PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (3) 555-561 March 2023

Anti-termite Activities of Extracts from *Euphorbia tithymaloides* and *Euphorbia tithymaloide* variegatus

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ABSTRACT: The objective of this work was to investigate the anti-termite properties of solvent extracts of two *Euphorbia* plant species, *Euphorbia tithymaloides* and *Euphorbia tithymaloides* "variegatus". Termiticidal activity of stem barks and leaf extracts were analysed by using anti-termite activity tests. All crude extracts obtained by petroleum ether and ethyl acetate exhibited 100% repellency activities at a concentration of 36 mg mL^{-1}. Average repellency time decreased as the concentration of crude extracts were increased. Contact bioassay tests revealed that crude extracts from stem barks are more potent than extracts from leaves; and non-polar solvents gave crude extracts which are more effective that those extracted by polar solvents. Crude extracts obtained by petroleum ether from stem barks of both *E. tithymaloides* and *E. tithymaloides* "variegatus" plants species gave dissimilar peaks in GC-MS chromatograms, except two peaks, which correspond to the compounds: 1-heptadecene and n-hexadecanoic acid. Crude extract from *E. tithymaloides* gave a prominent peak corresponding to the compound tetracontane as the principal constituent (73.7%) of the extract. Since this peak is absent in crude extracts from *E. tithymaloides* "variegatus" stem barks, its presence in crude extracts from *E. tithymaloides* stem barks can be attributed to its high termite lethality assay results. In general, from the results obtained from this work, it could be concluded that the *Euphorbia* species are potential sources of botanical pesticides against termites.

DOI: **https://dx.doi.org/10.4314/jasem.v27i3.20**

Cite this paper as: SULEIMAN, S. H; MAHADHY, A; PHILIP, J. Y. N. (2023). Anti-termite Activities of Extracts from *Euphorbia tithymaloides* and *Euphorbia tithymaloide* "variegatus". *J. Appl. Sci. Environ. Manage.* 27 (3) 555-561

Dates: Received: 02 January 2023; Revised: 12 March 2023; Accepted: 24 March 2023
Published: 31 March 2023

Keywords: *Euphorbia tithymaloides* species; Termiticidal activity; anti-termite activity; repellence potential

Termites are eusocial insects found in different places throughout the world. They are the largest portion of the insects of the order Blattodea with over 2600 species found on earth (Kambhampati and Eggleton, 2000; Engel et al., 2009). Ecological classification of termites includes dry wood termites that live in wood and require no contact with the soil; damp wood termites that inhabit in wood with moisture condition and do not need to be in contact with soil in order to survive; subterranean termites dwell in soils with low moist condition (Rasib et al., 2017). On the other hand, termites can be categorised into four clusters, depending on their feeding behaviour. Soil feeders-termites feed on soil with high organic contents. Wood-feeder termites feed on dry soft wood. Fungi-growing termites grow fungi of genus *Termitomyces* in carbon-dioxide rich environments of their nest mounds as their important food resource. Grass-feeder termites feed mainly on grasses (DeSouza and Cancello, 2010). Termites are known to cause massive loses in crops, plants and wood. They are able to infest at different phases of plant growth and cause 50-100% yield losses in different crops such as sugarcane, millet, maize, wheat, beans, pea, sunflower, groundnut, tomato, potato and cotton. They are

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extremely destructive to wooden structures including furniture and soft floorboards (Loko et al., 2017).

As reported in a review by Oi (2022) both inorganic and organic synthetic pesticides are extensively applied globally. Such synthetic pesticides are highly poisonous and kill both targeted and non-targeted organisms. The majority of synthetic pesticides and their residuals remain in the environment for a long time, eventually threatening biodiversity. Therefore, there has been a growing demand for more effective, selective and environmentally friendly pesticides. Various plant extracts have been used in pharmacology (Kumar and Saikia 2016) and to control household and agricultural pests. These extracts contain insecticides produced by plant as secondary metabolites to defend themselves against pests (Divekar et al., 2022; Rasib et al., 2017). One of the common groups of plants used to control termites in the gardens and houses in Dar es Salaam, Tanzania, is *E. tithymaloides* species. Local people grow these plant species close to their houses and as border partitions of garden fields. This study investigated on anti-termite activities of extracts of two *E. tithymaloides* species, i.e., *Euphorbia tithymaloides (Et)* and *E. tithymaloides* "variegatus" (*Et*V).

