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Repellent Bioactivity of *Lantana camara* Leaf Extract Against Culex Mosquitoes

Sutrisnawati Mardin[1*]

[1] Departement of Biology Education, Faculty of Teacher Training and Education, Universitas Tadulako, Palu, Indonesia

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Corresponding Author:
Sutrisnawati Mardin
watikmardin10@gmail.com

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Abstract: The increasing resistance of mosquitoes to chemical insecticides, which negatively impacts the environment, necessitates alternative plant extract usage to mitigate these effects. The objective of this study was to evaluate the bioactivity of *Lantana camara* leaf extract as a repellent against Culex mosquitoes. *L. camara* leaves were extracted using the maceration method. Concentrations of 1%, 2%, 3%, and 4% *L. camara* leaf extract were used to test repellent efficacy. The arm-in-cage method was used to assess effectiveness. Key parameters observed included the number of mosquito landings, time to first bite, and duration of protection. Results indicated that a 4% concentration of *L. camara* leaf extract provided optimal protection, reducing mosquito landings by up to 90% and offering a two-hour protection duration. This efficacy was comparable to commercial repellents, which provided 96% protection. These findings suggest that *L. camara* leaf extract has the potential to serve as an effective, safe, and environmentally friendly mosquito repellent.

Keywords: Bioactivity; Lantana camara; Mosquito; Repellent

Introduction

Mosquitoes are one of the primary vectors for disease transmission, significantly impacting public health, especially in tropical regions such as Indonesia. Several mosquito species from the genera Anopheles, Culex, and Aedes act as carriers of viruses that cause a range of diseases, including yellow fever, chikungunya, dengue fever, dengue hemorrhagic fever, filariasis, Japanese encephalitis, and malaria (Venu et al., 2023; Madhav et al., 2024). Culex mosquitoes are known vectors for diseases like filariasis and West Nile virus, often posing health risks in tropical areas (Bakri & Jadkarim, 2025) . Efforts to control mosquito populations using chemical insecticides have shown significant results. However, long-term use of these insecticides has led to adverse outcomes, including mosquito resistance to active ingredients and harmful impacts on the environment and human health (Venu et al., 2023). As a result, safer and more environmentally friendly control alternatives are urgently needed. One emerging alternative is the use of natural plant-based compounds with bioactivity as natural repellents or insecticides. Although various plant extracts have been explored for their mosquito repellent properties, research on the efficacy of *Lantana camara* leaf extract, particularly against *Culex* mosquitoes, remains limited. Most existing studies focus on its insecticidal properties rather than its potential as a repellent.

Lantana camara, commonly known in Indonesia as tembelekan, has long been used in traditional herbal medicine and is increasingly recognized for its potential as a natural vector control agent. The leaves of *L. camara* contain bioactive compounds such as flavonoids, alkaloids, terpenoids, steroids, polyphenols, and tannins (Edy & Parwanto, 2020). These leaves exhibit strong antimicrobial activity against Gram-positive bacteria and mild inhibition against Gram-negative bacteria (Wahyuningrum et al., 2021). *L. camara* leaf extracts have also shown potent antioxidant and anti-inflammatory activities (Hoang et al., 2024). Furthermore, ethanol extracts of *L. camara* leaves have demonstrated anti-

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inflammatory properties and the ability to inhibit cyclooxygenase-2 (COX-2), an enzyme responsible for inflammation (Ifora et al., 2020). Additionally, *L. camara* has insecticidal properties. Previous research has indicated that *L. camara* leaf extracts exhibit significant larvicidal bioactivity against *Anopheles arabiensis* and *Culex quinquefasciatus* mosquitoes (Alghamdi, 2021).

Methanol extracts of this plant have also shown fumigant and contact toxicity against *Sitophilus oryzae*, *Callosobruchus chinensis*, and *Tribolium castaneum* (Rajashekar et al., 2014). Various solvent extracts from *L. camara aculeata* leaves are effective against the larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*, with phytochemical screening revealing the presence of multiple bioactive compounds (Hemalatha et al., 2015). Furthermore, *L. camara* extracts have shown toxicity against *Musca domestica* at various developmental stages (Ordanza-Cortez, 2015).

