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Bio-larvicidal potential of leaf extracts of selected plant species against dengue vector, *Aedes aegypti* L.

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Abstract
The major vector for dengue disease, zika, chikungunya, and urban yellow fever is believed to be *Aedes aegypti*. Concerns about the environment hazards and pesticide resistance to conventional pesticides, plant extracts are getting more attention as natural insecticidal remedies for getting rid of mosquito vectors because of their rich quantity of bioactive components. The current study set out to determine the effectiveness of acetone extracts of leaves from four affordable and easily available native plant species: *Acacia nilotica* (Mimosaceae), *Eucalyptus rudis* (Myrtaceae), *Bignonia capreolata* (Bignoniaceae), and *Jasminum arborescens* (Oleaceae) as larvicides against *Aedes aegypti* mosquitoes. Three concentrations (1%, 1.5%, and 2%) of each plant extracts in acetone were applied to *Aedes aegypti* larvae in the fourth instar. Larval mortality was then measured after 24, 48, and 72 hours of treatment. With an increase in dosage concentration and treatment duration, the extracts demonstrate increase in larvae mortalty. Following 24 hours of treatment, the highest mortality rate (100%) was observed in 2% of *Jasminum arborescens*. This was followed by 93.35% and 85% mortality in 2% doses of *Eucalyptus rudis* and *Bignonia capreolata*, after 72 hours of treatment. Even after 72 hours of treatment, none of the extracts demonstrated significant mortality at 1% dose. To better understand the potency of these plant extracts, their LC_{50} and LC_{90} values were determined after 24, 48, and 72 h post-exposure. Among the tested extracts, *Acacia nilotica* exhibited the highest LC_{50} and LC_{90} values (4.89, 2.57, 1.94 and 24.54, 7.24, 5.37), suggesting lower toxicity, while *Eucalyptus rudis* showed the lowest LC_{50} values and LC_{90} (1.38, 1.20, 1.07 and 2.34. 2.17, 1.70), indicating high toxicity to *Aedes aegypti* mosquito larvae.

Keywords: *Aedes aegypti*, larvicidal activity, *Acacia nilotica*, *Eucalyptus rudis*, *Bignonia capreolata*, and *Jasminum arborescens*, LC^{50}, LC^{90}

Introduction

For infections like dengue fever, zika, chikungunya, and urban yellow fever, *Aedes aegypti* is considered to be the primary vector (Adams *et. al*., 2017) [2]. Although it originated in Africa, it is now widely distributed and found in tropical and subtropical areas worldwide. Due of its domestication and strong inclination for biting humans, *Aedes aegypti* is a particularly significant vector, acting as the main spreader and carrier of the infectious dengue virus (Brown *et. al*., 2011) [10]. Over 7.6 million dengue cases, including 3.4 million confirmed cases, over 16,000 severe cases, and over 3000 deaths, had been recorded to the WHO as of April 30, 2024 [33].

The conventional approach to vector control involves the application of environmentally hazardous chemical insecticides, such as pyrethroid and organophosphate pesticides (Marino, 2010) [20]. The emergence of novel mosquito species that are resistant to these widely used pesticides as a result of increased dosage and frequency (Simus *et. al*., 2004) [31]. The need for more environmentally acceptable alternatives to chemical pesticides is growing because to worries about the environment and pesticide resistance. With their abundance of bioactive components such as flavonoids, phenols, terpenoids, and alkaloids, plant extracts are receiving more attention as natural insecticidal solutions for eliminating mosquito vectors (Ahmed *et. al*, 2021; Iqbal *et. al*., 2021; Gosh *et. al*., 2012; Muhammed *et. al*., 2022) [4, 18, 16, 23]. According to Pavela, R. *et al*.'s assessment in 2019, 429 plant species from 101 botanical families exhibit exceptional larvicidal activity (LC_{50} values < 10 ppm) against key vectors, including those from the genera, *Anopheles*, *Aedes*, and *Culex*. [26] The larvicidal ability of 150 plant species from 52 families against *Aedes aegypti* and *Aedes albopictus* was reviewed by Rodrigues *et. al*., 2020 [28]. Wherein the development of commercial botanical larvicides in the form of extracts with the best LC_{50} values against mosquitoes appeared potential for the two families, Piperaceae and Annonaceae.