MATERIALS AND METHODS

Chemicals and Reagents: Petroleum ether (90%), n-hexane analytical reagent (85%), methanol (100 %), ethyl acetate (99.0 %), hydrochloric acid (35-38%, specific gravity 1.18), hydrogen peroxide (30%, specific gravity 1.11), artificial sea salt (sodium chloride), dimethylsulphoxide, ferric chloride, acetone (99.5 %), chloropyrifos (48%), Dragendoff's reagent, were purchased from Lab Chemicals Limited in Dar es Salaam. Brine shrimp eggs were obtained from Living World, Metaframe Inc. (USA). Distilled water was obtained from the Department of Chemistry, University of Dar es Salaam.

Collection and Processing of Plant Samples: The plant materials of *Euphorbia tithymaloides* and *Euphorbia tithymaloides* variegatus were collected on February 2019 from Kitunda area in Ilala district, Dar es Salaam Region. The plant species were identified by a taxonomist in the field from Botany Department of the University of Dar es Salaam and confirmed voucher specimens were deposited and assigned number SSH 2332. Figure 1 shows the photographs of the sampled plant species. In Chemistry Department of the University of Dar es Salaam, the plant samples were separately chopped into small fragments and shed dried at room temperature (at 28 ± 2 ºC) for 21 days.

Fig 1: Photographs of (a) *E. thithymaloides* (*Et*) and (b) *E. tithymaloides* "variegatus" (*EtV*)

Crude extracts from leaves and stem barks were prepared as described by Frezza et al., (2018). Separately, dried leaves and stem barks were pulverized to fine powder using an electric blender. Each powder was sifted through a sieve of 0.2 mm pore size. The process involving grinding and sieving was repeated until almost all the plant materials passed through the sieve. Thus, four different powdered samples were obtained, i.e., powders of *E. thithymaloides* leaves (*Et*-L), *E. thithymaloides* stem bark (*Et*-S), *E. tithymaloides* "variegatus" leaves (*EtV*-L) and *E. tithymaloides* "variegatus" stem bark (*EtV*-S). About 250 g powder of *Et*-L was soaked into 1500 mL methanol for 48 hours while agitating. Thereafter, the content was filtered through Whatman filter paper No. 1. The filtrate obtained was concentrated under reduced pressure using a rotary evaporator at 40 ºC, and then left at room temperature for further evaporation of the solvent. The crude extract was stored in an airtight container at 4 ºC until further use. The same protocol was used to obtain crude methanol extracts from the remaining powdered materials. In addition, an identical procedure described hitherto was repeated for each powdered material except that, instead of methanol, in each case a different solvent with different polarity was used. The solvents used distinctly were ethyl acetate, petroleum ether and hexane. Table 1 shows the summary of the crude extracts obtained.

Table 1: Summary of crude extracts obtained from *Et*-L, *Et*-S, *EtV*-L and *EtV*-S using different solvents, methanol (Me), ethyl acetate (Et), petroleum ether (P) and hexane (H)

Crude Extract	Code
Euphorbia tithymaloide Leaves Methanol extract	*Et*-L-Me
Euphorbia tithymaloide Stem bark Methanol extract	*Et*-S-Me
Euphorbia tithymaloide "variegatus" Leaves ethyl acetate extract	*EtV*-L-Et
Euphorbia tithymaloide "variegatus" Stem bark Methanol extract	*EtV*-S-Me
Euphorbia tithymaloide Leaves ethyl acetate extract	*Et*-L-Et
Euphorbia tithymaloide Stem bark ethyl acetate extract	*Et*-S-Et
Euphorbia tithymaloide "variegatus" Leaves ethyl acetate extract	*EtV*-L-Et
Euphorbia tithymaloide "variegatus" Stem bark ethyl acetate extract	*EtV*-S-Et
Euphorbia tithymaloide Leaves petroleum ether extract	*Et*-L-P
Euphorbia tithymaloide Stem bark petroleum ether extract	*Et*-S-P
Euphorbia tithymaloide "variegatus" Leaves petroleum ether extract	*EtV*-L-P
Euphorbia tithymaloide "variegatus" Stem bark petroleum ether extract	*EtV*-S-P
Euphorbia tithymaloide Leaves Hexane extract	*Et*-L-H
Euphorbia tithymaloide Stem bark Hexane extract	*Et*-S-H
Euphorbia tithymaloide "variegatus" Leaves Hexane extract	*EtV*-L-H
Euphorbia tithymaloide "variegatus" Stem bark Hexane extract	*EtV*-S-H

