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A novel plant-based larvicide against *Aedes aegypti*: Phytochemical profiling, bioefficacy, and molecular docking analysis

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Abstract
The necessity for environmentally safe choices for synthetic larvicides rises day by day due to severe global mosquito-borne ailments. In this concern, the larvicidal activity was conducted with the ethanolic leaf extract of *Cleome gynandra* against *Aedes aegypti* by bioassay and molecular mechanistic approaches. The phytochemicals present in the *Cleome gynandra* were identified by GC-MS techniques, and palmitic acid and linolelaidic acid were identified as the major and more bioactive constituents among all. Larvicidal bioanalysis explored a clear dose-dependent mortality with significant larval death, and the LC_{50} value was found to be 15.39 µg/mL indicates effective larval inhibition even at low doses. To validate this bioassay, a molecular docking study was performed with 4EY7 protein, and the interactions of palmitic acid and linolelaidic acid with binding energies of -7.0 kcal/mol and -7.8 kcal/mol corroborate this effective larval control. Conclusively, all the findings showed that the phytoconstituents present in the ethanolic leaf extract of *Cleome gynandra* act as an effective, eco-friendly, and sustainable mosquito vector control agent.

Keywords: Mosquito larvicidal activity, *Aedes aegypti*, GC–MS analysis, molecular docking, eco-friendly vector control

Introduction

Syndromes such as dengue, Zika, chikungunya, and yellow fever have shown a steady rise in incidence over recent decades, resulting in increased morbidity, mortality, and economic burden. Due to its close association with human habitats, *Aedes aegypti* plays a pivotal role in disease transmission, rapid life cycle, and high adaptability among the mosquito vectors [1]. To suppress adult mosquito populations and reduce disease spread, targeting mosquito larvae at the aquatic developmental stage is widely recognized as a successful approach. Chemical larvicides and insecticides have been extensively used for mosquito control; however, their unremitting application has led to the appearance of ecological imbalance, insecticide resistance, and adverse effects on non-target organisms. Moreover, emergent environmental concerns and regulatory restrictions have limited the use of synthetic chemicals. These challenges have exaggerated the search for eco-friendly, novel, and sustainable larvicidal agents that can offer effective mosquito control without compromising environmental safety [2].

In recent years, plant-based larvicides have engrossed increasing attention owing to their structural diversity, biodegradability, and wide spectrum of biological activities. Medicinal plants are rich sources of secondary metabolites such as terpenoids, phenolics, fatty acids, and antioxidants, many of which have been reported to exhibit insecticidal and larvicidal properties [3]. Despite extensive research on various plant species, a large number of medicinal plants remain unexplored for mosquito larvicidal relevance, highlighting the importance of identifying new botanical resources [4].

In this context, this study exposed the biochemically rich ethanolic leaf extract of *Cleome gynandra* as the medicinal plant for *Aedes aegypti* survival control [5]. To the best of our knowledge, this plant extract has not been previously investigated for larvicidal activity, making this work a novel contribution to the field of botanical mosquito control. Establishing such first-time evaluations is crucial for expanding the repertoire of plant-based larvicides and identifying new bioactive candidates [6]. This research provides a comprehensive assessment of a previously unexplored botanical resource by integrating GC–MS phytochemical profiling, larvicidal bioassays, and molecular docking studies. The findings highlight the potential of the plant extract as an eco-friendly and sustainable larvicidal agent, contributing valuable knowledge toward the expansion of alternative mosquito vector control strategies [7].

Materials and Methods

Cleome gynandra leaves were collected from Thanjavur, Tamil Nadu, India, and the third instar larvae of *Aedes aegypti* were collected from the stagnant water sources in the local background of Thanjavur. Ethanol and Whatmann No.1 filter paper was purchased from Technoscientific, Thanjavur. A chromatogram of the ethanolic leaf extract of *Cleome gynandra* was obtained using Gas Chromatography Mass Spectrometry (GC-MS).

