

Biopestcidal Potentials of *Erythrophleum Africanum* (Benth.) Harms Stembark and Leaf Extracts for the Management and Control of Termites

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Abstract

Background

Environmental concerns over the use of large quantities of synthetic insecticides for termite control owing to the huge pollution burdens and modern environmental health hazards (MEHH) of such chemicals on non-target living beings and other environmental element. To reduce the burden of such chemicals on the environment, the use of plant-based extracts to control termites could be an effective alternative if properly explored. One such plant with potential for anti-termite activity was assessed in this study.

Result

The phytochemical evaluation indicated high presence of alkaloids, cardiac glycosides, flavonoids and steroids in the stem-bark, while anthraquinones, saponins, tannins and terpenes were observed in the leaf extract. The GC-MS analysis revealed the presence of twenty-five compounds and thirty-four compounds in the leaf and stem bark extract respectively. On the other hand, the termiticidal activity of the leaf and stembark extracts was more against *Reticulitermes flavipes* Kollar (Subterranean) termites than *Reticulitermes tibialis* (Arid Land Subterranean) termites. Further study revealed that a concentration of 30.35 mg/L and 183.31 mg/L of the leaf extract was required to knock-off 50% and 90% of the *Reticulitermes tibialis* termites respectively, while a concentration of 40.76 mg/L was required to knock-off 50% and 66.19 mg/L is needed to knock-off 90% of the *Reticulitermes flavipes* Kollar termites. Similarly, for 50% of the *Reticulitermes tibialis* termites to be knocked-off, 48.20 mg/L of the stembark extract was required. To achieve higher mortality of the termites (90%) of the *Reticulitermes tibialis* termites, 243.26 mg/L of the stembark was required. Concentrations of 33.24 mg/L and 54.35 mg/L of the stembark were required to knock-off 50% and 90% of the *Reticulitermes flavipes* Kollar termites respectively. *E. africanum* ethanol leaf and stembark extracts can be classified as Category 4 which gives the indication of moderate acute toxicity hazard.

Conclusion

E. africanum extract will therefore, serve as a safe, efficacious and environmentally friendly pesticide for use against termites.

Background

Termites are among the oldest social insects on Earth,[1] as structural or agricultural pests. They are a serious menace to crops, forests, and various wooden structures. They are placed into common name functional groups associated with their nesting habitat, such as subterranean (e.g., *Reticulitermes*, *Coptotermes*), drywood (e.g., *Incisitermes*, *Cryptotermes*), dampwood (e.g., *Neotermes*, *Zootermopsis*), and arboreal termites (e.g., *Nasutitermes*) [2]. They are structurally destructive pests that can infest a

consumer's most important investment, most especially their home. The species that caused serious damage include *Coptotermes formosanus*, *Odontotermes formosanus*, *Macrotermes barneyi*, *Reticulitermes flaviceps*, and *Reticulitermes chinensis* [3]. The global economic impact of termites is estimated to be approximately USD 40 billion annually and subterranean termites are responsible for about 80% of the total impact [2, 3]. The intensity of a termite attack and the magnitude of the resulting damage can be very high [2]. direct economic losses from termites for Malaysia, India, Australia, China, Japan, and the United States have been reported to be USD 10, 35, 100, 375, 800, and 1000 million a year respectively. According to Arinana [5], economic losses from termite-related damage in residential buildings in Indonesia was estimated to be 8.7 trillion in 2015. The threat of termite attack on agriculture, buildings and household furniture will remain high. As a result, the control of termites through synthetic pesticides or chemicals which are harmful to mankind and nontarget pest, is commonly practiced nowadays. The use of soil termiticide barriers, which may act either by repellence or lethal contact depending on the active ingredient applied, used to be the most common curative method used by industry for termite control [6]. Environmental concerns over the use of large quantities of synthetic insecticides lead to a clear decline of these methods since the 1990s [7]. The persistence of these chemicals in the environment and their direct effect to mankind has resulted in stringent environmental regulations against their usage. However, prior to the discovery of these synthetic pesticides, most indigenous African communities were using various plant and animal-based extracts to control pests from attacking and destroying their crops both before and after harvest [8]. For instance, the *Amo*, *Mushere*, *Mwaghavul* and *Tarok*, communities of Plateau state in central Nigeria were using the Latex from *Canarium schweinfurthii* Engl., *Euphorbia unispina* N.E.Br. and *Euphorbia tirucalli* L.; extracts of *Erythrophleum africanum* (Benth.) Harms, *Parkia biglobosa* (Jacq.) R.Br. ex G.Don and *Azadirachta indica* A. Juss to control crops and post-harvest losses in cereals and leguminous grains caused by termites. These plant and animal-based extracts when properly formulated are called "biopesticides"[8].

The United Nations Organization for Food and Agriculture [9] defines pesticides as any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human disease or animal species, unwanted plants or animals that cause damage or otherwise interfere in the production, processing, storage, transport or marketing of food, agricultural products, wood and wood products or animal feeds or which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. Although pesticides are developed through very strict regulation processes to function with reasonable certainty and minimal impact on human health and the environment, serious concerns have been raised about health risks resulting from occupational exposure and from residues in food and drinking water [10]. Pesticides are referred to as "biopesticides" when they are formulated from bioresources.

The evolution of termite control is complex, involving people's understanding of termite biology and behaviour, building construction, the use of soil termiticides, baits, wood treatments, and physical barriers, as well as regulations from different industries (pest control and building construction). Soil termiticides have been the standard method of treatment for decades, but the concern for human and environmental health has driven the development of alternatives [3].

The plant *E. africanum* (*syn. Erythrophleum Suaveness*) known as *Gwaska* among the Hausa, *erunobo* among the Yoruba, *koro* among the Igbo and *kuchol* among the Amo of Nigeria belong to the genus *Erythrophleum*, specie *suaveolens* and family of fabaceae, which consist of about 10 species that are mainly native of Africa [11]. Medium-sized tree grow up to 25 – 30 m tall often branching low; bark finely fissured, scaly and grey; twigs glabrous. *Erythrophleum africanum* has numerous uses in Africa for example; the crushed stem bark is applied to swellings caused by filarial and the dried powdered is taken as snuff to cure headache in Democratic Republic of Congo (D.R.C) [12].

Erythrophleum africanum stem bark extract has been reported for the presence of appreciable high quantity of bioactive secondary metabolites, with significant excellent wood protection properties against; fungi, termites and including antibacterial activity [13]. Phytochemical studies of *E. africanum* stem bark and leaf extracts revealed the presence of triterpenoids, saponins, phenols, alkaloids, anthraquinine, flavonoids, tannins and terpenoids. [14]. The alkaloids found on the stem extracts has a stimulating effects on heart and it also increases contractions of the intestine and uterus. The genus is one of the few reported to contain alkaloid. *E. africanum* is widely used as an ordeal poison throughout its distributional range for executing capital punishment for witches and enemies, and the specie is also used as an insecticides [12]. Yusuf et al. [15] reported that Chlorophyll, proteins and common sugars are examples of primary constituents, while terpenoid, alkaloids, tannin, flavonoid and phenolic compounds are secondary constituents also called bioactive compound.

Emerhi et al. [13] pointed out that the use of multi-component extracts may represent a method for enhancing better protection while creating the potential for reduced extracts consumption. This study therefore sought to comparatively assess the termiticidal activity of *E. africanum* stembark and leaf extracts against termites and the formulation of termiticides from both the leaf and stembark extracts as shown in Fig. 1.

Figure 1: *Erythrophleum africanum* (Benth.) Harms (A) shoot (B) stembark

RESULTS

3.1 Identified Termites

The two species of termites collected were identified as shown in Table 1.

Table 1
Termites Species Used for Bioassay Analysis

Soil and Wood Termites Species		
	Soil	Wood
Order	Isoptera	Isoptera
Family	Rhinotermitidae	Rhinotermitidae
Genus	Reticulitermes	Reticulitermes
Species	<i>R. tibialis</i>	<i>R. flavipes</i> (Kollar)
Common name	Arid Land Subterranean Termites (soldiers and workers caste)	Subterranean Termites (soldiers and workers caste)

3.2 Percentage Yield, Density and Specific Gravity of Extracts

The percentage yield of extract was measured in weight of extract recovered to weight of sample fed into the Soxhlet Extractor as presented in Table 2. It was observed that percentage yield of extract was higher in stem bark (19.98%) and lower in leaf extract (18.12%). The density and specific gravity of the ethanol extracts of *E. africanum* stembark and leaf are presented in Table 2.

Table 2
Percentage Yield, Density and Specific Gravity of Extract.

Sample	Percentage yield (%w/w)	Density (kg/m ³)	Specific gravity
Leaf	18.12	1095	1.095
Stembark	19.98	1144	1.144

3.3 Preliminary Phytochemical Screening

The phytochemical analysis on the extract was carried out to determine the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, steroids, tanins and terpenes. The results obtained was presented in Table 3. The presence of the phytochemicals was noted by using the positive sign and the negative sign for its absence.

Phytochemicals in *E. africanum* were comparable in both the stembark and leaf extracts. High presence of alkaloids, cardiac glycosides, flavonoids and steroids were recorded in the stembark while anthraquinones, saponins, tannins and terpenes was observed in leaf extract. However, phenols, saponins

and terpenes was absent in *E. africanum* stem bark and absent in the leaf extract was carbohydrate, flavonoids and steroids as shown in Table 3.

