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Insecticidal Properties of *Euphorbia tirucalli* latex against Selected Household Pests (*Blattella germanica*, *Periplaneta americana* and *Acheta domesticus*) under Laboratory Settings

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ABSTRACT

Insects such as *Blattella germanica* and *Periplaneta americana* are disease spreading pests, while *Acheta domesticus* is a pest due to the male's disruptive chirping. Plants, unlike synthetic insecticides, have insecticidal properties with minimal negative effects. *Euphorbia tirucalli* is a plant that has insecticidal properties against cockroaches and crickets. This study aimed to determine the phytochemical compounds within *E. tirucalli* and to investigate the insecticidal properties of *E. tirucalli* latex against *B. germanica* and *P. americana*. The total saponin and alkaloid content within the plant was 122 ± 98.28 mg diosgenin g^{-1} and 271 ± 51.16 mg atropine g^{-1} , respectively. *Euphorbia tirucalli* twig extracts at concentrations of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1% (w/v) achieved mortalities of 30, 40, 40, 40, 50, 60, 60, 70, 80 and 100% for *A. domesticus*, respectively. *Euphorbia tirucalli* latex concentrations of 20, 40, 60, 80 and 100% (v/v) achieved mortalities of 20, 60, 70, 90 and 100%, respectively for *P. americana*. The same concentrations resulted in mortalities of 20, 50, 60, 80 and 100%, respectively for *B. germanica*. The LC_{50} of latex against *B. germanica* and *P. americana* were determined at 39.03 and 35.35, respectively. The LC_{50} extract against *A. domesticus* was 0.03. The 95% confidence interval was found to be 51.14-26.92, 45.63-25.07 and 0.018-0.042 for *B. germanica*, *P. americana* and *A. domesticus*, respectively. Therefore, it can be concluded that *E. tirucalli* has various phytochemical compounds and insecticidal properties against *B. germanica*, *P. americana* and *A. domesticus*.

INTRODUCTION

Insects are invertebrate organisms belonging to the class Insecta within the phylum Arthropoda (Wilkes, 2006). Scientific reports estimate that there are at least 5.5 million identified insect species globally with 100 species of these organisms considered household pests (Dhong, 2014; Stork, 2018). These insect pests feed on cultivated crops and are carriers of disease-causing pathogens (Sarwar, 2015; Skendžić *et al.*, 2021). Some of the most common household pests in the world are *Blattella germanica* (Linnaeus, 1767), *Periplaneta*

americana (Linnaeus, 1758) and *Acheta domesticus* (Linnaeus, 1758) among others (Fagerlund, 2004). These organisms thrive in human habitations as these places provide safety from outside elements and natural predators.

Blattella germanica, *P. americana* and *A. domesticus* within households are found living in kitchens, bedrooms, living rooms and basements. Additionally, *P. americana* and *A. domesticus* are also found living on the household grounds under tree branches, bark, stones, plant debris and garbage bins (Scholtz and Holm, 1985). They are omnivorous and feed on almost any materials including cardboard, plant matter, stored food, paper, wood and wool (Wilkes, 2006; Trematerra and Pinniger, 2018). *Blattella germanica* and *P. americana* cause social and health difficulties, as they are known to produce a foul odour and male *A. domesticus* creates irritating chirping noises. Heavy infestations of *B. germanica* and *P. americana* have been reported to carry and transmit harmful pathogens such as viruses, bacteria, protozoa and fungi (Roth and Willis, 1960; Mille and Peters, 2004).

A study by Barbosa *et al.* (2013) reported that cockroach allergy is associated with human diseases among people living in Brazil. The study showed how people who were hospitalized with hay fever and eczema were sensitized to cockroach allergens. Another study by Rosenstreich *et al.* (1997) stated that exposure to cockroach allergens in the home was linked with increased morbidity in children with asthma living in inner cities in the United States. A study by Baez *et al.* (2022) reported that *A. domesticus* extensively damages plants in the garden. Additionally, the authors stated that *A. domesticus* graduates from the garden and enters the household where they target fabrics such as clothing, bedding and leather. *Acheta domesticus* even infest wood in various forms such as furniture and structural timber of housing.

