



Green Solutions for Mosquito Control: *Carica papaya*, *Cascabela thevetia*, and *Caesalpinia bonduc* Extracts against *Aedes vittatus* Larvae

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ABSTRACT

Aedes vittatus (Bigot) mosquitoes, known to breed in diverse habitats, have been identified in Corsica, Europe, and have a geographical distribution across tropical Asia, Africa, and the Mediterranean region of Europe. These mosquitoes are potential vectors of arboviruses such as yellow fever, dengue, chikungunya, and Zika, raising concerns about public health risks. This research investigates the larvicidal activity of methanolic leaf extracts from three plants, namely *Carica papaya*, *Cascabela thevetia*, and *Caesalpinia bonduc*, against the fourth instar larvae of *Ae. vittatus*. The phytochemical analysis of the plant extracts revealed the presence of various secondary metabolites, including alkaloids, flavonoids, saponins, terpenoids, polyphenols, and glycosides, suggesting their potential as biopesticides. Larvicidal bioassays demonstrated significant larvicidal effects of the methanolic extracts on *Ae. vittatus* larvae. The extract from *C. thevetia* exhibited the highest efficacy, while *G. bonduc* extracts showed the lowest larvicidal activity. Probit regression analysis provided LC50 and LC90 values for each plant extract, allowing for a better understanding of their effectiveness. These findings highlight the potential of natural plant extracts as environmentally friendly alternatives for mosquito control, offering a safer approach compared to synthetic compounds. Utilizing these biopesticides may aid in reducing pesticide-related hazards to humans and the environment while effectively targeting *Ae. vittatus* mosquitoes, contributing to the management of arboviral diseases and protecting public health.

Keywords: *Aedes vittatus*, Larvicidal activity, Plant extracts, Mosquito control, Arboviral diseases

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INTRODUCTION

Aedes vittatus (Bigot) mosquitoes, previously identified as *Culex vittatus*, were first observed in Corsica, Europe and have a geographical distribution spanning tropical Asia, Africa, and the Mediterranean region of Europe, and they are known to breed in diverse habitats, including rock pools and discarded containers [1]. *Ae. vittatus* mosquitoes are commonly found in peridomestic environments and exhibit a preference for breeding in rock pools. They can be distinguished from other prevalent *Aedes* species by three pairs of small, round, silvery-white spots on the scutum. Additional characteristic features include narrow-scaled wings, dark tibiae with white spots, a white band at the base of the tibiae, white bands on tarsomeres 1–4, and a fully white fifth tarsomere [2].

Ae. vittatus mosquitoes have been implicated as potential vectors of arboviruses such as yellow fever, dengue, chikungunya, and Zika, with evidence of virus isolation and their ability to transmit these viruses in laboratory settings. Due to their high preference for biting humans (anthropophily), these mosquitoes are considered important species for studying and monitoring their potential role in the maintenance and transmission of arboviruses that pose public health risks [3]. Recently, they have gained attention in association with the Zika virus (ZIKV) [4].

The extensive use of pesticides in agriculture and public health activities poses hazards to humans and other organisms due to unintended exposure. Pesticide exposure has been linked to various diseases, including cancer, asthma, and birth defects. Determining the risks associated with pesticide exposure is challenging due to factors such as the specific pesticide used, the level and duration of exposure, and

environmental conditions [5]. To overcome the limitations associated with synthetic compounds used in mosquito control, there has been a shift toward the utilization of botanicals. The application of easily degradable plant compounds has emerged as a safe approach to controlling insect pests and vectors [6]. In a recent study, Madhavi and Mahesh [7, 8] demonstrated the efficacy of *Murraya paniculata* and *Pongamia pinnata* plant extracts in controlling the fourth instar larvae of *Ae. vittatus*. Similarly, Santhosh et al. [9] evaluated *Murraya koenigii* plant extracts against *Ae. vittatus* larvae.

