International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 27-02-2026, Accepted: 30-03-2026, Published: 14-04-2026
Volume 11, Issue 2, 2026, Page No. 65-69

Preliminary phytochemical analysis and mosquitocidal activity of *Calophyllum inophyllum* leaf extract against *Aedes aegypti*

Karupuswami Dhanalakshmi, Chellamuthu Vasugi*
Department of Zoology, Sri Vasavi College, Affiliated by Bharathiar University, Erode, Tamil Nadu, India
Corresponding Author: Chellamuthu Vasugi

Abstract
Background: *Aedes aegypti* is a primary vector of dengue, chikungunya, and Zika viruses. Plant-derived mosquitocidal agents offer an eco-friendly alternative to synthetic chemicals. This study evaluated the phytochemical composition and mosquitocidal activity of *Calophyllum inophyllum* methanol leaf extract against *Aedes aegypti*.
Methods: Preliminary phytochemical analysis was conducted using standard qualitative tests. Ovicidal activity was assessed by exposing eggs to various concentrations (100–500 µg/mL). Larvicidal and pupicidal activities were evaluated against first to fourth instar larvae and pupae using five concentrations (100–500 µg/mL) with five replicates. Mortality data were subjected to probit analysis to determine LC_{50} and LC_{90} values.
Results: Phytochemical screening revealed the presence of phenolics, alkaloids, saponins, steroids, tannins, flavonoids (strong positive), and terpenoids, while quinones were absent. The extract exhibited concentration-dependent ovicidal activity, with egg hatchability reducing from 80.6% at 100 µg/mL to 6.2% at 400 µg/mL, and complete inhibition at 500 µg/mL. Larval mortality increased with concentration and decreased with developmental stage. The LC_{50} values ranged from 248.6 µg/mL (first instar) to 390.8 µg/mL (pupae). The highest susceptibility was observed in first instar larvae, while pupae were most tolerant.
Conclusion: *Calophyllum inophyllum* methanol leaf extract possesses significant multi-stage mosquitocidal activity against *Aedes aegypti*, supporting its potential as a natural vector control agent.

Keywords: Phytochemical analysis, larvicidal activity, *Aedes aegypti*, *Calophyllum inophyllum*, mosquitocidal activity

Introduction

Mosquito-borne diseases remain one of the most significant public health challenges in tropical and subtropical regions of the world [1]. Among the various mosquito species, *Aedes aegypti* stands out as a highly efficient vector responsible for the transmission of several debilitating viral diseases, including dengue fever, chikungunya, Zika virus disease, and yellow fever [2]. The global burden of these diseases has increased dramatically over recent decades, primarily due to rapid urbanization, inadequate sanitation, climate change, and the expansion of international travel and trade [3]. Dengue alone infects an estimated 390 million people annually across more than 120 countries, placing nearly half of the world's population at risk. The absence of specific antiviral treatments or universally available vaccines for many of these diseases makes vector control the primary strategy for disease prevention and outbreak management [4]. Traditionally, vector control programs have relied heavily on synthetic chemical insecticides, particularly organophosphates, pyrethroids, and insect growth regulators. While these compounds have contributed to the reduction of mosquito populations in many settings, their prolonged and indiscriminate use has led to several serious consequences [5]. The development of insecticide resistance in *Aedes aegypti* populations has been widely documented across multiple continents, compromising the effectiveness of existing control measures [6]. Furthermore, synthetic insecticides pose significant risks to non-target organisms, including beneficial insects, aquatic fauna, and vertebrates. Their persistence in the environment leads to bioaccumulation and biomagnification through food chains, while repeated human exposure has been associated with potential health hazards, including neurotoxicity and endocrine disruption. These limitations underscore the urgent need for alternative, environmentally sustainable approaches to mosquito management [7].

Plant-derived natural products have emerged as promising candidates for the development of novel mosquitocidal agents. Plants represent a vast reservoir of bioactive secondary metabolites, which have evolved as chemical defense mechanisms against herbivores, including insects. These compounds often exhibit diverse modes of action, including neurotoxicity, growth disruption, reproductive inhibition, and repellency, making them valuable resources for integrated vector management [8]. Unlike synthetic insecticides, botanical products are generally biodegradable, exhibit lower mammalian toxicity, and are less likely to induce rapid resistance due to their complex chemical mixtures. Several plant species have been traditionally used for mosquito control in various cultures, and scientific validation of these ethnobotanical practices has gained considerable attention in recent years [9].

