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Evaluating the Larvicidal Efficiency of Selected Botanical Plant Extracts against Dengue Vector *Aedes* sp.

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Abstract – Growing numbers of mosquito-borne diseases worldwide, especially dengue fever, demand sustainable and environmentally friendly control measures. This study compared the larvicidal effects of five medicinal plant extracts-*Nicotiana tabacum* (Tobacco), *Acorus calamus* (Sweet flag), *Pongamia pinnata* (Pungam), *Calotropis* sp., and *Aristolochia bracteolata* (Worm killer)—against *Aedes* sp. larvae. Methanolic extracts were tested at concentrations ranging from 25 to 500 ppm, and larval mortality was recorded after 24 h of exposure. *Acorus calamus* exhibited the highest larvicidal efficacy, with 98.00 ± 2.45% mortality at 500 ppm and the lowest lethal concentration values (LC_{50} = 58.07 ± 0.80 ppm; LC_{90} = 557.82 ± 0.75 ppm; LC_{99} = 1103.22 ± 0.75 ppm). *Calotropis* sp. showed moderate activity with an LC_{50} of 97.27 ± 0.75 ppm. One-way ANOVA revealed significant differences in larval mortality among plant extracts ($p < 0.05$), and Tukey post hoc analysis indicated that *Acorus calamus* differed significantly from *Pongamia pinnata* and *Aristolochia bracteolata* ($p < 0.05$). Regression analysis showed a significant dose–response relationship for all extracts, with the highest regression coefficient for *Acorus calamus*. Phytochemical screening identified phenols, terpenoids, and alkaloids as major bioactive components. Overall, *Acorus calamus* demonstrates strong potential as a natural larvicide for mosquito control programs.

Keywords- *Aedes* sp., Dengue vector control, Larvicidal activity, Medicinal plants, Natural insecticides.

Recommended APA Citation

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Introduction

Global health and economy are at severe risk due to the danger of both community and economy since vectors are spreading harmful diseases with high risks of transmission. The alarming rate of the spread of mosquito-borne arboviruses prevalence and the resulting high morbidity and mortality rates have elevated it to a health priority issue on the global agenda (Beard et al., 2019). Among these diseases is dengue fever, which is an annual illness with over 390 million cases annually predicted to be transmitted by vector (*Aedes* mosquitoes) and caused the dengue viral fever. It is predominant in the towns and cities. (Wang et al., 2024; Wu et al., 2021).

Traditional approaches to dengue control principally involve controlling vectors, especially by elimination of breeding places and by elimination of mosquitoes through the application of chemical insecticides. The prolonged and continuous use of chemical pesticides has led to the development of genetic resistance in mosquitoes (Alphey et al., 2010; Wang et al., 2025), thereby reducing the effectiveness of these control measures. Synthetic pesticides are challenged by their sustainability and safety due to its environmental and non-target effects (Madhuri et al., 2024). This has in turn necessitated the need to explore other, sustainable, and affordable green ways of managing vectors as soon as possible.

Plant-based insecticides have also emerged as a feasible alternative to synthetic pesticides during the control of pests. Plant-based pesticides may also be thought to be safer compared to other products because they are less toxic and can be readily biodegraded (Ahmed et al., 2022; Iqbal et al., 2022). In addition, botanical pesticides are multifaceted blends of phytochemicals, which possess a variety of mechanisms of action, e.g., disruption of physiological and behavioural insect processes of interest (Isman, 1997; Isman, 2006; Melanie et al., 2022).

Therefore, the paper has made a comparison of the larvicidal potentials of five plant extracts for the prevailing need to seek alternative ways of controlling the *Aedes* sp. larvae in the fourth instar. In here the selected plant extracts were minimally used in scientific papers and because of the extensive history of use as a pest control agent in traditional system. The work is also a contribution to the existing quest of environmentally friendly methods of control of mosquitoes and gives novel facts on the effectiveness of the plants of ethnobotanical importance as larvicides.