A number of research investigations offer insightful information on plant extracts' potential as safe, efficient insecticides against *Aedes aegypti* (Ahmed *et. al*., 2023; Ajaegbu *et. al*., 2023; Alvarez-Valverde *et. al*., 2023) [3,5,6]. In order to investigate the potential of additional plant extracts, the current study aimed to investigate the potency of acetone extracts of leaves from four affordable and easily accessible native plant species: *Acacia nilotica* (Mimosaceae), *Eucalyptus rudis* (Myrtaceae), *Bignonia capreolata* (Bignoniaceae), and *Jasminum arborescens* (Oleaceae) as larvicides against *A. aegypti* mosquitoes. The results of this study will help design sustainable ways of controlling *Aedes aegypti* mosquitoes.

Material and methods

The mosquitoes (*Aedes aegypti*) were raised in a laboratory using cages of 30 x 30 x 30 cm, with a temperature of 26ºC ± 2ºC, a relative humidity of 70±5%, and a 12-hour light: dark cycle, in accordance with the protocol that Saxena and Yadav, 1983 used [29]. Leaves of test plant species were gathered in and around the district of Jaipur. The authenticity of the plant species was verified by a plant taxonomist, Department of botany, University of Rajasthan. Following a thorough washing under running water, the plant leaves were allowed to air dry in the shade for seven days at room temperature. The dried leaves were then mechanically powdered using a commercial electrical stainless-steel blender. To acquire a significant amount of active component from the plant leaves, 30 grams of leaf powder of each species were extracted using acetone as a solvent in a Soxhlet apparatus for eight hours. Whatman No. 1 filter paper was used to filter the extracts via a Buchner funnel. The extract was concentrated for three to five hours at room temperature (28 °C) using a rotating evaporator. After the solid product is combined with the appropriate solvent (acetone), a stock 10% (w/v) solution is made and kept at 4 °C until used. Three test concentration 1, 1.5 and 2 % were prepared by adding 1, 1.5 and 2 ml of stock solution in water to make 100 ml test solution in volumetric flask followed by vigorous stirring. Tween-80 was used as emulsifier at a 0.05% conc. in final test solution. For the control solution, the same amount of solvent was added to the water at each concentration. 25 fourth instar larvae of *A. aegypti*, all the same age, were released into a 500 ml glass beaker filled with 100 ml of test solution. For every conc. of each plant extract, three replicates and one control test were conducted. Using a small plastic dropper, the dead and living larvae were sorted during the data collection process. The number of dead and alive larvae after 24, 48, and 72 hours was carefully counted to assess the toxicity effect of the plant extract. The % mortality data was corrected using the following Abbott's formula, 1987 [1].

% corrected mortality = [PO – PC] / [100 – PC] × 100
Whereas PO = larval mortality in treated solution
PC = larval mortality in control solution

Statistical analysis: The LC_{50} and LC_{90} values of these plant extracts were determined at 24, 48, and 72 hours after exposure in order to gain a better understanding of their efficacy. A popular metric in toxicology, the LC_{50} and LC_{90} values show the concentration at which 50% and 90% of the test organisms are killed, respectively. Using the MS Excel software, the mortality data were examined by doing two-way analysis of variance (ANOVA) (Post Hoc/Turkey's HSD test) and probit analyses to determine lethal concentration (LC_{50} & LC_{90}) values. A significant threshold of $P < 0.05$ was used. (Finney, 1971)[15].

Results

Mortality data of different plant extracts against *A. aegypti* larvae is presented in Table no.1 and Figures no.1-4. At 1% dose concentration of various treatment after 24, 48 and 72 hrs of exposure, the % corrected mortality order is *Eucalyptus* (18.35, 30, 38,77%), followed by *Jasminum* (10.35, 11.22, 27.83%), *Bignonia* (10.65, 10.65, 28.57%) and *Acacia* (10, 10.35, 20%). At 1.5% dose concentration of various treatment after 24, 48 and 72 hrs of exposure, again *Eucalyptus* showed maximum toxicity recording 66.65, 77.19, 87.39 % corrected mortality followed by *Jasminum* (30, 63.65, 83.31), *Bignonia* (28.35, 40, 65.96%), *Acacia* (16.65, 30, 33.67%). At 2% dose concentration, *Jasminum* found to be most effective showing cent percent larval mortality within 24 hrs of exposure. Eucalyptus at 2% conc. was the next best in significant killing larvae with 76.65, 83.01, 93.21 % corrected mortality followed by *Bignonia* (30.35, 77.19 & 84.53) after 24, 48 and 72 hrs of treatment. *Acacia* at 2% conc. exhibited moderate effect with 23.35, 33.67 & 51.90 % corrected mortality respectively. Higher treatment concentrations have been shown to cause restlessness in the larvae, as evidenced by their constant abdomen flickering. They also display a semi-circular bending of the body, become drowsy and eventually perish.