Preparation of ethanolic leaf extract

Fresh *Cleome gynandra* leaves were collected from the Thanjavur district, and the collected leaves were washed with running tap water followed by distilled water to remove all the surface impurities. Subsequently, the washed leaves were dried for 10 days at room temperature and the completely dried leaves were ground into a fine powder. Further, 10 g of dried *Clome gynandra* leaf powder was mixed with 100 mL of ethanol, and the mixture was kept in a magnetic stirrer for 24 hours. After ensuring the maximum extraction of bioactive compounds, the extract was filtered through Whatmann No.1 filter paper to remove the unwanted plant residue. At last, a rotary evaporator was utilized to concentrate the extract under reduced pressure at 45°C and stored at 4°C for further use [8].

Larvicidal Bioassay

According to WHO guidelines with slight modifications, the larvicidal activity was performed with the stock solution of *Cleome gynandra* ethanolic leaf extract. To prepare 1000 ppm of stock solution, a small volume of ethanol was mixed with the crude extract and diluted with distilled water. Subsequently, 10, 20, 30, 40, and 50 µg/mL concentrations of test solutions were prepared to conduct the larvicidal bioassay [9]. After that, 10 healthy late third instar larvae were taken in plastic cups containing 100 ml of each test solution, and to ensure reproducibility, triplicates were conducted for each concentration. A control group was also conducted simultaneously without ethanolic leaf extract. The larval mortality was assessed after 24 hours of exposure, and no movement was considered as dead when gently probed with a glass rod [10]. The rate of mortality was calculated using the following formula (1)

$$\text{Rate of mortality (\%)} = \frac{\text{number of dead larvae}}{\text{number of total larvae}} \text{ X } 100 \text{ ------- (1)}$$

Statistical analysis

From triplicate experiments, the mortality percentage was determined for each concentration, and the outcomes were expressed as mean ± standard deviation. From the dose-mortality relationship, the LC_{50} value was determined at the mortality rate of 50% with statistical significance considered at $p<0.05$.

Results and Discussion

Larvicidal bioassay

Figure 1 reveals the potential inhibition of larval survival using *Cleome gynandra* ethanolic leaf extract at various concentrations. The mortality rate of the larvae progressively increases with increasing the concentration of the leaf extract, revealing a clear dose-dependent response [11].

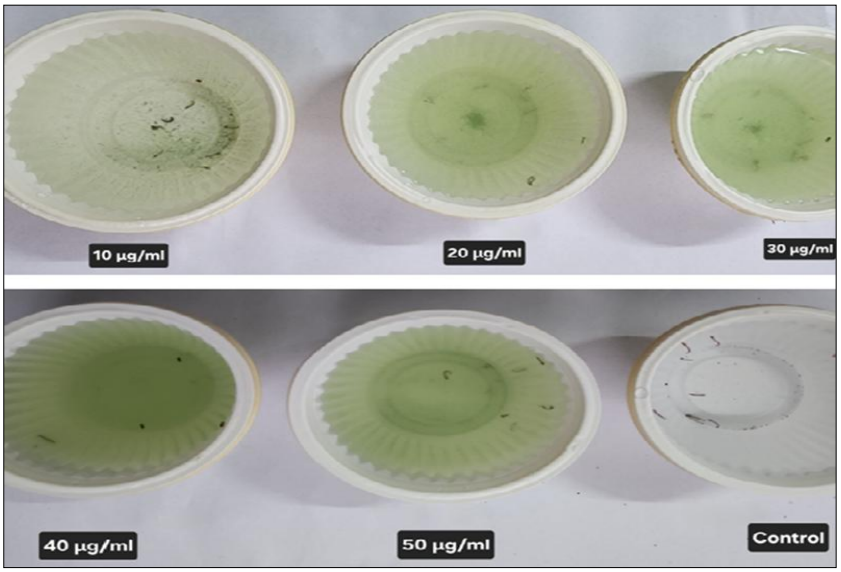

Fig 1: Larvicidal bioassay for the different concentrations of the ethanolic leaf extract of *Cleome gynandra*