Table 3
Preliminary phytochemical screening of the leaf and stembark of *Erythrophleum africanum* (Benth.) Harms

Phytochemical	Leaf Extract	Stembark Extract
Alkaloids	+	+++
Anthraquinones	++	+
Carbohydrate	-	+
Cardiac glycosides	+	+++
Flavonoids	-	++
Phenols	+	-
Saponins	++	-
Steroids	-	++
Tanins	++	+
Terpenes	+++	-

3.4 GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GC-MS) ANALYSIS

3.4.1 Leaf Extract

The result obtained by GC-MS analysis of leaf extract of *E. africanum* is presented in Table 4. A total of twenty-five (25) compounds were detected in the leaf extract of *E. africanum* as shown in Table 4. The major compound in the Leaf extract is 11-Octadecenoic acid, methyl ester (35.61%); other compounds in moderate amounts are: Hexadecanoic acid, methyl ester (7.90%), 12-Methyl-E,E-2,13-octadecadien-1-ol (6.99%), Ergosterol (6.11%), 4,4-dimethyl-, (3- β ,5- α)-Cholest-7-en-3-ol, (5.81%), β -Sitosterol (5.05%), decamethyl-Cyclopentasiloxane (4.76%) and 16- methyl ester Heptadecanoic acid, (4.70%) as shown in

Table 4
GC-MS Result of *Erythrophleum africanum* (Benth.) Harms Leaf Extract

S/NO	RT (min)	% Abundance	Compound
1	8.18	4.76	decamethyl-cyclopentasiloxane
2	12.75	3.57	dodecamethyl-cyclohexasiloxane
3	17.12	1.29	tetradecamethyl-Cycloheptasiloxane
4	26.74	7.90	methyl ester-hexadecanoic acid,
5	30.07	35.61	methyl ester-11-octadecenoic acid,
6	30.63	4.70	16-methyl-, methyl ester-heptadecanoic acid,
7	33.58	3.30	9-Octadecenal
8	33.79	1.41	Octadecanoic acid
9	35.89	6.11	Ergosterol
10	35.94	2.25	β -Sitosterol
11	36.03	5.05	β -Sitosterol
12	36.07	5.81	4,4-dimethyl-, (3- β ,5- α)-cholest-7-en-3-ol
13	36.48	6.99	12-Methyl-E,E-2,13-octadecadien-1-ol
14	36.95	1.69	Myristoleic acid
15	39.19	0.84	2-butyl-1-Octanol
16	39.26	0.14	17-Pentatriacontene
17	41.31	1.46	Tetradecanoic acid
18	41.35	2.99	(Z)-13-Octadecenal
19	41.48	1.13	Oleic Acid
20	41.9	1.17	(Z)-, methyl ester-9-octadecenoic acid
21	42.14	0.47	Z-8-Methyl-9-tetradecenoic acid
22	42.19	0.33	Oleic Acid
23	42.25	0.28	2-methyl-2-oxiranyl-cyclobutanone,
24	42.28	0.41	Cyclopentaneundecanoic acid
25	42.40	0.33	Cyclopentaneundecanoic acid

The result obtained by GC-MS analysis of stem bark extract of *E. africanum* is presented in Table 5. A total of thirty-four (34) compounds were detected in the stem bark extract. Some of the major compounds

in the stem bark extract are: methyl ester-trans-13-Octadecenoic acid, (29.43%) and Diisooctyl phthalate (10.72%). Others found in moderate amounts are: (Z)-9,17-octadecadienal, (6.72%), Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl- (5.73%), 16-methyl-, methyl ester-Heptadecanoic acid, (4.97%), decamethyl-cyclopentasiloxane, (4.84%) and bis(2-ethylhexyl) ester-hexanedioic acid, (4.39%).

Table 5
GC-MS Result of *Erythrophleum africanum* (Benth.) Harms Stem-bark Extract

S/NO	RT (min)	% Abundance	Compound
1	8.20	4.84	decamethyl-cyclopentasiloxane,
2	12.78	3.93	dodecamethyl-cyclohexasiloxane,
3	17.17	1.53	3,5-diethoxy-1,1,1,7,7,7-hexamethyl-3,5-bis(trimethylsiloxy)-tetrasiloxane,
4	26.91	3.33	methyl ester-hexadecanoic acid,
5	30.28	29.43	methyl ester-trans-13-Octadecenoic acid
6	30.78	4.97	16-methyl-, methyl ester-Heptadecanoic acid
7	33.18	0.30	1-Methyl-3,3-pentamethylenediaziridine
8	33.77	0.26	Oxacyclotetradecan-2-one
9	33.80	0.18	Chloromethyl 11-chlorododecanoate
10	33.83	0.14	Palmitoleic acid
11	35.55	4.39	Hexanedioic acid, bis(2-ethylhexyl) ester
12	35.57	3.04	Hexanedioic acid, bis(2-ethylhexyl) ester
13	35.61	3.62	2-Hexyldecyl isobutyrate
14	36.75	5.73	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-
15	36.80	6.72	(Z)-9,17-Octadecadienal
16	36.95	0.76	9-Methyl-Z-10-tetradecen-1-ol acetate
17	37.18	0.23	Oleic Acid
18	37.95	3.46	Diisooctyl phthalate
19	37.99	3.98	Diisooctyl phthalate
20	38.01	10.72	Diisooctyl phthalate
21	41.71	0.67	cis-9-Hexadecenal
22	41.77	0.36	(Z)-13-Octadecenal
23	41.80	0.34	heptadecyl ester-heptadecanoic acid,
24	42.01	3.66	β -Myrcene
25	42.05	0.62	(Z)-13-Octadecenal
26	42.10	0.53	undec-10-enyl ester-trichloroacetic acid

S/NO	RT (min)	% Abundance	Compound
27	42.15	0.26	9-Hexadecenoic acid
28	42.19	0.40	(Z)-9-eicosenoic acid,
29	42.22	0.30	9-Octadecenal
30	42.31	0.33	9-Octadecenal
31	42.35	0.28	(Z)-9,17-octadecadienal,
32	42.40	0.39	6-methyl-3-Octyne,
33	42.42	0.18	2-Methyl-Z,Z-3,13-octadecadienol
34	42.46	0.11	9-Octadecenal

3.5 MEAN MORTALITY OF THE ACTIVITY OF *E. AFRICANUM*

3.5.1 Mortality Rate of Leaf and Stembark Extracts

The potency of the leaf and stembark extract of *E. africanum* on *Reticulitermes flavipes* Kollar (Subterranean) termites and *Reticulitermes tibialis* (Arid Land Subterranean) termites was carried out and presented in Tables 6, 7, 8 and 9. Various concentrations of the extract were prepared using Tween 80 and distilled water. This was used to measure the mortality rate of both *R. tibialis* and *R. flavipes* termites at 2 hours intervals and then 24 hours interval. Physical count of the dead termites were recorded for each concentrations and also for the positive control using a synthetic pesticide (chlorphyropos) and for negative control without the application of any pesticide.

Table 6

The mean mortality of the activity of *E. africanum* leaf extract on *Reticulitermes flavipes* (Kollar) termites.

Conc. (mg/L)	Time/mean mortality			
	2hrs	4hrs	24hrs	48hrs
31.25	0.67 ± 0.33 ^d	1.00 ± 0.58 ^d	1.00 ± 0.58 ^c	3.67 ± 1.20 ^b
62.5	4.33 ± 0.88 ^c	4.33 ± 0.88 ^c	10.00 ± 2.08 ^b	13.00 ± 2.00 ^a
125	6.33 ± 0.68 ^b	7.00 ± 1.00 ^b	13.00 ± 1.16 ^a	15.00 ± 0.00 ^a
250	13.33 ± 1.67 ^b	14.67 ± 0.33 ^b	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
500	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
Standard	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
LSD	2.42			
P - Value	< 0.0001			

At $P \leq 0.05$ there was a significant difference in the mean mortality rate of the *R. flavipes* termites at the different concentration of the extracts used. Values are presented as means ± standard errors of means. Ranking was done across the concentration and value with same superscript are not significant.

Table 7

The mean mortality of the activity of *E. africanum* leaf extract on *Reticulitermes tibialis* termites.

Time/mean mortality = SEM				
Conc. (mg/L)	2hrs	4hrs	24hrs	48hrs
31.25	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	1.67 ± 0.88 ^e	7.67 ± 0.88 ^c
62.5	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	3.33 ± 0.67 ^e	8.33 ± 0.67 ^c
125	1.00 ± 1.00 ^d	1.67 ± 1.20 ^d	10.00 ± 0.00 ^d	12.67 ± 0.33 ^b
250	3.00 ± 0.58 ^c	7.33 ± 1.20 ^c	11.00 ± 0.58 ^c	13.67 ± 0.88 ^b
500	6.67 ± 0.33 ^b	9.67 ± 0.33 ^b	13.00 ± 0.58 ^b	15.00 ± 0.00 ^a
Standard	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
LSD	1.64			
P – Value	< 0.0001			

At $P \leq 0.05$ there was a significant difference in the mean mortality rate of the *R. tibialis* termites at the different concentration of the extracts used. Values are presented as means ± standard errors of means. Ranking was done across the concentration and value with same superscript are not significant.

Table 8
The mean mortality of the activity of *E. africanum* stem-bark extract on *Reticulitermes flavipes* (Kollar) termites.