Table 1: Percent corrected mortality of three dose levels (1, 1.5 & 2%) of test plant extracts after 24, 48 & 72 hrs exposure time

Plant Extracts	Percent corrected mortality								
	1% conc		.	1.5% conc.			2% conc.		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Acacia nilotica	10	10.35	20	16.65	30	33.67	23.35	33.67	51.90
Bignonia capreolata	10.65	10.65	28.57	28.35	40	65.96	30.35	77.19	84.53
Jasminum arborescens	10.35	11.22	27.83	30	63.65	83.31	100	-	-
Eucalyptus rudis	18.35	30	38.77	66.65	77.19	87.39	76.65	83.01	93.21

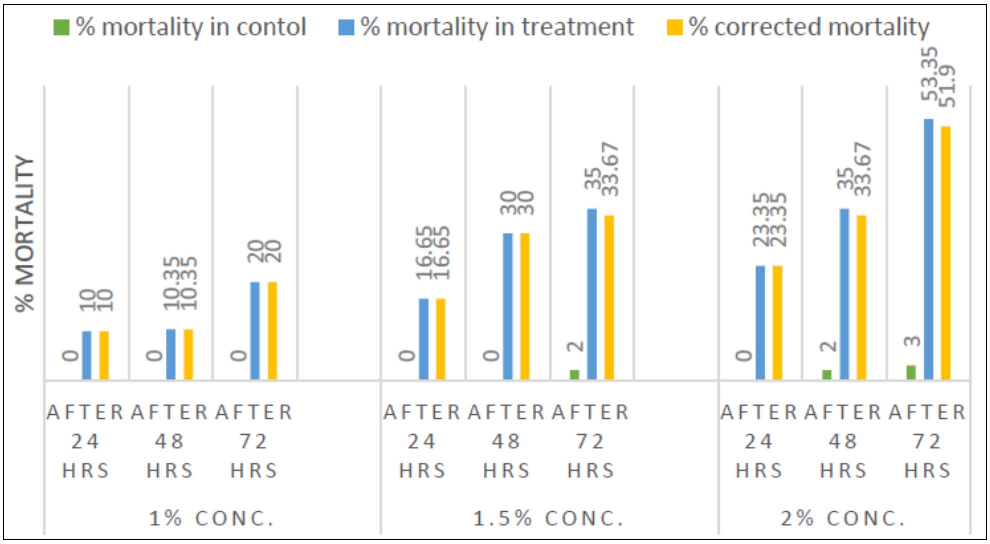

Fig 1: Effect of leaf extract of *Acacia nilotica* against *Aedes aegypti*

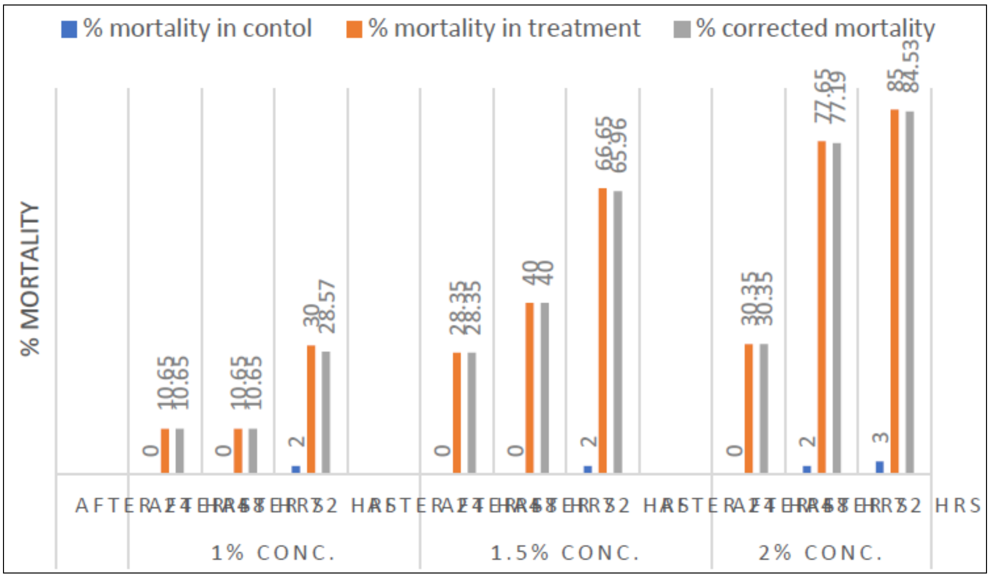

Fig 2: Effect of leaf extract of *Bignonia capreolata* against *Aedes aegypti*

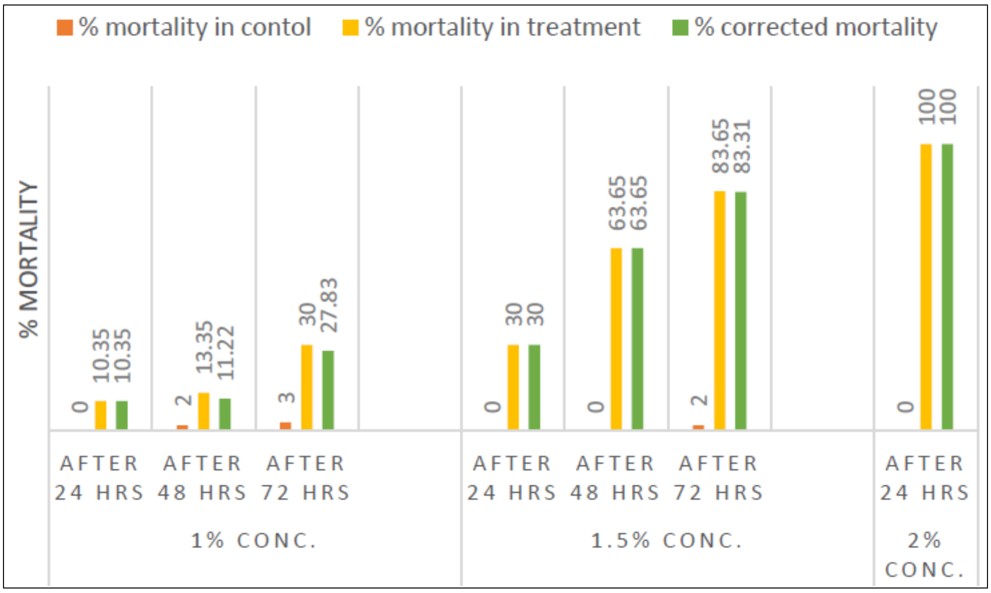

Fig 3: Effect of leaf extract of *Jasminum arborescens* against *Aedes aegypti*

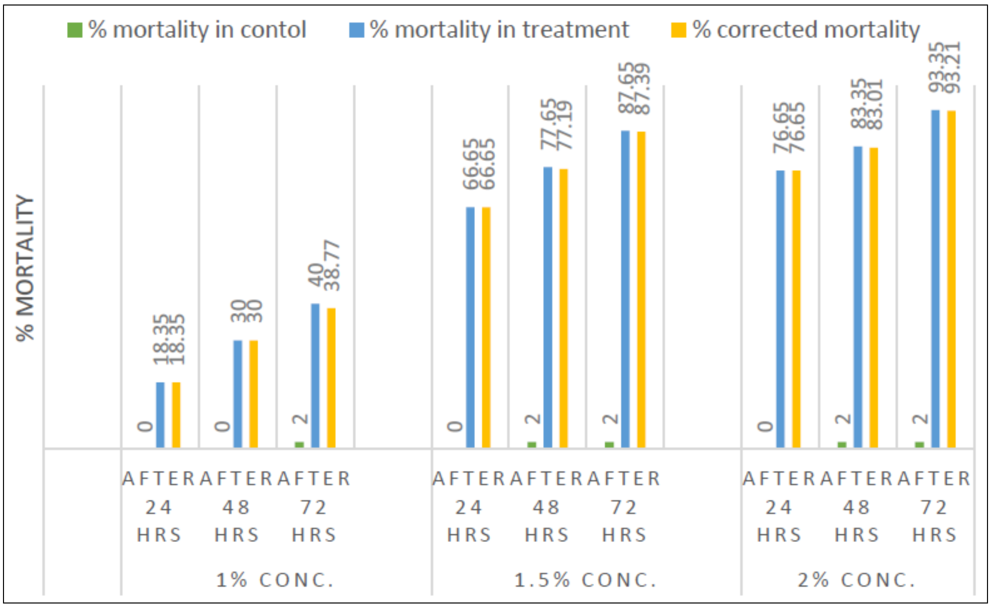

Fig 4: Effect of leaf extract of *Eucalyptus rudis* against *Aedes aegypti*