Materials and Methods

Plant Collection for Stock Solution Preparation

The native plant species were collected in Sammanthurai region, which included the rhizomes of *Acorus calamus* and the fresh leaves of *Nicotiana tabacum* (Tobacco), *Acorus calamus* (Sweet flag), *Pongamia pinnata* (Pungam), *Calotropis* sp., and *Aristolochia bracteolata* (Worm killer). The sterile distilled water was used to get rid of dust and dirt on the plant-materials. The plant parts were allowed to dry under the shade after cleaning and the process required a month thus the bioactive chemicals would not be lost. When the materials dried, they were crushed to make minute particles. The sieving of the powdered components was then repeated again by passing the material through a sieve with 210 µm diameters. The extracted plant powders were stored in room temperature, dark and sealed vials to be extracted later.

Methanol Extraction

To proceed, 250 mL of methanol, together with 25 g of seed powder of each plant was mixed in a beaker. The mixture was put in the shaker with 360 rpm over the two days. To dispose of the plant debris, the extract was mixed and filtered with filter paper. Then filtrates concentrated by the help of rotatory evaporator at 41°C and 80 rpm until all the solvent was blown off and only the crude extract remained. The extracts were stored in dark and sealed vials until use at 4 °C.

Larvicidal Bioassay

In Sri Lanka, Sammanthurai, Aedes sp. fourth-instar larvae were collected from rice fields and residential water containers. In the laboratory, larvae were maintained at 26 ± 3 °C, under a 13 h:11 h (light: dark) photoperiod, and 70–80 % relative humidity. The larvicidal bioassay was conducted following an adaptation of the World Health Organization procedure. Before the experiment, the larvae were treated by a 4:1 mixture of yeast powder. Plant extracts were used in seven concentrations; starting from 25 to 500 ppm. A total volume of 250 mL of distilled water was used for each test, to which 1 mL of plant extract was added. Each experiment was conducted using 20 fourth-instar larvae, while a control group was maintained simultaneously under identical conditions. Mortality was measured at 24 h and the mortality percentage was obtained by using Abbott formula. Each concentration is duplicated five times.

Phytochemical Analysis

Qualitative phytochemical screening of the methanolic extracts of each plant was conducted using standard methods to identify secondary bioactive compounds. (Bagheri et al., 2020; Rajendrasozhan et al., 2021; Rajiv et al., 2016; Sonam et al., 2017).

Statistical Analysis

To compare larval mortality at different concentrations of the plant extracts a one-way Analysis of Variance (ANOVA) was performed. Following ANOVA, a post-hoc Tukey's test was conducted to determine which of the individual plant extracts caused significantly higher larvicidal activity than others. Using Probit Analysis, lethal concentrations (LC_{50}, LC_{90}, and LC_{99}) of the plant extracts were calculated. To provide an additional measure of the relative variability in larval mortality among extracts, Effect Size calculations were used. Regression analysis was also performed to investigate the dose-response relationship between plant extract concentrations and larval mortality.

Results

Compare the Plant Extracts Efficiency Against Larvae

This study has shown the significant variation in the larvicidal potency among five methanolic plant extracts tested for their ability to kill fourth-instar *Aedes* sp. According to Table 1, all five extracts demonstrated an increase in larval mortality as the concentration of each extract increased which demonstrates that the amount of toxic material in each extract increases as the concentration of each extract is increased. Among the extracts, *Acorus calamus* had the greatest larvicidal potency at all tested concentrations, followed by *Calotropis* sp., *Nicotiana tabacum*, *Pongamia pinnata* and *Aristolochia bracteolata*. This indicates that different plant-derived compounds have varying degrees of effectiveness when applied to mosquito larvae.

Acorus calamus produced 98 % larval death after only 24 h of exposure to 500 ppm of the extract, demonstrating its very potent toxic effects to mosquito larvae. In addition to being most lethal to the larvae at the high dose tested, it also demonstrated considerable efficacy even at the lowest doses as evidenced by its low LC_{50} of 58.07 ppm.

Table 1

Mean larval mortality (% ± SD) of fourth-instar Aedes aegypti larvae exposed to different concentrations (25–500 ppm) of methanolic plant extracts after 24 h of exposure.

