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ORIGINAL ARTICLE

Larvicidal effectiveness of plant leaf extracts from *Mussaenda philippica* against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT
The mosquito species Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus are major vectors responsible for severe nuisance and the transmission of life-threatening diseases in urban environments. In the present study, the larvicidal efficacy of crude extracts of Mussaenda philippica was evaluated against these vector mosquito larvae. Crude extracts prepared using four different solvents, methanol, chloroform, ethyl acetate, and hexane were tested at varying concentrations against early third-instar larvae of the three mosquito species. Among the extracts tested, the methanolic extract exhibited the highest larvicidal activity, with LC_{50} values of 50.30, 55.56, and 60.37 µg/mL and LC_{90} values of 101.33, 110.57, and 119.98 µg/mL against Ae. aegypti, An. stephensi, and Cx. quinquefasciatus, respectively. The findings demonstrate that M. philippica possesses significant larvicidal potential and can effectively control mosquito vectors. This study highlights the promise of plant-based botanical extracts as eco-friendly and sustainable alternatives to synthetic insecticides for mosquito control and vector-borne disease management. Key words: Larvicidal activity, Mussaenda philippica, Crude extract, Dengue vector, Malaria vector, Filariasis vector.
Keywords: Larvicidal activity; *Mussaenda philippica*; Crude extract; Dengue; Malaria; Filariasis.

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INTRODUCTION

Vector-borne diseases (VBDs) transmitted by mosquitoes pose severe health problems to the global human population and represent a serious threat, particularly in tropical and subtropical regions [1,2]. Worldwide, these diseases are responsible for millions of deaths annually and contribute significantly to morbidity [3]. Among mosquito vectors, *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* play crucial roles in the transmission of major infectious diseases, including dengue fever, Zika fever, filariasis, Japanese encephalitis (JE), and malaria [4,5]. Globally, dengue fever has emerged as a major public health concern, with *Ae. aegypti* being the principal vector. In India, the transmission of all dengue virus serotypes has shown a steady increase among arboviral diseases [6]. It is estimated that 50–200 million dengue infections occur worldwide each year [7]. Since *Ae. aegypti* breeds predominantly in clean freshwater habitats, controlling its reproduction remains a significant challenge.

Similarly, *Anopheles stephensi*, recognized for its ecological adaptability and diverse feeding behavior, is a key vector of malaria across different geographic regions. It commonly breeds in wastewater disposal systems, fallow rice fields, and rainwater collections, highlighting the importance of understanding its ecological niche for developing effective vector control strategies. Although global malaria mortality has declined by approximately 45% since 2000, with a 49% reduction in the WHO African Region, malaria

continues to pose a significant health burden in many endemic areas. In parallel, *Culex quinquefasciatus* has emerged as a major vector of lymphatic filariasis in several Southeast Asian countries, including India, where the disease remains endemic. Lymphatic filariasis continues to affect over 40 million individuals in sub-Saharan Africa alone. The continued endemicity of these mosquito-borne diseases underscores the urgent need for targeted, sustainable, and environmentally safe vector control strategies [8].

Conventional mosquito control strategies rely largely on synthetic chemical insecticides; however, their extensive and indiscriminate use has raised serious concerns due to adverse environmental effects and potential risks to human health. These chemicals can disrupt ecological balance and may lead to bioaccumulation and genetic alterations in non-target organisms. In response to these challenges, increasing attention has been directed toward alternative mosquito control approaches based on botanical extracts. Plant-derived bioactive compounds offer promising opportunities for the development of environmentally benign larvicides capable of interrupting mosquito life cycles and reducing the transmission of vector-borne diseases [9].

Several studies have demonstrated the larvicidal potential of plant extracts against mosquito vectors. For instance, Rahuman et al. [10] reported significant larvicidal activity of leaf extracts from five cucurbitaceous plant species, *Citrullus colocynthis*, *Coccinia indica*, *Cucumis sativus*, *Momordica charantia*, and *Trichosanthes anguina* against early fourth-instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. These findings highlight the effectiveness of botanical resources as sustainable alternatives to synthetic insecticides for mosquito control.

