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Effectiveness of Kalamansi Leaves (*Citrofortunella microcarpa*) as a Natural Insecticide against Cockroaches (*Periplaneta americana*)

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Abstrak: Ketergantungan pada insektisida sintetis untuk membasmi kecoa memicu kerusakan ekosistem dan resistensi hama, sehingga sangat dibutuhkan insektisida alami yang lebih ramah lingkungan. Penelitian ini bertujuan untuk menguji efektivitas ekstrak daun jeruk kalamansi *(Citrofortunella microcarpa)* sebagai insektisida alami kecoa Amerika *(Periplaneta americana)*. Daun *C. microcarpa* diekstrak dengan menggunakan metode maserasi dengan pelarut etanol 96%. Ekstrak dari daun *C. microcarpa* dibuat dalam 5 konsentrasi yaitu 20%, 40%, 60%, 80%, 90%. Efektivitas ekstrak diuji dengan menggunakan metode semprot dan diamati selama 9 jam pada hewan uji. Pengamatan menunjukkan penurunan aktivitas *P. americana* dan perubahan morfologi sehingga berujung kematian. Data mortalitas dianalisis menggunakan analisis probit untuk menentukan LC_{50}. Hasil uji fitokimia ekstrak daun *C. microcarpa* menunjukkan adanya *tanin, saponin, flavonoid,* dan *alkaloid.* Ekstrak daun *C. microcarpa* efektif membunuh *P. americana* dengan persentase kematian masing-masing 33%, 83%, 100%, 100%, dan 100% pada konsentrasi 20%, 40%, 60%, 80%, dan 90% dengan LC_{50} 23,85%. Berdasarkan hasil penelitian, ekstrak daun *C. microcarpa* teridentifikasi mengandung senyawa *tanin, saponin, flavonoid,* dan *alkaloid*. Konsentrasi 60% menunjukkan efektivitas tertinggi dalam meningkatkan tingkat mortalitas *P. americana.*

Kata kunci: Daun *Citrofortunella microcarpa*; *Periplaneta americana*; Insektisida Alami; Mortalitas.

Abstract: Synthetic insecticides used to eradicate cockroaches leads to ecosystem damage and pest resistance, so there need environmental friendly natural insecticides. This study aims to evaluate the effectiveness of calamansi leaf extract (*Citrofortunella microcarpa*) as a natural insecticide against the American cockroach (*Periplaneta americana*). The leaves of *C. microcarpa* were extracted using the maceration method with 96% ethanol. The extract was prepared in 5 (five) concentrations: 20%, 40%, 60%, 80%, and 90%. Its effectiveness was tested using a spraying method and observed over 9 (nine) hours on the trial. The data shows decreased in the activity of *P. americana* and morphological changes that ultimately led to death. Mortality data were analyzed using probit analysis to determine the LC_{50} value. The phytochemical screening of leaf *C. microcarpa* extract revealed the presence of tannins, saponins, flavonoids, and alkaloids. The leaf *C. microcarpa* extract was effective in killing *P. americana*, with mortality rates of 33%, 83%, 100%, 100%, and 100% at concentrations of 20%, 40%, 60%, 80%, and 90%, respectively, and an LC_{50} of 23.85%. Leaf *C.*

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Microcarpa extract containing tannins, saponins, flavonoids, and alkaloids. The 60% concentration demonstrated the highest effectiveness in increasing the mortality rate of *P. americana*.
Keyword:*Citrofortunella microcarpa*; *Periplaneta americana*; Natural Insecticide; Mortality.

1. Introduction

The environment is one of the key factors that strongly determine public health in daily life. Unhealthy environmental conditions have a detrimental impact on both comfort and overall health. The continuously increasing population drives this situation due to rising birth rates, as well as urbanization, which makes the environment more densely populated [1]**.** Indonesia ranks fourth in the world as the country with the largest population, driven by an annual population growth rate of 1.00% [2]. As the population continues to grow, increasingly rising consumption patterns among society also trigger a surge in the volume of waste generated each day [3]. The largest proportion of waste consists of food waste, accounting for 41.55% of the total [4]. The lack of awareness in maintaining environmental sanitation contributes to the emergence of *P. americana* infestations in domestic areas, reflecting the fragility of cleanliness standards around the home. *P. americana* significantly disrupts comfort and causes distress for some individuals. It is one of the most common household pests and can act as a vector for diseases in humans, being widely found across Indonesia and around the world [5]. *P. americana* is considered a nuisance to human health because it typically breeds and forages in unsanitary environments such as garbage bins, drainage systems, and septic tanks. Health problems associated with this insect include asthma, allergies, and gastrointestinal infections resulting from contamination of food and household utensils [6].

