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QUANTITATIVE PHYTOCHEMICAL SCREENING AND TERMICIDAL ACTIVITIES OF *EUPHORBIA TIRUCALLI* L. EXTRACTS ON *DANIELLIA OLIVERI* (ROLFE) HUTCH. AND DALZIEL AND *FICUS CAPENSIS* THUNB. WOODS

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ABSTRACT

This study investigated effect of *Euphorbia tirucalli* extracts on *Daniellia oliveri* and *Ficus capensis* woods. Wood samples were purchased and processed into 10 cm x 2 cm x 2 cm dimensions, while plant parts collected were oven dried before extraction. *E. tirucalli* was screened for phytochemicals. Concentrations of 0.5%, 1% and 2% extracts were prepared by serial dilution. Soligum, methanol and untreated wood samples were used as control. Treated wood samples were laid within 6 x 12 metres field at 1 x 3 metres spacing in a Completely Randomized Design (CRD) in a termitarium and data were taken within 8 weeks. Phytochemical results indicated the presence of alkaloids, phenols, tannins, cardiac glycosides, flavonoids and saponins. Percentage absorption of extracts ranged from 47 - 86 % and 94.00 - 50.67 % in *D. oliveri* and *F. capensis,* respectively. Percentage retention of extracts ranged from 10.84 - 2.14 kg/m^3 and 11.62 - 7.01 kg/m^3 in *D. oliveri and F. capensis.* Soligum treated wood samples were not attacked throughout the period of study. *D. oliveri* and *F. capensis* woods treated with 0.5% *E. tirucalli* methanol extract were not attached on till the 6th and 8th week respectively. The least percentage weight loss of 5.49 % and 28.32 % were recorded for *D. oliveri* and *F. capensis* woods treated with soligum, while, 27.5 5 % and 52.50 % weight loss were recorded for *F. capensis* and *D. oliveri* woods treated with 0.5% methanol extract. It was concluded that the use of *E. tirucalli* extracts could be exploited to develop new wood presevatives to protect wood and wood products.

Keywords: Weight loss, phytochemicals, absorption, retention, termites, incidence of attack.

INTRODUCTION

In both tropical and subtropical regions of the world, termites remain as leading invertebrate decomposers of living and dead organic matter (Bignell and Eggleton, 2000). Their ecological dominance is commonly ascribed to the blend of their thriving social organization and exceptional capacity to feed on tough plant matters like wood (Bignell *et al.,* 2011). Termites are very essential in ecosystems where they majorly influence soil physical and chemical structure, plant disintegration, carbon and nitrogen cycling with microbial activity (Holt and Lepage, 2000).

However, termites are extremely devastating herbivorous insect pests of crop plants that harm plant foliages, wood, fibers, seedlings, and many other household wood and composite cellulose based materials. Crop species attacked by termites considerably decrease in yield and greatly infest post harvest stored products (Upadhyay, 2013).

Wood is one of the most essential renewable bio-resources used by mankind since prehistoric days (Gogoi, 2010).Wood consists largely in a decreasing order of cellulose, hemicelluloses and lignin. These lignocellulosic constituents differ from one species to the other. Nevertheless, lignocellulosic substances like wood is susceptible to degradation and dimensional changes when exposed to various environmental conditions as moisture, heat and biological organisms such as fungi, termites and wood boring insects which weaken wood for structural purposes (Gogoi, 2010).

Globally, termites constitute severe threat to wood and wood products that are not treated and many other lignocellulosic materials utilized in various constructions. To preserve wood from degradation and increase its durability, different methods have been adopted (Connell, 1991). These methods include physical, biological and chemical of which the use of synthetic chemicals has been very prominent. However, these chemicals are not friendly to human, animal and environment as they are persistently harmful. Demand for naturally durable biopesticides in the control of termites has increased because of concern for human safety and environmental impacts of chemically treated wood products (Taylor *et al.,* 2006).