The effective toxic capacity of the leaf extract was observed at the lower concentration of 10 µg/mL indicating the strong toxic nature of the leaf extract against the mosquito larvae. Moreover, Figure 2 shows the mortality rate of 40.13% at 10 µg/mL, while the optimum death rate of 93.97% at 50 µg/mL was observed indicating the efficient interaction and penetration of the phytoconstituents present in the leaf extract into the biological system of the larvae. The LC_{50} value of the larval bioassay was determined to be 15.39 µg/mL showing that half of the larval population was effectively suppressed at a relatively low dose. These outcomes highlight the biomolecules present in the *Cleome gynandra* leaf extract as a suitable and eco-friendly candidate for mosquito vector control [12].

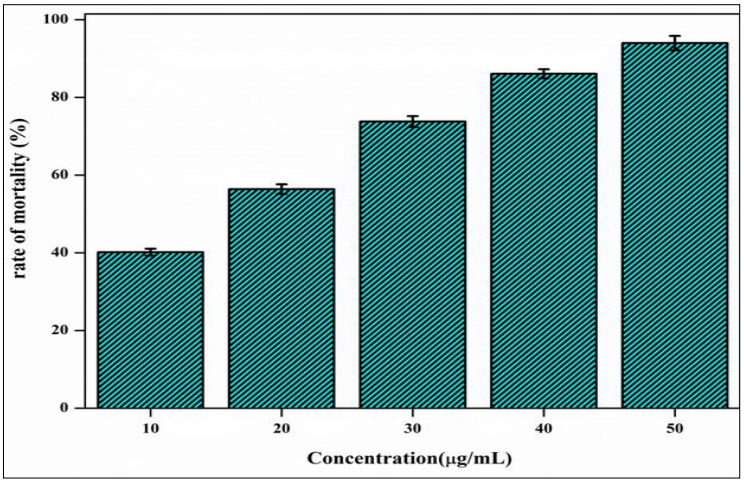

Fig 2: rate of mortality of the different concentrations of the ethanolic leaf extract of *Cleome gynandra*

GC–MS Analysis

The GC-MS analysis of the *Cleome gynandra* ethanolic leaf extract exposed 16 bioactive phytochemicals representing hydrocarbons, esters, fatty acids, antioxidant compounds, and alcohols, which are known to contribute insecticidal, larval, and other biological effects, as shown in Figure 3 and Table 1 [13]. The major constituent of the leaf extract was palmitic acid, accounting for 48.04% of abundance, which was widely reported for larvicidal activity and many biological activities, and the second most abundant one was linolelaidic acid with 18.11%, which was accountable for the membrane-disrupting activity of larval mortality [14].

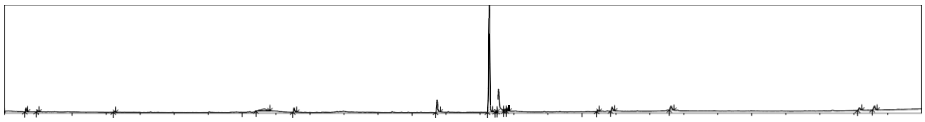

Fig 3: GC-MS chromatogram for the ethanolic leaf extract of *Cleome gynandra*

The other biologically significant constituents that are responsible for insecticidal and repellent effects were present at minor levels and included diethyl phthalate (6.65%), tridecane (5.64%), glycerol 1-palmitate (3.68%), and 1-heneicosanol (3.29%). Moreover, the presence of biomolecules such as fatty alcohols and ester derivatives further supports the larvicidal activity by the penetration through the larval cuticle [15].