Populations of *B. germanica*, *P. americana* and *A. domesticus* are controlled by practicing good hygiene coupled with the use of synthetic and organic insecticides (Passara *et al.*, 2025). However, synthetic insecticides are preferred over the latter. Synthetic insecticides are formulated in various forms, which include aerosols, sprays, dusts, baits, gels and foams (Wang *et al.*, 2021). These formulations belong to the prominent insecticide classes such as organochlorines, pyrethroids, organophosphates and carbamates (Stoytcheva, 2011; Stejskal *et al.*, 2021). Although synthetic insecticides are undeniably effective, they harm the environment and non-targeted species while also having disadvantages on human health (Stoytcheva, 2011). Pyrethroids such as permethrin, bifenthrin and cypermethrin have been reported to harm non-targeted freshwater organisms (Ranatunga *et al.*, 2023). Another report indicated that organophosphate pesticides used in common households are detrimental to the health of homeowners and their pets (Fagerlund, 2004). Additionally, scientific reports provide evidence of resistance development in *B. germanica* under constant exposure to permethrin (Oladipupo, 2022).

Scientific reports provide evidence that the latex of most plants has insecticidal properties against various pest insects. A study by Morais *et al.* (2021) reported that *Himatanthus drasticus* (Plumel, 1991) latex is toxic to *Callosobruchus maculatus* (Fabricius, 1775). Another study by Ileke *et al.* (2014) investigated the insecticidal properties of *Alstonia boonei* (De Wildeman, 1914), *Jatropha curcas* (Linnaeus, 1753) and *Argemone mexicana* (Linnaeus, 1753) latex against *Callosobruchus maculatus* and found them to be effective. Tripathi and Upadhyay (2023) also showed that *Ficus benghalensis* (Linnaeus, 1753) latex has insecticidal effects against *Odontotermes obesus* (Rambur, 1842). However, there is little scientific reports on the insecticidal properties of *Euphorbia tirucalli* (Linnaeus, 1753) latex against pest insects. Therefore, the current study aimed to investigate the insecticidal effects of the plant latex and twig extracts of *E. tirucalli* against *P. americana*, *B. germanica* and *A. domesticus*, respectively.

MATERIALS AND METHODS

Study Area:

The study was conducted at the Sefako Makgatho Health Sciences University (SMU), north-west of Pretoria, Gauteng province, South Africa (25°36'53"S, 28°01'25"E).

Collection of *Euphorbia tirucalli*:

Fresh *E. tirucalli* twigs were randomly collected from their natural environment within the SMU gardens. The collected specimens were transported to the Biology and Environmental Sciences laboratory in airtight plastic bags to prevent moisture loss. Voucher samples were submitted to the South African National Biodiversity Institute (SANBI) for official authentication.

Collection of *Euphorbia tirucalli* latex (de Souza *et al.*, 2019):

Euphorbia tirucalli latex was collected from matured plants. The stem of the plant was incised and the exuding latex was collected at dropwise into centrifuge tubes. The tubes were sealed, wrapped with aluminium foil and stored at 4°C for later use.

Collection and Rearing of *Blattella germanica*, *Acheta domesticus* and *Periplaneta americana* (Wang *et al.*, 2021):

Adult *A. domesticus* and *P. americana* were purchased from a pet shop and adult *B. germanica* were collected from a kitchen using harmless baited traps. The crickets and cockroaches were transported from the pet shop and kitchen to the laboratory of the Department of Biology and Environmental Sciences. *Blattella germanica*, *P. americana* and *A. domesticus* were kept in ventilated plastic containers of 50 l capacity and 57L x 41W x 34H centimetre dimensions. The plastic containers included egg carton boxes to add extra surface areas for the cockroaches and crickets to climb around and move freely. Environmental material such as wood chips and tree bark was provided as a type of bedding they could hide under. The insects underwent a 7 d acclimatization period with controlled 12 h light and 12 h dark (L:D) photoperiod conditions. The temperature of the laboratory was kept between 20-30°C. Fish flakes were provided as food to the specimens at ad libitum and carrots were used as a water source.

Chemicals:

All chemicals were of analytical grade (purity ≥ 90). Chloroform, Vanillin, Sulphuric acid, Methanol and Bromocresol green were purchased from Merck Life Science, USA.

Extraction of *Euphorbia tirucalli*:

Euphorbia tirucalli twigs were extracted using the maceration technique following the guidelines of Srikacha and Ratananikom (2020) with slight modifications. The collected twigs of the plant were washed thoroughly using distilled water. The specimens were air-dried for three weeks and in the fourth week, the twigs were ground into powder using an electric blender. The powder was sieved using a mesh of 250 μ m to separate the debris from the fine material. A weighted mass of 50 g of the plant sample was poured into a 2000 ml volumetric flask using a glass funnel.