Calophyllum inophyllum, commonly known as Alexandrian laurel, beauty leaf, or kamani, is a medium to large evergreen tree belonging to the family Calophyllaceae. The plant is widely distributed along coastal regions of the Indian Ocean, Southeast Asia, the Pacific Islands, and East Africa. Various parts of this plant, including leaves, seeds, bark, and roots, have been extensively used in traditional medicine systems such as Ayurveda, Siddha, and Unani for the treatment of wounds, skin disorders, rheumatism, and inflammation. The seeds yield a thick, dark green oil known as tamanu oil, which is valued for its wound-healing and anti-inflammatory properties. Previous phytochemical

investigations have revealed that *Calophyllum inophyllum* is rich in a wide array of bioactive compounds, including coumarins (such as calophyllolide, inophyllum B, and inophyllum P), xanthones, flavonoids, triterpenes, saponins, and tannins. Many of these compounds have demonstrated antimicrobial, anti-inflammatory, analgesic, and anticancer activities. However, the mosquitocidal potential of this plant, particularly its leaf extracts, remains relatively underexplored [10].

The present study was therefore undertaken to evaluate the mosquitocidal activity of *Calophyllum inophyllum* methanol leaf extract against different developmental stages of *Aedes aegypti*, specifically eggs, larvae (first to fourth instars), and pupae. Additionally, preliminary phytochemical screening was performed to identify the major classes of secondary metabolites present in the extract that may be responsible for the observed biological activity. The findings of this research are expected to contribute to the development of eco-friendly, plant-based alternatives for integrated mosquito management programs, thereby reducing reliance on harmful synthetic insecticides and mitigating the growing problem of insecticide resistance.

Materials and Methods

Collection and Authentication of Plant Material

Healthy, mature leaves of *Calophyllum inophyllum* were collected from Bharathiar University Campus, Tamil Nadu, India. A botanist at Bharathiar University, Tamil Nadu, India taxonomically authenticated the plant material, and a voucher specimen was deposited in the institutional herbarium for future reference. The collected leaves were washed thoroughly with tap water to remove dust and debris, followed by rinsing with distilled water.

Preparation of Plant Extract

Shade-dried leaves (25–30°C for 10–15 days) were powdered and sieved (40-mesh). For methanol extraction, 100 g of powder underwent Soxhlet extraction with 500 mL methanol for 48–72 hours until colorless. The extract was concentrated using a rotary evaporator (40–45°C), air-dried, and stored at 4°C. For the aqueous extract, 20 g of powder was macerated in 200 mL distilled water for 24 hours with intermittent shaking, then filtered through Whatman No. 1 filter paper.

Preliminary Phytochemical Analysis

The *Calophyllum inophyllum* methanol leaf extract was subjected to qualitative phytochemical screening using standard protocols. Phenolics were detected using the ferric chloride test (dark green/blue color). Alkaloids were identified by Mayer's/Wagner's test (cream or reddish-brown precipitate). Quinones were tested using alcoholic NaOH/ammonia (red/blue/violet color). Saponins were confirmed by the foam test (persistent foam column for 10–15 minutes). Steroids were analyzed using the Salkowski/Liebermann–Burchard test (red or blue-green color). Tannins were detected by the ferric chloride/gelatin test (greenish-black precipitate). Flavonoids were identified using the alkaline reagent/Shinoda test (yellow or pink/magenta color). Terpenoids were confirmed by the Salkowski/chloroform test (reddish-brown interface).