Methods for controlling *P. americana* pests have been implemented through the application of chemical agents as well as the use of mechanical traps; however, their effectiveness is often limited, leading to negative impacts on human health and the environment. Synthetic insecticides are effective in eliminating *P. americana*, but their long-term use can result in adverse effects, such as the accumulation of toxic residues in the environment [7]. Another

negative impact of the use of synthetic insecticides is that they can lead to contamination of water resources as well as pose health risks to humans and animals. Continuous exposure to insecticides creates high selective pressure, which in turn promotes the development of resistance in insects [8]. This condition necessitates comprehensive management and control over the use of and dependence on synthetic insecticide dosages [9].

Based on this phenomenon, more serious challenges arise in sustainable pest management, highlighting the need for innovative and environmentally friendly solutions, one of which is the use of natural insecticides. Natural insecticides contain compounds that are easily degraded in the environment compared to synthetic ones [10]. Natural insecticides are typically derived from plant extracts and specific bioactive compounds [11] . These natural materials are safer to use because they have high biodegradability, resulting in lower toxicity when they come into contact with non-target organisms [12]. The utilization of natural substances offers the advantage of a multitarget mechanism, which includes disruption of the nervous system, inhibition of reproduction, and repellent activity [13]. The integration of these various mechanisms contributes to improving the effectiveness of sustainable pest control. The development of natural insecticides is a highly strategic step toward creating more environmentally friendly and sustainable solutions for controlling *P. americana* [14].

Various natural substances have been used as insecticides by previous researchers; however, no studies have yet examined the leaves of *C. microcarpa* as a natural insecticide, particularly against *P. americana*. Earlier research has primarily focused on other natural materials, making this an opportunity as well as a challenge to utilize *C. microcarpa* leaves as a more environmentally friendly alternative insecticide. The leaves of *C. microcarpa* have potential as a natural insecticide because they contain secondary metabolite compounds that can be used for insecticidal purposes. The essential oil content of *C. microcarpa* leaves can be utilized as a natural insecticide. The compounds present in *C. microcarpa* leaves include citronellal, which is found in the essential oil and

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Home page: www.jurnal.ar-raniry.ac.id/index.php/biotik/index

belongs to a class of compounds that act as insect repellents [15]. Leaves of *C. microcarpa* also contain flavonoids, alkaloids, tannins, saponins, terpenoids, and steroids, which are toxic to insects and vectors [16]. The presence of these compounds suggests that *C. microcarpa* leaves have potential as a natural insecticide against *P. americana*. The effectiveness of the extract was evaluated over five consecutive days, and the LC_{50} value was determined to estimate the concentration required to kill 50% of the test population.

2. Research Method

a. Equipment and Chemicals

The equipment included a blender, an SF-400 digital scale, scissors, trays, tissue, a spatula, a stirring rod, a measuring cylinder, a beaker, a glass, a mini funnel, filter paper, test tubes, along with their rack, a rotary vacuum evaporator unit, and a roll of aluminum foil. For testing containers and the extract application, a thin-walled container (Victory Square, 3000 ml) and a 30 ml mini spray bottle were used. The research materials consisted of 3000 g of *C. microcarpa* leaves, 5 liters of 96% ethanol, distilled water (aquades), as well as various chemical reagents such as concentrated HCl, 2N HCl, 1% FeCl, amyl alcohol, CH_3COOH, Mayer's reagent, and magnesium metal. The test subject used in this study was *P. americana*.

b. Research Procedure

Sample preparation

Leaves of *C. microcarpa* were selected because they contain secondary metabolites with insecticidal potential and are environmentally friendly due to their biodegradability. Only mature leaves were used to ensure optimal and stable levels of secondary metabolites. Leaves of *C. microcarpa* were obtained from a community garden located in Pekik Nyaring Village, Central Bengkulu (-3.740980° S, 102.253896° E). The leaves were cleaned, cut into small pieces, and then air-dried indoors for four days. The dried samples were subsequently ground into a fine powder using a chopper.