Carica papaya extracts possess significant antioxidant properties, and can be employed as a natural remedy for treating inflammation, skin aging, chronic diseases, and cancers by activating antioxidant defense mechanisms [10]. The leaf extract of *C. papaya* exhibited potential as a candidate for increasing platelet count in dengue patients [11]. The methanolic and ethanolic leaf extracts of *Cascabela thevetia* showed low to moderate antibacterial activity against several bacterial strains, suggesting their potential as antibacterial agents [12]. Arasaretnam, et al. [13] reported the presence of carbohydrates, saponins, alkaloids, and terpenoids in the aqueous extract of the whole plant of *C. thevetia*. These secondary metabolites play a significant role in the biological activities exhibited by medicinal plants, including hypoglycemic, antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic, antimalarial, and antileprosy activities. Ali et al. [14] reported the beneficial effects of leaf extracts of *Caesalpinia bonduc* in treating various ailments, such as amenorrhea, asthma, body ache, chest pain, cough, diarrhea, dysmenorrhea, elephantiasis, fevers, headache, hepatomegaly, hydrocele, indigestion, intestinal worms, menstruation disorders, rheumatism, skin infections, smallpox, and splenomegaly. Different classes of chemical compounds, including flavonoids, diterpenes, and steroids, have been isolated from various species of *Caesalpinia* [15].

The present study aims to investigate the larvicidal activity of methanolic leaf extracts of *C. papaya*, *C. thevetia*, and *C. bonduc* at different concentrations (100, 200, 300, and 400 ppm) against the 4th instar larvae of *Ae. vittatus*.

MATERIAL AND METHODS

Mosquito Larvae: Early-stage larvae of *Ae. vittatus* were collected from the lake and rockpools situated in the Osmania University campus, Hyderabad, Telangana State, India. The larvae were reared in glass troughs with a diet consisting of a mixture of yeast and dog biscuits in a ratio of 3:1. Fourth instar larvae were selected for the larvicidal bioassay.

Test Plants: High-quality leaves of *C. papaya*, *C. thevetia*, and *C. bonduc* were collected from Zaheerabad town, Telangana State, India. The leaves were thoroughly washed with running tap water and then rinsed with distilled water. They were subsequently shade dried for a period of 15 days. Once dried, the leaves were powdered using an electrical blender. Methanolic extracts were obtained from the powdered samples by soaking 50 gr of powders separately in 200 ml of methanol for 4 days with frequent shakings. On the 5th day, the solutions were filtered using filter papers and then methanol was allowed to evaporate under a fan until a semisolid extract is obtained. The semi-solid extracts obtained thus were preserved in the refrigerator at 4°C until usage.

Phytochemical Analysis: Various tests were conducted to identify the presence of secondary metabolites in the prepared extracts. Alkaloids were detected using the Mayor's Test, wherein the addition of Mayor's reagent resulted in the formation of a cream-colored precipitate. The Alkaline Reagent Test was employed to identify flavonoids, which exhibited a yellow color upon the addition of NaOH. Terpenoids were identified using the Salkowski Test, where the addition of concentrated H₂SO₄ led to a red or orange color. The Froth Test was used to detect saponins, which produced foam upon vigorous shaking. The Keller-Killiani Test was performed to identify glycosides, with the formation of a red or violet color upon adding HCl and FeCl₃. Lastly, the NaOH Test was conducted to identify polyphenols, and the addition of NaOH resulted in yellow color.

Test solutions: 1 gr. of each extract was dissolved in 10 mL of methanol separately and then was added to 990 mL of distilled water to prepare 1000 ppm stock solutions. Four different concentrations (100, 200, 300, and 400 ppm) of the test solutions were prepared for each extract by serial dilution method. The control solution was prepared by adding the methanol and distilled water in the same ratios, excluding the extracts.

Larvicidal Bioassay: The larvicidal bioassay followed the guidelines set by the World Health Organization (WHO) [16]. The test solutions were placed in 250 mL test cups, and three batches of 20 larvae were introduced into each cup. After 6, 12, and 24 hours of exposure, the number of dead larvae was counted, and the percentage mortality was calculated as the average of five replicates using the following equation:

$$\%PM = (\text{Number of dead larvae} / \text{Total larvae population}) \times 100.$$

Corrected mortalities were calculated using Abbott's [17] formula.

$$\text{Corrected Mortality(\%)} = \frac{\%MT - \%MC}{100 - \%MC} \times 100$$

Statistical analysis: Microsoft Excel software was used to subject the results to one-way analysis of variance (ANOVA). The level of significance was set at $p < 0.05$. Probit analysis was conducted to calculate LC50 and LC90 concentrations.