Mussaenda philippica is recognized as a valuable natural source of antimicrobial and antioxidant compounds, largely due to its rich profile of bioactive secondary metabolites, and its hepatoprotective properties further support its relevance in traditional medicine [11,12]. Despite these documented pharmacological activities, its potential as a botanical larvicide against medically important mosquito vectors remains largely unexplored. Therefore, the present study, for the first time, systematically evaluates the larvicidal efficacy of different crude leaf extracts of *M. philippica* against *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*, aiming to identify eco-friendly and sustainable alternatives for mosquito vector control.

MATERIAL AND METHODS

Plant Material Collection and Processing

Fresh leaves of *Mussaenda philippica* were collected from in and around Kumbakonam, Thanjavur District, Tamil Nadu, India. The plant material was taxonomically authenticated by a plant taxonomist from the Department of Botany, Government College for Women (Autonomous). A voucher specimen was deposited in the research laboratory for future reference. The collected leaves were thoroughly washed with tap water followed by distilled water to remove dust and contaminants and were then shade-dried at room temperature (28 ± 2 °C) for 20 days. After complete drying, the leaves were finely powdered using an electric blender and stored in airtight containers until further use.

Preparation of Crude Extract

The powdered plant material (250 g) was extracted separately using four solvents—methanol, chloroform, ethyl acetate, and hexane (1 L each) in a Soxhlet apparatus for 8 h following standard procedures [13]. After extraction, the solvent extracts were filtered through Whatman No. 1 filter paper using a Buchner funnel. The filtrates were concentrated under reduced pressure using a rotary evaporator to obtain viscous, dark greenish crude extracts (Fig. 1). For larvicidal bioassays, the dried residues were dissolved in acetone to prepare 1% (w/v) stock solutions.

Testing Organisms

Pathogen-free laboratory strains of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* were maintained in the research laboratory of the Department of Zoology, Government College for Women (Autonomous). Larvae were reared in dechlorinated water and fed a mixture of dog biscuits and yeast powder in a 3:1 ratio. Prior to experimental use, larvae were starved for 12 h and were 3–4 days old at the time of the bioassays. Adult mosquitoes were provided with a 10% sucrose solution, and blood meals were supplied using a one-week-old chick. The insectary was maintained under controlled conditions at 28 ± 2 °C, 70–85% relative humidity, and a 12:12 h light–dark photoperiod [14].