Plant Extract	**Concentrations (ppm)**						
	25	50	100	200	300	400	500
Acorus calamus	42 ± 4.00	49 ± 3.74	59 ± 3.74	75 ± 3.16	85 ± 3.16	92 ± 4.00	98 ± 2.45
***Calotropis* sp**	41 ± 3.74	45 ± 3.16	58 ± 2.45	56 ± 5.83	68± 8.12	78 ± 5.10	93 ± 2.45
Nicotiana tabacum	31 ± 3.74	39 ± 3.74	43 ± 5.10	49 ± 3.74	58 ± 4.00	75 ± 4.47	84 ± 3.74
Pongamia pinnata	25 ± 3.16	29 ± 3.74	33 ± 2.45	40 ± 4.47	45 ± 4.47	58 ± 9.27	70 ± 3.16
Aristolochia bracteolata	21 ± 2.00	25 ± 3.16	30 ± 3.16	37 ± 2.45	44 ± 3.74	58 ± 2.45	67 ± 5.10

On the other hand, the two extracts Calotropis sp. and *Nicotiana tabacum* had much higher concentrations to produce comparable mortality than *Acorus calamus*; their respective LC_{50} values were 97.27 ppm and 181.87 ppm. As well, the two least effective extracts, *Pongamia pinnata* and *Aristolochia bracteolata*, caused only modest mortality even when exposed to the highest concentration of the two extracts.

Statistical Analysis in Larval Mortality

Statistical analysis using one-way ANOVA demonstrated a statistically significant difference in the larval mortality of the Aedes larvae exposed to the five plant extracts when comparing the different concentration levels of the plant extracts tested ($p < 0.05$). These findings confirm that the observed variability in larvicidal activity is likely a result of the potency of each plant extract as opposed to being randomly distributed.

The Tukey's post hoc multiple comparisons test further elucidated these statistical differences by determining which pairs of plant extracts were significantly different in terms of larvicidal activity. Specifically, the plant extracts of *Acorus calamus* produced significantly greater larval mortality than both the plant extracts of *Pongamia pinnata* and *Aristolochia bracteolata* at the highest tested concentrations. Similarly, the plant extracts of Calotropis sp. exhibited significantly greater larval mortality than the plant extracts of *Aristolochia bracteolate.* Overall, these findings support an ordering of the plant extracts based on their relative efficacy for controlling populations of *Aedes* larvae

Lethal Concentrations: Practical Implications for Mosquito Control

The LC_{50}, LC_{90}, and LC_{99} values used to determine the lethal concentration values, served as an objective measure to quantify how effective each plant extract was compared to one another (Figure 1). The highest potency in terms of lethal concentration was *Acorus calamus* with the lowest LC_{50} value at 58.07 ppm, which indicated that the smallest amount of the extract would result in 50% mortality in all larvae treated. Next in potency were the *Calotropis* sp., *Nicotiana tabacum*, *Pongamia pinnata*, and lastly, *Aristolochia bracteolate.*

In the same manner, both the LC_{90} and LC_{99} concentrations were also lower than that of the other plants; however, much higher concentrations were needed to achieve a nearly complete (99%) larval mortality. Specifically, it required over 2,200 ppm of *Pongamia pinnata*, and *Aristolochia bracteolate* extracts to reach an LC_{99}, and thus, these two had the least effectiveness as a larvicide