A volume of 500 ml methanol was added to the 2000 ml volumetric flask. The volumetric flask containing the mixture was shaken for 24 hr using an orbital shaker at 120 rpm. Hereafter, the twigs mixture was filtered using Whatman filter paper No. 1 into clean glass beakers. The various aqueous plant extracts within each glass beaker were dried using a rotary evaporator at 40°C. The residue of the twig extracts was weighed, recorded and then stored.

Quantitative analysis of dry methanolic twig extracts of *Euphorbia tirucalli*:

Alkaloids:

Euphorbia tirucalli extracts of 100 mg were dissolved in 100 ml of distilled water in volumetric flasks. Hereafter 5 ml of bromocresol green and 5 ml of the prepared buffer

solution were slowly pipetted into each volumetric flask. The sample was then washed with 5 ml of chloroform. The aliquot was transferred to a cuvette and the absorbance was measured at 470 nm. Estimation of the alkaloid content in the methanolic twig extract was carried out in triplicate. The total alkaloid content within *E. tirucalli* methanolic twig extracts was calculated using the following standard curve equation: $y = 0.0009x + 0.9352$ (Fig. 1).

Saponins:

The sample was prepared by dissolving 100 mg of methanolic twigs extracts with 100 ml of aqueous methanol in a volumetric flask. Hereafter, 0.25 ml of the vanillin reagent and 2.5 ml of 72% sulphuric acid were slowly added to the inner side of the volumetric flask. The sample was transferred to a test tube to be vortexed, where after it was incubated in a water bath at 60°C for 15 min, and then cooled down in a bucket of water for 5 min. The aliquot was taken to the spectrophotometer, and the absorbance was measured at 544 nm. The estimation of the saponin content in the methanolic twig extracts was carried out in triplicate. The total saponin content within *E. tirucalli* methanolic twig extracts was calculated using the following standard curve equation: $y = 0.0023x + 0.7144$ (Fig. 2).

Insecticidal effects of *Euphorbia tirucalli* Methanolic Twig Extracts on Adult *Acheta domesticus* by Using the Dipping Method:

The insecticidal activity of *E. tirucalli* against adult *A. domesticus* was evaluated using the dipping method of Paramasivam and Selvi (2017) with a slight modification. Ten adult *A. domesticus* of approximately 1.8 cm in length were placed within a mosquito mesh net and dipped for 5 sec in 11 plastic containers. The first 10 containers were filled with 5 ml of experimental concentration solutions of *E. tirucalli* twig extracts of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1% (w/v), respectively, while the 11th plastic container served as a negative control and received only distilled water. The crickets after exposure were transferred into recovery containers and provided with food and water and observed for a 24 hr period. The crickets were examined by being brushed with a fine camel brush and those that remained inactive were considered dead and removed from the setup and mortality percentages were recorded. The experimental trials for each concentration including the control were carried out in triplicate.

Insecticidal Effects of *Euphorbia tirucalli* latex on Adult *Blattella germanica* and *Periplaneta americana* Using the Dipping Method:

The insecticidal effects of *E. tirucalli* latex against adult *B. germanica* were evaluated using the dipping method described by Paramasivam and Selvi (2017) with slight modifications. Ten adult *B. germanica* of approximately 1.3 cm in length were placed within a mosquito mesh net and dipped for 5 sec in six plastic containers. The first five containers were filled with 5 ml of experimental concentration solutions of *E. tirucalli* latex of 20, 40, 60, 80 and 100% (v/v), respectively, while the sixth plastic container served as a negative control and received only distilled water. The experiment trials were carried out for 24 hr and cockroaches that remained inactive after being brushed with a fine camel brush were considered dead. Furthermore, the dead roaches were removed from the setup and mortality percentages were recorded. The experimental trials for each concentration including the control were carried out in triplicate and the same procedure was repeated for *P. americana* specimens, which were approximately 3.5 cm in length.

Data Analysis:

Mortality was assessed 24 hr after treatment and established using Abbott's formula (Abbott, 1925). The lethal concentration (LC₅₀) was determined using the probit analysis method by Finney and Stevens (1948), using Microsoft Excel. Thereafter, data was presented as mean values with the standard error of the mean (SEM).