E. tirucalli L. is an ever green plant species that belongs to the family Euphorbiaceae (Sauaia Filho *et al.,* 2013). It is a small tree that grows up to between 3 - 6 m tall with somewhat pencil-like branches. The plant is commonly called pencil tree in English. The tree is normally utilized for fence construction and boundary demarcation because domestic animals do not browse on it. *Euphorbia tirucalli* has limited pests and is not easily destroyed by extreme weather conditions such as drought and salt stress (Van Damme, 2001

E. tirucalli is recognized as medicinal plant locally and internationally which is similar to several other Euphorbiaceae species. The plant has been reported to possess remedial activities on many diseases (Kony *et al.,* 2013).). *E. tirucalli* contains white milky latex in every part of the shoot which is said to possess some chemical constituents that are attributed to the plant's low herbivore pressure, poisonous features, pesticidal and medicinal properties (Mwine, 2011). *E. tirucalli* has been used to treat snakebites, warts and cure for sexual impotence and syphilis and also to extract skin parasites in Africa. The plant is also commonly applied in the treatment of broken bones, hemorrhoids, pains, warts, swellings and ulcerations in Asia. Besides it is said the species heals scorpion bites, spasms, asthma, cancer, and others in Brazil (Cataluna and Rates, 1997; Van, 2001).

It has also been reported that *E. tirucalli* shows anti-fungal features (*Mohamed et al.,* 1996); piscicidal properties (Neuwinger, 2004; Tiwari, 2006); anti-viral characteristics (Betancur-Galvis *et al.,* 2002) and anti-bacterial features (Lirio *et al.,* 1998). Several authors have reported the pesticidal properties of *E. tirucalli* latex against *Brevicoryne brassicae* (aphids); *Aedes aegypti* and *Culex quinquefasciatus* (mosquitoes); *Staphylocococcus aureus*; *Lymneae natalensis* (mollusks) and *Biomphalaria gabrata* (Mwine and Van Damme, 2010; Lirio *et al.,* 1998; Rahuman *et al.,* 2008; Vassiliades, 1984; Tiwari, 2006) among others. Siddiqui *et al.* (2003) observed a dose-dependent of *E. tirucalli* latex toxicity to parasitic nematodes: *Helicotylenchus indicus, Tylenchus filiformis and Haplolaimus indicus* in vitro, with long exposure period. *E. tirucalli* latex is also reported killing agent applied on local fishing tools and poisoning of arrows in tropical Africa (Neuwinger, 2004).

Although *E. tirucalli* has been reported to be injurious to human and animals and possesses larvicidal, anti-fungal, anti-viral and anti-bacterial activities; no much has been reported on its termicidal properties. In this light, this study was conducted to evaluate the quantitative phytochemical content and termicidal activities of *E. tirucalli* extracts on *D. oliveri* and *F. capensis* wood species with a view to profiling its bioactive and eco-friendly activities that can be used in the control of termites.

MATERIALS AND METHODS

Plant collection and preparation

Experimental woods of *D. oliveri* and *F. capensis* were purchased (defect free sawn wood) from Timber Shed at New Bridge Makurdi and cross-cut into 2 x 2 x 4 cm (width x breadth x length) dimension. The fresh *E. tirucalli plant* was collected within north bank Makurdi, oven dried and pulverized (Figure 1).

Figure 1: *E. tirucalli* preparation for Extraction

[a] Fresh stand of *E. tirucalli*
[b] Oven dried sample of *E. tirucalli*
[c] Pulverized sample of *E. tirucalli* in three bottles for solvent extraction

Extraction of *Euphorbia tirucalli* .

Solvent extraction of *E. tirucalli* in n-Hexane, ethyl acetate and methanol solvents was carried out according to method described by Ekhuemelo *et al.* (2019). Two hundred grammes (200 g) of *E. tirucalli* powdered sample was measured and poured in three glass bottles labeled sample A, B and C containing 650mL hexane, ethyl acetate and methanol solvent respectively and kept for 48 hours for extraction to take place. At the end of 48 hours, the extracted crude were filtered with Whiteman 1 filter papers and evaporated to dryness under a standing fan (Figure 2). Dried extracts were pre-absolved in methanol to constitute three levels of concentrations of 0.5%, 1% and 2 % for each extracts respectively, by serial dilution method to treat wood sample for layout experiment.

Figure 2: Filtration process of extracts

[A] Mixture of pulverized solute of ***E.*** *tirucalli* and extraction solvents left to macerate
[B] Filtration of macerated sample
[C] Filtered crude extracts placed under electric fan to dry

Qualitative phytochemical screening of *E. tirucalli*
Qualitative test for alkaloids, tannins, flavonoids, saponins, cardiac glycosides and steroids were carried out according to standard procedures as adopted by Dominguez (1973); Tona *et al.* (2000).