Time/mean mortality				
Conc. (mg/L)	2hrs	4hrs	24hrs	48hrs
31.25	1.67 ± 0.33 ^c	2.33 ± 0.33 ^d	3.33 ± 1.33 ^c	6.00 ± 1.73 ^b
62.5	2.67 ± 2.19 ^c	4.33 ± 2.33 ^c	10.33 ± 3.18 ^c	15.00 ± 0.00 ^a
125	4.33 ± 0.88 ^{b^c}	5.00 ± 1.00 ^c	12.33 ± 1.76 ^a	15.00 ± 0.00 ^a
250	5.67 ± 0.33 ^b	9.33 ± 0.67 ^b	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
500	14.67 ± 0.33 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
Standard	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
LSD	3.22			
P - Value	< 0.0001			

At $P \leq 0.05$ there was a significant difference in the mean mortality rate of the *R. flavipes* termites at the different concentration of the extracts used. Values are presented as means ± standard errors of means. Ranking was done across the concentration and value with same superscript are not significant.

Table 9
The mean mortality of the activity of *E. africanum* stem-bark extracts on *Reticulitermes tibialis* termites.

Time/mean mortality = SEM				
Conc. (mg/L)	2hrs	4hrs	24hrs	48hrs
31.25	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	1.33 ± 0.88 ^e	7.00 ± 1.53 ^b
62.5	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	3.00 ± 1.00 ^e	8.00 ± 0.58 ^b
125	0.00 ± 0.00 ^c	0.33 ± 0.33 ^c	5.33 ± 2.60 ^b	9.33 ± 1.76 ^b
250	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c	9.33 ± 0.67 ^c	14.33 ± 0.33 ^a
500	2.67 ± 1.67 ^b	3.67 ± 1.45 ^b	12.00 ± 1.00 ^b	15.00 ± 0.00 ^a
Standard	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
LSD	2.32			
P – Value	< 0.0001			

At $P \leq 0.05$ there was a significant difference in the mean mortality rate of the *R. tibialis* termites at the different concentration of the extracts used. Values are presented as means $\pm$ standard errors of means. Ranking was done across the concentration and value with same superscript are not significant.

3.5.2 Mortality Rate of Formulations

The mean mortality of the activity of the emulsifiable concentrate of the stembark and leaf extracts formulated was tested on both the *R. flavipes* and *R. tibialis* termites and presented in tables 10, 11, 12 and 13. The test was carried out using various concentrations such as 100%, 50%, 25%, 12.5% and 6.25% of the GSC and GLC formulants.

Table 10
The mean mortality of the activity of GSC on *Reticulitermes flavipes* termites.

Time/mean mortality				
Conc. (%)	2hrs	4hrs	6hrs	24hrs
6.25	0.33 $\pm$ 0.33 ^d	1.00 $\pm$ 0.58 ^c	1.00 $\pm$ 0.58 ^c	4.67 $\pm$ 1.20 ^b
12.5	4.00 $\pm$ 0.88 ^b	6.67 $\pm$ 0.88 ^b	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a
25	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a
50	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a
100	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a
Standard	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a	15.00 $\pm$ 0.00 ^a
LSD	2.38			
P - Value	< 0.0001			

At $P \leq 0.05$ there was a significant difference in the mean mortality rate of the *R. flavipes* termites at the different concentration of the GSC used. Values are presented as means $\pm$ standard errors of means. Ranking was done across the concentration and value with same superscript are not significant.

Table 11
The mean mortality of the activity of GLC on
Reticulitermes flavipes termites.

Time/mean mortality				
Conc. (%)	2hrs	4hrs	6hrs	24hrs
6.25	0.00 ± 0.00 ^d	1.67 ± 0.58 ^c	2.67 ± 0.33 ^c	8.33 ± 1.20 ^b
12.5	1.67 ± 0.88 ^c	5.00 ± 0.88 ^b	5.33 ± 0.67 ^b	9.00 ± 0.00 ^b
25	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
50	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
100	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
Standard	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
LSD	2.44			
P - Value	< 0.0001			

At $P \leq 0.05$ there was a significant difference in the mean mortality rate of the *R. flavipes* termites at the different concentration of the GLC used. Values are presented as means $\pm$ standard errors of means. Ranking was done across the concentration and value with same superscript are not significant.

Table 12
The mean mortality of the activity of GSC on
Reticulitermes tibialis termites.

Time/mean mortality				
Conc. (%)	2hrs	4hrs	6hrs	24hrs
6.25	0.00 ± 0.00 ^d	0.00 ± 0.33 ^c	1.67 ± 0.58 ^c	1.67 ± 1.20 ^c
12.5	1.00 ± 0.88 ^c	4.00 ± 0.88 ^b	5.67 ± 0.00 ^b	7.33 ± 0.00 ^b
25	2.67 ± 0.00 ^c	9.00 ± 0.00 ^b	12.67 ± 0.00 ^a	15.00 ± 0.00 ^a
50	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
100	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
Standard	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
LSD	2.46			
P - Value	< 0.0001			

At $P \leq 0.05$ there was a significant difference in the mean mortality rate of the *R. tibialis* termites at the different concentration of the GSC used. Values are presented as means $\pm$ standard errors of means. Ranking was done across the concentration and value with same superscript are not significant.

Table 13
The mean mortality of the activity of GLC on *Reticulitermes tibialis* termites.

Time/mean mortality				
Conc. (%)	2hrs	4hrs	6hrs	24hrs
6.25	0.00 ± 0.00 ^d	0.67 ± 0.58 ^c	1.00 ± 0.33 ^c	2.00 ± 1.20 ^c
12.5	0.33 ± 0.88 ^d	2.00 ± 0.88 ^c	2.67 ± 0.67 ^b	3.00 ± 0.00 ^b
25	1.00 ± 0.00 ^c	4.67 ± 0.00 ^b	7.00 ± 0.00 ^b	10.00 ± 0.00 ^b
50	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
100	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
Standard	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a	15.00 ± 0.00 ^a
LSD	2.62			
P - Value	< 0.0001			

At $P \leq 0.05$ there was a significant difference in the mean mortality rate of the *R. tibialis* termites at the different concentration of the GLC used. Values are presented as means ± standard errors of means. Ranking was done across the concentration and value with same superscript are not significant.

3.6 PROBIT ANALYSIS

3.6.1 Probit Analysis of Leaf and Stembark Extracts

The raw data obtained by counting the number of dead termites per petri dish was subjected to Probit analysis to determine the lethal concentrations of the leaf and stembark extracts at 50% and 90%, is shown in the Tables 14, 15, 16 and 17.

Table 14
Probit analysis of the lethal concentration of the mortality of termites exposed to the leaf extracts of *E. africanum*

					Confidence limits	
Termite	Regression Equation	Chisquare (P > 0.005)	LC (50%)	LC (90%)	Lower	Upper
<i>R. tibialis</i> Termite	$y = 0.03858x + 28.13$	2.404	30.35	183.31	1.079	2.203
<i>R. flavipes</i> Termite	$y = 0.04344x + 28.58$	0.078	40.76	66.19	4.192	7.975

Table 15

Probit analysis of the lethal concentration of the mortality of termites exposed to the stembark extracts of *E. africanum*

Termite	Regression Equation	Chisquare (P > 0.005)	LC (50%)	LC (90%)	Confidence limits	
					Lower	Upper
<i>R. tibialis</i> Termite	$y = 0.05308x + 21.92$	11.275	48.2	243.26	1.287	2.359
<i>R. flavipes</i> Termite	$y = 0.03019x + 33.75$	2.615	33.24	54.35	3.552	8.451

3.6.2 Probit Analysis of Formulation on *Reticulitermes flavipes* and *Reticulitermes tibialis* Termite

The raw data obtained by counting the number of dead *R. flavipes* termites per petri dish was subjected to Probit analysis to determine the lethal concentrations at 50% and 90%.

Table 16

Probit analysis of the lethal concentration of the mortality rate of *Reticulitermes flavipes* termite on different formulations.

Extracts	Hours	Regression equation	Chi square (P > 0.05)	LC 50	LC90	Lower	Upper
GSC	2	$Y = 0.7686 * x + 12.25$	0.387	15.64	19.96	7.389	16.791
	4		1.482	12.64	18.69	5.120	9.982
	6	$Y = 0.6610 * x + 17.38$	14.099	8.82	14.70	4.152	7.933
		$Y = 0.4805 * x + 26.63$					
GLC	2	$Y = 0.7751 * x + 11.92$	0.468	15.95	20.36	7.771	16.387
	4		10.389	12.62	21.98	39.55	6.680
		$Y = 0.6529 * x + 17.45$					
	6	$Y = 0.6120 * x + 19.10$	12.718	11.86	22.47	3.435	5.797

Table 17

Probit analysis of the lethal concentration of the mortality rate of *Reticulitermes tibialis* termite on different formulations.

