

# The potential use of *Gomphrena celosioides* Mart. (Amaranthaceae) for the control of malaria, dengue, and filariasis vector: An eco-friendly botanical management of mosquitoes

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## ABSTRACT

**Background:** Mosquitoes pose a significant public health threat because they can transmit dreadful diseases such as Zika, dengue, and malaria. Hence, there is increased interest in using plant-derived pesticides as an alternative to chemical synthetic larvicides, adversely affecting overall human health and the environment. **Objectives:** This work aimed to assess the effects on larvae of vector mosquitoes *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* using crude extracts of *Gomphrena celosioides* derived from methanol, chloroform, ethyl acetate, and hexane. **Materials and Methods:** Various concentration of the plant crude extracts (from 6 to 75 µg/ml) were tested. The larvicidal activity was measured after 24 hours of treatment. **Results:** The results showed that larval mortality increased with dosage, with 100% mortality at the highest concentration. *G. celosioides* extracts were found to possess larvicidal properties. Among the four solvent extracts tested for efficacy against mosquito larvae, methanol extract showed the best performance against *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus* (LC<sub>50</sub> = 12.53, 13.47 and 14.31; LC<sub>90</sub> = 24.77, 25.67 and 26.97 µg/ml, respectively). **Conclusion:** This finding offers additional insight into the fact that plant extracts could eventually serve as a replacement for chemicals that control mosquito populations.

**Keywords:** *Gomphrena celosioides*, Crude extracts, Malaria, Filariasis, Dengue, Larval control.

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## INTRODUCTION

A wide range of diseases, including infections, are spread by arthropods, including mosquitoes. These animals are thought to be the most frequent carriers of many diseases and parasites that spread worldwide epidemics and pandemics. Numerous literature<sup>1-3</sup> show mosquitoes to be vectors for millions of sufferers of infectious diseases such as malaria, dengue, yellow fever, chikungunya, Zika virus, lymphatic filariasis, and encephalitis. Dengue fever is found in more than 129 countries and affects more than 2.9 billion people globally. The incidence and mortality due to dengue in India seem to increase yearly.<sup>3</sup> Malaria is a public health problem affecting 46% of the African population.<sup>4</sup> According to the baseline estimate, lymphatic filariasis was estimated to have affected approximately 15 million people with lymphoedema and 25 million men with hydrocele worldwide.<sup>5</sup>

Numerous strategies have been developed and implemented to decrease the burden of these diseases in endemic countries. From the time human disease vector control began, chemical-based pesticides truly underpinned the system. The development of pesticide resistance, with adverse environmental implications, constitutes a serious challenge to chemical approaches to vector control. The change of focus from pesticides to natural phytochemicals is good news since the former are biodegradable, and the latter does not affect non-target species at a controlled low concentration. Other articles regarding phyto-insecticides for mosquito vector control have been published.<sup>6-8</sup>

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There is a new wave of green, plant-derived insect repellents coming. Some secondary metabolites in the extract may affect mosquito behavior and physiological processes. Some of the phytochemicals can be used as mosquito larvicides. Govindarajan<sup>9</sup> tested larvicidal and

ovicidal activities of certain different solvent extracts of *Cardiospermum halicacabum* against *Culex quinquefasciatus* and *Aedes aegypti*. Certain plant extracts have shown toxicity towards *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. The plants used in their study included *Acacia nilotica*, *Argemone mexicana*, *Citrullus colocynthis*, *Jatropha curcas*, and *Withania somnifera*.<sup>10</sup> In addition, the curry leaf or *Murraya koenigii* is rich in bioactive alkaloids and flavonoids that are highly potent larvicides.<sup>11</sup> Kannathasan *et al.*<sup>12</sup> conducted a generalization of the larvicidal activity of the extracts of leaves of *Vitex negundo* on the various species of mosquitoes. Khan and Qamar<sup>13</sup> state that the essential oils of *Cymbopogon citratus*, *Mentha piperita*, and *Ocimum gratissimum* possess high activity against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* larvae. Making them effective and ecologically friendly in the fight against vector-borne diseases, plant extracts have the potential to replace synthetic pesticides, and evidence is accruing on the efficacy of plant extracts as a larvicidal agent. This study aimed to provide mosquito control in an environmentally friendly manner using extracts of *G. celosioides*.