RESULTS AND DISCUSSION

(1) Phytochemical Analysis: The results of the phytochemical analysis (Table No.1) show that methanolic leaf extracts of *C. papaya*, *C. thevetia*, and *C. bonduc* plants contain a wide range of phytochemicals. Alkaloids were present in high quantities in *C. papaya* and *C. thevetia* extracts whereas they were present in less quantity in *C. bonduc* extracts. Flavonoids were present in moderate quantities in *C. papaya* and *C. thevetia* extracts. However, they were found to be in less quantity in *C. thevetia* extracts. Saponins quantity was excess in *C. thevetia* extracts, moderate in *C. bonduc* extracts, and less in *C. papaya* extracts. Terpenoid test results confirmed the abundance of Terpenoids in *C. thevetia* extracts, moderate presence of them in *C. papaya* extracts, and less quantity in *C. bonduc* extracts. Polyphenols were found to be in excess quantity in *C. thevetia* extracts while in less quantity in *C. papaya* and *C. bonduc* extracts. However, Glycosides were present in less quantity in all three tested extracts.

Larvicidal Bioassay:

The results of the bioassay conducted with the methanolic extracts of *C. papaya* leaves (Table 2) revealed that they possess significant larvicidal activity against *Ae. vittatus* larvae with mortality percentages of 26.09 ± 0.48 , 47.83 ± 0.51 , 65.22 ± 0.46 , and 82.61 ± 0.34 at 100, 200, 300, and 400 ppm concentrations, respectively. Probit regression analysis of the results (Figure 1) indicated the LC 50 and LC 90 values to be 227.71 and 423.50 ppm, respectively.

On the other hand, the methanolic extracts of *C. thevetia* exhibited the highest larval mortalities of all three bioassays (Table 3). larvicidal activity against *Ae. vittatus* larvae, with a mortality percentage of 35.35 ± 0.62 , 48.48 ± 0.60 , 62.63 ± 0.67 , and 88.89 ± 0.83 at 100, 200, 300, and 400 ppm concentrations, respectively. Probit regression analysis of the results (Figure 2) indicated the LC 50 and LC 90 values to be 214.23 and 409.26 ppm, respectively.

However, the methanolic extracts of *C. bonduc* leaves exhibited low larvicidal activity of all tested extracts yet, significant activity against *Ae. vittatus* larvae. with a mortality percentage of 18.39 ± 0.69 , 33.33 ± 0.64 , 48.28 ± 0.97 , and 75.86 ± 0.94 at 100, 200, 300, and 400 ppm concentrations, respectively. Probit regression analysis of the results (Figure 3) indicated the LC 50 and LC 90 values to be 269.00 and 489.27 ppm, respectively.

In summary, the methanolic extracts of all the tested extracts showed significant larvicidal effects with slight variations, against the 4th instars of *Ae. vittatus* (Figure 4). Of them, *C. thevetia* extracts exhibited the highest efficacy, whereas *G. bonduc* extracts showed the lowest larvicidal efficacy.

Phytochemical analysis of the tested extracts in the present study showed various secondary metabolites such as alkaloids, terpenoids, flavonoids, phenols, saponins, and glycosides. The same results were reported in previous studies with *C. papaya* extracts, *C. thevetia* extracts, and *C. bonduc* extracts [18, 19, 20].