Extracts	Hours	Regression equation	Chi square (P > 0.05)	LC 50	LC90	Lower	Upper
GSC	2	$Y = 0.6641 \cdot x + 6.420$	20.358	28.76	46.22	4.611	7.826
	4		5.582	19.25	36.41	3.508	5.755
	6	$Y = 0.6692 \cdot x + 11.91$	1.571	13.84	28.79	3.072	4.986
		$Y = 0.6164 \cdot x + 17.21$					
GLC	2	$Y = 0.6604 \cdot x + 5.09$	249.280	32.19	44.50	6.642	11.587
	4	$Y = 0.6418 \cdot x + 9.08$	20.830	24.24	49.85	3.199	4.985
	6	$Y = 0.6408 \cdot x + 11.10$	12.097	20.92	45.39	2.984	4.641

3.7 Acute Toxicologic Analysis: General Sign and Behavioural Analysis

Oral administration of ethanol extracts of *E. africanum* leaf and stem-bark extracts showed changes in fur, lethargy and death of all the mice administered 2000 mg/kg body weight of the extracts after 24 hours. However, no treatment-related mortality in group 3 and 4 of mice were observed throughout the study (Table 18a and 18b). Physical observation of the 300 mg/kg *E. africanum* leaf no death case was recorded but one death was recorded for the stem-bark treated mice. Observation of the surviving mice throughout this study indicated that none of them showed signs of toxic effects such as changes in skin and fur, eyes and mucous membrane, behaviour pattern, tremors, diarrhoea, salivation, and coma.

Table 18a: Toxicity tests on Mice using leaf extract of *E. africanum*.

Days	Sex	Control	2000 mg/kg	300 mg/kg
1	F (n = 3)	3	0	3
7	F (n = 3)	3	0	3
14	F (n = 3)	3	0	3

Table 18b: Toxicity tests on Mice using stembark extract of *E. africanum*.

Day	Sex	Control	2000 mg/kg	300 mg/kg
1	F (n = 3)	3	0	2
7	F (n = 3)	3	0	2
14	F (n = 3)	3	0	2

DISCUSSION

4.1 Percentage Yield

The yield of the Soxhlet extraction of *E. africanum* leaf and stem bark with ethanol was 18.12% and 19.98% respectively. The yield recorded in this study was higher than 4.45% hot water extraction and 6.1% cold water extraction reported previously [13, 16]. It was found to be lower than 33.0% methanol and 37.8% combined chloroform/methanol extractions reported by Adedeji et al. [17]. The difference between this study and previous investigations' findings could most likely be due to the variation in extraction solvent and conditions at which the yields were evaluated. However, the yield obtained in this extraction was sufficient for the termiticidal activity test of *E. suaveolens* stem bark and leaf extracts suggesting that ethanol could be applied as an effective solvent for *E. suaveolens* leaf and stem bark extraction.

4.2 Preliminary Phytochemical Screening

The preliminary phytochemical screening carried on the leaf and stem bark extracts of *E. africanum* showed the presence of seven (7) out of the ten (10) phytochemicals tested in each of the leaf and stem bark extracts (Table 3). The phytochemicals present in the leaf extract include alkaloids, anthraquinones, cardiac glycosides, phenols, saponins, tannins and terpenes while alkaloids, anthraquinones, carbohydrate, cardiac glycosides, flavonoids, tannins and steroids are present in the stem bark extract. The reasons of this variability could be due the age of the plant part, availability for photosynthesis and structure. All of this variability influences the chemical composition and the relative concentration of each constituent. Carbohydrates, flavonoids and steroids were absent in the leaf extract while phenols, saponins and terpenes was absent in stem-bark of *E. africanum*.

Few studies that have been reported on anti-degradation efficacies of *E. africanum* stem-bark extracts against bacteria, fungi and termites lacked quantitative analysis of its phyto-constituents [18]. Adedeji [17] discovered Phyto-constituents composition of stem-bark extract of *E. africanum* with total saponins content of 395.52 ± 3.045 mg/g, tannins (106.16 ± 0.420 mg/g), phenol (91.90 ± 0.335 mg/g), alkanoids (40.85 ± 0.050 mg/g) and flavonoids (1.57 ± 0.105). This higher degree of bioactive compounds content of saponins, tannin and phenol might be responsible for the woods preservative efficacies of the bark extracts against fungi and termite [17]. Yusuf et al. [15] showed that the phytochemical qualitative analysis of *E. africanum* stem-bark extracts contain tannins, steroids, phenols, terpenoids, saponin, flavonoid, phlobatannins and cardiac glycosides; the presence of tannins was measured at a concentration of 2.47 mg/g, flavonoids at 1.328 mg/g, saponin at 2.72 mg/g, phenols at 0.838 mg/g. Another study [19] revealed that bioactive components from different plant parts can be extracted and used in the management of termites. The research further revealed that flavonoids, terpenes, alkaloids, tannins and anthraquinones can act as bioactive termiticides against *Coptotermes gestroi*, *Globitermes sulphurues*, *Heterotermes indicola* (wasmann), *Macrotermes bellicosus*, *Odontotermes* and *Odontotermes obesus* termites. The extracts can target the *Formosan subterranean* termites hind-gut and

by destroying the microbes, thereby play the role of anti-feedants and deterrents. The anti termite activities have been reported in extracts of hexane, ethanol, methanol and various solvents of different plants like *Juniperus* species and of tarbush *Flourensia cernua* [20]. Investigation by Cornelius et al [21], found that terpenes and monoterpenoid alcohols are toxic to *Coptotermes formosanus* termites. The monoterpenoid alcohols, particularly eugenol were the most effective as termiticides to *C. formosanus*. Eugenol caused 50% mortality of *C. formosanus* at a concentration of only 0.04mg/g of sand when termites were continuously exposed to treated sand in test tubes [21]. Phenolic compounds are a group of plant's bioactive components that are omnipresent in nature [22]; flavonoids produced by plants are hydroxylated phenol substances, and have been reported to have antimicrobial effect against some microorganisms and this ability could be attributed to complexity with extracellular and soluble proteins. On the other hand, saponins have an inhibitory effect, alkaloids have cytotoxicity biological properties, terpenoids show some vital pharmacological activities as an anti-malarial, anti-inflammatory, antiviral, anticancer, antibacterial activities and inhibition in cholesterol synthesis. The bark, leaves, seeds and stems of *E. africanum* contain alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins and terpenoids. Pharmacological research revealed that the leaf and twig extracts of *E. africanum* exhibited antibacterial, antifungal, antidote, antioxidant and toxicity activities. There is need for clinical and toxicological evaluations of crude extracts and compounds isolated from the species since *E. africanum* contains potentially toxic compounds [23].

4.3 Gas Chromatography Mass Spectroscopy (GC-MS)

The results obtained by GC-MS analysis shows that each of the leaf and stem bark extract of *E. africanum* plant species has a specific quantitative and qualitative chemical composition. Table 4 revealed a total of twenty-five (25) compounds found in the leaf extract of *E. africanum*. The major compounds in the Leaf extract include: 11-Octadecenoic acid, methyl ester (35.61%), Hexadecanoic acid, methyl ester (7.90%), 12-Methyl-E,E-2,13-octadecadien-1-ol (6.99%), Ergosterol (6.11%), 4,4-dimethyl-, (3- β ,5- α -cholest-7-en-3-ol, (5.81%), beta.-Sitosterol (5.05%), decamethyl-cyclopentasiloxane, (4.76%) and 16-methyl-, methyl ester-heptadecanoic acid, (4.70%). On the other hand, a total of thirty-four (34) compounds were detected in the stem-bark extract (Table 5). Some of the major compounds in the stem-bark extract are: methyl ester-trans-13-Octadecenoic acid, (29.43%), Diisooctyl phthalate (10.72%), (Z)-9,17-Octadecadienal, (6.72%), 4-(4-ethylcyclohexyl)-1-pentyl-cyclohexene, (5.73%), 16-methyl-methyl ester-heptadecanoic acid, (4.97%), decamethyl-cyclopentasiloxane, (4.84%) and bis(2-ethylhexyl) ester-hexanedioic acid, (4.39%). It was reported that treatments of ester and 2-Iodo Octadecanoic acid were highly lethal and low consumption when applied as both *R. flavipes* and *R. tibialis* termites, therefore high activity was seen as compared to their 2-chloro analogs [19]. Methyl, ethyl, and isopropyl-2-haloctadecanoates were similar or more toxic than their specific halo acids. Slow-acting insect-toxicants used in termite baits are noviflumuron (C₁₄H₉ClF₉N₂O₃), bistrifluron (C₁₆H₇ClF₈N₂O₂), hexaflumuron (C₁₆H₈Cl₂F₆N₂O₃), and diflubenzuron (Crompton-Dimilin C₁₇H₇Cl₂F₂N₂O₃). Karr et al. and King et al. [24, 25] found that Noviflumuron is responsible for the higher mortality in *Reticulitermes speratus* compared with diflubenzuron and hexaflumuron. Kubota et al. [26] (2006) reported that Bistrifluron

demonstrates a speedier action rate against *C. formosanus* than hexaflumuron. Su and Scheffrahn, observed that hexaflumuron is a bait toxicant against both *Reticulitermes flavipes* and *C. formosanus* [27]. The anti-termite activity of these toxicants is increasing because their chemical structure also increases the number of fluorine molecules. Furthermore, saponins isolated from *Pometia pinnata*, with a single sugar chain had more termite toxicity efficacy as compared to two sugar chains [28]. The major components found in the seed oil is citral (62.37%) and squalene (20.07%). These are oxygenated compounds and its isomeric composition are 59.3% α -citral and 40.7% β -citral [22].