RESULTS

The total saponin and alkaloid content within *E. tirucalli* methanolic twig extracts were determined at 122 ± 98.28 mg diosgenin g^{-1} and 271 ± 51.16 mg atropine g^{-1} , respectively (Table 1). The above-mentioned phytochemical compounds were calculated using the following standard curve equations: $y = 0.0023x + 0.7144$ (Fig. 1) and $y = 0.0009x + 0.9352$ (Fig. 2), respectively.

Table 1. Standard deviation table representing the concentration of *Euphorbia tirucalli* in equivalence to the various phytochemical standards in mg/ml.

Sample name	Absorbance (λ)	Concentration (mg/ml)	Deviations ($xi - \underline{x}$)	$(xi - \underline{x})^2$	$(xi - \underline{x})^2 + (xi - \underline{x})^2 + (xi - \underline{x})^2$	$\sigma = \sqrt{\sum \frac{(xi - \underline{x})^2}{n-1}}$
<i>Euphorbia tirucalli</i> (Saponins)	0.758	18.95	-103.33	10677.08	19321.5	98.28
	1.021	133.30	11.02	121.44		
	1.208	214.60	92.32	8522.98		
		Ave $\pm$ S.E= 122 $\pm$ 98.28				
<i>Euphorbia tirucalli</i> (Alkaloids)	1.1400	227.55	-43.33	1877.48	5235.95	51.16
	1.2298	327.33	56.45	3186.60		
	1.1672	257.77	-13.11	171.87		
		Ave $\pm$ S.E= 271 $\pm$ 51.16				

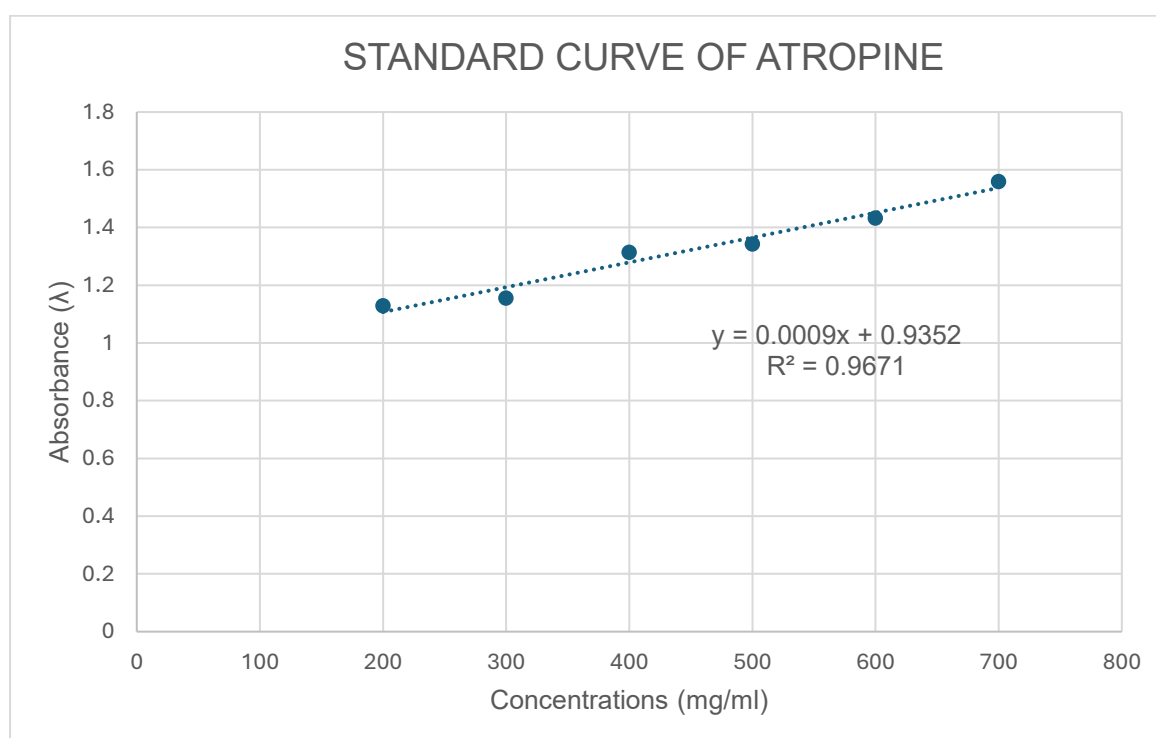


Fig. 1. Standard calibration curve of atropine for estimation of total alkaloid content for standard atropine (AE/mg).