The results of the larvicidal bioassays of the present study also are supported by previous studies. Kovendran, et al. [21] reported the highest mortality rates with LC50 values of 375.89 ppm in their study with the methanolic extracts of *C. papaya* against the 4th instars of *Ae. aegyptii*. Borah, et al. [22] studied the efficacy of plant extracts of *C. thevetia* against different mosquito species. They reported LC 50 values of 95.19, and 140.99, and LC 90 values of 267.73 and 428.86 ppm for *Ae. aegyptii* and *C. quinquefasciatus*, respectively. Saravanan, et al. [23] evaluated the larvicidal efficacy of *C. bonduc* leaf extracts against *C. quinquefasciatus*. They reported 100% mortality with 1% concentrations of petroleum ether and ethanolic extracts. The aqueous extract at 2.5% concentration had a mortality rate of 55%, while the fixed oil at the same concentration had a mortality rate of 92.6%.

Table 1. Identified secondary metabolites in the selected plants' methanolic leaf extracts. Absent: - ; Slightly Present: +; Moderately present: ++; Heavily present: +++.

Extracts	Alkaloids	Flavonoids	Saponins	Terpenoids	Polyphenols	Glycosides
<i>C. papaya</i>	+++	++	+	++	++	+
<i>C. thevetia</i>	+++	+	+++	+++	+++	+
<i>C. bonduc</i>	+	++	++	+	++	+

Table 2. *C. papaya* Methanolic leaf extracts larval mortality percentages \pm Standard Deviations (SD) against the 4th Instar larvae of *Ae. vittatus*. LC50 – 50% Lethal Concentration; LC90 – 90% Lethal Concentration- 95% CL – 95% Confidence Limits; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

Conc. In ppm	Mortality % \pm SD	LC50 95% CL (LCL – UCL)	LC90 95% CL (LCL – UCL)
0	0 \pm 0.75		
100	22.99 \pm 0.67	263.91 (263.77	480.01 (479.87
200	37.93 \pm 0.58	-	-
300	51.72 \pm 0.88	264.14)	480.24)
400	78.16 \pm 0.71		

Table 3. *C. thevetia* Methanolic leaf extracts larval mortality percentages \pm Standard Deviations (SD) against the 4th Instar larvae of *Ae. vittatus*. LC50 – 50% Lethal Concentration; LC90 – 90% Lethal Concentration- 95% CL – 95% Confidence Limits; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

Conc. In ppm	Mortality % \pm SD	LC50 95% CL (LCL – UCL)	LC90 95% CL (LCL – UCL)
0	0 \pm 0.80		
100	35.35 \pm 0.62	214.23 (213.54	409.26 (408.56
200	48.48 \pm 0.60	-	-
300	62.63 \pm 0.67	215.31)	410.34)
400	88.89 \pm 0.83		

Table 4. *C. bonduc* Methanolic leaf extracts larval mortality percentages \pm Standard Deviations (SD) against the 4th Instar larvae of *Ae. vittatus*. LC50 – 50% Lethal Concentration; LC90 – 90% Lethal Concentration- 95% CL – 95% Confidence Limits; LCL – Lower Confidence Limit; UCL – Upper Confidence Limit

Conc. In ppm	Mortality % \pm SD	LC50 95% CL (LCL – UCL)	LC90 95% CL (LCL – UCL)
0	0 \pm 0.75		
100	18.39 \pm 0.69	269.00 (268.10	489.27 (488.37
200	33.33 \pm 0.64	-	-
300	48.28 \pm 0.97	270.14)	490.40)
400	75.86 \pm 0.94		

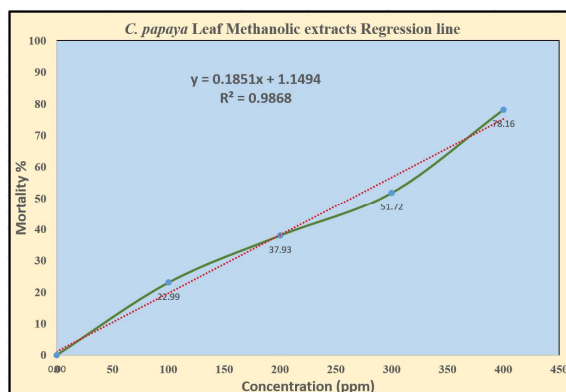


Figure 1. Regression analysis of *C. papaya* methanolic leaf extracts against the 4th instar larvae of *Ae. vittatus*.