4.4 Mortality of *E. africanum* Extracts and Formulations

The results of the mean mortality tests carried out on both *R. flavipes* and *R. tibialis* termites using the leaf and stem-bark extracts of *E. africanum* are presented in Tables 6, 7, 8 and 9 which showed the comparative termiticidal effects of the two extracts.

The results in Tables 6 and 7 indicated that the mean mortality rate of the leaf extract on the *R. flavipes* and *R. tibialis* termites increases with increase in concentration and time of exposure. The leaf extract apparently showed different degrees of termiticidal effects at all the extracts' concentrations tested on the *R. flavipes* and *R. tibialis* termites. At a concentration of 31.25 mg/L, the mean mortality rate of 24.5% after 48 hrs was lower than the mortality rate of 28.9% after 2hrs for a concentration of 62.5 mg/L as shown in Fig. 2a. At a concentration of 125 mg/L, the mortality rate of 100% after 48 hrs was the same as that of the concentration of 500 mg/L and the standard (*Perfect Killer*) after two 2 hrs of exposure which showed that all the 15 termites were knock-off or 100% mortality was observed. The leaf extract was more effective against *R. flavipes* termites than *R. tibialis* termites (Figs. 2a and 2b). At a lower concentration of 125 mg/L, the test resulted in 100% mortality of the *R. flavipes* termites after 48 hrs of exposure.

Figure 2: Percentage mortality of leaf extract on (a) *R. flavipes* termite (b) *R. tibialis* termite

On the contrary, an average of 12.67 termites (84.5%) mortality was recorded at the same concentration of 125 mg/L when applied to *R. tibialis* termites as shown in Fig. 2b. This values were statistically significant as shown on the Table 6 and Table 7. At a general level, the leaf extract's concentration of 125mg/L may provide a better means of controlling *R. flavipes* termite, while a much higher concentration; twice as much will be required when dealing with *R. tibialis* termites. This imply that the use of leaf extracts may represent a method for enhancing better termite control and protection using natural products obtained from plants while creating the potential for reduced extracts consumption. The trend of these results recorded in this study were contrary to those of Emerhi et al.[13] who reported a higher resistance performance of *Lagenaria breviflora* (Benth.) Roberty fruit juice extract treated wood at higher concentration. These results are interesting, implying that the threshold efficacy might be achieved using small quantities.

Similarly, the test results on the same termites using the stem-bark extracts produce better results on *R. flavipes* termites, but lower in *R. tibialis* termites as shown in Tables 8 and 3.9. At a concentration of

31.25 mg/L, 40% of the *R. flavipes* termites were knock off (Figs. 3a) compared to 24.5% mortality recorded using the leaf extract (Fig. 2a). For the leaf extract, the least concentration that achieved 100% mortality was 125 mg/L while a lower concentration of 62.5 mg/L of the stem-bark extract recorded a 100% mortality rate. On the other hand, the mean mortality rate on the *R. tibialis* termites using the stem-bark extract revealed a relatively lower percentage compared to the leaf extract. For instance, a concentration of 31.25 mg/L of the leaf extract knock off 51.1% of the *R. tibialis* termites after 48 hrs exposure (Fig. 2b) while a lower mortality rate (46.7%) was observed (Fig. 3b) using the stem-bark extract. These results are in agreement with a study by Emerhi *et al.*[13] who suggested that good utilization of *E. africanum* stem-bark extracts singly for wood protection can be achieved at lower concentrations. These results implied greater advantages in conservation of natural resources because small quantity of extracts formulations effected positive result on both *R. tibialis* and *R. flavipes* termites.

Figure 3: Percentage mortality of stembark extract on a) *R. flavipes* termite (b) *R. tibialis* termite

Based on the results recorded in this research, one can easily infer that stem-bark extracts are better candidates for *R. flavipes* termite control formulations while leaf extracts are better candidates for the *R. tibialis* termites control formulations.

Effects of naturally occurring compounds on termites have been investigated by several authors [21]. Azadirachtin, the active component of neem oil, is currently one of the most effective botanical insecticides according to a survey on ethnobotanical survey of plants used as biopesticides by indigenous communities of Plateau State, Nigeria [8]. However, another research [21] shows that neem extract was not effective on *C. formosanus* as a sand barrier and showed a feeding deterrent activity at thresholds much higher than that for other insects.

The termiticidal activity of *E. africanum* is due to the presence of different compounds such as alkaloids, anthraquinones, phenols, tannins and terpenes found in the leaf extract and alkaloids, anthraquinones, flavonoids, tannins and steroids found in the stem bark extract (Table 3). This supports the findings of Manzoor *et al.* [29] that activity of crude plant extracts against termites is often attributed to complex mixture of active compounds and that Ethyl acetate extract of *Ocimum sanctum* leaves resulted in higher mortality of termite (*Heterotermes indicola* (W.)) than methanol, butanol, hexane, water and chloroform extracts in this order. Supriana [30] found that most of the monoterpenes tested on seven termite species (including *C. formosanus*) were repellent but not toxic, whereas quinones and anthraquinones had both a repellent and toxic effect, acting as feeding inhibitors and toxins. Among the natural products tested by Cornelius *et al.* [21], monoterpenoid alcohols were the most effective termiticides against Formosan subterranean termite. Eugenol in particular was an effective fumigant and prevented tunneling through sand for at least 5 days, but it was not effective as a feeding deterrent, and its repelling activity against termites only lasted for a short time. Another report [31] also revealed that different plant parts are known to show termiticidal properties. For instance, essential oil of *Tagetes erecta* leaf has termiticidal activity due to presence of (Z)- ocimene (42.2%); aerial parts of *Lepidium meyenii* Walp. contains essential oil which acts as a feeding deterrent to termites; essential oil obtained from aerial part of *Nepeta cataria* L.

function as an obstacle against termites; essential oils from coniferous species, *Calocedrus macrolepis* and *Cryptomeria japonica* (heartwood and sapwood) and *Chamaecyparis obtusa* (leaf) showed significant anti-termite action against *C. formosanus* (shiraki). Water extracts of the bark of *Carpobrotus edulis* (L.) N.E. Br presented low anti-termite activities with termite's survival rates of 80 and 71.7% and mass losses of 41.6 and 37.3% at 500 ppm and 1000 ppm, respectively [32]. Stigmasterol and β -sitosterol present in the dichloromethane extract could explain this activity. Indeed, according to literature, terpenes and terpenoids have been reported to present toxic, anti-feeding and repellent properties against termites and other insects [33].

The test results on the mortality rate of both *R. tibialis* and *R. flavipes* termites using the formulations GSC and GLC shows different degree of mortality at the various concentrations. The stem-bark extract formulation (GSC) gave the highest mortality on the *R. flavipes* and *R. tibialis* termites tested.

Figure 4: Percentage mortality of stembark extract emulsifiable concentrate (GSC) on (a) *R. flavipes* termite (b) *R. tibialis* termite

As shown in table 10 and Fig. 4, all the *R. flavipes* termites were knock-off at a concentration of 25% after 2 hrs exposure and a concentration of 12.5% after 6 hours exposure (100% mortality was recorded). At a concentration of 6.25% of the GSC formulation, 31.13% of the *R. flavipes* termites were knock-off while 11.13% of the *R. tibialis* termites were knock-off after 24 hours. This implies that the GSC emulsifiable concentrate showed more efficacy on *R. flavipes* termites compared to *R. tibialis* termites. A lower mortality rate was recorded when the same termites were exposed to the emulsifiable concentrate formulated from the leaf extract (GLC). In this case, only 60% of the *R. flavipes* termites were knock-off after 24 hours exposure to a concentration of 12.5% compared to 100% mortality recorded after 6 hours when exposed to the GSC formulation. Also, a closer look at Fig. 5 showed that the GLC formulation was more potent on the *R. flavipes* termites compared to the *R. tibialis* termites. In general, a similar trend was observed in the efficacy of GSC and GLC formulations as both were more potent on the *R. flavipes* termites. The startling difference in this research work is that the extracts and the formulations do not agree in terms of their efficacy on the various termites tested. For instance, the emulsifiable concentrate produced from stem-bark crude extract (GSC) was more potent on all termites tested while the leaf crude extract was more potent on all the termites. This difference is attributed to the solubility of the extracts in the ethanol used for the formulation of the ECs. It was observed that the stem-bark extract was more soluble than the leaf extract. However, both the crude extracts and the EC showed similar superiority in their ability to knock-off *R. flavipes* compared to *R. tibialis* termites. In general, the use of leaves is more sustainable compared to the use of stembark in ethnomedicine.

Figure 5: Percentage mortality of leaf extract emulsifiable concentrate (GLC) on (a) *R. flavipes* termite (b) *R. tibialis* termite

4.5 Probit Analysis

4.5.1 Leaf and Stem-bark Extracts

The Probit test carried out using the leaf and stem-bark extracts as presented in Tables 14 and 15 indicate various degree of termiticidal activity for both *R. tibialis* and *R. flavipes* termites. The study revealed that a concentration of 30.35 mg/L and 183.31 mg/L of the leaf extract was required to knock-off 50% and 90% of the *R. tibialis* termites respectively while a concentration of 40.76 mg/L and 66.19 mg/L was required to knock-off 50% and 90% of the *R. flavipes* termites respectively. Similarly, a concentration of 48.20 mg/L and 243.26 mg/L of the stem-bark extract was required to knock-off 50% and 90% of the *R. tibialis* termites respectively while a concentration of 33.24 mg/L and 54.35 mg/L was required to knock-off 50% and 90% of the *R. flavipes* termites respectively. This imply that much lower concentrations were required to knock-off the *R. flavipes* termites compared to the *R. tibialis* termites. There is no general conclusion on the termiticidal activity of the leaf and stembark extracts based on these results. The leaf extract was more potent on the *R. tibialis* termites while the stem-bark extract was more potent on the *R. flavipes* termites as shown by the LC₅₀ and LC₉₀ results.