Smoke derived from *L. camara* leaves, specifically methanol extracts at 400 ppm, effectively repelled Anopheles mosquitoes for over 300 minutes (Edwin et al., 2014). Essential oils extracted from *L. camara* demonstrated repellency when applied to human skin at concentrations ranging from 0.08 to 3.33 mg/cm² (Bhargava et al., 2013). Further isolation of repellent compounds from *L. camara* flowers yielded a chloroform-eluted fraction that provided 100% protection for 2 hours and 75.8% protection after 7 hours against Aedes mosquitoes (Dua et al., 2003).

This study suggests that *L. camara*-based products could be developed as effective natural mosquito repellents, potentially contributing to the control of mosquito-borne diseases. This research also examines various extract concentrations to identify the optimal formulation for use as a natural repellent, while considering the tropical environmental conditions in Indonesia that may affect repellent efficacy. Thus, this study aims to offer a more sustainable alternative for Culex mosquito control, reducing dependence on chemical insecticides and contributing to the development of environmentally friendly natural repellent products.

Method

Sample Collection and Preparation

Fresh *L. camara* leaves were collected from a clean, pollution-free location. Only healthy, pest-free leaves were selected. The leaves were washed with clean water, then dried in an oven at low temperatures (around 40-50°C) to preserve their bioactive compounds. Once dry, the leaves were ground into powder, ready for extraction (Bhuvaneswari & Giri, 2018).

Extraction

Extraction was conducted using the maceration method with ethanol as the solvent. A total of 500 grams of *L. camara* leaf powder was macerated with 70% ethanol for 48-72 hours in a sealed container. After maceration, the mixture was filtered using filter paper to separate the liquid from the leaf residue. The obtained extract was evaporated using a rotary evaporator to remove the solvent, and the thick extract was then weighed. The yield was calculated by comparing the weight of the extract with the initial weight of the simplicia using Formula 1.

$$\text{Yield (\%)} = \frac{\text{Extract Weight}}{\text{Simplicia Weight}}\ x\ 100 \quad (1)$$

Phytochemical Screening

To detect the presence of alkaloids—heterocyclic nitrogen compounds found in *L. camara* leaf extract—an alkaloid test was conducted using the Dragendorff reagent. Several drops of Dragendorff's reagent were added to the L. camara leaf extract. The presence of alkaloids was indicated by the formation of an orange or cloudy precipitate. Additionally, flavonoids, a group of polyphenol compounds known for their antioxidant and anti-inflammatory properties, were detected using the NaOH Test by adding a few drops of 10% NaOH solution to the extract. A stable yellow color indicated the presence of flavonoids.

Preparation of Concentrations

The *L. camara* extract concentrations used were 1%, 2%, 3%, and 4%. Concentration preparation was done using Formula 2.

$$\text{Concentration (\%)} = \frac{\text{Mass of Solute (g)}}{\text{Mass of Solute (g)}}\ x\ 100 \quad (2)$$

Mosquito Repellent Testing

Adult Culex mosquitoes were bred in the Biology Education Laboratory. Mosquitoes aged 3-5 days post-adulthood were selected for testing. The mosquitoes were starved for 12-24 hours before testing to enhance biting response. Testing was conducted using the arm-in-cage method, where the subject's arm was used to evaluate the repellent's effectiveness. The arm was cleaned with neutral soap and rinsed with water prior to testing (WHO, 2009). *Lantana camara* leaf extract at concentrations of 1%, 2%, 3%, and 4% was evenly applied to the subject's arm. The application area was around 25-50 cm². The arm was then placed in a test cage containing 100 adult mosquitoes for 3-5 minutes. A commercial repellent served as the positive control, and a solvent without extract was used as the negative control. Both controls were used for comparison. To ensure consistency, the experiment was conducted in a

controlled environment with a temperature of 26-28°C and a relative humidity of 70-80%, mimicking natural mosquito activity conditions.

Observed Parameters

The observed parameters included the number of mosquitoes landing on the subject's arm, the time to the first bite (measured from the moment the arm was introduced until the first bite occurred), and the duration of protection, assessed every 30 minutes over a period of 2-3 hours to evaluate the extract's efficacy. Additionally, skin reactions were monitored to detect any allergic or irritative responses following application. The optimal concentration parameter was also assessed, with results from varying extract concentrations analyzed to identify the most effective concentration for protection.