*G. celosioides* is a perennial herbaceous species first found in South America (Family: Amaranthaceae), but it is now prevalent in Asia and the extensive areas of Africa.<sup>14</sup> It treats some diseases, such as respiratory infections, skin infections, sexually transmitted infections (STIs), asthma, kidney dysfunctions, and colds.<sup>15,16</sup> *G. celosioides* contains alkaloids, flavonoids, tannins, saponins, amino acids, terpenoids, steroids, and glycoside compounds.<sup>17</sup> Phytochemical components of *G. celosioides* exhibit well-known antibacterial activity, immunomodulatory and antioxidant properties, and gastroprotective effects.<sup>18-20</sup> The present work shows the effectiveness of crude extracts of *G. celosioides* in methanol, chloroform, ethyl acetate, and hexane on larvicidal action against *An. stephensi*, *Ae. aegypti*, and *Cx.*

*quinquefasciatus* larvae. Extracts were also screened against the non-target organism *Gambusia affinis* to allow for a more environmentally sustainable lab evaluation.

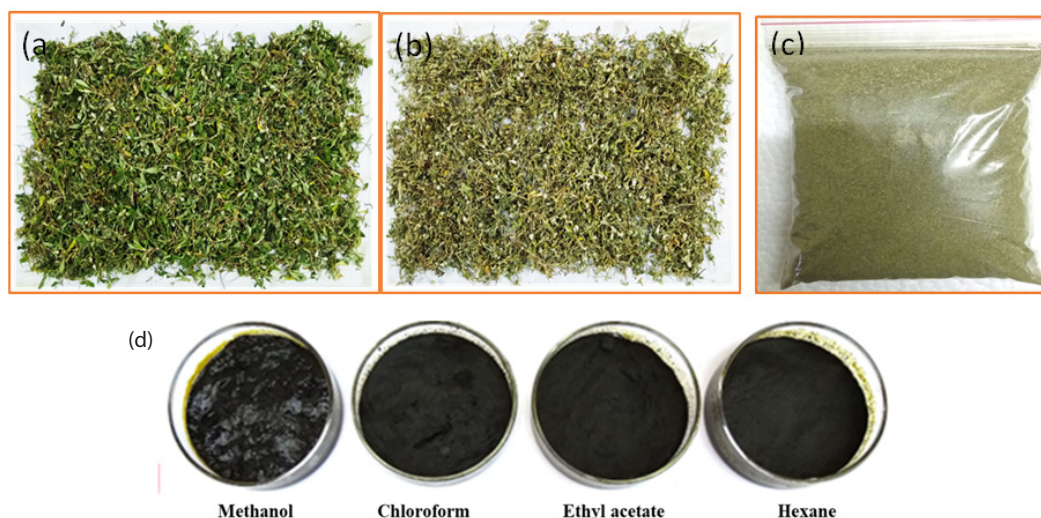
## MATERIALS AND METHODS

### Target Organism

Eggs of *Ae. stephensi*, *Ae. Aegypti* and *Cx. quinquefasciatus* species were collected from the Vector Control Research Centre, Pondicherry, and thereafter reared at the Department of Animal Health Management at Alagappa University in Tamil Nadu, India. The fed powder mixture was a combination of dog biscuits and yeast pills (3:1 ratio).<sup>21</sup> Adult female mosquitoes were fed a blood meal via a membrane-feeder, and adult male mosquitoes were fed honey and a 5.0% glucose solution. The mosquitoes were maintained under a temperature of  $28 \pm 2^\circ\text{C}$ , a relative humidity of 70 to 85% and 12 hours of light/dark cycle.