The Probit test carried out using the GSC and GLC formulations as presented in Tables 16 and 17 also indicate various degree of termiticidal activity on both *R. tibialis* and *R. flavipes* termites. It was generally observed that a lower concentration of the GSC formulation was required to knock-off the *R. tibialis* and *R. flavipes* termites compared to the GLC formulation. For instance, 8.82% of the GSC formulation was required to knock-off 50% of the *R. flavipes* termites after 6 hours exposure while 11.86% of the GLC formulation was needed for the same test condition. Similarly, 13.84% of the GSC formulation was required to knock-off 50% of the *R. flavipes* termites after 6 hours exposure while 20.92% of the GLC formulation was needed for the same test condition. As stated earlier, it is suggested that termiticidal formulation from the stembark of *E. Africanum* provides a better result than formulation from the leaf extract.

4.5.2 Toxicology Test

Herbal medicines have become vital substitute to orthodox therapy as studies are now focused on searching for new and safer molecules from natural resources [34]. With the increase in the use of medicinal plants, screening plant products to evaluate their toxic characteristics is an important initial step [35]. Furthermore, data from the acute toxicity study may serve as the basis for classification and labelling of the test material [36]. The oral route administration is the most useful and normally used route of administration for carrying out toxicity study. The absorption may be slow; however, this method is less expensive and painless to the animals. All the procedures were done based on the appropriate Organisation for Economic Cooperation and Development (OECD) guideline [37]. Test method with a starting dose of 300 mg/kg body weight primarily used in situations where the investigator has no information indicating that the test material is likely to be toxic [37]. From the Globally Harmonized Classification System, *E. africanum* ethanol leaf and stem-bark extracts can be classified as Category 4, with an LD₅₀; 300–2000 mg/kg.

The limit test is primarily used in situations where the investigator has information indicating that the test material is likely to be non-toxic or of low toxicity [38]. This finding, therefore, suggests that the extract at the limit dose tested is essentially non-toxic in oral formulations when given in small doses. The mice were monitored daily until the last day of the experiment (day 14th) for any toxic signs and mortality, the clinical symptom is one of the most important observations to indicate the toxicity effects on organs within the treated groups [38]. During the 14 days of acute toxicity observation period, all surviving mice orally administered with leaf and stem-bark of *E. Africanum* ethanol extract at a single dose of 300 mg/kg showed no obvious signs of distress, and there were no noticeable symptoms of either toxicity or deaths, this indicate that the extract did not cause acute toxicity in doses below 2000 mg/kg. Based on OECD guidelines 423 (Annex 2d), the results of this test allow the substance to be ranked and classified according to the Globally Harmonized System of Classification and Labelling of Chemicals. Thus, the *E. africanum* ethanol leaf and stembark extract can be classified as category 4 with moderate acute toxicity hazard [37]. Acute toxicity information is of limited clinical application because cumulative toxic effects do occur even at very low doses. Consequently, multiple dose studies are necessary in assessing long-term safety profile of phytomedicines. Hassan et al. [38] evaluated the acute and sub-acute toxicity activities of *E. africanum* aqueous leaf extracts by orally administering 1 mL of 1000 mg/kg, 2000 mg/kg and 3000 mg/kg body weight to Wister albino rats once daily for 28 days and toxicological effects were assessed. The lethal dose (LD₅₀) was greater than 3000 mg/kg and sub-acute administration of the extract resulted in some changes in renal and liver in the form of moderate and marked infiltration with necrosis and perivascular lymphocytic cuff [23].

CONCLUSION

Investigation into the application of *Erythrophleum africanum* (Benth.) Harms leaf and stem-bark extracts for the management and control of termites was carried out. Despite the key role synthetic pesticides played in providing reliable supplies of agricultural produce at prices affordable to consumers, improving the quantity and quality of produce, and ensuring high profits to farmers, they still impose health challenges to both farmers, the environment and other end-users. The results obtained from this study revealed that *E. africanum* leaf and stem-bark extracts and their formulations possess potential termiticide activity and can be used in the management and control of termites. The probit analysis indicated that the leaf extract was more potent on the *R. tibialis* termites while the stem-bark extract was more potent on the *R. flavipes* termites as shown by the LC₅₀ and LC₉₀ results. However, formulation from the stem-bark showed a better activity than that produced from the leaf extract on both termites. This activity is due to the presence of secondary metabolites in both the leaf and stem-bark extracts. From sustainability stand point, it is advisable to use leaves for ethnomedicine than the use of stem-bark. Toxicological test carried out on the *E. africanum* ethanol leaf and stem-bark extracts can be classified as category 4 with moderate acute toxicity hazard, thus it can be said to be efficacious and safer than most synthetic pesticides and more environmentally friendly.

MATERIALS AND METHODS

Animals

Female Swiss albino mice (25–30 g), acquired from the Animal experimental unit of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos were used. All animals were kept in clean cages under standard conditions of temperature (25°C) and light (12/12 h light/dark cycle), and fed with standard feed and water ad libitum under hygienic conditions in conformity to protocol for good laboratory practice and animal handling, which is in agreement with the National Institutes of Health Guide for the Care and use for Laboratory animals (NIH Publications No. 80 – 23, revised 1996).

2.1 COLLECTION OF PLANT MATERIALS AND TERMITES

2.1.1 Study Area

The Leaf and Stem-bark of *E. africanum* was collected from Kawam village (10°02', 8°52') 61 km northwest of Jos City, a suburb of Jengre in Bassa Local Government Area of Plateau State. This was identified in the Taxonomy Unit of the Department of Plant Science and Biotechnology, University of Jos, Jos Nigeria. The voucher number JUHN21000383 was obtained from the plant and specimen of the leaf representing plant kept in the herbarium.

2.1.2 Preparation of Plant Material

The plant samples collected was air-dried for six weeks in the shade in the National Research Fund (NRF) 2019 Research Laboratory, Department of Science Laboratory Technology, University of Jos, Main Campus, ground into powder and stored in sealed plastic containers.

2.1.3 Collection and Rearing of Termite

Termites were systematically collected when they were most active, that is, during the beginning of the rainy season, within University of Jos Community, Jos Plateau State, Nigeria (9°57', 8°53'). The collected termites were kept in a special box for preservation with a portion of the termite resting materials. The box was covered with wool to maintain a constant temperature. In addition, 10% glucose sugar solution was placed where sterile cotton was absorbed into the sugar solution and suspended in a flask above the cardboard feeding medium as a source of nourishment for the termites, which also provides the required moisture and nutrition [39]. The termites were incubated at $48 \pm 2^\circ\text{C}$ in order to provide a medium close to that of the oasis. The termites were identified in the Entomology unit of the Department of Pest Management, Federal College of Forestry, Jos Plateau State, Nigeria.

2.2 EXTRACTION AND SOLVENT RECOVERY

2.2.1 Extraction

The dried sample (50g/400ml) of both stembark and leaf of *E. africanum* extract were obtained successively using the Soxhlet setup with ethanol as the solvent. The extract was filtered through a Whatman No. 42 filter paper. The extract was transferred into a pre-weighed beaker and placed on a water

bath at 40°C until a constant weight of the extract was obtained (soft extract). All the crude extracts were then weighed to determine the percentage yield of each. The percentage yield was calculated using the following equation:

$$\text{Percentage (\%) yield} = \frac{\text{mass of extract}}{\text{mass of sample}} \times 100$$

2.2.2 Solvent Recovery

The ethanol extract was recovered at a temperature of 79°C using a rotary evaporator. It was further left open in a clean cupboard at room temperature to allow more solvent to evaporate.

2.3 PURIFICATION OF EXTRACT.

A glass micro packed column was used to purify the extracts obtained after solvent recovery. The extract was made to pass through silica gel and sodium sulphate. To ensure purity of the extract, the column used was properly washed and dried. This was done by introducing 5-6mg of the silica gel (60–120 mesh) into the column and a ready pre-inserted cotton wool. After which anhydrous sodium sulphate was added into the column to serve as a drying agent. The extract was added and allowed to stand. The purified extract was collected in a glass bottle and covered with cotton wool to avoid contamination during purification. The extracts collected were properly labelled and used for the Phytochemical Screening, bioassay test and Gas Chromatography Mass Spectrometer (GC-MS) detection.

2.4 PHYTOCHEMICAL SCREENING

The purified crude extracts of both leaf and stem-bark of *E. africanum* was subjected to phytochemical screening by modifying and using standard procedures [40–42] to test for alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, phenols, saponins, steroids, tannins.

2.4.1 Test for Alkaloids

5 ml of each extract was mixed with 5 ml of 1% hydrochloric acid in steam bath and filtered, then 1 ml of Mayer's reagent added. The presence of turbidity shows the presence of alkaloids. Further addition of a few drops of olive oil to form an emulsion confirmed the presence of alkaloids.