Collection of Termites: Termites were collected at Mbala – Kijiweni around 5 km from the main road. This village is located at Bagamoyo District – Pwani Region in Tanzania. The termites were obtained from the anthill by digging progressively deeper from the edges of the anthill towards the anthill centre. Hence, the termites were collected randomly and released into the plastic container together with soil from the same sampling point. During collection, the installing traps method was used at termites' infestation areas in order to avoid their bites. The contents were sprinkled with small amount of water in a container and covered with raw white rag above in order to maintain the moisture condition as described by Ali et al., (2014). The sample was transferred to the Chemistry laboratory at UDSM, maintained at 28 ± 2 ºC and 80 ± 5% relative humidity in a plastic container. The termites were left to acclimate for 24 hours before subjecting them in the experiments. The acclimatisation of the termites was important to ensure that active subterranean termites are obtained before subjecting them into the assay experiments (Arango et al., 2021).

Termite's Repellency Bioassay: Repellency bioassay was performed using the procedure reported in the literature (Upadhyay, 2013). Repellence response experiments was performed in a plastic tube by using successive concentrations of extracts (3, 5, 8, and 10 mg/ml). Different solutions of crude extract (1 mL each) were applied on separate cotton wools (0.4 g each), and air-dried to remove the solvent. The treated cotton wool and untreated cotton wool were inserted in the right side and left sides of a plastic tube, respectively. Five active termites were transferred into the tube through the hole at the centre of the plastic tube. After transferring the termites, the hole was covered by pin-perforated transparent Teflon tape to ventilate the setup and keeping the termites inside the tube. The three replicates were made for each tested sample. The same tests were performed when positions of treated and untreated cotton wools were changed test directional bias. A number of termites moved towards untreated cotton wool areas were counted as repelled. The maximum observed time was 10 minutes.

Contact Bioassay: Anti-termites bioassay was performed by using similar protocol described by Upadhyay (2013). Different concentrations of the extracts (10, 8, 5, 3 and 0 mg/ml) were prepared in acetone. From the prepared extract samples, 1 mL of each was introduced on a separate 8.5 cm diameter Whatman® filter papers (positioned on Petri dishes) by using sterilized syringe. Thereafter, the solvent was air-dried. To maintain moist condition, 1 mL of distilled water was added on each filter paper. Ten active termites were placed on each filter paper and fresh leaves were provided as food. The dishes were covered with a black papers and then incubated at room temperature 28 ± 2 ºC and 75 ± 5% relative humidity. A drop of distilled water was periodically added on the bottom edge of each Petri dish to maintain the moisture condition. The three replicates were made for each test concentration and the numbers of survived termites were counted after 24 hours. The termite was considered dead if movements of its legs and antennae were not observed when the termite (viewed under the electric magnified lens) was touched with a tip of pencil.

Phytochemicals Analysis: About 1 µL of each sample dissolved in dichloromethane was used to analyse phytochemicals present in ethyl acetate and petroleum ether extracts from stem barks and leaves using Gas

Chromatography-Mass Spectrometry (GC-MS). The GC-MS spectra were recorded using GC/MS-QP 2010 Ultra Shimadzu (Tokyo, Japan) instrument, operating in Electron Ionization (EI) mode (MS) at 70 ev, and Flame Ionization Detector for GC. A Restek-5MS column (30 m × 0.25 mm × 0.25 μm) was used. The oven temperature program was kept at 90 ºC to 280 ºC, and held at 90 ºC for four minutes. The temperature was increased to 280 ºC for 12 minutes (hold time) at the rate of 7 ºC per minute. The injection temperature was 250 ºC with split injection mode. The flow rate of carrier gas (helium) was 1.21 ml min^{-1}. The ion source temperature and interface temperatures in MS were 230 ºC and 300 ºC, respectively. Identification of compounds in the samples was achieved by comparing a query mass spectrum with reference mass spectra in a library *via* spectrum matching. This was done by scanning method, which involved the use of Mass Spectral Library & Search Software (NIST). The quantification of compounds in the samples was done using peak area integration method whereby ion allowance was 20%.