Treatment of wood materials
The test wood samples were correctly labeled and soaked in the different treatments for 72 hours, removed and air dried for another 24 hours before field layout experiment (Figure 3). Absorption and retention of extracts were calculated and expressed volumetrically using formulae (Equations 1 and 2):

$$\text{Percentage Absorption} = \frac{W2-W1}{W1} x100 \ldots\ldots\ldots\ldots\ldots\ldots [1]$$

$$\text{Percentage Retention } (kgm-3) = [(G \text{ x } C)/V] \text{ x } 10 \ldots [2]$$

Where:
G = (W2-W1) = amount of the treating solution absorbed by the test wood blocks (g),
W1 = the oven dried weight of the conditioned wood blocks before treatment (g),
W2 = the weight after treatment,
V = volume of wood test block (cm^3).
C = grams of preservative in 100 g of treating solution/concentration of extract


Figure 3: Wood samples after soaking in extracts

Experimental Design
The treated samples were laid down in a Completely Randomized Design (CRD) with two (2) wood samples, 9 treatments, 2 positive and 1 negative controls. The treatments were replicated three times as R1, R2 and R3. Three wood samples were laid for each treatment to give a total of nine (9) test samples for a replicate. A grand total of 108 wood samples

were used for the three replicates and the controls. The treatments and controls include:

i. 0.5 % concentration of hexane extract.
ii. 1 % concentration of hexane extract.
iii. 2 % concentration of hexane extract.

iv. 0.5 % concentration of ethyl acetate extract.
v. 1 % concentration of ethyl acetate extract.
vi. 2 % concentration of ethyl acetate extract.

vii. 0.5 % concentration of methanol extract
viii. 1 % concentration of methanol extract
ix. 2 % concentration of methanol extract

Control

a) Methanol solvent (+ positive control)
b) Synthetic chemical (+ control)
c) Untreated wood sample (- control)

The treated wood samples were buried in a termitarium for a period of 8 weeks. At each position of test block, the soil was excavated according to Trinity, (2009) to a depth of 10 to allow test blocks to be completely buried. Spacing was 1m between holes and 3 m between replicates according to Ekhuemelo *et al.* (2017).

Data collection

Inspection and evaluation of the test wood samples were made on weekly basis for a period of eight weeks for any sign of termite attack. At each visit, specimens were removed from the soil and cleaned while attack on each of the wood specimen assessed in two ways as follows:

(a) Incidence of termite attacked was recorded as:
- Not attacked and,
+ Attacked.

(b) Severity of termite attack was recorded by weighing the wood samples. Equation 3 was used to determine percentage weight loss of treated wood sample after period of the experiment.

$$\% WL = [(W1 - W2)/W1]\, x\, 100 \ldots\ldots\ldots\ldots [3]$$

Where:
% WL = Percentage weight loss,
W_1 = the air dry weight before field exposure tests,
W_2 = the air dry weight after field exposure tests.

Data analysis

Data from the study was analyzed using descriptive statistics and one-way Analysis of Variance (ANOVA) to determine significant effects of treatment on wood sample. A follow up test was carried out using Duncan Multiple Range Test (DMRT) where significant differences were found.

RESULTS

Quantitative screening of phytochemicals in *E. tirucalli*

The result indicated that methanol extract had the highest (0.6 %) value of alkaloids, while, ethyl acetate extract had the least (0.03%). Phenol was highest (0.08%) in n'hexane extract and lowest (0.05%) in ethyl acetate extract. Tannins was highest (0.02%) in methanol extract, while, in both n' hexane and ethyl acetate extracts it was (0.01 %) respectively. Cardiac glycoside was only present in n' hexane with 0.01 % and absent in methanol and ethyl acetate extracts. Methanol extract had the highest (0.03 %) flavonoids and was least (0.01 %) in ethyl acetate. Ethyl acetate extract had the highest (0.02 %) value of saponins, while, methanol had the least (0.01 %) value (Figure 4).