### Plant Materials and Extraction

The plant leaves (*G. celosioides*) were collected in and around the Thanjavur District in Tamil Nadu. The herbarium specimen voucher (BDUGC-201) was laid for reference, and the plant materials were authenticated. We rinsed off dirt or debris with running water, then removed the leaves from the plant and allowed them to air dry for two weeks in the shade. After, they were pulverized in an electric blender to obtain the fine powder (Figure 1a-d). The Soxhlet apparatus was used to extract the plant powder. The solvents used for extracting the plant material include methanol, chloroform, ethyl acetate, and hexane. After 8 hours of extraction, samples were filtered through Whatman No.1 filter paper. Filtrates were then placed in a rotary vacuum evaporator. As before, the crude pulled from the evaporator is air-dried to remove any residual solvent. After that, those were stored in a vial for future studies.



**Figure 1:** Processing of *Gomphrena celosioides* plant. (a) Fresh leaves, (b) Shade dried leaves, (c) Powder of the dried leaves, and (d) Crude extracts

## Larvicidal Bioassay

The larvicidal activity was tested using the method described by the World Health Organization.<sup>22</sup> Different amounts of various extracts were tested on the mosquito larvae, including hexane (15—75 µg/mL), ethyl acetate (12—60 µg/mL), chloroform (10—50 µg/mL), and methanol (6—30 µg/mL). About 25-instar *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* larvae were introduced individually. They set up five individual experiments per concentration and five control groups that did not implement any concentration and instead used tap water. The experiment was conducted at 27 ± 3°C in a controlled laboratory environment.

## Non-target Biotoxicity Assay

The methods of Sivagnaname and Kalyanasundaram<sup>23</sup> were employed to assess the biotoxic effects for non-target species. The bioassay also demonstrated the biotoxicity of *G. celosioides* plant extracts such as methanol (3000—15000 µg/mL), chloroform (4000—20000 µg/mL), ethyl acetate (5000—25000 µg/mL) and hexane (6000—30000 µg/mL) to aquatic non-target *G. affinis* species with different concentrations. A cement tank (85 × 30 cm) was prepared in the laboratory to keep specimens of this species collected from their natural habitat. Water at 27 ± 3°C and 85% relative humidity filled the tank. Concentrations of *G. celosioides* extract were fifty times the LC<sub>50</sub> mosquito larva dosages. Thus, there were a total of ten replicates of each concentration, plus four control samples that have been treated as controls. After 48 hours of treatment, we assessed the non-target species for inactivity,

swimming impairment, and mortality.

## Statistical Data Analysis

The collected data were processed and analyzed using SPSS software (version 26). LC<sub>50</sub> and LC<sub>90</sub> values were determined by probit statistical analysis of mortality data.<sup>24</sup> To assess biotoxicity to non-target organisms, the method applied for calculating the Suitability Index (SI) for non-target organisms is articulated following the method described by Deo *et al.*<sup>25</sup> The degree of significance was assessed at p < 0.05.

# RESULTS

## Larvicidal Activity

The mosquito larvicidal efficacy of the methanol crude extract of *G. celosioides* against three important mosquitoes, viz., *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* with the LC<sub>50</sub> values were 12.53, 13.47, and 14.31 µg/mL, respectively, indicating the highest degree of toxicity. The results indicated the larval toxicities of chloroform, ethyl acetate, and hexane extracts were moderate, and the value of the lowest was in hexane extract (LC<sub>50</sub> = 31.59, 34.75, and 37.97 µg/mL; Table 1).

## Biotoxicity to Non-target Species

Herein, the impact of *G. celosioides* leaf extracts on non-target aquatic mosquito predator *G. affinis* was investigated. The biotoxicity effects of *G. celosioides* solvent crude extracts against *G. affinis* are presented in Table 2, after 48 hours of exposure using LC<sub>50</sub> values of 7013.24 µg/mL for methanol,

**Table 1:** Effect of methanol, chloroform, ethyl acetate, and hexane extract of *Gomphrena celosioides* against the larvae of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*.