Data Analysis: The contact anti-termite bioassay test was analysed with the aid of Microsoft® word Excel 2007 version to obtain the mean mortality percentage mortality. The mean results of the percentage mortality were plotted against the logarithms of concentrations and the regression equations obtained from the graphs were used to obtain LC_{100}. Termite's repellency activity was analysed by counting the number of repelled and non- repelled termites followed by calculating the percentage of repellence activity.

RESULTS AND DISCUSSION

Repellence Activity Test: Before performing, the termite repellence activity tests of the crude extracts, their toxicity tests against nauplii larvae of brine shrimp were executed to screen extracts that are more potent (Siddiqui et al., 2013). The general observation was that, for both *Euphorbia tithymaloides* (*Et*) and *Euphorbia tithymaloides* 'variegatus' (*Et*V) crude extracts, the fractions extracted using non-polar solvents were more toxic than their corresponding fractions obtained using polar solvents. In addition, the non-polar fractions from *Euphorbia tithymaloide* (*Et*) showed more toxicity than the *Et*V fractions. Subsequently, only crude extracts obtained using petroleum ether and ethyl acetate were subjected to termite repellency activity tests. The results for 12 samples are presented in Table 2.

Table 2: The percentage repellence of termites using *Et* and *Et*V crude extracts

Sample Code	Concentration (mg mL^{-1})	Average Repellency Time (min.)	Repelled Termites	Average Repellence (%)
Et-S-P	12	3.6 ± 0.4	4, 4, 5	86
Et-L-P	12	3.5 ± 0.5	5, 4, 4	86
*Et*V-S-Et	12	3.6 ± 0.3	3, 4, 4	73
*Et*V-S-P	12	3.5 ± 0.4	3, 4, 4	73
Et-S-P	24	2.6 ± 0.1	4, 5, 5	93
Et-L-P	24	2.7 ± 0.2	5, 4, 4	93
*Et*V-S-Et	24	2.5 ± 0.3	5, 4, 5	93
*Et*V-S-P	24	2.6 ± 0.2	5, 4, 4	93
Et-S-P	36	2.0 ± 0.3	5, 5, 5	100
Et-L-P	36	2.0 ± 0.0	5, 5, 5	100
*Et*V-S-Et	36	2.1 ± 0.3	5, 5, 5	100
*Et*V-S-P	36	2.0 ± 0.2	5, 5, 5	100

It can be seen from Table 2 that at a concentration of 12 mg mL^{-1} all crude extracts exhibited over 70% repellency activity. As the concentration of the crude extract was increased to 24 mg mL^{-1}, the repellency activities increased to over 90%, and with a concentration of 36 mg mL^{-1}, repellency activities were 100%. From this table, one can observe that average repellency time decreases as the concentration of crude extracts were increased. In addition, all cr

ude extracts registered identical repellency activities.

Contact Activity Test: The results of the contact activity test for the samples *Et*-S-P, *Et*-L-P, *Et*V-S-Et and *Et*V-S-P are presented in Figure 2, which was obtained by plotting percentage mortality of anti-termite as a function of logarithm of various concentrations of the extracts from two *Euphorbia* species. It can be seen from Figure 2 that all linear regression equations had coefficient of determination, R^2, greater than 0.8, except *Et*V-S-Et. This means that the linear regression for the three crude extracts obtained using petroleum ether, i.e., *Et*-S-P, *Et*-L-P, and *Et*V-S-P, predicted well the actual data points. One can observe from this figure that all crude extracts obtained using petroleum ether showed significantly high activities against termites, which increased with increase concentration of extracts.

