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Insecticidal Properties of *Euphorbia tirucalli* Against the German Cockroach (*Blattella germanica*) and American Cockroach (*Periplaneta americana*) Under Laboratory Settings

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ABSTRACT

Blattella germanica and *Periplaneta americana* are cockroach species known to carry disease causing pathogens. They are commonly controlled by synthetic insecticides, which are often associated with negative effects. Various plant species are documented to possess insecticidal properties against *B. germanica* and *P. americana* with minimal negative effects. *Euphorbia tirucalli* (Euphorbiaceae) is a succulent plant with reported insecticidal properties against various pest insects. The current study examined phytochemical compounds within *E. tirucalli* using qualitative analysis and determined the plant's insecticidal properties against *B. germanica* and *P. americana* using the dipping method. Qualitative analysis revealed that the plant has a moderate presence of glycosides and a strong presence of saponins, tannins, alkaloids, phenols and terpenoids. *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 mortality percentages of 10, 20, 50, 50, 70, 70, 80, 90, 100 and 100%, respectively for *P. americana*. The same concentrations also achieved mortality percentages of 10, 20, 30, 30, 30, 40, 60, 70, 70 and 80%, respectively against *B. germanica*. The LC_{50} of *E. tirucalli* extracts against *B. germanica* and *P. americana* were determined at 0.056 and 0.031, respectively. The 95% confidence interval was 0.021-0.091 and 0.02-0.042 for *B. germanica and P. americana*, respectively. Therefore, it can be concluded that *E. tirucalli* has various phytochemicals and insecticidal properties against *B. germanica* and *P. americana*.

INTRODUCTION

Cockroaches are invertebrate insects commonly known as roaches, in the order Blattodea and are globally regarded as pests. There are approximately 4,300 identified cockroach species worldwide and 30 are associated with human habitations (Wang *et al*., 2021). *Periplaneta americana* (Linnaeus, 1758) and *Blattella germanica* (Linnaeus, 1767) are the most common cockroach species that are known to thrive in human dwellings (Mullen and Durden, 2009; Picker, 2012). These species thrive in human habitations because they have access to unlimited supply of resources vital for their survival such as food, water and shelter. Additionally, human habitations are preferred by these cockroach species because they have little to no natural predators (Bell *et al*., 2007).

Periplaneta americana and *B. germanica* are found thriving in human habitations such as commercial store buildings, sewers, households, healthcare centers, food storage facilities and establishments such as hotels and restaurants (Wang *et al*., 2021). These cockroaches are omnivorous and feed on various materials such as stovetop food splatters, leftovers, staple foods, produce, cardboard packaging, paper and soap (Bell *et al*., 2007; Jensen *et al*., 2015). Heavy infestations of these organisms, however, poses potential social and health risks. These organisms have a foul bodily smell, release fecal matter that has an unpleasant odor and are associated with poor hygienic practices (Oldenburg *et al*., 2008; Wang *et al*., 2021). They are also known to act as mechanical vectors to various disease-causing pathogens such as viruses, bacteria, protozoa and fungi (Roth and Willis, 1960).

Cockroach allergens derived from feces, saliva and bodies of *B. germanica* and *P. americana* have been reported to induce asthma in children. A review study by Do *et al*. (2016) report that within United States inner cities, children allergic to cockroach allergens and residing within cockroach infested areas are hospitalized at a 3.4 times higher rate due to asthma. The study further reported that children without cockroach allergies and stay in low cockroach infestations suffer less asthma hospitalizations than their counterparts. Additionally, the former children have 78% more hospital visits and more missed school days as compared to children living in non-infested areas. A study by Kalantari *et al*. (2023) implicated cockroaches in the transmission of SARS-CoV-2 (COVID-19). According to the authors, *P. americana* and *B. germanica* mechanically transmit COVID-19 by coming in contact with the virus from contaminated surfaces, human feces and feeding on said feces.