Two-way ANOVA results of each test plant extract (Table no.2) clearly indicated that mortality in larvae increase with the increase in dose concentration and duration of exposure is statistically significant. After the analysis of results applying Post hoc Turkey's HSD, it is revealed that at 1% conc., all the plant extracts did not show promising larvicide effects. *Jasminum* and *Eucalyptus* was found to be most effective followed by *bignonia*. Acacia showed moderate effects even at higher concentration (2%).

Table 2: Two-way ANOVA test results of different plant extracts according Dose level (I%, 1.5% & 2%) and Treatment duration (24hrs, 48 hrs & 72 hrs)

	Acacia nilotica		***Bignonia capreolata***		***Jasminum arborescens***		***Eucalyptus rudis***	
	p-value	F-value	p-value	F-value	p-value	Fvalue	p-value	F-value
Conc.	0.018	12.65	0.04	7.14	0.005	25.77	2.91E-06	1170.14
Time	0.037	8.28	0.02	12.08	0.24	2.05	0.0002	120.052

F crit-6.944 (F-value > F-crit= significant), (p-value > 0.05= significant)

Table no.3 summarized the LC_{50} and LC_{90} values of three dose levels viz- 1%, 1.5% & 2% conc. of each test plant extract after 24, 48 and 72 hrs of treatment. It clearly showed that *Jasminum* and *Eucalyptus* have the lowest LC_{50} and LC_{90} values (1.38% & 1.78%, 2.34%) followed by Bignonia (4.20% & 15.84%), *Acacia* (4.89% & 