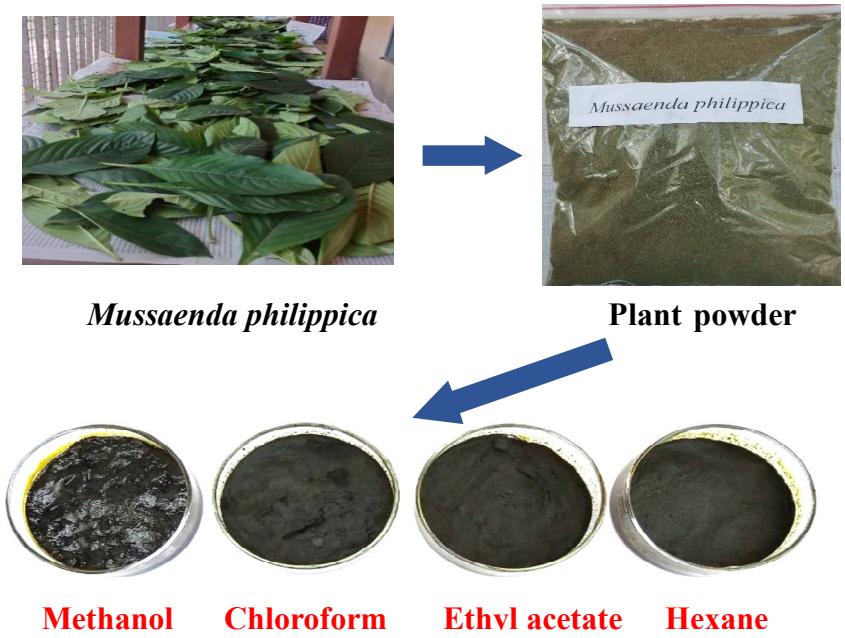

Fig 1. Process of plant extraction

Assessment of Larvicidal Activity

Larvicidal bioassays were conducted using a modified version of the World Health Organization [15] standard protocol for evaluating plant extracts against mosquito larvae. Bioassays were performed in 250 mL plastic cups containing 200 mL of dechlorinated water. Exactly 25 early third-instar larvae were introduced into each cup to standardize test conditions. From the respective stock solutions, a series of test concentrations were prepared for each solvent extract: 25, 50, 75, 100, and 125 ppm for methanol; 30, 60, 90, 120, and 150 ppm for chloroform; 40, 80, 120, 160, and 200 ppm for ethyl acetate; and 50, 100, 150, 200, and 250 ppm for hexane. One milliliter of the appropriate plant extract dilution was added to each test cup, and five replicates were maintained for each concentration. Control groups were set up using dechlorinated water without plant extract. All test cups were maintained under standard laboratory conditions and incubated for 24 h. After the exposure period, larval mortality was observed and recorded, and larvae were considered dead if they showed no movement when probed gently.

Statistical Analysis

Statistical analyses were performed using SPSS software version 26.0 (Statistical Package for the Social Sciences, Chicago, IL, USA). Statistical significance was determined at a probability level of $P < 0.05$.

RESULTS AND DISCUSSION

The larvicidal activity of crude extracts of *Mussaenda philippica* was evaluated against the larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Table 1,2,3,4). Among the solvent extracts tested, the methanolic extract exhibited the highest larvicidal efficacy, with LC_{50} values of 50.30, 55.56, and 60.37 µg/mL and LC_{90} values of 101.33, 110.57, and 119.98 µg/mL against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, respectively. The chloroform extract also showed considerable larvicidal activity, with LC_{50} values of 63.12, 69.73, and 75.80 µg/mL and LC_{90} values of 124.33, 134.77, and 143.81 µg/mL, respectively. Moderate larvicidal activity was observed with the ethyl acetate extract, which recorded LC_{50} values of 80.21, 87.47, and 100.94 µg/mL and LC_{90} values of 165.13, 180.37, and 193.38 µg/mL, respectively. The hexane extract showed comparatively lower toxicity, with LC_{50} values of 103.40, 117.64, and 131.57 µg/mL and LC_{90} values of 204.99, 233.78, and 248.29 µg/mL against the three mosquito species, respectively. No larval mortality was observed in the control groups. Chi-square analysis indicated that the mortality rates were statistically significant at $P < 0.05$. Notably, 100% larvicidal mortality was achieved against *Ae. aegypti* larvae at higher concentrations of the methanolic extract.

Table 1. Larvicidal activity of leaf extracts of *Mussaenda philipica* against the third instar of *Aedes aegypti*

Solvents	LC_{50} (µg/ml) (LCL - UCL)	LC_{90} (µg/ml) (LCL – UCL)	Regression equation	χ^2*
Methanol	50.30 (44.17 – 55.64)	101.33 (93.87 – 111.17)	y=13.18+0.7176x	5.243*
Chloroform	63.12 (56.01 – 69.42)	124.23 (94.05 – 110.87)	y=10.40+0.6120x	6.156*
Ethyl acetate	80.21 (46.10 – 102.68)	165.13 (137.30 –227.32)	y=14.16+0.4390x	7.844*
Hexane	103.40 (91.45 – 113.93)	204.99 (190.04 –224.66)	y=11.36+0.3648x	5.886*

*p≤0.05, Level of significance.

Table 2. Larvicidal activity of leaf extracts of *Mussaenda philipica* against the third instar of *Anopheles stephensi.*

Solvents	LC_{50} (µg/ml) (LCL - UCL)	LC_{90} (µg/ml) (LCL – UCL)	Regression equation	χ^2*
Methanol	55.56 (49.38 – 61.06)	110.57 (102.36 – 121.48)	y=8.760+0.7248x	1.191*
Chloroform	69.73 (62.64 – 76.17)	134.77 (124.93 – 147.82)	y=5.800+0.6187x	3.249*
Ethyl acetate	87.47 (76.94 – 96.71)	180.37 (166.48 –199.04)	y=10.98+0.4365x	1.079*
Hexane	117.64 (105.09 – 128.95)	233.78 (215.99 – 257.68)	y=6.840+0.3592x	0.445*

*p≤0.05, Level of significance.