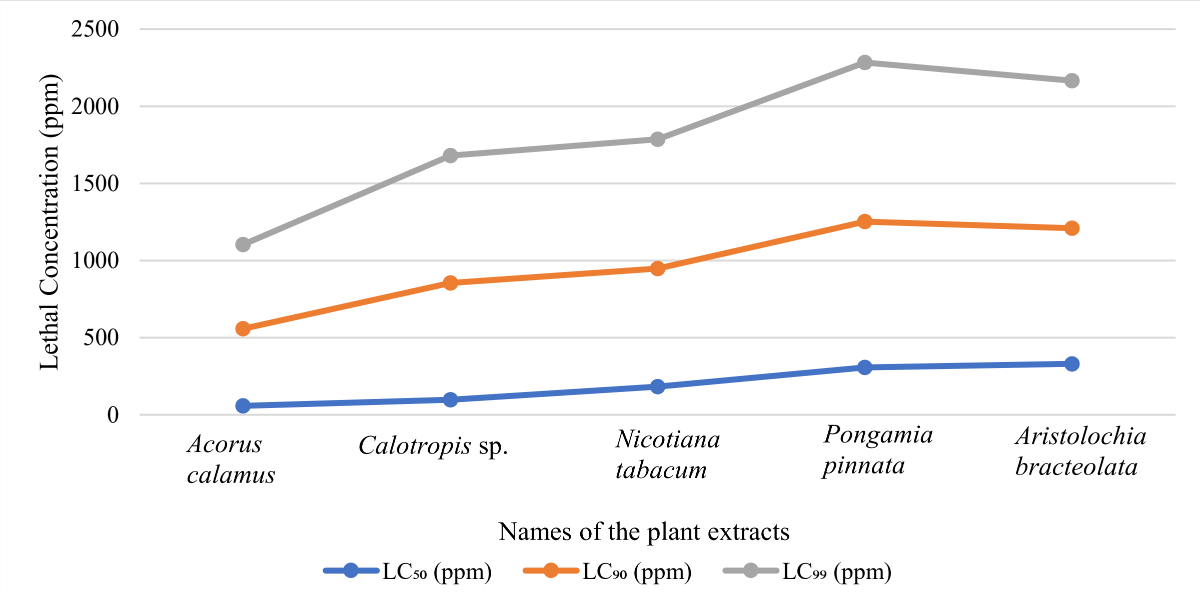

Figure 1: *Lethal concentration values (LC_{50}, LC_{90}, LC_{99}) for plant extracts*

Regression and Analysis

The regression analysis results showed a positive dose response relationship with all the plant extracts, which means that as the concentration increase, larval mortality increased. The coefficients of the regression of *Acorus calamus* (0.22) and *Calotropis* (0.18) were the most significant, indicating that the extracts have a strong dose-response relationship. Minor changes in concentration cause large changes in mortality of larvae, which effectively makes them larvicides. The dose-response relationship was appreciably weaker with *Aristolochia bracteolate* as it had the smallest regression coefficient (0.08), which suggests that it might not be good enough to induce the same rate of death as more potent extracts.

The d of Cohen defining the extent of differences between two groups demonstrated that the magnitude of the effects between *Acorus calamus* and *Aristolochia bracteolate* (d = 1.72) is relatively large and indicates that the difference between the larvicidal activity of the two groups is statistically significant and has practical meaning. This shows that *Acorus calamus* is a much more effective larvicide, which makes it a better option to be further developed into commercial insecticidal preparations. *Calotropis* and *Pongamia pinnata* (d = 1.24) had a significant effect size, which further supports the advantage of *Calotropis* over *Pongamia pinnata*as a mosquito larvicide.

Phytochemical Analysis

Qualitative phytochemical screening indicated that there is a lot of variability within the secondary metabolites present in the plant extracts tested (Table 2). *Acorus calamus* had phenols, terpenoids, saponins, and glycosides which have been shown to have many insecticidal properties. *Nicotiana tabacum* had alkaloids, which have been proven to be neurotoxic to insects as well. *Calotropis* sp., had glycosides and saponins while *Pongamia pinnata* had a broader phytochemical profile than most other plants which included flavonoids, terpenoids, and saponins. *Aristolochia bracteolata*, had alkaloids and terpenoids but was found to be one of the lowest larvicidal efficacies in the plant extracts. The variability in phytochemical composition may have contributed to the observed variation in larvicidal efficacy of the plant extracts.

Table 2

Phytochemical screening results among the plant extracts

Plant Extract	Phenols	Carbohydrates	Terpenoids	Saponins	Flavonoids	Alkaloids	Glycosides	Tannins	Steroids
Acorus calamus	+	-	+	+	-	-	+	-	-
Nicotiana tabacum	+	+	-	-	-	+	-	-	-
Aristolochia bracteolata	+	+	+	-	-	+	-	+	-
***Calotropis* sp)**	+	+	-	+	-	-	+	-	-
Pongamia pinnata	+	+	+	+	+	-	+	-	-

Discussion

The current research demonstrates that methanol extracts from selected medicinal plants show varied larvicidal activities for *Aedes* sp. larvae. Among the assessed medicinal plants, *Acorus calamus* was the most active, due to its high mortality, lower lethal concentration, and higher dose-response association. Several researchers previously have reported that *Acorus calamus* extracts exhibit good insecticidal activities, attributed to the presence of bioactive compounds such as terpenoids and saponins (Hung et al., 2019; Lozano & Dussán, 2013).