2.4.2 Test for Saponins

Each of extract (0.2 g) was dissolved in 5 ml distilled water. The mixture was shaken vigorously, and formation of stable persistent froth shows the presence of saponins. A further addition of 6 drops of olive oil while shaking forms an emulsion, confirming the presence of saponins.

2.4.3 Test for Tannins

A portion (0.2 g) of the extract was dissolved in 10 ml of distilled water, then a few drops of 1% ferric chloride solution added to obtain a brownish green or blueblack precipitate, which confirms the presence of tannin.

2.4.4 Test for Anthraquinones

A few drops of magnesium acetate was added to 0.2g of each of the extract. Pink colour formation indicates the presence of anthraquinone.

2.4.5 Test for Cardiac Glycoside

A portion (0.2g) of each extract was dissolved in 70% alcohol and filtered, and 3 drops of lead sub-acetate introduced into the filtrate and all mixtures filtered. The filtrate was further extracted with 10mls of chloroform in a separating funnel and concentrated to dryness. The resulting residue was dissolved in 1ml of glacial acetic acid containing one drop of Ferric chloride solution. This was underplayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interphase indicates the presence of a deoxysugar characteristic of cardenolides.

2.4.6 Test for Steroids

About 0.2g of the extract was dissolved in 2ml of chloroform, and concentrated sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interphase indicate the presence of steroidal ring.

2.4.7 Test for Terpenes

A little quantity (specify quality) of each extract was dissolved in chloroform, and 1ml of acetic anhydride added. This was followed by addition of two drops of concentrated Sulphuric acid was added. A pink colour which changes to bluish green when left to stand undisturbed for 5–10 minutes is indicative of the presence of steroid and terpenes.

2.4.8 Test for Flavonoids

Dilute ammonia (5ml) was added to 5 ml of each extract and then followed by addition of 5 ml concentrated sulfuric acid. Formation of yellow colour shows the presence of flavonoids.

2.4.9 Test for Carbohydrates

A portion (0.2g) each of the extract was dissolved in 10 ml of distilled water. This extract was boiled with Fehling solution A and B in a test tube and colour changes were observed. Presence of brick red colour is an indication reducing sugar.

2.4.10 Test for Phenols

2 ml of each extract was dissolved in 4 ml of distilled water and few drops of 10% FeCl₃ was added. Appearance of blue or green colour indicates presence of phenols.

2.5 GAS CHROMATOGRAPHY MASS SPECTROSCOPY (GC-MS) ANALYSIS

The Gas Chromatography-Mass Spectroscopy (GC-MS) of the purified extracts of the leaf and stembark of *Erythrophleum africanum* was carried out in the Multi User Research Laboratory, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

2.6 FORMULATION OF TERMITICIDE EMULSIFIABLE CONCENTRATE (EC)

The stembark and leaf extracts of *E. africanum* was used for the formulation of Termiticides for the control of termites. The emulsifiable concentrate (EC) was formulated according to the method of Knowles [43]. The stem-bark and leaf extracts formulations were code named GSC (Gwaska stembark concentrate) and GLC (Gwaska leaf concentrate). Various concentrations of the EC were then used to determine the mortality rate of both the *Reticulitermes tibialis* and *Reticulitermes flavipes* Kollar termites.

2.7 ASSAYS ANALYSIS

A 48hrs acute toxicity assay was carried out on the leaf extract, stembark extract and a 24 hrs acute toxicity assay was carried out on the the emulsifiable concentrate (EC) produced from the leaf extract (GLC) and stembark extract (GSC) to determine the LC₅₀ and LC₉₀, which gave a clue to the minimum concentrations of the extract to be used. Concentrations of 31.25 mg/L, 62.5 mg/L, 125 mg/L, 250 mg/L, and 500 mg/L were used thereafter for the extracts and percentage concentration of 100, 50, 25, 12.5 and 6.25 were used for the EC. The survival assay was carried out using three replicate vials with 15 termites per vial for each concentration of the extract and the EC. Cumulative 2 hrs, 4 hrs, 24 hrs and 48 hrs mortality of the termites was obtained. A positive control using chlorpyrifos (*Perfect Killer™*), a synthetic pesticide obtained from Jos Main Market, Jos, Plateau State) and negative control (Distilled water) were also carried on both *Reticulitermes tibialis* and *Reticulitermes flavipes* Kollar termites for the same time interval. The extract concentrations on termites were treated with appropriate solvent and Tween 80 for the survival assay. The locomotor activity of the termites after treatment was investigated using the negative assay by modifying the method of Feany and Bender [44]. Fifteen (15) termites for each group were separately placed in labelled petri dish (9 cm diameter), and allowed to acclimatize, after which they were gently tapped to the bottom of the disc. The termites were cultured and mortality rate was taking from 2 hrs to 48 hrs intervals and results were recorded. The score for each group was an average of three trials for both control and repellent groups.

2.8 ACUTE ORAL TOXICITY

The mice were kept in their cages for 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. The animals were randomly selected, weighed and marked to permit individual identification,

and Eighteen female Swiss albino mice were weighed and randomly divided into 6 groups of 3 female rats each according to the Organisation for Economic Cooperation and Development (OECD) guideline 423 [37]. Ethanol leaf and stem bark extracts of *E. africanum* were administered to the female mice following 3–4 hour fasting through oral gavage at a volume of 10 ml/kg body weight. Groups 1 and 2 were administered 300 mg/kg of the ethanol leaf and stem-bark extracts respectively dissolved in 10% tween 80 and group 3, which served as control were administered 10% tween 80. The mice were observed for general behavioural changes; symptoms of toxicity and mortality after treatment for the first 4 h, then over a period of 48 h. The number of mortality was noted, and the next higher dose of 2000 mg/kg body weight of extracts were administered to Group 4, 5 and 6, observations were carried out as stated earlier. The surviving mice were then kept in clean cages and fed with food and water for 14 days and observed at least once daily, the observations carried out included changes in fur and skin, mucous membranes and eyes, and behavioural pattern, which were systematically recorded and maintained with an individual record. In addition, consideration was given for observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and mortality. At the end of the observation period the mice were humanely killed and disposed accordingly.

2.9 STATISTICAL ANALYSIS

The data were expressed as mean $\pm$ SEM (standard error of mean), and the statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Turkey's post-hoc test. The results were considered statistically significant at $p < 0.0001$. Probit analysis was also carried out [45]. SPSS computer program was used for data analysis to estimate LC_{50} and LC_{90} values, regression coefficient (slope) and its standard error, intercept and its standard error, Pearson goodness of fit Chi-square, expected mortality and its residual, 95% confidence limits (CLs) for effective level of concentrations, and the heterogeneity factor in calculation of the confidence limits.

Abbreviations

GC-MS: Gas Chromatography Mass Spectrometer, NIH: National Institute of Health, OLAW: Office of Laboratory Animal Welfare, OECD: Organisation for Economic Cooperation and Development, LC_{50} : Lethal Concentration at 50%, LD_{50} : Lethal Dose at 50%, LC_{90} : Lethal Concentration at 90%, LD_{90} : Lethal Dose at 90%, GPS: Global Positioning System; LGAs: Local Government Areas; NRF: National Research Fund; TETFund: Tertiary Education Trust Fund; UN OFA: United Nations Organization for Food and Agriculture, DRC: Democratic Republic of Congo (D.R.C), SEM: Standard Error of Mean, ANOVA: Analysis of Variance, SPSS: Statistical Package for Social Sciences, ppm: parts per million, mg/L: milligram per liter, ml/L: milliliter per liter, mg/kg: milligram per kilogram, EC: Emulsifiable Concentrate, MEHH: Modern environmental health hazards.

Declarations

Ethical Approval

The investigation conformed to the Guide for the Care and Use of Laboratory Animal Published by the US National Institute of Health (NIH No. 85 - 23, revised 1996). All protocols were also approved by the University of Jos Animal Care and Use in collaboration with the Office of Laboratory Animal Welfare (OLAW), with Reference: Assurance Approval-UJ/FPS/F17 -00379.

Competing Interests: The authors declare no competing interests

Author's Contributions:

Conceptualization, John Stephen Gushit; Data curation, John Israila Joshua; Formal analysis, John Israila Joshua; Funding acquisition, Ahmed Difa Ali, Lydia Doosuur Ior, Goni Abraham Dogo, John Israila Joshua and John Stephen Gushit; Investigation, Lydia Doosuur Ior, Goni Abraham Dogo, Ahmed Difa Ali, John Israila Joshua and John Stephen Gushit; Methodology, John Israila Joshua and Lydia Doosuur Ior; Project administration, John Stephen Gushit; Supervision, Ahmed Difa Ali and John Stephen Gushit; Validation, John Israila Joshua and Lydia Doosuur Ior; Visualization, Goni Abraham Dogo; Writing – original draft, John Israila Joshua; Review & editing, John Stephen Gushit and Lydia Doosuur Ior.

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Availability of data and Materials:

All raw data generated and materials consulted are readily available from the corresponding author on reasonable request.

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Consent for publication: Not applicable.