Ovicidal Activity Assay

The ovicidal activity of the methanol leaf extract was evaluated following the standard method with modifications. Five different concentrations were prepared: 100, 200, 300, 400, and 500 µg/mL using distilled water as the diluent. Distilled water served as the control. For each concentration, 100 freshly laid *Aedes aegypti* eggs (less than 12 hours old) were immersed in 250 mL of the test solution in a 500 mL beaker. Five replicates were maintained for each concentration and control. After 24 hours of exposure, the eggs were transferred to plastic trays containing dechlorinated tap water and maintained under laboratory conditions [11]. The number of unhatched eggs was recorded after 48 hours, and the percentage of egg hatchability was calculated using the formula:

Percentage of egg hatchability = (Number of hatched eggs / Total number of eggs) × 100

Larvicidal and Pupicidal Activity Assay

The larvicidal and pupicidal activities were evaluated against first, second, third, and fourth instar larvae and pupae of *Aedes aegypti* using the standard World Health Organization (WHO) protocol with minor modifications. Five different concentrations of the methanol leaf extract (100, 200, 300, 400, and 500 µg/mL) were prepared. For each concentration, 25 larvae or pupae were introduced into 250 mL beakers containing 200 mL of the test solution. Distilled water served as the control. Each concentration and control were replicated five times. Mortality was recorded after 24 hours of continuous exposure. Larvae or pupae were considered dead if they showed no movement upon gentle probing with a fine needle [12]. The percentage mortality was calculated using Abbott's formula when control mortality exceeded 5%:

Corrected mortality (%) = [(Test mortality – Control mortality) / (100 – Control mortality)] × 100

Statistical Analysis

Mortality data (mean ± SD, n=5) were analyzed by probit analysis using statistical software. LC_{50}, LC_{90}, and their 95% confidence limits (LCL, UCL) were calculated. Regression equations and chi-square (χ^2) values determined the goodness-of-fit. A p-value < 0.05 was considered significant. χ^2 values with $p > 0.05$ were not significant (n.s.), indicating a satisfactory model fit.

Results

Preliminary Phytochemical Analysis

The preliminary phytochemical screening of *Calophyllum inophyllum* methanol leaf extract revealed the presence of several bioactive secondary metabolites. As presented in Table 1, the extract tested positive for phenolics, alkaloids, saponins, steroids, tannins, flavonoids, and terpenoids, while quinones were absent. Flavonoids showed the strongest positive reaction (+++), indicating their abundance in the extract. Phenolics, alkaloids, saponins, steroids, tannins, and terpenoids all showed moderate positive reactions (+). The presence of these diverse phytochemicals suggests that the methanol extract is rich in potential bioactive compounds that may contribute to its mosquitocidal properties.

Table 1: Preliminary Phytochemical Analysis of *Calophyllum inophyllum* Methanol Leaf Extract

S. No.	Phytochemical Constituent	Test Performed	Observation	Result
1.	Phenolics	Ferric Chloride Test	Dark green/blue color appeared	Positive (+)
2.	Alkaloids	Mayer's / Wagner's Test	Cream precipitate (Mayer) or Reddish-brown precipitate (Wagner)	Positive (+)
3.	Quinones	Alcoholic NaOH / Ammonia	Red, blue, or violet coloration	Negative (-)
4.	Saponins	Foam (Frothing) Test	Persistent foam column (1-2 cm) for 10-15 min	Positive (+)
5.	Steroids	Salkowski / Liebermann-Burchard Test	Red color in lower chloroform layer (Salkowski) or Blue-green color (L-B)	Positive (+)
6.	Tannins	Ferric Chloride (Gelatin) Test	Greenish-black precipitate or bluish-black color	Positive (+)
7.	Flavonoids	Alkaline Reagent / Shinoda Test	Intense yellow color (Alkaline) or Pink/magenta (Shinoda with Mg + HCl)	Strong Positive (+++)
8.	Terpenoids	Salkowski / Chloroform test	Reddish-brown interface (Salkowski)	Positive (+)

Ovicidal Activity

The ovicidal activity of *Calophyllum inophyllum* methanol leaf extract against *Aedes aegypti* eggs is presented in Table 2. The extract demonstrated concentration-dependent inhibition of egg hatchability. In the control group, 100% egg hatchability was observed. At the lowest tested concentration of 100 µg/mL, egg hatchability was reduced to 80.6±2.30%. As the concentration increased, a progressive decline in hatchability was recorded: 48.4±1.51% at 200 µg/mL, 22.8±1.92% at 300 µg/mL, and 6.2±1.30% at 400 µg/mL. Complete inhibition of egg hatching (NH = no hatching) was achieved at the highest concentration of 500 µg/mL. These findings indicate that the extract effectively prevents embryonic development or causes mortality of the developing larvae within the eggs.