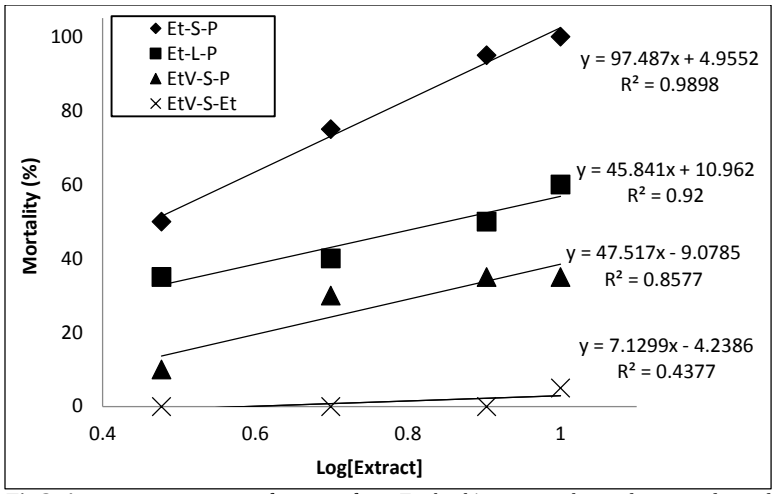

Fig 2: Anti-termite activities of extracts from *Euphorbia* species obtained using polar and non-polar solvents

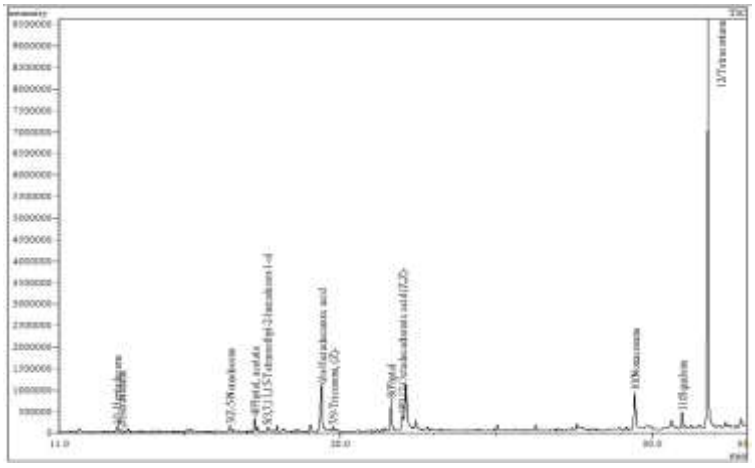

Fig 3: Chromatogram of the petroleum ether extract from *E. tithymaloides* stem barks

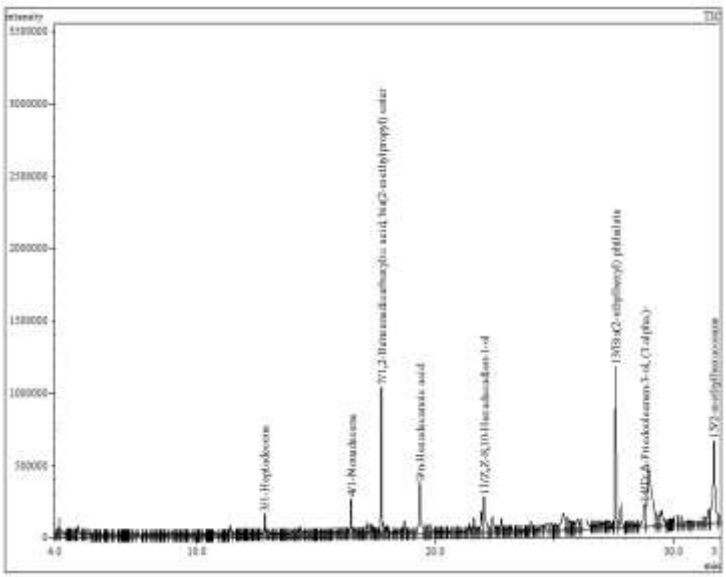

Fig 4: Chromatogram the petroleum ether extract from *E. tithymaloides* "variegatus" stem barks

With the concentration of 10 mg $mL^{\square 1}$ of each extract, *Et*-S-P registered 100% mortality, while *Et*-L-P and *Et*V-S-P gave 60% and 35% mortality, respectively. It is obvious that extracts from stem barks were more potent (Et-S-P gave 100% mortality) than extracts from leaves (Et-L-P gave 60% mortality). On the other hand, non-polar solvents extracted compounds which were more effective (*EtV*-S-P caused 35% mortality) that those extracted by polar solvents (*EtV*-S-Et caused 5% mortality). Linear regression equations of the three crude extracts obtained using petroleum ether were used to calculate LC_{100} and the obtained values are reported in Table 3.