Table 1: Phyto chemicals present in the ethanolic leaf extract of *Cleome gynandra*

Peak#	R. Time	Area%	M.W	Name
1	3.642	1.79	170	Mesitylene
2	3.974	0.75	120	Dodecane
3	6.236	0.44	120	1,2,4-trimethyl-Benzene
4	10.648	5.64	184	Tridecane
5	11.529	2.41	132	Butoxyacetic acid
6	15.738	6.65	222	Diethyl Phthalate
7	17.276	48.04	256	n-Hexadecanoic acid
8	17.475	0.7	296	Phytol
9	17.547	18.11	280	Linoelaidic acid
10	17.745	1.64	264	(Z, Z, Z)-9,12,15-Octadecatrien-1-ol
11	17.804	1.6	312	Eicosanoic acid
12	20.466	0.7	306	Eicosatrienoic acid
13	20.89	2.67	313	Fumaric acid, 2-dimethylaminoethyl nonyl ester
14	22.621	3.68	330	Glycerol 1-palmitate
15	28.164	1.9	366	(9Z,12Z,15Z)-1-Hydroxy-3-methoxypropan-2-yl octadeca-9,12,15-trienoate
16	28.611	3.29	312	1-Heneicosanol
		100	430	Vitamin E

Molecular Docking Analysis of Bioactive Compounds with 4EY7 Protein

Based on the GC-MS analysis, palmitic acid and linolelaidic acid were selected for molecular docking analysis due to the combination of abundance, structural diversity, and biological relevance [16]. This molecular docking study is critical for understanding the larvicidal mechanism at the molecular level. The mosquito larval protein (PDB code: 4EY7) was selected for conducting this theoretical investigation with the above-mentioned biomolecules [17].

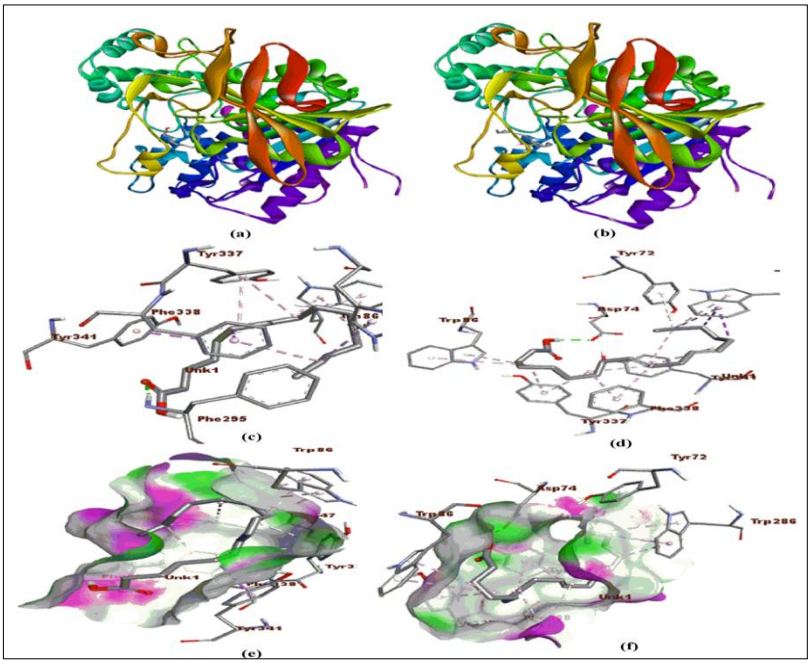

Fig 4: Three dimentional Molecular docking maps for 4EY7 with palmitic acid and linolelaidic acid