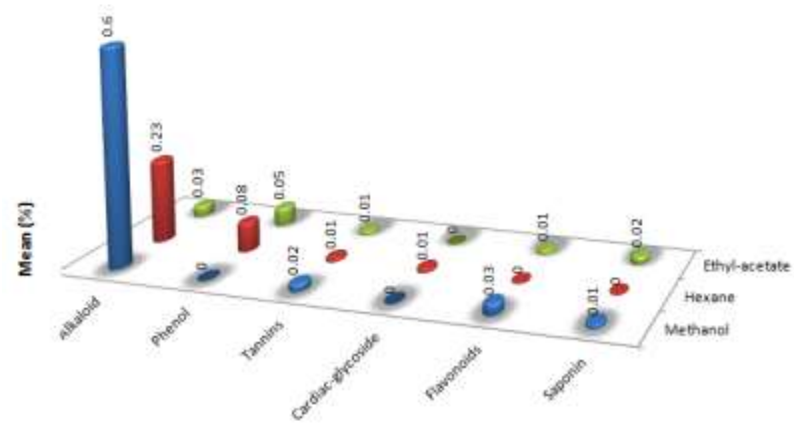

Figure 4: Phytochemicals screened from *E. tirucalli*

The percentage absorption of *E. tirucalli* extracts by *D. oliveri* and *F. capensis* treated wood samples. For *D. oliveri* wood, percentage solvent absorption ranged from 47 – 86 %, highest in samples treated with 0.5% *E. tirucalli* methanol extract and lowest in wood samples treated 1 % *E. tirucalli* methanol extract. There was no significant difference (p>.05) among *D. oliveri* wood sample treated with different concentrations of extracts but they differ significantly from soligum treated *D. oliveri* wood. Percentage solvent absorption in *F. capensis* treated wood is in the descending order of 94 % > 79.67 % > 77.33 % > 75.33 % > 67.67 with 2% methanol, 0.5% ethyl acetate, 2% methanol, 1 % ethyl acetate, and 1 % *E. tirucalli* n' hexane extracts, respectively among others. The mean percentage absorption among *F. capensis* treated wood were not significantly different ($p > 0.05$) but they differ significantly ($p < 0.05$) from 2 % n' hexane extract treated wood samples (Table 1).

Table1: Percentage absorption of *Daniellia oliveri* and *Ficus capensis* treated wood

Euphorbia tirucalli extract	Concentration (%) of *Euphorbia tirucalli* extract	*Daniellia oliveri* Mean±Std	*Ficus capensis* Mean±Std
Methanol extract	0.5	86.00 ± 14.53^{b}	56.00 ± 13.89^{a}
	1	47.67 ± 30.11^{b}	50.67 ± 12.06^{a}
	2	70.00 ± 15.40^{b}	77.33 ± 19.35^{a}
Hexane extract	0.5	58.67 ± 18.82^{b}	62.00 ± 0.00^{a}
	1	52.67 ± 11.24^{b}	67.67 ± 8.33^{a}
	2	76.67 ± 13.58^{b}	94.00 ± 14.00^{b}
Ethyl Acetate extract	0.5	63.33 ± 14.05^{b}	79.67 ± 18.16^{a}
	1	80.00 ± 17.059^{b}	75.33 ± 5.13^{a}
	2	66.00 ± 7.000^{b}	61.67 ± 13.20^{a}
Methanol Control		42.00 ± 5.29^{b}	72.67 ± 17.90^{a}
Soligum Control		15.67 ± 4.51^{a}	62.00 ± 20.66^{a}

Means with the same alphabet as superscripts within each column are not significantly different.

The percentage retention of *D. oliveri* and *F. capensis* treated wood samples after soaking with *E. tirucalli* extracts

The percentage retention of extracts in *D. oliveri* ranged between 10.84 and 2.14; highest in wood treated with 2 % ethyl acetate extract and least in synthetic chemical (+ control). In *F. capensis* treated wood, the mean percentage retention of extracts was from 11.62 - 7.01; peak in methanol solvent (+ control) and lowest in chemical (+ control). The mean percentage retention among *F. capensis* and *D. oliveri* treated woods were not significantly different ($p > 0.05$) (Table 2).

The incidence of termite attack on treated wood samples

From the results, wood samples treated with synthetic chemical (+ control) were not attacked (-) throughout the period of the experiment. *D. oliviri* wood samples treated with 0.5% *E. tirucalli* methanol extract were not attached on till the 6th week of the layout. However, wood samples treated with 2 % methanol, 0.5 % hexane and 0.5 % ethyl acetate extracts were attacked (+) from the 2nd week to the 8th week. For *F. capensis* test wood, 0.5% *E. tirucalli methanol* extract treated samples were not attacked throughout 8 weeks. Also, there was no attack (-) on any of the treated wood till the 6th week of the experiment. However, from 6th week test wood with all other treatments were attacked (+) (Table 3).

