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Antioxidant activity and mosquitocidal activity of *Calophyllum inophyllum* aqueous leaf against *Aedes aegypti*

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Abstract
The present study evaluated the antioxidant and mosquitocidal activities of *Calophyllum inophyllum* aqueous leaf extract against the dengue vector, *Aedes aegypti*. Antioxidant potential was assessed using DPPH and ABTS radical scavenging assays, while mosquitocidal efficacy was determined through ovicidal, larvicidal, and pupicidal bioassays. The DPPH scavenging activity increased dose-dependently, with percentage inhibition ranging from 22–28% at 100 µg/mL to 86–85% at 500 µg/mL, compared to 90–92% for ascorbic acid (standard). Similarly, ABTS scavenging activity ranged from 16–18% at 100 µg/mL to 74–77% at 500 µg/mL, with ascorbic acid showing 80–82% inhibition. These results indicate moderate antioxidant capacity. For mosquitocidal activity, the extract exhibited significant ovicidal effects, with egg hatchability decreasing from 97.8±1.92% in controls to 84.0±1.87%, 54.2±1.78%, 24.2±1.92%, and 8.6±1.67% at 100, 200, 300, and 400 µg/mL, respectively; complete inhibition (NH) was observed at 500 µg/mL. Larvicidal and pupicidal assays revealed concentration-dependent mortality across all instars and pupae. The 1st instar larvae showed the highest susceptibility (LC_{50} = 252.94 µg/mL; LC_{90} = 455.39 µg/mL), while pupae were most tolerant (LC_{50} = 385.62 µg/mL; LC_{90} = 665.83 µg/mL). Mortality rates ranged from 7.2±1.92% (pupae at 100 µg/mL) to 97.0±1.30% (1st instar at 500 µg/mL). Regression equations showed positive concentration–response relationships, and chi-square values indicated non-significant deviations ($p > 0.05$). These findings suggest that *C. inophyllum* aqueous leaf extract possesses promising antioxidant properties and potent mosquitocidal activity, making it a potential eco-friendly alternative for integrated vector management programs targeting *A. aegypti*. Further studies on phytochemical characterization and field applicability are warranted.

Keywords: Antioxidants, larvicidal activity, *Aedes aegypti, Calophyllum inophyllum,* ovicidal activity

Introduction

Mosquito-borne diseases represent a critical global public health challenge, with *Aedes aegypti* serving as the primary vector for debilitating arboviral infections including dengue fever, Zika virus, chikungunya, and yellow fever [1]. The World Health Organization has documented a dramatic surge in dengue cases, with over 5 million reported worldwide in 2019, highlighting the urgent need for effective vector control strategies [2]. Conventional mosquito management has relied heavily on synthetic insecticides such as organophosphates and pyrethroids; however, the indiscriminate use of these chemicals has precipitated widespread insecticide resistance in *Ae. aegypti* populations and raised serious environmental and health concerns [3]. These challenges have catalyzed a paradigm shift toward exploring eco-friendly, sustainable alternatives, particularly plant-derived bioactive compounds [4].

Calophyllum inophyllum L. (*Calophyllaceae*), commonly known as nyamplung or mastwood, is a medium-sized mangrove-associated tree native to tropical regions, reaching heights up to 20 meters with rough bark and fragrant white flowers [5]. This plant has been utilized for generations in traditional medicine across the Pacific Islands and Southeast Asia, with different parts—leaves, roots, stems, seeds, and flowers—being employed to treat various ailments including skin diseases, arthritis, wounds, eye irritation, dysentery, and rheumatism [6, 7]. The therapeutic versatility of *C. inophyllum* is attributed to its rich repertoire of bioactive phytochemicals, encompassing flavonoids, coumarins, xanthones, triterpenoids, steroids, fatty acids, saponins, and phenolic compounds [8]. Notable compounds include pyranoxanthones such as inophyllin B and brasilixanthone B, friedelin, phytol, and various coumarin derivatives [9, 10]. These phytoconstituents have demonstrated a remarkable spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, antiviral (including anti-HIV), antidiabetic, and mosquitocidal properties [11, 12].