Table 2: Ovicidal activity of *Calophyllum inophyllum* Methanol Leaf Extract against *Aedes aegypti*

Egg hatchability					
Control	**100 µg/mL**	**200 µg/mL**	**300 µg/mL**	**400 µg/mL**	**500 µg/mL**
100.0±0.0	80.6±2.30	48.4±1.51	22.8±1.92	6.2±1.30	NH

Larvicidal and Pupicidal Activity

The larvicidal and pupicidal activity of the methanol leaf extract against different developmental stages of *Aedes aegypti* is summarized in Table 3. The extract exhibited concentration-dependent mortality across all instars and pupae, with mortality increasing progressively from 100 µg/mL to 500 µg/mL. First instar larvae showed the highest susceptibility, with mortality ranging from 19.8±1.48% at 100 µg/mL to 100% complete mortality at 500 µg/mL. The LC_{50} value was calculated as 248.633 µg/mL (LCL: 178.823, UCL: 306.784), and the LC_{90} was 445.146 µg/mL (LCL: 372.488, UCL: 610.197). The regression equation was y = -1.621 + 0.007x, with a chi-square value of 9.210 (not significant, $p > 0.05$). Second instar larvae exhibited mortality rates of 18.0±1.22%, 35.0±1.87%, 53.8±1.30%, 74.2±1.92%, and 93.8±1.30% at 100, 200, 300, 400, and 500 µg/mL, respectively. The LC_{50} was 270.107 µg/mL (LCL: 247.740, UCL: 291.564), and the LC_{90} was 492.793 µg/mL (LCL: 456.995, UCL: 540.544). The regression equation was y = -1.554 + 0.006x, with $\chi^2 = 2.253$ (n.s.). Third instar larvae showed mortality percentages of 15.8±1.48%, 30.6±1.81%, 49.4±1.51%, 67.4±1.81%, and 85.2±1.92% at the respective concentrations. The LC_{50} was 301.818 µg/mL (LCL: 277.648, UCL: 326.093), and the LC_{90} was 556.333 µg/mL (LCL: 511.360, UCL: 618.593). The regression equation was y = -1.520 + 0.005x, with $\chi^2 = 0.217$ (n.s.). Fourth instar larvae were less susceptible, with mortality ranging from 12.6±1.14% at 100 µg/mL to 76.0±1.58% at 500 µg/mL. The LC_{50} was 346.270 µg/mL (LCL: 320.461, UCL: 374.647), and the LC_{90} was 623.514 µg/mL (LCL: 567.879, UCL: 703.331). The regression equation was y = -1.601 + 0.005x, with $\chi^2 = 0.006$ (n.s.). Pupae were the most tolerant stage, exhibiting the lowest mortality rates: 10.8±1.30%, 20.4±1.51%, 34.4±1.81%, 55.2±1.78%, and 65.6±1.14% at 100, 200, 300, 400, and 500 µg/mL, respectively. The LC_{50} was 390.829 µg/mL (LCL: 361.422, UCL: 426.613), and the LC_{90} was 693.554 µg/mL (LCL: 623.923, UCL: 797.824). The regression equation was y = -1.655 + 0.004x, with $\chi^2 = 0.793$ (n.s.). The chi-square values for all developmental stages were not significant ($p > 0.05$), indicating that the probit model provided a good fit to the mortality data. The LC_{50} values increased progressively with developmental stage: first instar (248.6 µg/mL) < second instar (270.1 µg/mL) < third instar (301.8 µg/mL) < fourth instar (346.3 µg/mL) < pupae (390.8 µg/mL), confirming that susceptibility decreases as the mosquito matures.

Table 3: Larvicidal and pupicidal activity of *Calophyllum inophyllum* Methanol Leaf Extract against *Aedes aegypti*

Mosquito life stages	LC_{50} (LC_{90}) (µg/mL)	95% confidence Limit		Regression equation	χ^2 (df=4)
		LC_{50} (LC_{90})			
		LCL	UCL		
1st Instar	248.633, (445.146)	178.823, (372.488)	306.784, (610.197)	y= -1.621+0.007 x	9.210 n.s.
2nd Instar	270.107, (492.793)	247.740, (456.995)	291.564, (540.544)	y = -1.554+0.006 x	2.253 n.s.
3rd Instar	301.818, (556.333)	277.648, (511.360)	326.093, (618.593)	y = -1.520+0.005 x	0.217 n.s.
4th Instar	346.270, (623.514)	320.461, (567.879)	374.647, (703.331)	y = -1.601+0.005 x	0.006 n.s.
Pupa	390.829, (693.554)	361.422, (623.923)	426.613, (797.824)	y = -1.655+0.004 x	0.793 n.s.