Table 2: Percentage retention of *Daniellia oliveri* and *Ficus capensis* treated wood samples

Euphorbia tirucalli extract	Concentration of *Euphorbia tirucalli* extract	*Daniellia oliveri* Mean±Std	*Ficus capensis* Mean±Std
Methanol extract	0.5%	10.47 ± 3.09^{b}	7.50 ± 2.15^{a}
	1%	6.05 ± 3.44^{ab}	6.93 ± 2.39^{a}
	2%	8.97 ± 2.25^{b}	7.68 ± 1.93^{a}
Hexane extract	0.5%	10.10 ± 3.73^{b}	$7.21 \pm .91^{a}$
	1%	8.41 ± 3.57^{b}	8.24 ± 1.32^{ab}
	2%	9.58 ± 2.13^{b}	$8.92 \pm .70^{ab}$
Ethyl Acetate extract	0.5%	8.81 ± 2.37^{b}	7.10 ± 1.54^{a}
	1%	9.77 ± 1.73^{b}	6.47 ± 1.73^{a}
	2%	10.84 ± 2.79^{b}	6.56 ± 2.80^{a}
Methanol Control		9.38 ± 1.75^{b}	11.62 ± 2.81^{b}
Chemical Control		$2.14 \pm .39^{a}$	7.01 ± 1.88^{a}

Means with the same alphabet as superscripts within each column are not significantly different.

Table 3: Incidence of termite attack on *Daniellia oliveri* and *Ficus capensis* treated wood

Treatment	Con. (%)	*Daniellia oliveri*					*Ficus capensis*			
		Week 1	Week 2	Week 4	Week 6	Week 8	Week 2	Week 4	Week 6	Week 8
Euphorbia tirucalli methanol extract	0.5	-	-	-	+	+	-	-	-	-
	1	-	-	+	+	+	-	-	-	+
	2	-	+	+	+	+	-	-	+	+
Euphorbia tirucalli Hexane extract	0.5	-	+	+	+	+	-	-	+	+
	1	-	-	+	+	+	-	-	+	+
	2	-	+	+	+	+	-	-	+	+
Euphorbia tirucalli ethyl acetat extract	0.5	-	+	+	+	+	-	-	+	+
	1	-	-	+	+	+	-	-	+	+
	2	-	+	+	+	+	+	+	+	+
Methanol (+ control)		-	-	+	+	+	-	-	+	+
Chemical (+ control)		-	-	-	-	-	-	-	-	-
Untreated wood (- control)		-	+	+	+	+	+	+	+	+

The percentage weight loss of treated wood samples at the end of the experiment

D. oliveri treated with 2 % methanol extract, 1 % ethyl acetate extract and the untreated had 100 % weight loss. However, the test wood samples treated with synthetic chemical had the least percentage weight loss of 5.49 %, followed by 0.5 % methanol extract (52.50 %) and 0.5 % ethyl acetate extract (74.48 %) among others. For treated *F. capensis* wood, samples treated with synthetic chemical had the least weight loss of 28.32% followed by 0.5% methanol extract of mean weight loss of 27.55% and 1% methanol extract of 31.34%. However, samples treated with 2% methanol extract had the highest weight loss of 69.28%. The mean weight loss among *F. capensis* and *D. oliveri* treated woods were not significantly different ($p > 0.05$).

Table 4: Percentage weight loss of *Daniellia oliveri* treated wood samples

Solvents	Concentration (%) of *Euphorbia tirucalli*	*Daniellia oliveri*	*Ficus capensis*
		Mean±Std	**Mean±Std**
Methanol extract	0.5	52.50 ± 41.28^{ab}	27.55 ± 87^{a}
	1	74.73 ± 43.77^{b}	31.34 ± 19.54^{a}
	2	100.00 ± 00^{b}	69.28 ± 29.99^{a}
Hexane extract	0.5	74.48 ± 44.21^{b}	47.27 ± 25.31^{a}
	1	86.89 ± 22.70^{b}	60.62 ± 34.35^{a}
	2	80.54 ± 18.52^{b}	40.85 ± 5.41^{a}
Ethyl acetate	0.5	90.29 ± 16.81^{b}	48.54 ± 5.29^{a}
	1	100.00 ± 0.00^{b}	47.04 ± 9.37^{a}
	2	77.14 ± 39.58^{b}	58.03 ± 36.42^{a}
Methanol control		80.62 ± 33.56^{b}	44.76 ± 23.58^{a}
Chemical control		5.49 ± 6.80^{a}	28.32 ± 20.49^{a}
Untreated wood		100.00 ± 00^{b}	41.19 ± 11.26^{a}

Means with the same alphabet as superscripts within each column are not significantly different.