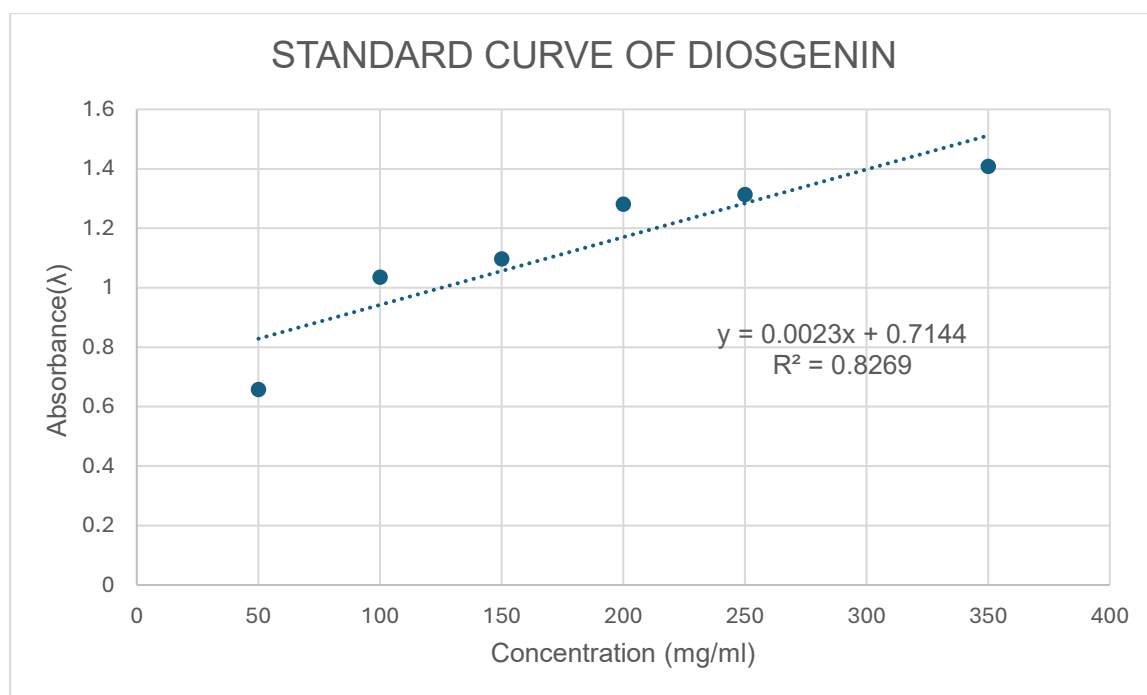


Fig. 2. Standard calibration curve of diosgenin for estimation of total saponin content for standard diosgenin (DE/mg).

The mortality of the crickets exposed to methanolic twig extracts of *E. tirucalli* increased with increasing concentrations. The highest concentration of the plant achieved 100% mortality of *A. domesticus*. *Acheta domesticus* in the control group survived for more than 24 hr (Table 2). *Euphorbia tirucalli* methanolic twig extract concentrations of 0.01 to 0.04% (w/v) yielded mortalities of 30 to 40% against *A. domesticus*, whereas 0.05% (w/v) achieved 50% mortality. Subsequent plant concentrations of 0.06 to 0.07% (w/v) resulted in 60% death of all exposed *A. domesticus*. Additionally, concentrations of 0.08 and 0.09% (w/v) yielded respective mortalities of 70 and 80% against tested *A. domesticus*. Finally, the highest concentration of 0.1% (w/v) resulted in 100% mortality of all tested *A. domesticus* (Table 2 and Fig. 3).

Table 2. Effects of *Euphorbia tirucalli* methanolic extracts on *Acheta domesticus*.

Sample no	Concentration (w/v) %	No of tested crickets	No of dead <i>A. domesticus</i>	Mortality (%)
0	0	10	0	0
1	0.01	10	3	30
2	0.02	10	4	40
3	0.03	10	4	40
4	0.04	10	4	40
5	0.05	10	5	50
6	0.06	10	6	60
7	0.07	10	6	60
8	0.08	10	7	70
9	0.09	10	8	80
10	0.1	10	10	100

