90 ± 55.24[a]
	L. leucocephala	500 ppm	28.00 ± 25.07[a]	9.32 ± 27.89[a]	−52.22 ± 28.31[ab]
		5000 ppm	64.00 ± 22.27[a]	42.33 ± 19.22[a]	33.33 ± 32.06[b]
		10,000 ppm	−32.00 ± 37.74[a]	−45.76 ± 16.14[a]	−61.73 ± 13.27[a]
		Control	40.94 ± 26.11[a]	33.33 ± 66.67[a]	66.67 ± 33.33[b]
Water	*P. niruri*	500 ppm	35.00 ± 41.53[a]	1.76 ± 34.77[a]	−10.00 ± 33.17[a]
		5000 ppm	35.00 ± 38.41[a]	6.67 ± 45.22[a]	71.52 ± 18.54[a]
		10,000 ppm	16.24 ± 41.22[a]	44.29 ± 37.28[a]	2.22 ± 2.22[a]
		Control	−10.65 ± 26.62[a]	35.87 ± 43.95[a]	51.39 ± 42.51[a]
	A. paniculata	500 ppm	33.56 ± 37.80[a]	8.00 ± 45.43[a]	46.63 ± 29.19[a]
		5000 ppm	25.19 ± 36.30[a]	58.85 ± 21.54[a]	73.04 ± 17.85[a]
		10,000 ppm	37.26 ± 27.93[a]	59.90 ± 18.60[a]	20.00 ± 48.99[a]
		Control	−69.23 ± 30.77[a]	−17.65 ± 50.84[a]	−42.86 ± 54.78[a]
	A. indica	500 ppm	40.00 ± 40.00[a]	46.00 ± 10.93[a]	54.51 ± 28.54[ab]
		5000 ppm	96.92 ± 3.08[a]	70.00 ± 8.94[b]	100.00 ± 0.00[b]
		10,000 ppm	100.00 ± 0.00[a]	20.00 ± 6.32[ab]	100.00 ± 0.00[b]
		Control	44.44 ± 29.40[a]	5.56 ± 27.78[a]	−11.36 ± 7.31[a]
	L. leucocephala	500 ppm	21.23 ± 24.99[ab]	36.15 ± 38.72[a]	68.70 ± 31.30[a]
		5000 ppm	35.00 ± 26.93[ab]	−4.44 ± 4.44[a]	60.00 ± 40.00[a]
		10,000 ppm	−70.00 ± 20.00[a]	−29.78 ± 36.28[a]	24.38 ± 27.26[a]
		Control	66.67 ± 33.33[b]	66.67 ± 33.33[a]	−66.67 ± 33.33[a]

Means followed by different letters within the same column for each plant are significantly different (Kruskal-Wallis multiple Range Test; $P < 0.05$).
Negative sign showed no repellent activity.

extraction solvents and the termite species of which plants extracted with methanol showed a better termiticidal activity compared to those extracted with hexane and water. In the no-choice bioassay, methanolic extract of *P. niruri* showed a greater termiticidal activity compared to the other three plant extracts (*A. paniculata*, *Az. indica*, *L. leucocephala*) used in this study. In addition, *P. niruri* showed a great mortality percentage of both termite species compared to water and hexane. Methanolic extract of *P. niruri* contains a large number of compounds [26]. A total phenolic and flavonoid contents were higher in methanolic extract of *P. niruri* compared to water extract due to the different polarity of the solvents [27]. In insects, several classes of phytochemicals, including the flavonoids, interfere with molting, reproduction, feeding, and

Table 6 – Mean percentage repellency of termites by different plant extracted with different solvent in *G. sulphureus*.