Extraction of C. microcarpa leaves

Leaves of *C. microcarpa* simplicia were macerated in 96% ethanol for 3 × 24 hours and filtered. The filtrate was then concentrated using a vacuum rotary evaporator to remove the remaining ethanol and obtain a concentrated *C. microcarpa* leaf extract.

Phytochemical screening

Flavonoid test

Dilute the leaf *C. microcarpa* extract, add 1–2 pieces of Mg metal and a few drops of concentrated HCl, then add amyl alcohol and shake vigorously. After phase separation, a yellow to reddish color indicates the presence of flavonoids.

Saponin test

Take a small sample of the extract, place it into a test tube, add 10 mL of distilled water, and shake it vigorously.

Tannin test

Place 0.5 g of the sample into a test tube, then add 10 mL of distilled water, followed by 2–3 drops of 1% $FeCl_3$ solution. A positive result is indicated by the formation of a green, greenish-blue, or bluish-black color.

Alkaloid test

Weigh 0.5 g of the sample and place it into a beaker. Add 5 mL of 2N HCl, then filter the mixture. Transfer the filtrate into a test tube and add Mayer's reagent dropwise. A positive result is indicated by the formation of a white precipitate, which confirms the presence of alkaloids.

Terpenoid and steroid test

Add one drop of anhydrous CH_3COOH and one drop of concentrated H_2SO_4 to the extract. A positive result for steroids is indicated by the appearance of a blue to purple color, while a positive result for terpenoids is indicated by the appearance of a red color.

Preparation of experimental animals

A total of 30 *P. americana* were collected from houses around Bengkulu University and divided into five transparent containers (six individuals each).

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The prepared solution was sprayed into each container, and observations were conducted over five days (one container per day) to assess mortality.

Preparation of the extract solution

Table 1. Concentration of Leaf *C. microcarpa* Extract

No	Treatment	Leaf *C. microcarpa* extract (ml)	Aquadest (ml)	Total (ml)
1	P1(20%)	3	12	15
2	P2(40%)	6	9	15
3	P3(60%)	9	6	15
4	P4(80%)	12	3	15
5	P5(90%)	13,5	1,5	15

Data analysis

Observations included mortality, activity, and morphological changes in *P. americana*. Healthy, active adults (2–5 cm) with no physical defects were selected. Each treatment was applied by spraying 2 mL of insecticide, and responses were monitored for 9 hours. Mortality was indicated by immobility, stiff legs, and complete cessation of movement after stimulation, while living *P. americana* were identified by their characteristic unpleasant odor. The effectiveness of calamansi lime leaves was evaluated based on these mortality, activity, and morphological changes after spraying.

To distinguish true death from thanatosis, *P. americana* was examined for leg and antenna stiffness, subtle movement after gentle stimulation, pheromone odor, and escape response. True death was indicated by curled legs, immobile antennae, a stiff and drying body, and no response to touch. Morphological changes were assessed by comparing photographs taken before and after spraying with the natural insecticide.

The data were analyzed using probit analysis to determine the LC_{50} value, which represents the concentration required to kill 50% of the test population. The analysis was based on $\log_{10}$ concentration and probit values, and the results were presented as a graph showing the relationship between *C. microcarpa* leaf extract concentration and the percentage of *P. americana* mortality. Probit analysis is primarily applied in the field of toxicology; this method produces a simple linear relationship between probit and dose response, derived from the percentage of affected organisms versus the dose

response [17]. A substance concentration meets the LC_{50} parameter if it is capable of causing 50% mortality in the test organism population [18]. The method for calculating LC_{50} is:

a. Percentage of mortality

$$\% \text{ mortality} = \frac{Number\ of\ dead\ insects}{Total\ number\ of\ insects} \text{ x } 100\%$$

b. Mortality Test (MT)

$$\% \text{ MT} = \frac{\%\ treatment\ mortality - \%\ control\ mortality}{100 - Number\ of\ dead\ insects\ in\ the\ control\ group}$$

c. The probit values of corrected mortality were determined and then used to construct a linear graph relating these probit values to the $\log_{10}$ of the extract concentration.

d. Determine the LC_{50} value by solving for x by substituting the values into the obtained equation. Then, calculate the LC_{50} value by taking the antilog of (x) or 10x.