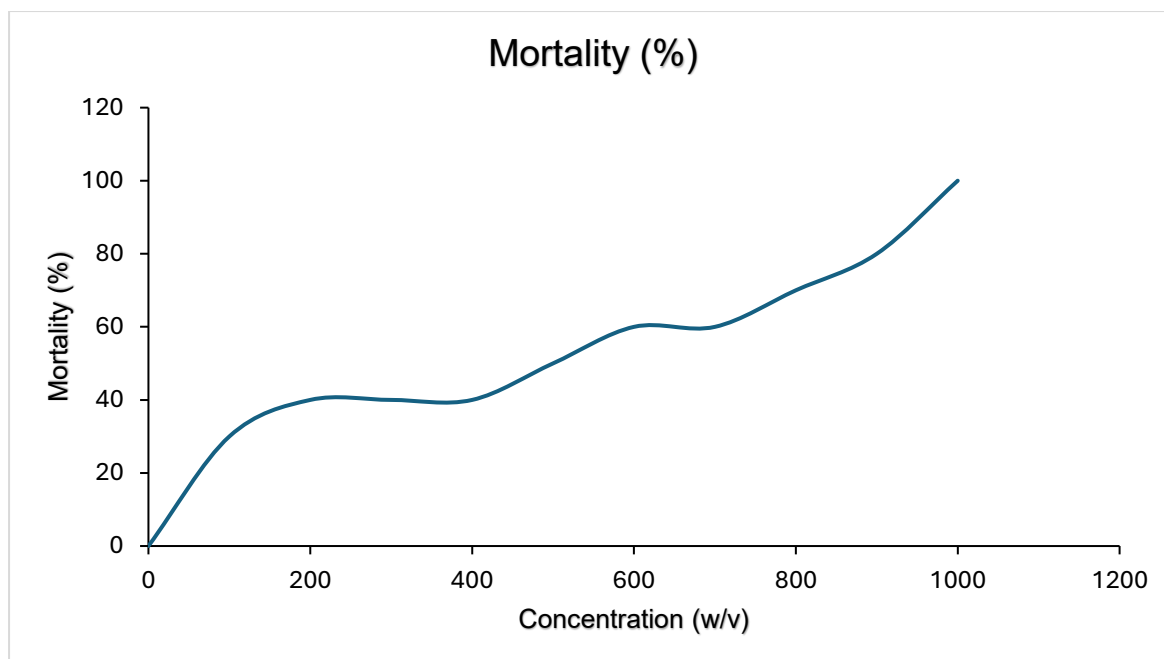


Fig. 3. Mortality of various concentrations of *Euphorbia tirucalli* methanolic twig extracts on *Acheta domesticus*.

The LC_{50} of methanolic twig extracts against *A. domesticus* was found to be 0.03% (w/v). The standard error of mean LC_{50} of methanolic twig extracts was determined at 0.03 ± 0.012 , with a 95% confidence interval of 0.018-0.042 (Table 3).

Table 3. LC_{50} values, standard error of LC_{50} and 95% confidence interval for effects of *Euphorbia tirucalli* methanolic twig extracts against *Acheta domesticus*.

Insect	$LC_{50} \pm S.E$	95% confidence interval
<i>Acheta domesticus</i>	0.03 ± 0.012	0.018-0.042

The mortality of *B. germanica* and *P. americana* exposed to *E. tirucalli* latex increased with increasing concentrations. The latex of the plant was more effective towards *P. americana* than *B. germanica*. *Blattella germanica* and *P. americana* in the control groups survived for more than 24 hr (Table 4).

Euphorbia tirucalli latex concentrations of 20, 40, 60, 80 and 100% (v/v) achieved mortalities of 20, 60, 70, 90 and 100%, respectively for *P. americana*. The same concentrations resulted in mortalities of 20, 50, 60, 80 and 100%, respectively for *B. germanica* (Table 4 and Fig. 4).

Table 4. Effects of *Euphorbia tirucalli* latex on *Blattella germanica* and *Periplaneta americana*.

Sample no	Concentration % (v/v)	No. of tested cockroaches	Mortality (%) of <i>P. americana</i>	Mortality (%) of <i>B. germanica</i>
0	0	10	0	0
1	20	10	20	20
2	40	10	60	50
3	60	10	70	60
4	80	10	90	80
5	100	10	100	100