Solvent	Plant	Concentration (ppm)	Time exposure		
			24 h	48 h	72 h
Methanol	*P. niruri*	500 ppm	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}
		5000 ppm	73.33 ± 19.44^{b}	80.00 ± 20.00^{b}	100.00 ± 0.00^{b}
		10,000 ppm	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}	97.50 ± 2.50^{b}
		Control	−19.71 ± 27.07^{a}	11.52 ± 36.67^{a}	6.35 ± 14.11^{a}
	A. paniculata	500 ppm	83.59 ± 12.91^{b}	100.00 ± 0.00^{b}	100.00 ± 0.00^{a}
		5000 ppm	40.00 ± 40.00^{ab}	56.00 ± 27.13^{b}	76.00 ± 24.00^{a}
		10,000 ppm	100.00 ± 0.00^{b}	100.00 ± 0.00^{b}	80.00 ± 20.00^{a}
		Control	−19.44 ± 10.02^{a}	−24.79 ± 21.06^{a}	33.33 ± 38.49^{a}
	A. indica	500 ppm	50.00 ± 22.36^{b}	46.67 ± 38.87^{a}	72.00 ± 19.60^{b}
		5000 ppm	85.00 ± 15.00^{b}	0.00 ± 44.72^{a}	100.00 ± 0.00^{b}
		10,000 ppm	100.00 ± 0.00^{b}	94.29 ± 5.71^{a}	100.00 ± 0.00^{b}
		Control	−44.44 ± 29.40^{a}	−31.11 ± 32.28^{a}	−15.87 ± 28.88^{a}
	L. leucocephala	500 ppm	16.67 ± 33.33^{a}	80.00 ± 20.00^{a}	73.33 ± 26.67^{a}
		5000 ppm	46.67 ± 22.61^{a}	80.00 ± 20.00^{a}	90.00 ± 10.00^{a}
		10,000 ppm	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	80.00 ± 20.00^{a}
		Control	−14.14 ± 48.18^{a}	44.44 ± 29.40^{a}	−11.11 ± 58.79^{a}
Hexane	*P. niruri*	500 ppm	−2.44 ± 27.12^{a}	4.57 ± 37.64^{a}	−1.99 ± 32.69^{a}
		5000 ppm	−17.58 ± 28.84^{a}	39.45 ± 23.94^{a}	61.37 ± 17.21^{a}
		10,000 ppm	73.33 ± 19.44^{a}	100.00 ± 0.00^{a}	61.21 ± 25.61^{a}
		Control	7.26 ± 28.42^{a}	62.50 ± 31.46^{a}	33.33 ± 33.33^{a}
	A. paniculata	500 ppm	40.83 ± 10.41^{a}	0.84 ± 29.53^{a}	35.50 ± 20.59^{a}
		5000 ppm	2.59 ± 35.67^{a}	51.52 ± 14.39^{a}	64.04 ± 16.96^{a}
		10,000 ppm	33.33 ± 18.26^{a}	80.00 ± 20.00^{a}	51.31 ± 21.24^{a}
		Control	−4.12 ± 48.06^{a}	−8.89 ± 27.31^{a}	0.00 ± 0.00^{a}
	A. indica	500 ppm	70.83 ± 26.15^{a}	86.67 ± 8.16^{b}	90.00 ± 10.00^{b}
		5000 ppm	100.00 ± 0.00^{a}	93.33 ± 6.67^{b}	92.00 ± 8.00^{b}
		10,000 ppm	100.00 ± 0.00^{a}	93.33 ± 6.67^{b}	100.00 ± 0.00^{b}
		Control	36.39 ± 20.66^{a}	6.67 ± 6.67^{a}	6.67 ± 6.67^{a}
	L. leucocephala	500 ppm	71.67 ± 17.40^{b}	65.33 ± 14.97^{a}	78.67 ± 13.73^{a}
		5000 ppm	23.67 ± 11.26^{ab}	52.98 ± 9.74^{a}	52.43 ± 11.79^{a}
		10,000 ppm	72.98 ± 9.22^{b}	42.89 ± 15.71^{a}	91.79 ± 5.64^{a}
		Control	1.15 ± 1.15^{a}	53.33 ± 29.06^{a}	33.33 ± 33.33^{a}
Water	*P. niruri*	500 ppm	−2.44 ± 27.12^{a}	4.57 ± 37.64^{a}	−1.99 ± 32.69^{a}
		5000 ppm	−17.58 ± 28.84^{a}	39.45 ± 23.94^{a}	61.37 ± 17.21^{a}
		10,000 ppm	73.33 ± 19.44^{a}	100.00 ± 0.00^{a}	61.21 ± 25.61^{a}
		Control	7.26 ± 28.42^{a}	62.50 ± 31.46^{a}	33.33 ± 33.33^{a}
	A. paniculata	500 ppm	40.83 ± 10.41^{a}	0.84 ± 29.53^{a}	35.50 ± 20.59^{a}
		5000 ppm	2.59 ± 35.67^{a}	51.52 ± 14.39^{a}	64.04 ± 16.96^{a}
		10,000 ppm	33.33 ± 18.26^{a}	80.00 ± 20.00^{a}	51.31 ± 21.24^{a}
		Control	−4.12 ± 48.06^{a}	−8.89 ± 27.31^{a}	0.00 ± 0.00^{a}
	A. indica	500 ppm	70.83 ± 12.03^{ab}	86.67 ± 2.11^{b}	90.00 ± 4.47^{b}
		5000 ppm	100.00 ± 0.00^{b}	93.33 ± 1.83^{b}	92.00 ± 2.00^{b}
		10,000 ppm	100.00 ± 0.00^{b}	93.33 ± 1.83^{b}	100.00 ± 0.00^{b}
		Control	27.16 ± 27.87^{a}	−31.43 ± 35.69^{a}	−4.44 ± 15.56^{a}
	L. leucocephala	500 ppm	71.67 ± 15.25^{a}	65.33 ± 4.39^{a}	78.67 ± 9.58^{ab}
		5000 ppm	23.67 ± 4.62^{a}	52.98 ± 4.35^{a}	52.43 ± 6.17^{ab}
		10,000 ppm	72.98 ± 4.46^{a}	42.89 ± 5.30^{a}	91.79 ± 8.21^{b}
		Control	16.95 ± 40.21^{a}	42.22 ± 39.50^{a}	22.22 ± 40.06^{a}