Data Analysis

The repellent testing data were analyzed using descriptive and inferential statistical methods. Significant differences in the efficacy of various *L. camara* leaf extract concentrations were tested using ANOVA. If significant differences were found, post-hoc (Tukey) analysis was performed to identify the optimal concentration of *L. camara* leaf extract. The percentage of bite reduction was calculated using Formula 3.

$$\text{MRB} = \frac{(\text{Bites on Negative Control})}{(\textit{Bites on Negative Control} - \textit{Bites in Test})} x\ 100\% \qquad (3)$$

MRB: Mosquito Bite Reduction (WHO, 2009)

Result and Discussion

Extraction Results of Lantana camara

The extraction of *L. camara* leaves was conducted to obtain active compounds for further testing. This process yielded a thick extract after separation and solvent evaporation. The final results of the extraction include the weight of the simplicia, the weight of the obtained extract, and the percentage yield.

Table 1. Extraction Results of *L. camara* Leaf Maceration.

Parameter	Value
Weight of Simplicia (g)	500
Weight of Extract (g)	55
Yield (%)	11

This table summarizes the extraction yield, indicating a percentage yield of 11% based on the weight of the extract relative to the initial simplicia weight. Based on Table 1, the maceration extraction results of *L. camara* leaves show that an initial simplicia weight of 500 grams yielded a thick extract weighing 55 grams after the filtration and solvent evaporation processes. The percentage yield, calculated as the ratio of the extract weight to the simplicia weight, is 11%. This yield value indicates the efficiency of the extraction method in isolating bioactive compounds and serves as an initial reference for further phytochemical analysis of the extract. The results of the phytochemical tests on *L. camara* leaf extract are presented in Table 2.

Table 2. Phytochemical Test Results of *L. camara* Leaf Extract.

Test Type	Reagent	Positive Result	Observation on Extract
Alkaloid	Dragendorff	Formation of orange precipitate	Positive - Orange precipitate formed upon reaction with Dragendorff reagent, indicating the presence of alkaloids.
Flavonoid	NaOH	Stable yellow color	Positive - Yellow color formed with NaOH reagent, indicating the presence of flavonoids.

This table displays the results of the phytochemical analysis, confirming the presence of alkaloids and flavonoids in *L. camara* leaf extract. The formation of specific colors or precipitates upon reaction with each reagent indicates the presence of these bioactive compounds.

Results of Repellent Testing on Culex Mosquitoes

The following data presents the results of the repellent efficacy test on Culex mosquitoes. This data includes key observed parameters: the number of landing mosquitoes, time to first bite, and the duration of repellent effectiveness across different concentrations of *L. camara* leaf extract (Figure 1).

The data illustrate the effectiveness of various extract concentrations (1%-4%) in repelling mosquitoes, compared to a negative control (ethanol) and a positive control (commercial repellent). Based on the graph, the higher the extract concentration, the fewer the mosquitoes that landed. The negative control (ethanol) had the highest number of landings, averaging 50 mosquitoes, while the positive control had only 2. The time to first bite increased with higher extract concentrations. For the negative control, the first bite occurred after 2 minutes, whereas with a 4% extract concentration, it occurred after 30 minutes. The positive control yielded the longest time to first bite, at 40 minutes.

In terms of protection duration, higher extract concentrations offered longer-lasting protection, ranging from 0.5 hours at 1% concentration to 2 hours at 4%. The commercial repellent provided the longest protection duration, lasting 2.5 hours. Observations also showed no skin irritation reported across all treatment

groups, including controls. This data indicates that higher extract concentrations provide more effective protection against mosquitoes, although they are still not as effective as commercial repellents in terms of protection duration and the number of mosquito landings.