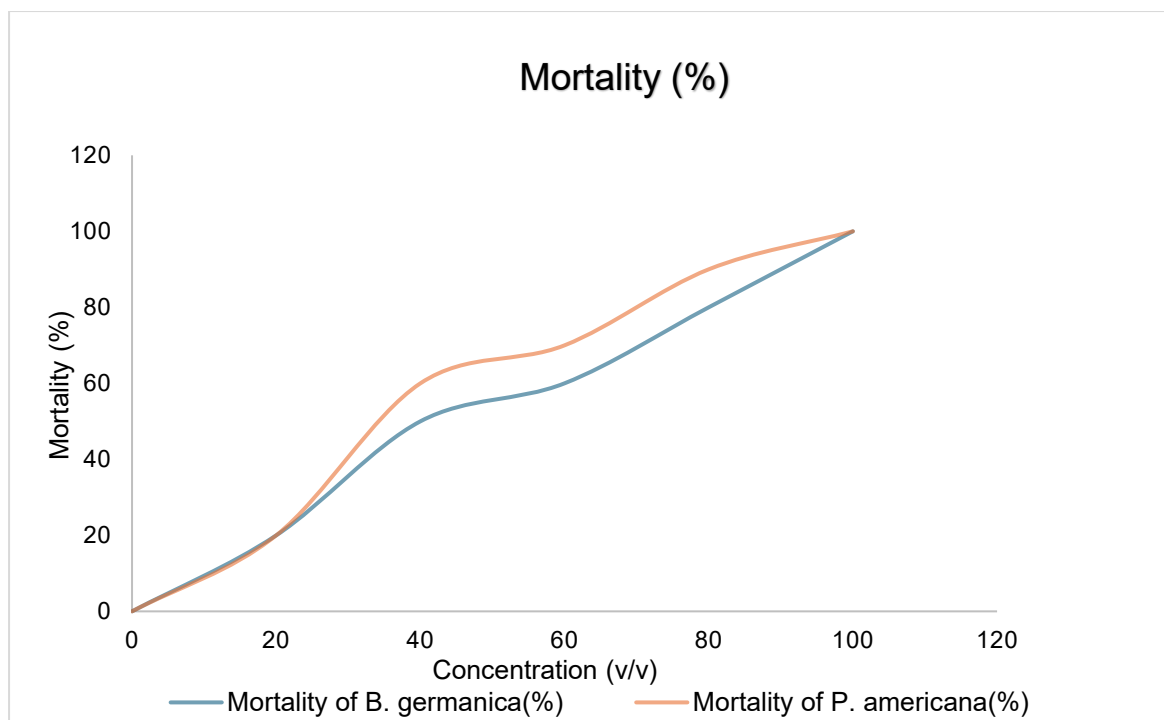


Fig. 4. Mortality of various concentrations of *Euphorbia tirucalli* latex on *Blattella germanica* and *Periplaneta americana*.

The LC_{50} of *E. tirucalli* latex against *B. germanica* and *P. americana* was found to be 39.03 and 35.35% (v/v), respectively. The standard error of mean LC_{50} of *E. tirucalli* latex was determined at 39.03 ± 12.11 , with a 95% confidence interval of 51.14-26.92 for *B. germanica*, whereas for *P. americana* it was found to be 35.35 ± 10.28 , with a 95% confidence interval of 45.63-25.07 (Table 5).

Table 5. LC_{50} values, standard error of LC_{50} and 95% confidence interval for effects of *Euphorbia tirucalli* latex against *Blattella germanica* and *Periplaneta americana*.

Insect	$LC_{50} \pm S.E$	95% confidence interval
<i>Blattella germanica</i>	39.03 ± 12.11	51.14-26.92
<i>Periplaneta americana</i>	35.35 ± 10.28	45.63-25.07

DISCUSSION

The quantitative analysis results of the current study reveal that *E. tirucalli* has considerable amounts of saponins and alkaloids. The quantitative analysis results of this study further confirms that plants of the genus *Euphorbia* have phytochemical compounds such as saponins and alkaloids. El-Amier *et al.* (2021) reported a total saponin and alkaloids estimation of 29.14 and 12.33 mg g⁻¹, respectively after investigating the methanolic aerial plant part extracts of *Euphorbia retusa* (Forsskål, 1775) sampled from Wadi Arabah, North-Eastern Desert, Egypt. The results of our study estimated the total saponin and alkaloids content of *E. tirucalli* to be 122 ± 98.28 mg diosgenin g⁻¹ and 271 ± 51.16 mg atropine g⁻¹, respectively, which are significantly higher as compared to compounds reported by El-Amier *et al.* (2021). The discrepancy between the two studies may be attributed to the different plant parts and species that were used in the respective studies.