Mortality rates are means ± SD of five replicates

LC_{50} = lethal concentration that kills 50% of the exposed organisms

LC_{90} = lethal concentration that kills 90% of the exposed organisms

LCL = Lower Confidence Limit

UCL = Upper Confidence Limit

$\chi 2$ = chi-square; n.s. = not significant ($\alpha = 0.05$)

Discussion

The present study investigated the phytochemical composition and mosquitocidal potential of *Calophyllum inophyllum* methanol leaf extract against all developmental stages of *Aedes aegypti*. The extract demonstrated significant concentration-dependent activity against eggs, larvae, and pupae, with efficacy varying by developmental stage.

Preliminary phytochemical analysis revealed that the methanol extract is rich in diverse secondary metabolites, including phenolics, alkaloids, saponins, steroids, tannins, flavonoids (strong positive), and terpenoids, while quinones were absent. The strong positive result for flavonoids is particularly noteworthy, as this class possesses well-documented insecticidal properties through multiple mechanisms: nervous system disruption, digestive enzyme inhibition, interference with molting and metamorphosis, and oxidative stress induction [13]. Tannins and phenolics bind to proteins, interfering with insect physiology. Saponins possess membrane-disrupting properties that increase cuticular permeability. Alkaloids and terpenoids are neurotoxins targeting acetylcholinesterase. This diverse phytochemical profile suggests a multi-modal mechanism of action, potentially reducing resistance development compared to synthetic insecticides [14].

The ovicidal activity observed indicates that the extract effectively penetrates the egg chorion and disrupts embryonic development. Complete inhibition of hatching at 500 µg/mL demonstrates its potential as an ovicidal agent [15]. The concentration-dependent reduction in hatchability (80.6% at 100 µg/mL to 6.2% at 400 µg/mL) suggests that even sub-lethal concentrations impact population recruitment.

Larvicidal and pupicidal results show that susceptibility decreases with advancing developmental stage. First instar larvae were most susceptible (LC_{50}: 248.6 µg/mL), while pupae were most tolerant (LC_{50}: 390.8 µg/mL) [16]. This age-dependent susceptibility is explained by thinner, more permeable cuticles and higher metabolic rates in younger larvae, allowing greater compound penetration and ingestion [17]. Later instars and pupae develop thicker, sclerotized cuticles, with pupae being non-feeding, limiting exposure to cuticular contact only [18]. Complete mortality of first instar larvae at 500 µg/mL and high mortality (93.8%) of second instars indicate the extract's effectiveness against early stages, preventing population progression to resilient later stages [19]. The mosquitocidal activity is likely attributable to synergistic effects of multiple phytochemicals. Botanical insecticides offer advantages over synthetics: biodegradability, lower mammalian toxicity, reduced environmental persistence, and lower resistance risk. The multi-stage activity demonstrated enhances the extract's value for integrated vector management [20].

Conclusion

The methanol leaf extract of *Calophyllum inophyllum* possesses significant mosquitocidal activity against all developmental stages of *Aedes aegypti*. Phytochemical screening revealed the presence of phenolics, alkaloids, saponins, steroids, tannins, flavonoids, and terpenoids, which likely contribute to the observed bioactivity. The extract exhibited concentration-dependent ovicidal, larvicidal, and pupicidal effects, with first instar larvae being the most susceptible (LC_{50} = 248.63 µg/mL) and pupae the most tolerant (LC_{50} = 390.83 µg/mL). Complete ovicidal inhibition occurred at 500 µg/mL. These findings support the potential of *Calophyllum inophyllum* as an eco-friendly, plant-based alternative for integrated mosquito vector management.