Periplaneta americana and *B. germanica* are commonly controlled by the practice of good hygiene and the usage of synthetic and organic insecticides (Yağci *et al*., 2016; Passara *et al*., 2025). Synthetic insecticides are preferred over their organic counterparts. Synthetic insecticide products are manufactured as aerosols, chalks and powders with most of the products belonging to major insecticide classes such as organophosphates, pyrethroids and carbamates (Zhu *et al*., 2020; Wang *et al*., 2021). These products, though effective, have been reported to harm non-targeted organisms and affect human health while also being expensive (Curl *et al*., 2020). The prolonged usage and abuse of synthetic insecticides such as cypermethrin also causes resistivity in targeted pests (Zhu *et al*., 2020). Organophosphates and pyrethroids commonly used by fumigation and pest control companies have been reported to pose health complications such as neurological symptoms to human pesticide applicators (Hoppin *et al*., 2008; Araujo *et al*., 2024). These products also run off into the soil and water, leading to environmental contamination (Sidhu *et al*., 2019).

Plants and plant-based products have been reported to have insecticidal properties against cockroaches. Gülmez *et al*. (2021) evaluated the insecticidal activity of *Euphorbia bupleuroides* (Desfontaines, 1798) latex against the German cockroach and found that it was efficient against males and nymphs. Manzoor *et al*. (2012) observed high repellent action of *Cymbopogen citratus* (Stapf, 1906) against *P. americana. Euphorbia tirucalli* (Linnaeus, 1753) is an evergreen plant from the Euphorbiaceae family and the plants from this family also have insecticidal properties against cockroaches (Azoui *et al*., 2016). Unfortunately, there is no indication that the plant *E. tirucalli* has been utilized to control *B. germanica* or *P. americana*. Therefore, the current study investigated the insecticidal properties of *E. tirucalli* against *P. americana* and *B. germanica*.

MATERIALS AND METHODS

Study Area:

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

Collection of *Euphorbia tirucalli*:

Euphorbia tirucalli twigs were randomly collected from their natural habitat in the gardens of SMU. The collected plant specimens were carried to the Biology and Environmental Sciences laboratory in sealed plastic bags to avoid dehydration. Voucher plant samples were sent to the South African National Biodiversity Institute (SANBI) for authentication and verification.

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

Blattella germanica were collected at SMU residences while *P. americana* were purchased from a local pet store in Ga-Rankuwa, Pretoria, South Africa. The cockroaches were transported from the SMU residences and pet store to the laboratory in ventilated plastic containers (41L x 28.6W x 27.6H centimeter dimensions). *Blattella germanica* and *P. americana* were transferred into respective ventilated plastic containers (41L x 28.6W x 27.6H centimeter dimensions) fitted with egg cartons and provided with bedding comprising of wood chips, corrugated cardboard and dried moss. The cockroaches were allowed to acclimatize for a 7 d period and a 12:12 (L:D) photoperiod was set. The temperature in the laboratory was kept between 25-30°C. Dry dog food was provided as food at *ad libitum* for the specimens and a container with water and a cotton roll was used as a water source.

Chemicals:

All chemicals were of highest purity (≥ 90) and well within the expiry date. Chloroform, Acetic acid, Sulfuric acid, Iron (III) chloride, Methanol and Acetic anhydride were purchased at 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 twigs were thoroughly washed with distilled water and air dried for a period of three weeks and grounded using an electrical blender on the fourth week. Thereafter, the grounded plant material was sieved using a mesh of 250 μm to separate the debris from the fine material and weighed at 100 g. The fine plant material was then soaked in 1000 ml of methanol, and the mixture was shaken for 24 hr using an orbital shaker at 110 rpm. Hereafter, the methanol 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 rotatory evaporator at 40ºC. The dried substrate was weighed at a mass of 15 g and stored in a sealed container at room temperature.

Phytochemical Screening of Dry Methanolic Twig Extracts of *Euphorbia tirucalli*:

Phytochemical screening of glycosides, saponins, terpenoids, alkaloids, tannins and phenols within *E. tirucalli* twig extracts were performed using methods described by Yadav and Agarwala (2011), Indumathi *et al*. (2014) and Shah and Yadav (2015) with slight modifications.