The antioxidant potential of *C. inophyllum* has garnered particular attention, as oxidative stress is implicated in numerous pathological conditions. Previous studies have reported significant DPPH and ABTS radical scavenging activities in extracts derived from its leaves, seeds, and flowers [13, 14]. Concurrently, preliminary investigations have revealed promising larvicidal effects of *C. inophyllum* extracts against mosquito vectors, with bioactive components such as phytol, eugenol, caryophyllene oxide, and β-caryophyllene potentially contributing to this activity [15, 16]. However, comprehensive evaluations that simultaneously assess both antioxidant capacity and mosquitocidal activity across all life stages of *Ae. aegypti* remain limited. Given the pressing need for integrated vector management strategies that target multiple mosquito developmental stages while minimizing environmental impact [17], this study was undertaken to evaluate the antioxidant activity and mosquitocidal efficacy of aqueous leaf extract of *C. inophyllum* against eggs, larvae (all instars), and pupae of *Ae. aegypti*. The findings from this investigation may contribute to the development of a sustainable, plant-based biocontrol agent as a viable alternative to synthetic pesticides in dengue vector management [18].

Methodology

Plant Collection and Authentication

Fresh, healthy leaves of *Calophyllum inophyllum* were collected from the Bharathiar University campus, Coimbatore, Tamil Nadu, India (11°02'N latitude, 76°52'E longitude) during the flowering season. The plant material was taxonomically authenticated by a taxonomist, and a voucher specimen was deposited at the university herbarium for future reference. Collected leaves were thoroughly washed under running tap water to remove surface contaminants, followed by rinsing with distilled water. The cleaned leaf material was shade-dried at ambient temperature for 7–10 days until constant weight was achieved, after which it was pulverized into a fine powder using an electric grinder and stored in airtight containers at 4°C until further use [19].

Preparation of Aqueous Extract

The aqueous leaf extract was prepared by macerating 100 g of powdered leaf material in 1000 mL of distilled water in a conical flask at room temperature for 72 hours with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary vacuum evaporator at 45°C under reduced pressure. The resulting crude extract was lyophilized to obtain a dry powder, which was weighed and stored at -20°C in sealed vials. Stock solutions of varying concentrations (100, 200, 300, 400, and 500 µg/mL) were prepared by dissolving appropriate amounts of the extract in distilled water for bioassay experiments [20].

Antioxidant Activity Assays

DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was determined following the method of Brand-Williams *et al*. [21]. Briefly, 0.1 mM DPPH solution in methanol was prepared, and 2.0 mL of this solution was mixed with 2.0 mL of extract at varying concentrations (100–500 µg/mL). The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid was used as the positive control. All assays were performed in triplicate, and the percentage inhibition was calculated using the formula: % Inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is absorbance of control and A_1 is absorbance of the sample [22].

ABTS Radical Scavenging Assay

The ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assay was performed according to Re *et al*. [23]. ABTS was dissolved in water to 7 mM concentration, and potassium persulfate was added to achieve 2.45 mM final concentration. The mixture was incubated in the dark at room temperature for 16 hours to generate the ABTS•+ radical cation. The solution was diluted with ethanol to obtain an absorbance of 0.700 ± 0.020 at 734 nm. Then, 2.0 mL of diluted ABTS solution was mixed with 2.0 mL of extract at various concentrations (100–500 µg/mL), and absorbance was recorded after 6 minutes at 734 nm. Ascorbic acid served as the reference standard, and all measurements were performed in triplicate [24].

Mosquito Rearing and Maintenance

Colonies of *Aedes aegypti* were maintained in the insectary under controlled environmental conditions (27±2°C, 75±5% relative humidity, 14:10 h light: dark photoperiod). Larvae were reared in enamel trays containing dechlorinated tap water and fed with a 1:1 mixture of yeast powder and dog biscuits. Pupae were collected daily and transferred to cages for adult emergence. Adult mosquitoes were maintained in 30×30×30 cm mosquito cages and provided with 10% sucrose solution ad libitum. For blood feeding, females were fed on anesthetized Swiss albino mice, and eggs were collected on filter paper strips placed in water-filled oviposition cups. Eggs were air-dried and stored for use in ovicidal assays [25].

Ovicidal Activity Assay

The ovicidal activity of the aqueous leaf extract was evaluated following the method of Su and Mulla [26]. Approximately 100 freshly laid *Ae. aegypti* eggs (24–48 h old) were immersed in 250 mL of test solutions at concentrations of 100, 200, 300, 400, and 500 µg/mL, with distilled water as the control. Each treatment was replicated five times. After 48 hours of exposure, eggs were transferred to dechlorinated water for hatching, and the number of hatched larvae was counted daily for seven days. Egg hatchability percentage was calculated as: (Number of hatched larvae / Total number of eggs) × 100 [27].