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Figures



Figure 1

Erythrophleum africanum (Benth.) Harms (A) shoot (B) stembark

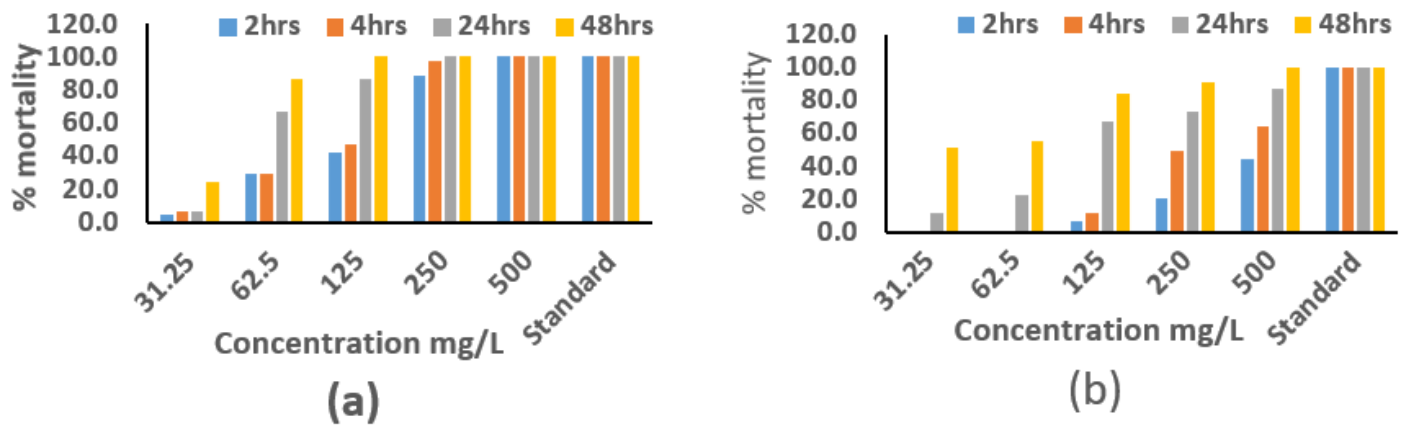
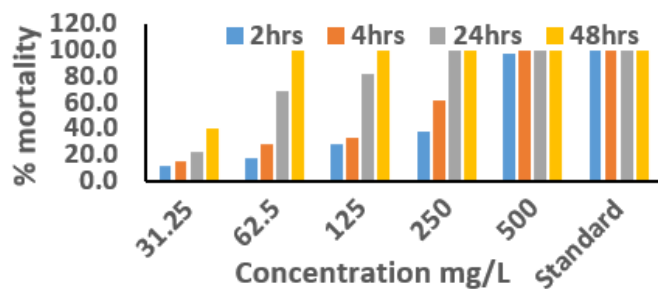
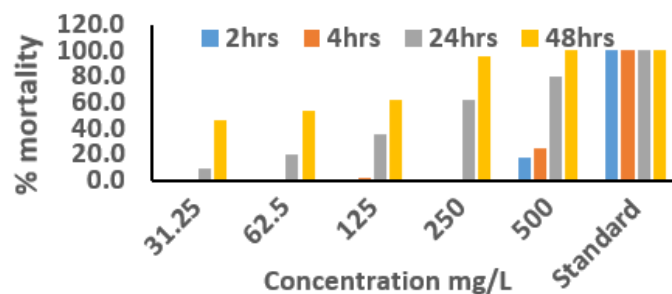


Figure 2

Percentage mortality of leaf extract on (a) *R. flavipestermite* (b) *R. tibialis* termite



(a)



(b)

Figure 3

Percentage mortality of stembark extract on a) *R. flavipestermite* (b) *R. tibialis* termite

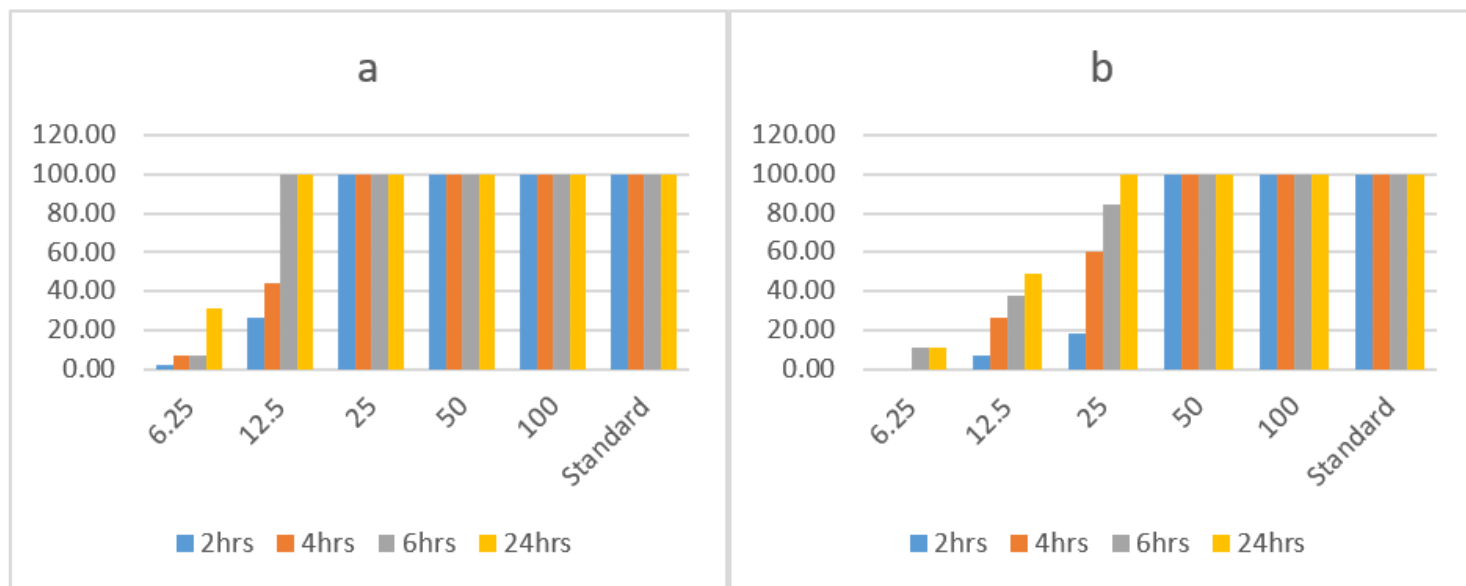


Figure 4

Percentage mortality of stembark extract emulsifiable concentrate (GSC) on (a) *R. flavipes* termite (b) *R. tibialis* termite

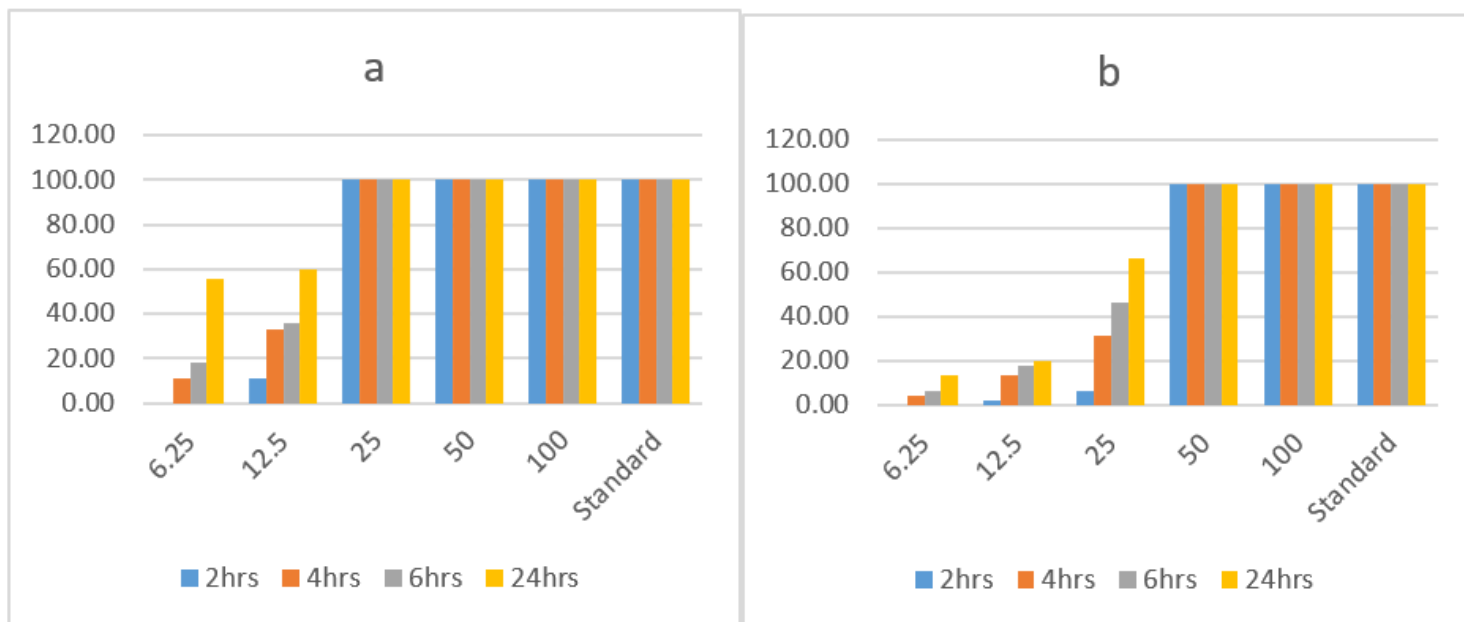


Figure 5

Percentage mortality of leaf extract emulsifiable concentrate (GLC) on (a) *R. flavipes* termite (b) *R. tibialis* termite