Table 3: The calculated LC_{100} values for *Et*-S-P, *Et*-L-P *and EtV*-S-P crude extracts

Extract	*Et*-S-P	*Et*-L-P	*Et*V-S-P
LC_{100} (mg mL^{-1})	9.4	87.6	197.5

One can perceive from Table 3 that *Et*-S-P had the lowest LC_{100} value (9.4 mg mL^{-1}) followed by *Et-L-P* (87.6 9.4 mg mL^{-1}) and *Et*V-S-P (197.5 mg mL^{-1}). Since the LC_{100} value registered by stem bark extract from *E. thithymaloides* (*Et*-S-P) was lower than leaf extract of the same plant species, it can be concluded that the stem extracts were more potent than the leaf extracts. The variation in lethality assay results is a clue of the present of different amount and type of compounds in different parts of the same plant species. Thus, GC-MS analysis was used to ascertain this indication.

GC-MS analysis: With the intention of getting a glimpse of the types of compounds which are likely to be responsible for the anti-termite activities of extracts from *Euphorbia* species, GC-MS analysis was carried out for the two samples, one with the lowest LC_{100} value (*Et*-S-P), and that with the highest LC_{100} value (*Et*V-S-P).

The GC-MS chromatograms of the two samples are shown in Figures 3 and 4, respectively. From Figure 3, one can observe that the chromatogram contains 12 prominent peaks and the chromatogram in Figure 4 have nine prominent peaks. Only two peaks appeared in both chromatograms, which correspond to the compounds 1-heptadecene and n-hexadecanoic acid. Other peaks in Figure 3 correspond to the compounds: hexacosane, Z-5-nonadecene; phytol-acetate; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; 9-tricosene-(Z)-; phytol; 9,12-octadecadienoic acid (Z,Z)-; nonacosane; squalene and tetracontane. In Figure 4, the additional peaks correspond to the compounds: 1-nonadecene; 1,2-benzenedicarboxylic acid; bis(2-methylpropyl) ester; Z,Z-8,10-hexadecadien-1-ol; bis(2-ethylhexyl) phthalate; D:A-friedooleanan-3-ol, (3.alpha.)-; and 2-methylhexacosane. In addition, one peak in Figure 3, corresponding to the compound tetracontane, was more pronounced than the rest. This means that tetracontane was the leading component (73.7% – obtained from peak area) of the petroleum ether extract from *E. tithymaloides* stem barks. Since the peak for tetracontane did not appear in petroleum ether extract from *E. tithymaloides* "variegatus" stem barks, this observation may explain the high termite lethality assay results registered by the extract from the stem barks of *E. thithymaloides*. Other researchers on efficacy of medicinal plant extracts termite, (Elango *et al*. 2011) reported tetracontane to be one of the major compounds extracted from *Tagetes erecta* and *Tagetes patula*, which exhibited insecticidal properties. Since contact activity test were executed using crude extracts, it is likely that, apart from tetracontane, the presence of other minor constituents had synergistic influence that resulted to the high termite lethality assay unveiled by the extract from the stem barks of *E. thithymaloides*.

Conclusion: Polar and non-polar solvent extracts of *E. tithymaloides* and *E. tithymaloides* "variegatus", used in this study, revealed high termite repellency activities. Crude extracts from stem barks were found to be more toxic to termites than extracts from leaves; and extracts obtained by non-polar solvents were more effective that those extracted by polar solvents. The results achieved in this work advocate *Euphorbia* species as potential sources of botanical pesticides against termites.

Acknowledgements: The authors thank the International Science Programme (ISP) for financial support via Materials Science and Solar Energy Network for Eastern and Southern Africa (MSSEESA).

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