Larvicidal and Pupicidal Activity Assay

The larvicidal and pupicidal assays were performed according to WHO standard protocols [28]. For each concentration (100, 200, 300, 400, and 500 µg/mL), twenty early fourth-instar larvae (or twenty pupae) were placed in 500 mL glass beakers containing 250 mL of test solution. Control groups received distilled water only. For larval instars (1st, 2nd, 3rd, 4th), separate experiments were conducted with twenty individuals per stage per concentration. Mortality was recorded after 24 hours of exposure. Larvae were considered dead when no movement was observed upon probing with a needle. All experiments were conducted in five replicates, and mortality percentages were corrected using Abbott's formula when control mortality exceeded 5% [29]. The lethal concentrations (LC_{50} and LC_{90}) were calculated using probit analysis [30].

Statistical Analysis

All data were expressed as mean ± standard deviation (SD) of five replicates. Statistical analysis was performed using SPSS version 20.0 (IBM Corp, Armonk, NY, USA). Probit analysis was used to calculate LC_{50} and LC_{90} values with 95% confidence limits. Regression equations and chi-square (χ^2) values were determined to assess the concentration–response relationship. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed to determine significant differences among treatment groups. A probability level of $p < 0.05$ was considered statistically significant [31].

Results and Discussion

Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH assay revealed a concentration-dependent increase in radical scavenging activity of *C. inophyllum* aqueous leaf extract (Figure 1). At 100 µg/mL, the percentage inhibition ranged from 22–28%, which increased substantially to 45–44% at 200 µg/mL, 55–57% at

300 µg/mL, 66–68% at 400 µg/mL, and reached 86–85% at 500 µg/mL. The standard antioxidant ascorbic acid exhibited 90–92% inhibition at equivalent concentrations. These findings demonstrate that the extract possesses considerable electron-donating capacity, effectively neutralizing the stable DPPH free radical by transferring hydrogen atoms or electrons [32].

The observed activity may be attributed to the presence of phenolic compounds and flavonoids in the extract, which are well-known for their redox properties and ability to act as hydrogen donors [33]. The dose-dependent nature of the scavenging activity aligns with previous reports on *C. inophyllum* seed and leaf extracts, where IC_{50} values ranging from 62–185 µg/mL were documented [34, 35].

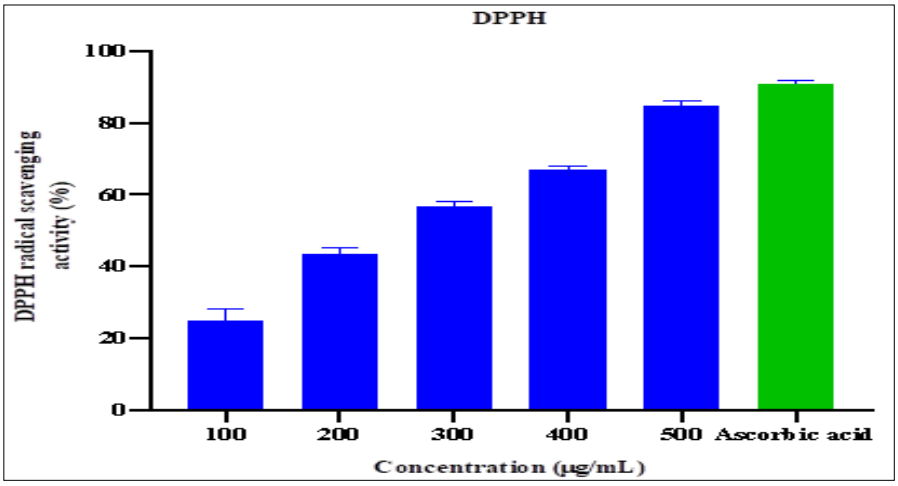

Fig 1: DPPH free radical scavenging activity of *C. inophyllum* aqueous extract

ABTS Radical Scavenging Activity

The ABTS assay further corroborated the antioxidant potential of the extract, showing a similar concentration-dependent trend (Figure 2). The percentage inhibition at 100 µg/mL was 16–18%, increasing to 37–39% at 200 µg/mL, 52–50% at 300 µg/mL, 61–63% at 400 µg/mL, and 74–77% at 500 µg/mL. Ascorbic acid, used as reference, demonstrated 80–82% inhibition. The ABTS radical cation scavenging assay measures the ability of antioxidants to quench the blue-green ABTS•+ radical, which is applicable to both hydrophilic and lipophilic antioxidants [36].