Glycosides:

Two mg of *E. tirucalli* twig extracts were mixed with 2 ml of $CHCl_3$ and CH_3COOH in a test tube maintained within an ice bath. Hereafter, 1 ml of concentrated H_2SO_4 was slowly added to the mixture. A green colour change indicated the presence of glycosides.

Saponins:

Five ml of distilled water was mixed with 2 mg of extract within a test tube and vigorously shaken. A stable foam indicated the presence of saponins.

Tannins and Phenols:

Two mg of *E. tirucalli* twig extracts were mixed with 2 ml of $FeCl_3$ (2% solution) in a test tube containing. A blue-green or black colour indicated the presence of phenols and tannins.

Terpenoids:

Two mg of *E. tirucalli* twig extracts were mixed with 5 ml of C_2H_5OH and 2 ml of CHCl3 in a test tube maintained within a warm water bath. Hereafter, 3 ml of concentrated H_2SO_4 was added after cooling. A reddish-brown precipitate indicated the presence of terpenoids.

Alkaloids:

Two mg of *E. tirucalli* twig extracts were mixed with a few drops of concentrated HCl and Mayer's reagent in a test tube. A green colour or white precipitate indicated the presence of alkaloids.

Insecticidal Effects of *Euphorbia tirucalli* on adult *Blattella germanica* and *Periplaneta americana*:

Insecticidal effects of *E. tirucalli* on adult *B. germanica* was conducted using the dipping method described by Yu (2011) with slight modifications. Ten adult *B. germanica* specimens of approximately the same length of 1.3 cm were placed within 11 plastic containers. The first 10 containers were filled with 5 ml experimental concentration solutions of methanolic twig extracts of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1% (w/v) and the 11th plastic container served as a negative control and was filled with only 5 ml of aged water. The cockroaches were dipped for 5 sec and removed from the concentrations and observed for a period of 24 hr with provision of food in recovery containers. Dead cockroaches were removed from the set-up and the data was recorded. The experiment trials were performed in triplicate and the same procedure was followed for *P. americana* specimens of approximately the same length of 3.5 cm.

Data Analysis:

Mortality percentages were recorded 24 hr post-treatment and corrected using Abbott's formula (Abbott, 1925). The median lethal concentration (LC_{50}) values were calculated using probit analysis, following the method described by Finney and Stevens (1948), using Microsoft Excel. The final mortality data of cockroaches exposed to *E. tirucalli* were presented as mean values ± standard error of the mean (SEM).

RESULTS

Qualitative analysis revealed that the methanolic twig extracts of *E. tirucalli* have a high presence of saponins, tannins, phenols, alkaloids, terpenoids and a moderate amount of glycosides (Table 1).

Table 1. Qualitative results for methanol extracts of *Euphorbia tirucalli*.

Secondary metabolites	Presence
Glycosides	++
Saponins	+++
Tannins	+++
Phenols	+++
Terpenoids	+++
Alkaloids	+++
(Note that motility is indicated as follows: +++ (strong presence), ++ (moderate presence), + (slight presence), - (faint presence), 0 (absent).	

The mortality of *B. germanica* and *P. americana* exposed to methanolic twig extracts of *E. tirucalli* increased with increasing concentrations. The methanolic twig extracts of the plant were more effective towards *P. americana* than *B. germanica*. *Blattella germanica* and

P. americana in the control groups survived for more than 24 hr (Table 2). *Euphorbia tirucalli* methanolic twig extract 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 mortality percentages of 10, 20, 50, 70, 70, 80, 90, 100 and 100%, respectively for *P. americana*. The same concentrations achieved mortality percentages of 10, 20, 30, 30, 30, 40, 60, 70, 70 and 80%, respectively for *B. germanica* (Table 2 and Fig. 1).

Table 2. Effects of *Euphorbia tirucalli* methanolic extracts on *Blattella germanica* and *Periplaneta americana*.