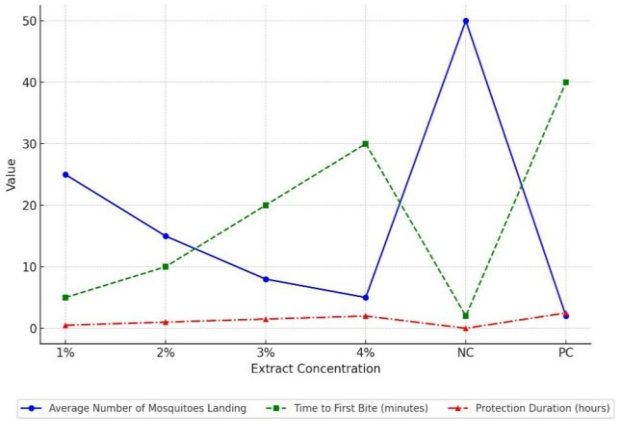

Figure 1. Effectiveness of *L. camara* Leaf Extract as a Repellent Against Culex Mosquitoes

The ANOVA test showed an F-value of 2389.89 with a p-value of 1.52×10^{-31}. Since the p-value is extremely low (less than 0.05), it can be concluded that there is a significant difference between the various *L. camara* leaf extract concentration groups and the control groups tested.

The results of the Tukey post-hoc analysis indicated statistically significant differences between the various concentration groups of *L. camara* extract and the control group, with all pairwise comparisons yielding p-values below 0.05. Notably, the 4% concentration exhibited a marked increase in repellency relative to the 3% concentration, suggesting a dose-dependent effect. Both concentrations also showed significant efficacy compared to the negative control, supporting the conclusion that higher concentrations of *L. camara* leaf extract serve as an effective mosquito repellent.

The findings of this study indicate that *L. camara* leaf extract exhibits significant repellent activity against Culex mosquitoes, particularly at higher concentrations (4%). Testing demonstrated a clear correlation between increased extract concentration and reduced mosquito bites, consistent with prior studies that have verified the efficacy of *L. camara* as both a repellent and a larvicide (Rajan & Varghese, 2017; Ghosh et al., 2024; Alghamdi, 2021). In this study, a 4% concentration of *L. camara* leaf extract reduced mosquito landings by up to 90%, closely approaching the 96% protection offered by commercial repellents. This aligns with a study by Abbas et al. (2024), which showed that *L. camara* essential oil provided 100% protection against Culex quinquefasciatus at specific concentrations. Such results support findings that *L. camara* exhibits potent larvicidal activity against mosquito larvae, with increased efficacy at higher extract concentrations. Other studies have reported Culex quinquefasciatus larvae mortality rates reaching 96-100% at high concentrations after 72-96 hours of exposure, further reinforcing the potential of *L. camara* as an eco-friendly insecticide alternative (Zeeshan & Kukshal, 2023).

Moreover, research by Mondal et al. (2023) demonstrated that *L. camara* leaf extracts had a higher larvicidal potential than flower extracts, with an LC50 of 5.01 ppm for Culex quinquefasciatus larvae. These findings support the current study's results, emphasizing the effectiveness of *L. camara* leaves as a primary ingredient in repellent formulations. The duration of protection at a 4% concentration reached 2 hours, slightly lower than the 2.5 hours provided by commercial repellents. This finding is consistent with Dua et al. (2003), who also observed that the repellent effects of *L. camara* are effective but typically require more frequent reapplication than chemical-based products. Abbas et al. (2024) found that *L. camara* essential oil effectively repelled *Culex quinquefasciatus*, with a longer protection duration compared to other species, such as *Aedes aegypti*. This suggests that while effective, the sensitivity differences among mosquito species lead to variations in protection duration. When compared to other mosquito species, such as *Aedes aegypti* and *Anopheles gambiae*, *L. camara* extract appears more effective against *Culex quinquefasciatus*.

Additionally, this study showed that *L. camara* leaf extract did not cause skin irritation during testing, indicating its safety for human use. Sharma et al. (2020) also identified bioactive compounds in *L. camara*, such as lantadenes and lantaniline, which exhibit repellent efficacy and are safe for human application. These findings reinforce the potential for developing *L. camara*-based products as safe, environmentally friendly natural repellents.

Conclusion

The study successfully demonstrated that *L. camara* leaf extract is effective as a repellent against Culex mosquitoes, particularly at a 4% concentration. While its efficacy is slightly lower than that of commercial repellents, this natural extract presents a safe, environmentally friendly alternative for mosquito control. Further testing in field settings is recommended to evaluate its real-world efficacy and to refine product formulations for optimized performance.

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Conflicts of Interest
The authors declare no conflict of interest.

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