DISCUSSION

Phytochemical screening of *E. tirucalli* indicated that alkaloids, tannins, saponins and flavonoids were more present in the extracts. In this study, phenol was highest in n'hexane extract and lowest in ethyl acetate extract. Plant phenolics are secondary metabolites concerned in the defense activities against microbial pathogens, many environmental stresses and insect herbivores (Kumar *et al*., 2014). Phenols have been reported to have an effective insecticide (Gojo-Cruz *et al.,* 2018). Plant phenolics like pure L-DOPA, gallic acid and tannic acid have been established to be poisonous to lots of insects (Wu *et al.,* 2015).Saunders, (1982) reported tannins as an effective insecticide on soft-bodied insects like spider mites, mealy bugs, white flies, termites, insect eggs and larvae. Gojo-Cruz *et al.*(2018) reported flavonoids as possible insecticide synergists. Wang *et al.,* (2016) applied an insecticide against Colorado potato beetle (CPB), and found it very effective and attributed the killing of the insects to flavonoids present in conifer. Ujvary, (1999) found out that plant extracts that containing alkaloids as bioactive properties have displayed a significant role in reducing insects of agricultural and public health importance for centuries. Tannins were reported to have a strong poisonous effect to phytophagous insects and possess principal feeding restraints against some insect species (Easwar *et al.,* 2017). Flavonoids have a major role in protecting plants against a variety of biotic stresses, organisms that cause plant disease and insect pests. Most alkaloids are toxic and function principally in protecting against microbial infection and plants attack by herbivores (Easwar *et al.,* 2017). Saponins are reported to have prospective use as natural insecticides and they put forth a powerful and rapid-working action against a wide variety of pests (De Geyter *et al.,* 2007). This finding proves that *E. tirucalli* could be propective source of pesticides.

Percentage of absorption and retention of extracts and solvents was influenced by percentage of extract and wood species used. Percentage of absorption and retention were highest in *D. oliveri* wood than *F. capensis* wood. The reascon may be because *D. oliveri* is softer and more porous wood than *Ficus capensis*. This agrees with the finding of Gunduz *et al.* (2009) that there is an appreciable relationship between weight loss and compression strength of wood while Esteves *et al.* (2007) also reported that there is a considerable connection between weight loss and equilibrium moisture content of wood.

D. oliveri wood was more susceptible to termite attack than *F. capensis* wood samples for all treatments. This agrees with Schaffer and Morrell (1998) who reported that resistance of wood to termite attack differs from species to species. *D. oliveri* is naturally a non-resistant wood as classified by Sotannde *et al*. (2010). This implies that *D. oliveri* requires adequate treatment to protect it before utilization.

Although synthetic chemical was most active against termites attack in this study it was not significantly different from *E. tirucalli* extracts. However, 0.5% methanol extract was the most active of all treatments. This confirms the effectiveness of plant extracts in as bioactive pesticides (Goktas *et al*., 2007). Previous studies have shown that *E. tirucalli* possess larvicidal properties (Rahuman *et al.,* 2008; Yadav *et al.,* 2002). *Mwine, (2011) established that E. tirucalli* latex *was efficient against the bean Brevicoryne brassicae and larvae of Anopheles gambiae and Anopheles fenestus as well as numerous nematodes.*

CONCLUSION

This study has revealed the presence of alkaloids, tannins, saponins and flavonoids in *E. tirucalli* extracts which prove that the species could be a very good source of insecticides. It is important to not that, 0.5% *E. tirucalli* methanol extract offered effective treatment and was not significantly from different the effect of sythehic chemical. *Ficus capensis* wood showed was more resistance to termite attack compared to *D. oliveri* wood. *E. tirucalli* extracts have proved to be useful source of bioactive agents against termites. The use of *E. tirucalli* extracts could be exploited to develop new wood presevatives to protect wooden structures, timbers as these are less harmful to the environment and humans and would as well reduce cost.

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