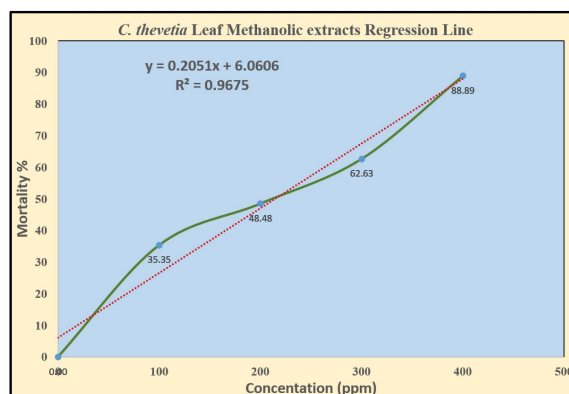


Figure 2. Regression analysis of *C. thevetia* methanolic leaf extracts against the 4th instar larvae of *Ae. vittatus*.

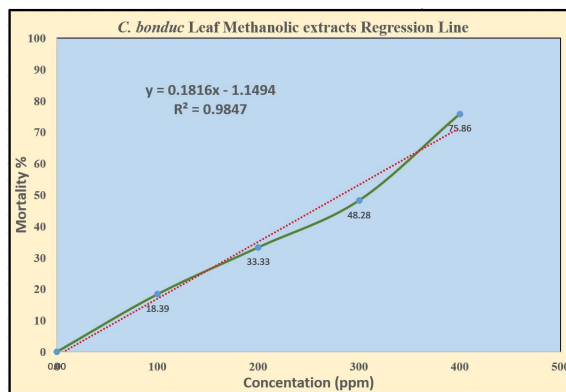


Figure 3. Regression analysis of *C. bonduc* methanolic leaf extracts against the 4th instar larvae of *Ae. vittatus*.

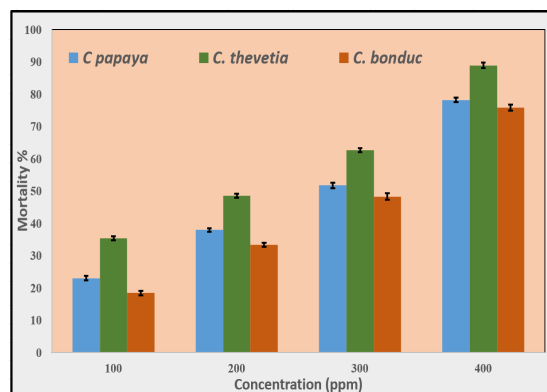


Figure 4. Comparative Larvicidal activity of *C. papaya*, *C. thevetia*, and *C. bonduc* methanolic leaf extracts against the 4th instar larvae of *Ae. vittatus*.

CONCLUSION

In conclusion, the phytochemical analysis of methanolic leaf extracts of *C. papaya*, *C. thevetia*, and *C. bonduc* plants revealed the presence of a diverse range of phytochemicals, with the methanol extract showing the highest abundance of phytochemicals. The larvicidal bioassay demonstrated significant larvicidal activity of the methanolic extracts of *C. papaya*, *C. thevetia*, and *C. bonduc* plant leaves against *Ae. vittatus* larvae, with the methanolic extract of *C. thevetia* leaves exhibiting the highest activity. The other two extracts also exhibited significant larvicidal efficacy against the larvae. The *C. thevetia* leaf extracts displayed the lowest LC50 and LC90 values, while the other two extracts had slightly higher LC50 and LC90 values. These findings suggest that the *C. papaya*, *C. thevetia*, and *C. bonduc* plant extracts have the potential as a natural pesticide against *Ae. vittatus* larvae.

Conflict of Interest:

The authors don't have any sort of financial or non-financial conflicts with any person or firm that can affect the results of this research.

Ethics of Human and Animal Experimentation: Not applicable

Informed Consent: Not applicable

Author Contributions: Srija conceptualized, investigated
Mahesh Supervised, and wrote the manuscript
Madhavi curated the data and edited the manuscript.
Vanaja investigated, and analyzed data.

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