Table 3. Larvicidal activity of leaf extracts of *Mussaenda philipica* against the third instar of *Culex quiquefasciatus.*

Solvents	LC_{50} (µg/ml) (LCL - UCL)	LC_{90} (µg/ml) (LCL – UCL)	Regression equation	χ^2*
Methanol	60.37 (54.04 – 66.11)	119.98 (110.71 – 132.53)	y=6.340+0.7096x	1.682*
Chloroform	75.80 (68.75 – 82.36)	143.81 (133.16 – 158.05)	y=2.180+0.6193x	2.061*
Ethyl acetate	100.94 (91.36 – 109.83)	193.38 (178.90–212.80)	y=2.720+0.4600x	1.376*
Hexane	131.57 (119.74 – 142.72)	248.29 (229.63 – 273.39)	y=0.8000+0.3688x	1.265*

*p≤0.05, Level of significance.

The present findings are consistent with earlier reports demonstrating the strong larvicidal potential of methanolic plant extracts against mosquito vectors. For instance, Asmaey et al. [16] reported exceptional larvicidal activity of methanolic crude extracts of *Melissa officinalis* and *Rosmarinus officinalis* against *Culex pipiens*, with LC_{50} values of 26.505 and 9.795 µg/mL, respectively. Similarly, Louis et al. [17] demonstrated significant larval mortality induced by methanolic extracts of unripe fruit peel of *Persea americana*, recording LC_{50} and LC_{90} values of 6.65 and 71.62 ppm for *Anopheles stephensi*, 7.12 and 86.59 ppm for *Aedes aegypti*, and 10.78 and 69.39 ppm for *Culex quinquefasciatus*.

In agreement with these observations, Araujo et al. [18] reported notable larvicidal efficacy of *Acmella oleracea* crude extracts prepared using methanol, hydroethanol, and hexane against *Ae. aegypti*, with comparatively low LC_{50} values of 39.67 µg/mL (methanol), 28.42 µg/mL (hydroethanol), and 23.2 µg/mL (hexane). Collectively, these studies support the present results and further confirm that methanol-based plant extracts are particularly effective in extracting bioactive compounds responsible for mosquito larvicidal activity.

Mussaenda philippica exhibits significant larvicidal activity against mosquito vectors, which may be attributed to its rich phytochemical composition, including alkaloids, flavonoids, and saponins, while also offering the advantage of being environmentally benign [19]. Comparable larvicidal effects of plant-derived extracts have been reported in earlier studies. For example, *Aloe vera* leaf extract showed pronounced larvicidal toxicity against *Aedes aegypti* larvae across all instars (I–IV), with LC_{50} values of

162.74, 201.43, 253.30, and 300.05 ppm and LC_{90} values of 442.98, 518.86, 563.18, and 612.96 ppm, respectively [20].

Similarly, Govindarajan and Angelina [21] demonstrated the larvicidal efficacy of *Ficus benghalensis* extracts against early second-, third-, and fourth-instar larvae of *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*, recording LC_{50} values of 41.43, 58.21, and 74.32 ppm; 56.54, 70.29, and 80.85 ppm; and 60.44, 76.41, and 89.55 ppm, respectively. These findings, together with the present results, reinforce the potential of phytochemical-rich botanical extracts as effective and eco-friendly larvicidal agents for sustainable mosquito vector control.

CONCLUSION

The findings of the present investigation clearly demonstrate that crude extracts of *Mussaenda philippica* possess strong larvicidal activity against three medically important mosquito species, *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* and may serve as effective natural alternatives to hazardous and environmentally harmful synthetic insecticides. These results suggest that *M. philippica*-based formulations could complement existing mosquito vector control strategies within integrated management programs. However, further studies are required to elucidate the precise mode of action of *M. philippica* bioactive compounds and to evaluate their larvicidal efficacy under field conditions. In addition, comprehensive assessments of their effects on non-target organisms are essential to ensure environmental safety and support their practical application in sustainable mosquito control.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they do not have any conflict of interest.

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