Means followed by different letters within the same column for each plant are significantly different (Kruskal-Wallis multiple Range Test; $P < 0.05$).
Negative sign showed no repellency activity.

behavior [28–30]. A previous study on ethanolic extract of another species from the genus *Phyllanthus* i.e. *P. amarus* has reported that the extract required 140 min to cause more than 90% mortality in a topical bioassay using *Macrotermes bellicosus* [30].

Repellent activity was observed in methanolic and hexane extracts of *L. leucocephala* but none in *L. leucocephala* extracted with water. These results indicated that the solvents used in this study had a different efficacy performance against the termites.

Plants with repellent properties possess a minimum negative impact on the environment as they deter the pests by stimulating their sensory organs before invading the plants [31]. Many studies have reported that application of plant

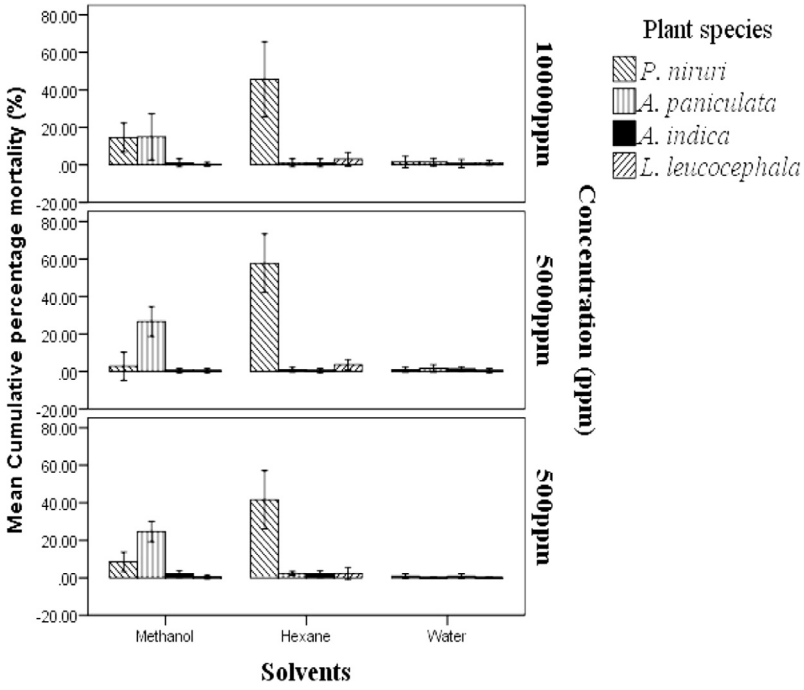

Fig. 2 – Percentage mean mortality of *C. gestroi* from repellency test in response to different plant extracts.