24.54%) after 24 hrs of exposure time. In case of after 48 hrs of treatment, the results revealed that lowest LC_{50} and LC_{90} values was found in *Eucalyptus* (1.20% & 2.17%), followed by Jasminum (1.30% & 1.66%), *Bignonia* (1.58% & 3.90%), and *Acacia* (2.57% & 7.24%). Similarly, 72 hrs post exposure results recorded *Eucalyptus* extract with lowest LC_{50} and LC_{90} values (1.07% & 1,70%) followed by *Jasminum* (1.15% &1.55%), *Bignonia* (1.17% & 2.12%), and *Acacia* (1.94% & 5.37%).

Table 3: LC_{50} and LC_{90} values (in %) of plant extracts applying three Dose concentrations (1%. 1.5% & 2%) after 24, 48 and 72 h of exposure time

Plant species	**After 24 hrs**		**After 48 hrs**		**After 72 hrs**	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Acacia nilotica	4.89	24.54	2.57	7.24	1.94	5.37
Bignonia capreolata	4.20	15.84	1.58	3.90	1.17	2.12
Jasminum arborescens	1.38	1.78	1.30	1.66	1.15	1.55
Eucalyptus rudis	1.38	2.34	1.20	2.17	1.07	1.70

Based on the comparative analysis of toxicity, the extracts' efficiency order was established as follows: *Eucalyptus* extract > *Jasminum arborescens* extract > *Bignonia capreolata* extract > *Acacia nilotica* extract.