The relatively higher activity observed in the DPPH assay compared to ABTS at equivalent concentrations suggests that the extract contains predominantly hydrogen-donating antioxidants rather than electron-transfer mechanisms [37]. The promising antioxidant activity of *C. inophyllum* leaves may be linked to the presence of bioactive compounds such as inophyllum D, calanolide A, and various flavonoids, which have been previously identified through phytochemical screening [38, 39]. These findings support the traditional use of this plant in managing oxidative stress-related disorders and highlight its potential as a natural source of therapeutic antioxidants [40].

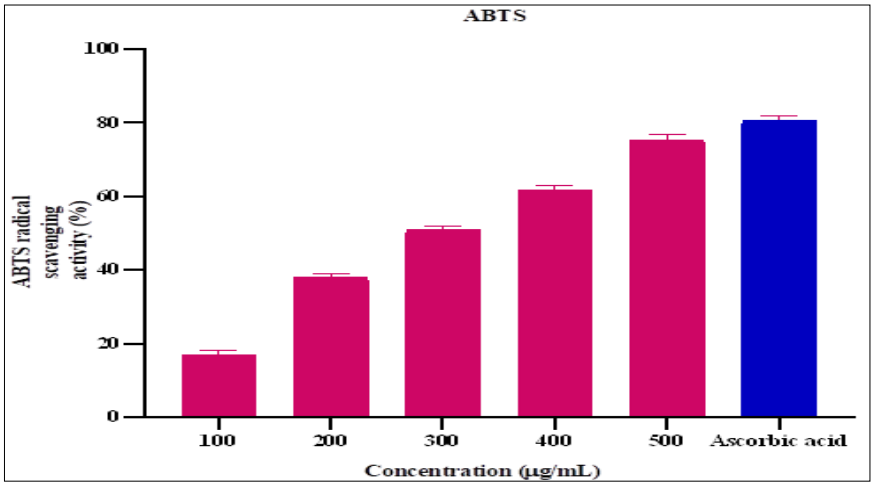

Fig 2: ABTS radical scavenging activity of *C. inophyllum* aqueous extract

Ovicidal Activity

Table 1: Ovicidal activity of *Calophyllum inophyllum* Aqueous Leaf Extract against *Aedes aegypti*

Egg hatchability					
Control	**100 µg/mL**	**200 µg/mL**	**300 µg/mL**	**400 µg/mL**	**500 µg/mL**
97.8±1.92	84.0±1.87	54.2±1.78	24.2±1.92	8.6±1.67	NH

* NH- No Hatchability

The ovicidal assay demonstrated remarkable efficacy of the aqueous leaf extract in inhibiting egg hatchability in a concentration-dependent manner (Table 1). The control group exhibited 97.8±1.92% hatching, indicating optimal viability of the eggs. Upon treatment with 100 µg/mL extract, hatching was reduced to 84.0±1.87%, followed by 54.2±1.78% at 200 µg/mL, 24.2±1.92% at 300 µg/mL, and a substantial reduction to 8.6±1.67% at 400 µg/mL. Complete inhibition of hatching (NH) was observed at the highest concentration of 500 µg/mL. The progressive reduction in hatchability suggests that the extract contains ovicidal compounds capable of penetrating the egg chorion and interfering with embryonic development [27]. The eggshell of *Ae. aegypti* is composed of a highly resistant chorion layer; however, plant-derived phytochemicals such as saponins and terpenoids have been reported to disrupt chorion integrity, leading to desiccation and embryonic mortality [41]. The complete ovicidal effect at 500 µg/mL is particularly noteworthy, as it indicates the potential for using this extract as a preventive measure to reduce mosquito population emergence from breeding sites. Earlier studies have reported similar ovicidal activity from other plant extracts, but the complete inhibition observed here with *C. inophyllum* at relatively low concentrations underscores its superior efficacy [42]. This ovicidal property is crucial for integrated vector management, as targeting the egg stage prevents the emergence of larvae that would subsequently develop into adults [43].

Larvicidal and Pupicidal Activity

Table 2: Larvicidal and pupicidal activity of *Calophyllum inophyllum* Aqueous 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	252.940 (455.387)	231.692 (424.166)	272.965 (496.223)	y= -1.601+0.006 x	2.950 n.s.
2nd Instar	270.258 (488.227)	248.225 (453.542)	291.354 (534.222)	y = -1.589+0.006 x	0.832 n.s.
3rd Instar	291.415 (541.205)	267.337 (498.361)	315.078 (600.110)	y = -1.495+0.005 x	0.779 n.s.
4th Instar	327.312 (599.732)	302.006 (547.541)	354.062 (673.949)	y = -1.540+0.005 x	1.497 n.s.
Pupa	385.618 (665.831)	358.353 (603.821)	418.034 (756.517)	y = -1.764+0.005 x	2.450 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 ($\alpha = 0.05$)