Sample no	Concentration %(w/v)	No. of tested cockroaches	Mortality (%) of *B. germanica*	Mortality (%) of *P. americana*
0	0	10	0	0
1	0.01	10	10	10
2	0.02	10	20	20
3	0.03	10	30	50
4	0.04	10	30	50
5	0.05	10	30	70
6	0.06	10	40	70
7	0.07	10	60	80
8	0.08	10	70	90
9	0.09	10	70	100
10	0.1	10	80	100

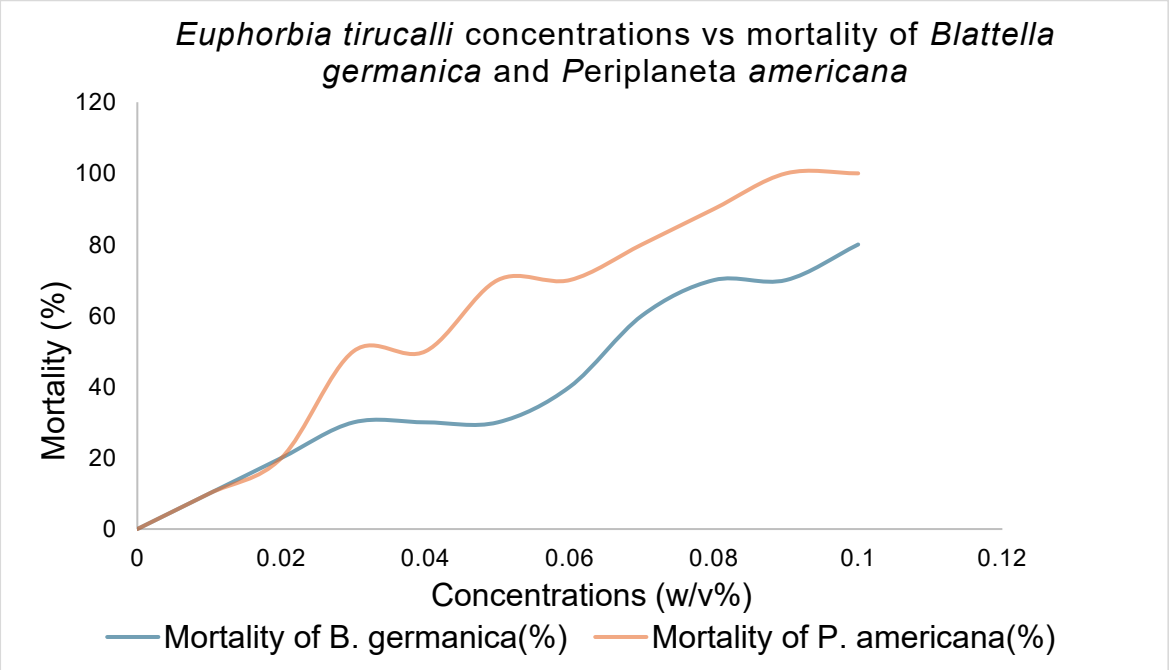

Fig. 1. Mortality of various concentrations of *Euphorbia tirucalli* methanolic twigs extracts on *Blattella germanica and Periplaneta americana.*

The LC_{50} value of *E. tirucalli* extracts against *B. germanica and P. americana* were determined at 0.056 and 0.031, respectively. The 95% confidence interval was found to be 0.198-0.162 and 0.081-0.049 for *B. germanica* and *P. americana*, respectively (**Table 3**).

Table 3. LC_{50} values, standard error of LC_{50} and 95% confidence interval for effects of *Euphorbia tirucalli* methanolic extracts against *Blattella germanica* and *Periplaneta americana*.

Insects	LC_{50}	Standard error of LC_{50}	95% confidence interval
B. germanica	0.056	0.056±0.035	0.021-0.091
P. americana	0.031	0.031±0.0.011	0.02-0.042

DISCUSSION

The current study examined the phytochemical compounds found within the twigs of *E. tirucalli* and revealed that the plant has various phytochemical compounds such as glycosides, saponins, alkaloids, tannins, phenols and terpenoids. The current study revealed similar results as Bang *et al*. (2024), who investigated the antibacterial activity of *E. tirucalli* against *Lactobacillus acidophilus* (Moro, 1900) and found that the plant contains tannins and saponins. Madane *et al*. (2013) reported the presence of phytochemicals within *E. tirucalli* leaves such as glycosides, saponins, phenols and alkaloids which align with the results of the current study.