Acorus calamus larvicidal effectiveness is likely due to the synergy between its phytochemical components. Terpenoids affect mitochondrial respiration and enzymes that lead to cellular damage and energy loss in the larvae of insects (De Geyter et al., 2012; Isah et al., 2018; Wang & Liu, 2016). Saponins destroy the structure of cell membranes causing them to become more porous and eventually killing the larvae (De Geyter et al., 2012; Osbourn et al., 1994). This explains the high larval mortality observed at low concentrations of the *Acorus calamus* extract

Nicotiana tabacum and *Calotropis* sp. displayed modest larvicidal activity when compared to *Acorus calamus*. The larvicidal activity of *Nicotiana tabacum* has been attributed to alkaloids, specifically nicotine, which acts as a neurotoxin through disruption of transmission of signals in insects (Zhang et al., 2024). Glycosides, cardenolides, and saponins found in *Calotropis* sp. can inhibit metabolic pathways and larval growth in mosquito larvae (Kumrungsee et al., 2023; Lauzon et al., 2009; Stumpp et al., 2013). However, the larger LC_{50} values indicate that the extracts of these two species would need to be used at higher concentrations than *Acorus calamus* to produce the same level of larval mortality.

Low larvicidal activities of *Pongamia pinnata* and *Aristolochia bracteolata* indicate limited potency toward *Aedes* sp larvae under tested conditions. Although *Pongamia pinnata* has terpenoids and flavonoids with well-documented insecticidal activities (Agliassa & Maffei, 2018), the concentrations used in this study may not have produced enough larval mortality quickly. Additionally, although *Aristolochia bracteolata* includes alkaloids and terpenoids, it showed poor larvicidal activity, suggesting that its bioactive compounds may work slowly or need to be given in large doses to produce a notable effect (Wu et al., 2004).

Positive dose-response relationships among the various plant extracts provide additional evidence that the larvicidal activity is dose-dependent. In addition, the extracts with the largest regression coefficient, i.e., *Acorus calamus* and *Calotropis* sp., indicated they had a stronger response to the increase in dose than the other extracts, thus producing a stronger toxic effect per unit increase in dose. The results of this study are consistent with other research showing that while the efficacy of botanical insecticides depend primarily upon their phytochemical content, they also depend upon the concentration and how they interact with the target organism (Isman, 2006).

In general, the results of this study demonstrate that *Acorus calamus* has potential as an effective and environmentally-friendly larvicide. *Acorus calamus*'s high efficacy, statistical significance, and diverse phytochemical profiles suggest that it is a promising alternative to synthetic chemical insecticides for use in integrated mosquito management programs. Additional research should focus on the development of formulations, field trials, and assessments of non-target toxicity to make its practical applications feasible.

Conclusion

This experiment demonstrated that the *Acorus calamus*, *Calotropis* sp., *Pongamia pinnata*, *Nicotiana tabacum*, and *Aristolochia bracteolata* have varying levels of larvicidal efficacy on *Aedes* sp. larvae with *Acorus calamus* showing the most potential applicability with the highest 98.00 ± 2.45 reduction and lowest LC_{50} of 500 ppm ± 0.80, LC_{90} of 511.48 ± 0.75 and LC_{99} at 500 ppm and with strong dose response relationship ($p < 0.05$). Conversely, *A. Bracteolate* was observed to be least effective, therefore, it could be used in practical sense with little benefit of concentration or synergy. Such findings have placed *Acorus calamus* as an excellent applicant to develop botanical larvicide and recommend on further development through optimization of extraction and formulation, total toxicological study and large-scale field validation. Such plant-based larvicides would be included in an integrated mosquito management program, which would provide an environmentally harmless and sustainable alternative to the synthetic insecticides and would address the growing issue of the insecticide resistance in controlling mosquitoes.

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