Vector species	LC <sub>50</sub> (µg/mL) (LCL-UCL)	LC <sub>90</sub> (µg/mL) (LCL-UCL)	Regression equation	χ <sup>2</sup>
Solvent: Methanol				
<i>An. stephensi</i>	12.53 (10.89 - 13.63)	24.77 (22.94 - 27.19)	y=12.08+3.000x	5.063
<i>Ae. aegypti</i>	13.47 (12.11 - 14.70)	25.67 (23.84 - 28.08)	Y=6.160+3.160x	4.776
<i>Cx. quinquefasciatus</i>	14.31 (12.95 - 15.56)	26.97 (25.04 - 29.53)	y=3.36+3.173x	3.663
Solvent: Chloroform				
<i>An. stephensi</i>	21.12 (18.8 - 23.19)	41.2 (38.22 - 45.11)	y=9.81+1.851x	6.012
<i>Ae. aegypti</i>	22.47 (20.06 - 24.63)	44.09 (40.84 - 48.41)	y=7.76+1.832x	3.134
<i>Cx. quinquefasciatus</i>	24.13 (21.82 - 26.26)	45.78 (42.47 - 50.15)	y=3.36+1.888x	0.875
Solvent: Ethyl acetate				
<i>An. stephensi</i>	26.44(23.73 - 28.88)	50.43 (46.85 - 55.13)	y=6.96+1.580x	4.785
<i>Ae. aegypti</i>	27.87 (25.12 - 30.38)	53.08 (49.27 - 58.12)	y=4.96+1.573x	3.711
<i>Cx. quinquefasciatus</i>	29.71 (26.84 - 32.36)	57.09 (52.83 - 62.79)	y=3.52+1.533x	1.159
Solvent: Hexane				
<i>An. stephensi</i>	31.59 (28.2 - 34.62)	60.76 (56.43 - 66.43)	y=9.30+1.252x	6.256
<i>Ae. aegypti</i>	34.75 (31.43 - 37.79)	64.95 (60.4 - 70.91)	y=3.84+1.291x	2.877
<i>Cx. quinquefasciatus</i>	37.97 (34.66 - 41.07)	69.66 (64.77 - 76.1)	y=0.48+1.301x	0.963



**Table 2:** Biotoxicity of hexane, ethyl acetate, chloroform, and methanol extracts of *Gomphrena celosioides* against non-target organism *Gambusia affinis*.

Test materials	LC <sub>50</sub> (µg/mL) (LCL-UCL)	LC <sub>90</sub> (µg/mL) (LCL-UCL)	Regression equation	χ <sup>2</sup>
Hexane	13335.24 (11945.1—14587.21)	25708.82 (23866.6 - 28128.35)	y=7.04 + 0.003x	1.273
Ethyl acetate	10957.52 (9692.59—12075.32)	22114.46 (20454.53 - 24326.2)	y=9.92 + 0.003x	0.867
Chloroform	8873.20 (7889.58—9749.93)	17640.35 (16329.51 - 19382.21)	y=8.77 + 0.004x	1.296
Methanol	7013.24 (6320.55—7644.64)	13361.51 (12407.35 - 14616.40)	y=4.47 + 0.006x	0.198

8873.20 µg/mL for chloroform, 10957.52 µg/mL for ethyl acetate, and 13335.24 µg/mL for hexane. According to the suitability Index (SI) (Table 3), extracts of *G. celosioides* were much less toxic than the aquatic non-specific species and the populations of mosquito larvae studied.