3. Results and discussion

a. Phytochemical screening

Phytochemical screening of the extract of *C. microcarpa* leaves (Table 2) identified secondary metabolites with potential insecticidal activity, including flavonoids, tannins, saponins, and alkaloids. These bioactive compounds can be extracted from various plant parts, making them a sustainable source of natural pesticides [19].

Table 2. Phytochemical screening test results of leaf *C. microcarpa* extract

Phytochemical compounds	Method	Screening (positive)	Identification
Alkaloid	0,5 g sample + HCl 2N + Mayer's	A white precipitate was formed	+
Flavonoid	Extract + 1–2 pieces of magnesium metal + concentrated HCl + amyl alcohol	An orange to red color was formed	+
Tanin	0.5 g sample + distilled water + 1% $FeCl_3$ solution	A green, blue-black color was formed	+
Saponin	Extract + Aquadest	A stable foam was formed	+
Terpenoid dan Steroid	Extract + CH_3COOH + H_2SO_4	A reddish-brown color and a blue to purple color were formed	-

Caption: (+) Contains secondary metabolite
(-) Not-contain secondary metabolite c

Alkaloids, flavonoids, saponins, and tannins were positively detected in Kalamansi lime leaf extract, indicated by the formation of a white precipitate (Mayer's reagent), orange color, stable foam, and dark greenish-black color ($FeCl_3$), respectively. In contrast, steroids and terpenoids were not detected due to the absence of a red color. The phytochemical test results are presented in Figure 1.

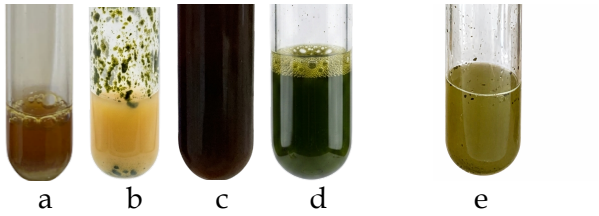


Figure 1. Positive test (a. *Alkaloid,* b. *Flavonoid,* c. *Tannin,* d. *Saponin)* Negative test (e. *Terpenoid dan steroid)*

b. Mortality, activity, and morphology of *P. americana* tested

Mortality of *P. americana* was identified by the absence of movement after stimulation, an inverted body position, and morphological changes such as a rigid body, inward-bent legs, discoloration, or detached body parts. Based on the 9-hour observation, the extract of *C. microcarpa* leaves showed significant effectiveness in reducing the *P. americana* population.

Table 3. Mortality Test of *P. americana*

Insecticide dosage	Insecticide concentration	N	Number of Mortality/hour									Amount mortality	% Lethal
			1	2	3	4	5	6	7	8	9		
15 ml (3 times sprayed)	P1 (20%)	6						1			1	2	33%
	P2 (40%)	6						1	2	1	1	5	83%
	P3 (60%)	6			2	1		1	2			6	100%
	P4 (80%)	6	4	1	1							6	100%
	P5 (90%)	6	6									6	100%

The data in Table 3 confirm a linear relationship between concentration level and the rate of insect mortality. *P. americana* that showed no movement was further tested by shaking the container and touching *P. americana* with a stirring rod; if it still did not move, it was considered mortality. [20]. At 20% concentration, *P. americana* mortality reached only 33%, whereas concentrations of 60% and above resulted in 100% mortality. Therefore, the 60% concentration was considered the optimal natural insecticide, achieving

complete mortality with lower residue exposure than the 80% and 90% concentrations. The mortality results are presented in Figure 2.

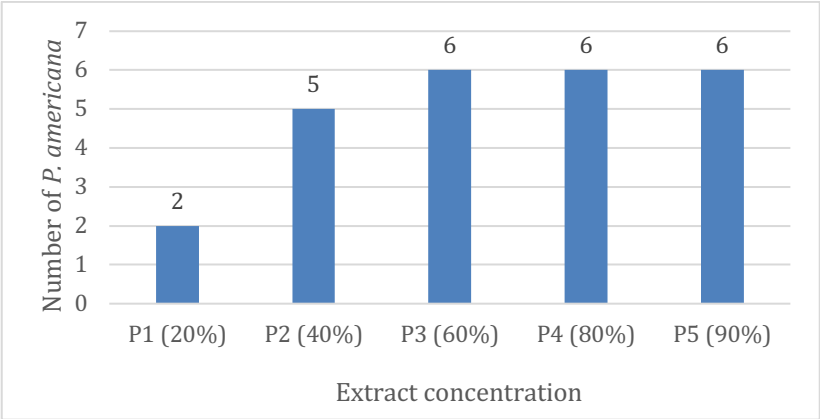
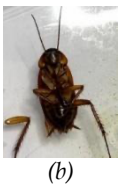


Figure 2. Mortality of *P. americana*

Figure 2 shows the higher the concentration of leaf *C. microcarpa* extract, the higher the mortality rate of *P. americana*. This indicates that increasing extract concentration leads to a greater accumulation of active compounds exposed to the target organism, resulting in mass organ failure in a shorter period of time [21].