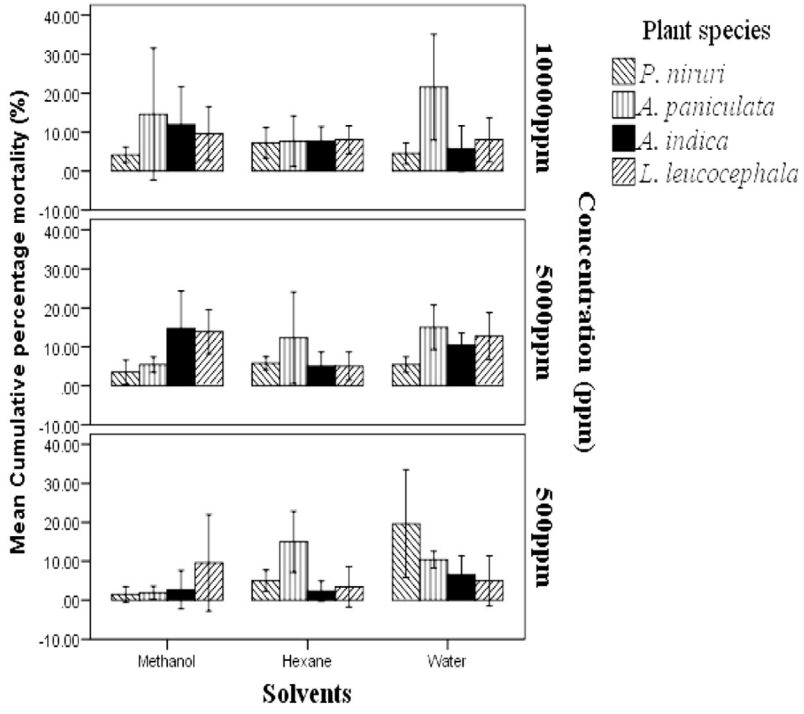

Fig. 3 – Percentage mean mortality of *G. sulphureus* from repellency test in response to different plant extracts.

extracts on a filter paper or mixed with the soil could cause a mortality or a repellency against several termite species [26,32,33]. Contact and inhalation are two main factors determining the effectiveness of toxic plant extracts [34]. In the choice bioassay, almost all methanolic plant extracts exhibited a strong repellent activity on *G. sulphureus* and *C. gestroi* even after 72 h, although their mortality percentage was slightly lower compared to those in the no-choice bioassay.

From the observation, all treated filter papers for all concentrations in the choice bioassay were covered with sand,

verifying the strong repellent activity between the treatments except for the untreated filter paper (respective solvent) and control. The avoidance behavior of both termites toward the treated filter papers resulting in low mortality percentage, which was probably due to the presence of strong toxic compounds in all methanolic plant extracts. The strong repellency by the toxic plant would slowly lead to death due to the starvation, while the close contact to the extract would cause the termitesbecome disoriented and eventually die [35]. Methanolic extracts of *P. niruri* and *Az. indica* exhibited good repellent activities against *G. sulphureus* and *C. gestroi* compared to *L. leucocephala* and *A. paniculata* extracted from the same solvent. *Phyllanthus niruri* composes of chemical compounds such as tannins, lignans, coumarins, flavonoids, terpenes, alkaloids, phenylpropanoids and saponins widely distributed in the stem, leaf and root [36]. A previous study demonstrated that the leaf part of the methanolic extract of *P. amarus* exhibited high repellent activity (100%) against two mosquitoes i.e. *Anopheles stephensi* and *Culex. quinquefasciatus* for 150 min [37]. In addition, strong repellency by the extraction of *P .amarus* oil was observed against *Heterotermes indicola* (Wasmann) [38].

Generally, plants possess biological activity against different insects and other organisms [21]. Insects that fed on secondary metabolites would encounter toxic effects which in turn would affect their physiology such as abnormality in the nervous system [25]. The strong termiticidal activity of *P. niruri* was probably explained by the presence of phytochemicals such as alkaloids, phenolics and flavonoids in that plant [38]. Flavonoids, for instance, have been previously reported to interfere with the molting process, reproduction, feeding and behavior of termite species [29]. Apart from flavanoids, rutin and quercetin have also been detected in the methanolic extract of *P. niruri*. Quercetin was reported to have an antifeedant activity [10] as well as antifungal and antibacterial activities [39]. This compound caused 40% mortality against *C. formosanus* through oral application [40]. Meanwhile, strong repellency in *Az. indica* might be induced by various active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin [41]. Azadirachtin is known as a feeding deterrent, insect-growth regulator, repellent, sterilant and inhibits oviposition of insect pests [42]. Other physiological effects that could be caused by azadirachtin are growth reduction, increased mortality or abnormal and delayed molts [43].

In the present study, both termites exhibited similar susceptibility levels to all plant extracts. However, the mortality activity for *G. sulphureus* was slightly lower compared to *C. gestroi* when treated with the methanolic extract of *P. niruri* which may be due to differences in physiological characteristics of the two species. It has been proven that compounds with antitermiticidal properties induce different activity in different termite species [44].

5. Conclusions

In summary, all methanolic plant extracts were potent at all concentrations tested. The methanolic extract of *P. niruri* exhibited an excellent termiticidal activity due to its high toxicity and repellency against *G. sulphureus* and *C. gestroi* over 72 h of exposure. The other extracts were found to be moderately toxic to both termite species. The results also showed that the termite mortality was time- and concentration-dependent. A further study should be conducted to understand the mode of action of chemical compounds existed in these plant extracts. A better understanding of botanical insecticides activity, advanced methods of compartmentalization and formulation are thus necessary to improve the effectiveness of these naturally-occurring insecticides.

Conflict of interest

We (author) declare no conflict of interest.

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