## DISCUSSION

The larvicidal efficacy of plant extracts against mosquitoes has attracted significant attention as an environmentally sustainable approach to mosquito management. Various plant-derived bioactive compounds, including alkaloids, flavonoids, tannins, and saponins, exhibit considerable toxicity toward mosquito larvae by disrupting their physiological and biochemical processes. Plant extracts operate through multiple mechanisms, such as inhibiting larval molting, damaging the midgut epithelium, and impairing essential enzyme activities for larval survival. Unlike synthetic insecticides, plant-derived larvicides offer a safer alternative with minimal environmental impact and a reduced risk of resistance development. Further research into optimizing extraction methods and formulation strategies may enhance their efficacy, rendering them a valuable component of integrated mosquito control programs.

Asmaey *et al.*<sup>26</sup> corroborated our research by indicating that methanol extracts of the medicinal herbs *Melissa officinalis* and *Rosmarinus officinalis* had significant larvicidal efficacy against third-instar *Cx. pipiens* larvae, with LC<sub>50</sub> values of 26.505 µg/mL and 9.795 µg/mL, respectively. An aqueous extract of *Solanum torvum* plants served as a natural biocontrol agent for mosquito control.<sup>27</sup> Nasir *et al.*<sup>28</sup> focus on a biopesticide of plant origin for mosquito control, evaluating the larvicidal capability of an acetone extract of *Allium sativum* (garlic) against *Ae. aegypti*. However, studies have previously found botanical extracts to be useful for vector control, with their evaluation affirming these findings. The *A. bouvieri*, *D. indicus*, and *G. affinis* efficacy LC<sub>50</sub> values for the non-target organisms were extremely low (415.61, 633.51, and 1056.04 µg/mL, respectively) when Ag nanoparticles synthesized from the *Merremia emarginata* plant were tested in a study done previously.<sup>29</sup> Mahaheswaran *et al.*<sup>30</sup> showed in their study that the *Couroupita guianensis* plant was proven not harmful to the non-target fish *Cyprinus carpio*. According to Researchers Vasantha-Srinivasan *et al.*<sup>31</sup> the methanolic extract of the *Swietenia mahagoni* plant had only a slight effect on a non-target mosquito, *Toxorhynchites splendens*. In addition, when Ag nanoparticles were synthesized using

**Table 3:** Suitability index of non-target organism *Gambusia affinis* over young instars of mosquitoes exposed to *Gomphrena celosioides* crude extracts.

Treatment	<i>An. stephensi</i>	<i>Ae. aegypti</i>	<i>Cx. quinquefasciatus</i>
Hexane crude extract	422.13	383.74	351.20
Ethyl acetate crude extract	414.42	393.16	368.81
Chloroform crude extract	420.13	394.89	367.72
Methanol crude extract	559.71	523.76	490.09

*Pimenta dioica*, they did not toxicize this non-target species, *Mesocyclops thermocyclopoides*.<sup>32</sup>

## CONCLUSION

The problem of insecticide-resistant mosquito populations has reached critical levels, making it more urgent than ever to identify safer and more sustainable solutions. In contrast, plant phytochemicals are broad-spectrum, non-target species friendly, and biodegradable. Therefore, they are promising candidates for natural control agents. In addition, this study demonstrated that *G. celosioides* leaf extracts efficiently killed *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* larvae, indicating that this plant could be utilized as a sustainable biocontrol agent in the future. These natural pesticides are an excellent alternative to synthetic ones because they are toxic to mosquito larvae while not harmful to other non-target organisms. Therefore, to enhance sustainable vector control and reduce ecologic hazards, integrated mosquito management programs must incorporate insecticides that target plant larvae. Further exploration into the formulation and application of *G. celosioides* extracts may improve its efficacy in mosquito control.

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## PEER-REVIEWED CERTIFICATION

During the review of this manuscript, a double-blind peer-review policy has been followed. The author(s) of this manuscript received review comments from a minimum of two peer-reviewers. Author(s) submitted revised manuscript as per the comments of the assigned reviewers. On the basis of revision(s) done by the author(s) and compliance to the Reviewers' comments on the manuscript, Editor(s) has approved the revised manuscript for final publication.