Figures 4a and 4b indicate the docking poses of the target protein with palmitic acid and linolelaidic acid, respectively. Subsequently, Figure 4c exposes the key amino acid residues such as Phe295, Tyr337, Tyr337, Tyr341, Phe338, and Trp81 forming hydrogen bonds toward the polar region of the binding pocket and hydrophobic interactions with the binding energy of -7.0 kcal/mol [18]. Likewise, Figure 4d shows the protein-linolelaidic acid complex adopts the flexible conformation within the binding site, with key residues such as Asp74, Asp74, Tyr124, Tyr337, Tyr341, and Phe338 further supporting the suppressed activity of mosquito survival with a binding energy of -7.8 kcal/mol. The aforementioned interactions were clearly shown in the hydrogen bond interaction of Figure 4e for palmitic acid and 4f for linolelaidic acid [19]. Furthermore, the two-dimensional interaction map exposes the detailed insights into the binding behavior of palmitic acid and linolelaidic acid within the binding pocket of the larvicidal receptor 4EY7, as shown in figures 5a and 5b, respectively [20].

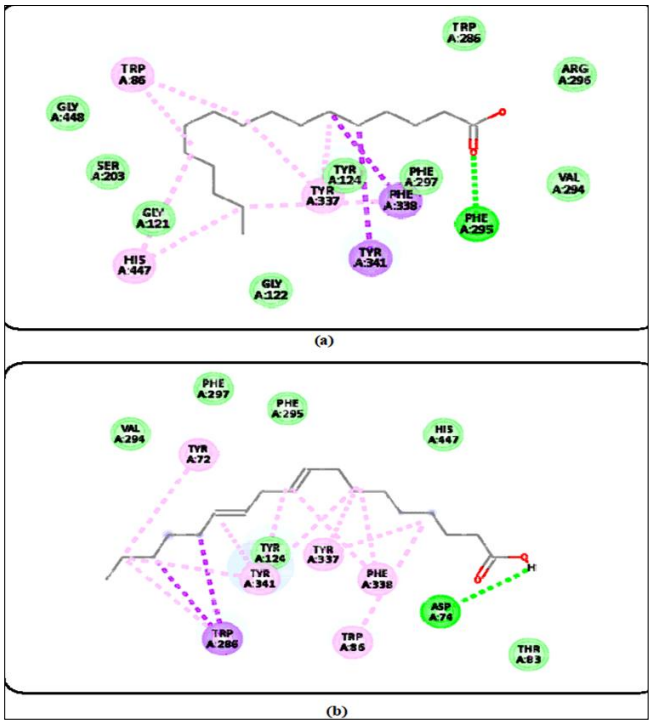

Fig 5: Two dimentional Molecular docking maps for 4EY7 with (a) palmitic acid and b) linolelaidic acid

Overall, the 3D and 2D interaction profiles of the palmitic acid and linolelaidic acid suggest a dominant role in mosquito larvicidal toxicity. Hence, the in-silico findings provide the molecular-level evidence for the larvicidal potential of the biomolecules and validate the *in vitro* study [21]. Hence, the molecular docking results are consistent with larvicidal observations and endow strong mechanistic support for the biological activity of the palmitic acid and linolelaidic acid compounds. The findings highlight linolelaidic acid as a key contributor to larvicidal activity, and this study underscores the potential of plant-derived bioactive compounds as eco-friendly and sustainable alternatives to synthetic mosquito control agents [22].

Conclusion

This study demonstrated the larvicidal potential of the ethanolic leaf extract of *Cleome gynandra* against *Aedes aegypti* by an integrated experimental and computational approach. GC-MS analysis provided 16 bioactive molecules with palmitic acid and linolelaidic acid as the major compounds that may contribute to larvicidal activity. Larvicidal examinations showed a clear dose-dependent increase in larval mortality with an LC_{50} value of 15.39 µg/mL, revealing potent larval suppression activity at lower concentrations. Moreover, the molecular docking study was conducted with the high abundance biomolecules, such as palmitic and linolelaidic acids, with mosquito target protein 4EY7, suggesting a dominant role in larvicidal action and other phytochemicals may contribute synergetically. Conclusively, the findings underscore the larvicidal potential of the ethanolic leaf extract of *Cleome gynandra* as a sustainable, eco-friendly, and effective larvicidal representative.

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