Discussio

For all plant extracts, current analysis showed a tendency of increase in larval mortality over time. Crucially, this study demonstrated that there was either no larvae death or very little larval mortality in the control group at all time points, demonstrating the plant extracts' significant larvicidal effectiveness. These botanicals' larvicidal ability is supported by the rising larval mortality that occurs with increasing extract concentration and exposure time. Our findings corroborate those of Ahmed *et al.* in 2023, showing a dose-dependent response in mosquito larvae to plant extracts.[3]. Baeshen and Baz (2023) [8] also revealed dose and time depended, promising larvicidal activity of acetone leaf extracts of *Acacia* and *Eucalyptus* sp. against *Aedes aegypti*. Rahman studied the effect of *Eucalyptus* leaf extract against larvae of *Anopheles* mosquitos in 2016[27]. He also suggested that larvicidal activity can be enhanced by applying maximum concentration of leaf extracts along with an extended time frame. Further dose levels of plants have toxic effects that cause larval mortality. Moreover, there are behavioural changes that occur as a result of these toxic effects, including restlessness, sluggishness, the development of cruciform larval shape, and a strong attempt to reach the water's surface for gaseous exchange but eventual failure to do so and death. Present study observed the formation of larval-pupal intermediates and the occasional occurrence of mortality during ecdysis. These outcomes are consistent with earlier findings in *Culex quinquifasciatus* by Neraliya and Srivastava (1996) [25], who used some acetone plant extracts that functioned as growth regulators.

Earlier studies have shown the efficacy of *Eucalyptus* oils as a larvicide against mosquitoes (Chenge *et. al.*, 2009; Lucia *et. al.*, 2012; Medhi *et. al.*, 2010; Senthil, 2007) [11, 19, 21, 30]. Additionally, we corroborate Nair *et al.*'s research (2014) [24] showcasing the effectiveness of *Eucalyptus* leaf extract in causing *Aedes aegypti* larval mortality. Cheng *et. al.*, 2009[11], separated the major constituents of leaf essential oils of two species of *Eucalyptus*, such as α-phellandrene, limonene, *p*-cymene, *γ*-terpinene, terpinolene, and α-terpinene using GC-GM analysis and found the excellent larvicidal effects of these active components against *A. aegypti* and *A. albopictus*.

Hidayah *et al*., 2020 [17] studied bio-larvicidal effectiveness of leaf extract of *Jasminum* sp and stated that *Jasminum* extract positively contains alkaloids, flavonoids, saponins and tannins. These toxic substances including saponins and flavonoids cause stomach poisoning in *Aedes aegypti* larvae, which can lead to digestive system problems and even death. Eugeni Anitha Preethi G and associates (2014)[14] support the present results with *Jasminum* extract reporting the significant larvicidal activity of the crude chloroform, methanol and aqueous flower extracts of *Jasminum officinale, Jasminum auriculatum* and *Jasminum grandthree* against *Aedes aegypti*. Our findings with *Bignonia* extract can be supported by study of Mohanraj and Sasikala, 2023[22], who demonstrated the promising insecticidal effects of leaf extract of *Millingtonia hortensis* belonging same family of *Bignonia* species as Bignoniaceae against *Aedes aegypti*.

Extracts from *A. nilotica* have been demonstrated in earlier research to be useful in killing mosquito larvae (Edriss et. al., 2012; Elkhidir et. al., 2020; Taura et. al., 2004) [12, 13, 32].These extracts can disrupt the larvae's life cycle and prevent them from developing into adult mosquitoes. Baddepudi Kamalababu (2023) [7] has registered the toxicity of *Acacia arabica* leaf extract as effective bio-larvicide with an LC_{50} of 3303 ppm or equivalent to a concentration of 0.33% against *Aedes aegypti*. Baz and other researchers (2024) [9] demonstrated a high level of insecticidal efficacy of aqueous and methanol extracts of leaf *Acacia* sp. against mosquito larvae. By using molecular hybridization and docking simulation mode of action, they found active component in *Acacia* leaf extracts having structure similarity to OLA co-crystallized ligand, which present a reasonable interpretation for its insecticidal activity via deactivating the FABP protein.

Conclusion

Present research concluded that the aforementioned plant extracts can all be processed into environmentally safe larvicides. However, *Jasminum arborescens* and *Eucalyptus rudis* exhibit promising potential larvicide activity against *Aedes aegypti*. Additionally, our findings pave the way for additional research on the effectiveness of natural product extracts' larvicidal qualities. Such a strategy would not only lessen the negative environmental effects of chemical pesticides but also encourage rural people to use locally accessible bioresources in a sustainable manner.

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