In the current study, the insecticidal effects of *E. tirucalli* methanolic twig extracts were investigated against *A. domesticus* and found to be effective. These findings support scientific studies which demonstrate that plants in the genus *Euphorbia* have insecticidal effects against crickets. A study by Okonkwo and Ohaeri (2018a) examined how the essential oils from the leaves of *Euphorbia milii* (Des Moulins, 1826) exert insecticidal activity through disruption in ionic composition and found it to be effective against *Tettigonia viridissima* (Linnaeus, 1758). The insecticidal effects of hexane oil extracts from *E. milii* leaves increased with higher concentrations which is a phenomenon also witnessed in the current study. Okonkwo and Ohaeri (2018b) achieved an LD₅₀ at a concentration of 0.681 mg/kg after 24 hr. The authors achieved 66.67%±5.77 mean mortality for *Tettigonia viridissima* treated with 0.5 g of *E. milii* hexane oil extracts 24 hr after the initial exposure. The current study achieved an LC₅₀ at a concentration of 0.031% (w/v) and 100% mortality at concentrations of 0.09 and 0.1% (w/v) after 24 hr. The LD₅₀ value from the referenced study, reported as 0.681 mg/kg, is significantly lower than the LC₅₀ value of 0.03% (w/v) determined in the current study. The LC₅₀ of the current study suggests that methanolic twig extracts of *E. tirucalli* are less effective compared to hexane oil extracts of *E. milii* leaves, however, they still proved to have effective insecticidal properties. The discrepancy between the two studies could be the result of the different plant and insect species that were used in the respective studies. Another factor could be the different plant parts employed in the respective studies. The current study used methanolic twig extracts to assess the *E. tirucalli* insecticidal potency, whereas Okonkwo and Ohaeri (2018b) used hexane oil leaves extracts of *E. milii*.

The present study also examined the insecticidal effects of *E. tirucalli* latex against *B. germanica* and *P. americana* and found it to be effective. The results of this study corroborate previous reports on the effectiveness of *E. tirucalli* against cockroaches. A study by Azoui *et al.* (2016) tested *Euphorbia bupleroides* (Croizat, 1934) latex against *B. germanica* and observed a concentration-dependent mortality, which is an occurrence also observed in the current study. Azoui *et al.* (2016) reported an LC₅₀ value of 15.94 and 47.22% for *B. germanica* males and females, respectively. The authors revealed a 100% mortality at a concentration of 75% after 21 d. In contrast, the present study achieved an LC₅₀ value of 39.03% and 100% mortality at the highest concentration of 100% (v/v) within 24 hr.

The findings of the present study suggests that *E. tirucalli* latex insecticidal activity against *B. germanica* is fast acting compared to *E. buperlroides* latex. *Euphorbia tirucalli* achieved 100% mortality against *B. germanica* within 24 hr, while *E. buperlroides* only achieved the same results after a 21 d period. The discrepancy between the two studies can be attributed to the different plant species that were used in the respective studies.

CONCLUSION

The present study found that *E. tirucalli* methanolic twig extracts consist of phytochemicals such as saponins and alkaloids. The study also discovered that *E. tirucalli* methanolic twig extracts have significant insecticidal effects against *A. domesticus*, whereas *E. tirucalli* latex has satisfactory effects against *B. germanica* and *P. americana*. Therefore, it can be concluded that the insecticidal activity of *E. tirucalli* twigs and latex suggests that the plant has the potential to be developed as an organic insecticide suitable for controlling cockroach and cricket species. Additional studies such as chemical, toxicological and biodegradability assessment can be explored to potentially lead to the development of *E. tirucalli* extracts into an effective, safe, biodegradable and inexpensive botanical insecticides.

Declarations:

Ethical Approval: This research paper was approved by the research ethics committee from Sefako Makgatho Health Sciences University (SMUAREC/S/07/2024: PG_O).

Competing interests: The authors report no conflict of interest.

Authors' Contributions: I hereby verify that all authors (K.S.M, P.H.K and M.C.M) mentioned on the title page have made substantial contributions to the conception and design of the study. K.S.M wrote the main manuscript text, while P.H.K and M.C.M edited the article and thoroughly reviewed it, ensuring clarity and accuracy. Additionally, K.S.M, P.H.K and M.C.M collaborated on the interpretation of the data and provided critical feedback throughout the process. All authors have reviewed the manuscript, confirmed the accuracy and authenticity of the data and its interpretation and consent to its submission.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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