References

1. Naik BR, Tyagi BK, Xue RD. Mosquito-borne diseases in India over the past 50 years and their global public health implications: a systematic review. Journal of the American Mosquito Control Association,2023:39(4):258–277.
2. Das B, Ghosal S, Mohanty S. Aedes: What do we know about them and what can they transmit? In Vectors and vector-borne zoonotic diseases, 2018.
3. Wei YL, Wu Z, Li RL, Tang F. Review of selected mosquito-borne diseases: arboviruses (dengue, chikungunya, Zika, West Nile, Japanese encephalitis, yellow fever) and parasitic diseases (malaria, lymphatic filariasis). Frontiers in Public Health, 2026, 13.
4. World Health Organization. Dengue situation updates,2026.
5. Shroff S, Mir S, Naik B, Baitharu I, Behera AK. Chemical methods for control of mosquito vector. In Molecular Identification of Mosquito Vectors and Their Management, 2021.
6. Zulfa R, Lo WC, Cheng PC, Martini M, Chuang TW. Updating the insecticide resistance status of *Aedes aegypti* and Aedes albopictus in Asia: a systematic review and meta-analysis. Tropical Medicine and Infectious Disease, 2022, 7(10).
7. Ray S, Shaju ST. Bioaccumulation of pesticides in fish resulting toxicities in humans through food chain and forensic aspects. Environmental Analysis Health and Toxicology, 2023, 38.
8. Thomas E, Stewart LE, Darley BA, Pham AM, Esteban I, Panda SS. Plant-based natural products and extracts: potential source to develop new antiviral drug candidates. Molecules, 2021, 26(20).
9. Ngegba PM, Cui G, Khalid MZ, Zhong G. Use of botanical pesticides in agriculture as an alternative to synthetic pesticides. Agriculture, 2022, 12(5).
10. Gunawan S, Hapsari S. Phytochemistry and biological activities of *Calophyllum inophyllum*. In Bioactive Compounds in Mangroves and their Associates, 2025.
11. Rajaganesh R, Murugan K, Panneerselvam C, Jayashanthini S, Aziz AT, Roni M *et al*. Fern-synthesized silver nanocrystals: towards a new class of mosquito oviposition deterrents? Research in Veterinary Science,2016:109:40–51.
12. Murugan K, Panneerselvam C, Samidoss CM, Madhiyazhagan P, Suresh U, Roni M *et al*. *In vivo* and *in vitro* effectiveness of Azadirachta indica-synthesized silver nanocrystals against Plasmodium berghei and

Plasmodium falciparum, and their potential against malaria mosquitoes. Research in Veterinary Science,2016:106:14–22.
13. Gupta S, Gupta P. The genus Calophyllum: review of ethnomedicinal uses, phytochemistry and pharmacology. Bioactive Natural Products in Drug Discovery, 2020.
14. Banerjee A, Manna S, Saha SK. Effect of aqueous extract of Azadirachta indica A. Juss (neem) leaf on oocyte maturation, oviposition, reproductive potentials and embryonic development of a freshwater fish ectoparasite Argulus bengalensis Ramakrishna 1951. Parasitology Research,2014:113(12):4641–4650.
15. Kaura T, Mewara A, Zaman K, Sharma A, Agrawal SK, Thakur V, *et al*. Utilizing larvicidal and pupicidal efficacy of Eucalyptus and neem oil against Aedes mosquito: an approach for mosquito control. Tropical Parasitology,2019:9(1):12–17.
16. Limboo N, Saha D. Assessment of sublethal effects of permethrin on adult life characteristics and resistance dynamics in Aedes albopictus. Pesticide Biochemistry and Physiology, 2024, 203.
17. Kaur N, Kocher DK. Ovicidal, larvicidal and pupicidal potential of neem seed kernel extract against *Aedes aegypti*. International Journal of Tropical Insect Science,2023:43(3):1089–1096.
18. Sarkar I, Sarkar SK. Lethal efficacy and mode of action of Indian medicinal plant extracts against dengue mosquito vectors with an overview of the disease burden in India. Green Health, 2026, 2(1).
19. Rahman MM, Morshed MN, Adnan SM, Howlader MTH. Assessment of biorational larvicides and botanical oils against Culex quinquefasciatus larvae in laboratory conditions. Heliyon, 2024, 10(11).