Table 4. Data from the mortality testing of *P. americana*

Treatment	Activity	Morphology	Figure
P1 (20%)	Praying: *P. americana* is still actively moving and avoiding. T+1: still actively moving. T+2: *P. americana* ID 2 and ID 6 began to weaken, with reduced movement response. T+3: no deaths observed; activity of *P. americana* ID 2, ID 3, and ID 6 decreased. T+6: *P. americana* ID 3 and ID 2 were died while others were still active. T+9: A total of 2 individuals were died, while the remaining ones were still able to move.	T+2: one leg of *P. americana* ID 3 detached. The weakened *P. americana* showed a decreased movement response. Not all *P. americana* exhibited body rigidity.	 *(a)*  *(b)*
P2 (40%)	Spraying: *P. americana* actively ran to escape. T+1: still active, although some began to be affected. T+3: ID 1, ID 2, and ID 5 remained active, while ID 3, ID 4, and ID 6 were more often immobile. T+6: Two *P. americana* became very weak and then died. T+9: five individuals were died, and one remained in a very weak condition.	T+1: a leg of *P. americana* ID 3 detached. The weakened *P. americana* was characterized by weak leg movements. Toward the end, the body was weak, and not all individuals had become fully rigid.	 *(a)*

			 (b)
P3 (60%)	Spraying: *P. americana* showed minimal movement. T+1: activity decreased significantly. T+3: four individuals were died, (ID 1, ID 3, ID 4, and ID 5). T+6: All *P. americana* were died.	Initial response: antennae and legs were still slightly moving. T+6: The bodies of all *P. americana* were rigid. *P. americana* ID 1 lost one antenna. Some *P. americana* were difficult to turn over due to body rigidity.	 (a)  (b)
P4 (80%)	Spraying: still able to move, but with limited activity. T+1: only ID 3 and ID 6 remained active. T+2: five individuals were died. T+3: All *P. americana* were died.	Mortality *P. americana* exhibited body rigidity. Response to touch was completely lost at T+3.	 (a)  (b)
P5 (90%)	Spraying: still moving, but without displacement. T+1: all *P. americana* were ortality (no movement observed).	The bodies of all *P. americana* were rigid. Some *P. americana* experienced antenna detachment.	 (a)  (b)

Captions: T = Observation time
(a) *P. americana* before extract exposed
(b) *P. americana* after extract exposed

Activity and morphological observations (Table 4) showed concentration-dependent effects. At 20% (P1), *P. americana* remained active, with initial leg detachment at T+2 and mortality limited to two individuals by T+6. At 40% (P2), toxin accumulation caused the death of five individuals by T+9. At higher concentrations (60–90%), rapid nervous system impairment resulted in complete mortality, with most individuals dying by T+3 at 60% and 80%, and within 60 minutes at 90%. Morphological changes included leg

and antenna detachment, permanent body rigidity (rigor), and other structural damage, as shown in Table 4.

The mortality of *P. americana* resulted from the penetration of secondary metabolites in leaf C. microcarpa extract into the insect's body. Saponins dissolved the cuticular wax layer, facilitating the entry of toxic compounds into the trachea and sensory glands, while the accumulation of bioactive compounds disrupted the respiratory and nervous systems, causing paralysis, leg detachment, and eventual death [14]. These findings confirm that C. microcarpa extract is an effective contact poison against *P. americana*.

Saponins reduce nutrient intake by inhibiting protease enzymes and forming protein complexes, thereby decreasing nutrient absorption. Alkaloids act as contact poisons that penetrate the cuticle and accumulate in the nervous system, disrupting cardiac activity and gradually reducing the activity of *P. americana*. The heart function within the circulatory system of *P. americana* is impaired, ultimately leading to death [22].