The current study examined the insecticidal effects of *E. tirucalli* against *B. germanica* and *P. americana* and found it to be effective. The results of the current study validate and support scientific reports that provide evidence on the insecticidal effects of *E. tirucalli* against cockroaches. A study by Azoui *et al*. (2016) investigated the insecticidal effects of *E. bupleuroides* latex on *B. germanica*. The insecticidal effects of *E. bupleuroides* latex increased with higher concentrations, which is a phenomenon also observed in the current study. Azoui *et al*. (2016) achieved LC_{50} at concentrations of 15.94 and 47.22% for males and female *B. germanica*, respectively. The authors also achieved 100% mortality at their highest concentration of 75% after 21 days. The current study achieved LC_{50} at a concentration of 0.056% and achieved 80% mortality at a concentration of 0.1% after a 24 hr period. The results of the current study suggest that methanolic twig extracts of *E. tirucalli* are more effective against *B. germanica* compared to *E. bupleuroides* latex because higher concentrations are required to achieve similar results, as indicated by the current study. The discrepancy between the two studies could be the result of the different plant species used in the respective studies. Another factor could be that methanolic twig extracts were used in the current study, whereas Azoui *et al*. (2016) used *E. bupleuroides* latex.

The results of the current study further confirm scientific reports revealing that plants in the genus *Euphorbia* have insecticidal properties. A study by Jaouadi *et al*. (2022) evaluated the antioxidant and insecticidal properties of *Euphorbia helioscopia* (Linnaeus, 1753) aqueous flower extracts against *Tribolium castaneum* (Herbst, 1797). The insecticidal effects of *E. helioscopia* aqueous extracts increased with higher concentrations, an occurrence also observed in the current study. Jaouadi *et al*. (2022) achieved LC_{50} at a concentration of 2.90 mg/ml. The authors also achieved 100% mortality at their highest concentration of 10% 4 d after treatment. The current study achieved LC_{50} at concentrations of 0.056 and 0.031% (w/v) for *B. germanica* and *P. americana* respectively, 24 hr after treatment.

The current study achieved 80 and 100% mortality for *B. germanica* and *P. americana* at concentrations of 0.1 and 0.09% (w/v) after a 24 hr period, respectively. The experimental concentrations of the current study are significantly lower compared to those used by the above-mentioned authors. The results of the current study suggest that methanolic twig extracts of *E. tirucalli* are more effective compared to aqueous flower extracts of *E.*

helioscopia as Jaouadi *et al*. (2022) only achieved a 100% mortality at a concentration of 10%, against *T. castaneum*, whereas in the current study the same results were achieved at concentrations of 0.09 and 0.1% (w/v) against *B. germanica* and *P. americana*, respectively. The discrepancy between the two studies could be the result of the different plant and insect species that were used in the respective studies.

CONCLUSION

The present study revealed that various phytochemicals such as glycosides, saponins, tannins, phenols and terpenoids are present in the methanolic twig extracts of *E. tirucalli*. This present study further demonstrated that methanolic twig extracts of *E. tirucalli* exhibit insecticidal properties against *B. germanica* and *P. americana*. Notably, the plant shows greater effectiveness against *P. americana* compared to *B. germanica*. The twigs of *E. tirucalli* could be a promising prospect for the development of organic insecticides targeting these cockroach species. Utilizing this plant may lead to the production of effective, safe, biodegradable and cost-effective botanical insecticides for vector control, which could enhance resistance management strategies against *B. germanica* and *P. americana* in South Africa and beyond. Consequently, further chemical analyses are crucial for identifying, preparing and formulating bioactive compounds derived from this plant.

DECLARATIONS

Ethical Approval: This research paper was approved by the research ethics committee of the 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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