The larvicidal and pupicidal activity data revealed that *C. inophyllum* aqueous leaf extract exerts potent concentration-dependent mortality across all mosquito life stages (Table 2). The first instar larvae were the most susceptible, with mortality rates increasing from 17.2±1.92% at 100 µg/mL to 97.0±1.30% at 500 µg/mL, yielding an LC_{50} of 252.94 µg/mL (LCL: 231.69, UCL: 272.97) and LC_{90} of 455.39 µg/mL (LCL: 424.17, UCL: 496.22). The regression equation y = -1.601 + 0.006x with χ^2 = 2.950 (n.s.) demonstrated a strong positive correlation between concentration and mortality. Second instar larvae showed slightly reduced susceptibility (LC_{50} = 270.26 µg/mL; LC_{90} = 488.23 µg/mL), with mortality ranging from 15.0±1.58% to 92.4±1.14% across the concentration gradient. Third instar larvae exhibited LC_{50} of 291.42 µg/mL and LC_{90} of 541.21 µg/mL, while fourth instar larvae demonstrated LC_{50} of 327.31 µg/mL and LC_{90} of 599.73 µg/mL. Pupae were the most tolerant stage, with LC_{50} of 385.62 µg/mL and LC_{90} of 665.83 µg/mL, displaying mortality from 7.2±1.92% at 100 µg/mL to 71.2±1.30% at 500 µg/mL.

The decreasing susceptibility from early instars to pupae is consistent with previous studies on plant-derived larvicides and reflects the physiological and morphological changes that occur during mosquito development [44]. Young larvae are generally more vulnerable due to their smaller size, higher surface-to-volume ratio, and less developed cuticular barriers [45]. The observed mortality may be attributed to the extract's phytochemical constituents interfering with the larval nervous system, midgut epithelium, or respiratory structures [46]. Previous studies have identified phytol, eugenol, and caryophyllene oxide as major compounds in *C. inophyllum* leaf extracts, all of which have demonstrated insecticidal properties [47]. The chi-square values (ranging from 0.779 to 2.950, all non-significant at $\alpha = 0.05$) confirm the goodness of fit of the probit model, validating the calculated lethal concentrations. The LC_{50} values obtained in this study compare favorably with those reported for other plant extracts against *Ae. aegypti*, which typically range from 300–600 µg/mL [48]. Furthermore, the pupicidal activity of the extract, although lower than larvicidal effects, is significant as pupae are often overlooked in vector control programs, yet they represent the final aquatic stage before adult emergence [49]. The broad-spectrum activity across all life stages positions *C. inophyllum* as a promising candidate for integrated vector management, potentially reducing the frequency of insecticide applications and delaying the development of resistance [50].

Conclusion

The present investigation conclusively demonstrates that the aqueous leaf extract of *Calophyllum inophyllum* possesses significant antioxidant and mosquitocidal activities against *Aedes aegypti*, the primary vector of dengue and other arboviral diseases. The extract exhibited robust DPPH and ABTS radical scavenging activity in a concentration-dependent manner, with maximum inhibition of 86–85% and 74–77% respectively at 500 µg/mL, compared to ascorbic acid standards. These findings validate the traditional medicinal use of *C. inophyllum* and highlight its potential as a natural source of antioxidant agents. Notably, the mosquitocidal assays revealed remarkable efficacy across all developmental stages, with complete inhibition of egg hatchability at 500 µg/mL and significant larval/pupal mortality at lower concentrations. The first instar larvae were the most susceptible stage (LC_{50} = 252.94 µg/mL), while pupae were comparatively tolerant (LC_{50} = 385.62

µg/mL), indicating stage-specific sensitivity. The non-significant chi-square values confirmed the reliability of the concentration–response relationships. The broad-spectrum activity of this eco-friendly botanical extract across multiple life stages of *Ae. aegypti*, combined with its antioxidant properties, offers a dual advantage for public health applications. This study contributes valuable baseline data for the development of sustainable, plant-based alternatives to synthetic insecticides, aligning with global efforts to mitigate insecticide resistance and environmental pollution. Future research should focus on isolation and characterization of the specific bioactive compounds responsible for these activities, elucidation of their mode of action, evaluation of non-target toxicity, and field-based validation. Phytochemical profiling and formulation development will be essential to translate these laboratory findings into practical vector control interventions. Given the escalating burden of mosquito-borne diseases worldwide, *C. inophyllum* emerges as a promising candidate for integrated vector management strategies that are both effective and environmentally sustainable.

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