Tannins also enter the insect's body and cause a decrease in cellular activity. [23]. The mechanism of action of *tannins* compounds involves shrinking the cell wall or cell membrane of *P. americana*, thereby disrupting cell permeability. This prevents the cells from carrying out their normal activities and inhibits insect growth. *Tannins* can also induce the formation of *tannins*–metal ion complexes and increase *tannin* toxicity.

P. americana that inhales leaf *C. microcarpa* extract through the trachea will also be exposed to toxins in the respiratory system. *Flavonoid* compounds enter the body of *P. americana* along with oxygen through the spiracles. Oxygen and vapor enter the tracheoles and reach the respiratory system through the tissue, resulting in a reduction of oxygen function and damage to the spiracles, as well as nervous system disturbances that ultimately lead to death [24].

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c. Determination results of the lethal concentration-50 (LC_{50})

Average *P. americana* mortality was 33%, 83%, 100%, 100%, and 100% at concentrations of 20%, 40%, 60%, 80%, and 90%, respectively. The LC_{50} value was determined using log_{10} concentration and probit analysis (Table 5). The relationship between leaf C. microcarpa extract concentration and *P. americana* mortality after 9 hours is presented in Figure 3.

Table 5. Mortality data analysis of *P. americana*

Concentration (%)	ppm	log (ppm)	probit	% Lethal	mortality	amount	LC_{50} value(%)
20	200000	5,301	4,45	33%	2	6	
40	400000	5,602	5,55	83%	5	6	
60	600000	5,778	8,09	100%	6	6	23,85%
80	800000	5,903	8,09	100%	6	6	
90	900000	5,954	8,09	100%	6	6	

Average mortality of *P. americana* was 33%, 83%, 100%, 100%, and 100% at concentrations of 20%, 40%, 60%, 80%, and 90%, respectively. Relationship between concentration and the mortality percentage of *P. americana* at 9 hours after application is shown in Figure 3.

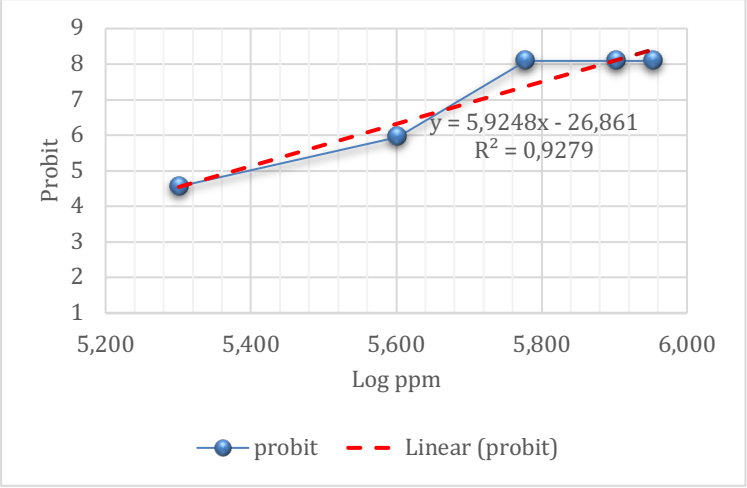


Figure 3. Relationship between concentration and mortality percentage of *P. americana* at 9 hours after application.

Figure 3 shows the regression equation $y = 5.9248x - 26.861$ ($R^2 = 0.9279$), from which the LC_{50} value was calculated as 23.85%, indicating that this concentration caused 50% mortality of *P. americana*. These findings are consistent with previous research using *Citrus aurantifolia* leaf ethanol extract as a larvicide against *Aedes aegypti*, where the fastest LT_{50} (27.91 min) was

obtained at a 50% concentration. The slope of the probit regression line indicates that the rate of mortality increases with dose; a steep slope means that a small change in dose causes a large change in mortality [25].

4. Conclusion

The leaf *C. microcarpa* extract contains secondary metabolite compounds such as *flavonoids, tannins, saponins*, and *alkaloids* that are effective in killing *P. americana*, showing mortality rates at concentrations of P1 20% (33%), P2 40% (83%), P3 60% (100%), P4 80% (100%), and P5 90% (100%), with an LC_{50} value of 23.85%. The results also show that a 60% concentration is the most optimal as a natural insecticide against *Periplaneta americana*, as it achieves 100% mortality with lower residue exposure compared to the 80% and 90% concentrations.

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