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# Evaluation of larvicidal effect of *Prosopis cineraria against Aedes aegypti* (Diptera: Culicidae)

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**Abstract**
Mosquito species *Aedes aegypti* (*Ae. aegypti*) act as a carrier for dengue and chikungunya virus, particularly throughout tropical regions. Concerning the growing resistance to chemical insecticides, researchers need to find safe plant-based options for mosquito control. This study evaluated the larvicidal potential of *Prosopis cineraria* (*P. cineraria*) against the 3rd and 4th instar larvae of *Ae aegypti*. Moreover, plant leaves and roots were extracted using aqueous, methanol, acetone, and hexane through soxhlet method, followed by their phytochemical screening and larvicidal assay. We found that methanolic extracts especially showed the richest profile of phytochemicals like strong presence of flavonoids and phenols. All plant extracts showed larvicidal activity that increased with higher dose and more time exposure, but leaf extracts performed better than root extracts only. Remarkably, the methanolic leaf extract demonstrated the highest efficacy, achieving a median lethal concentration ($LC_{50}$) of 223.27 ppm and $LC_{90}$ of 566.51 ppm after 48 hours of exposure. This leaf extract showed a marked increase in toxicity from the 24-hour exposure, which had an $LC_{50}$ of 393.80 ppm. In comparison, the aqueous leaf extract was less potent, with a 48-hour $LC_{50}$ of 240.63 ppm, while the methanolic root extract was significantly weaker, with a 48-hour $LC_{50}$ of 404.94 ppm. The overall solvent efficacy for leaf extracts was ranked as methanol > aqueous > acetone > hexane. Additionally, combined methanolic extracts of leaf and root showed enhanced synergistic effects. These findings, particularly the potent larvicidal values of the methanolic leaf extract, establish their importance against dengue vector *Ae. aegypti*.

**Keywords:** *Larvicidal activity, Toxicity assessment, Aedes aegypti, Dengue vector, Prosopis cineraria*

## Introduction
*Ae. aegypti* (Diptera: Culicidae) is the primary vector of dengue and chikungunya diseases responsible for millions of infections and thousands of mortality each [1]. As per latest reports, dengue virus infection is now a major public health problem India including Uttar Pradesh (UP) [2]. Basically, the local health records showed Dengue cases increased eight times from 2000 to 2019, with numbers rising from 0.51 million to 2.4 million in 2010 and further reaching 5.2 million in 2019 itself [3]. Seasonal dengue outbreaks keep happening in the same region every year. *Ae. aegypti* population appears to have achieved ecological stability in the Ganga-Jamuna doab areas as well as our lowland area of eastern UP [4]. Female *Ae. aegypti* are an autogenous and exhibit distinct behavioral shifts during adulthood, requiring a blood meal for egg development [5]. Vector control remains a key strategy to reduce disease transmission, with larvicides offering particular benefits by targeting the immature stages of mosquitoes, thereby preventing their emergence as adults [6]. Across many developing nations, mosquito-borne diseases keep causing serious illness and death rates, hitting the working population hard. However, we are seeing that mosquitoes are becoming strong against synthetic chemicals, which has pushed both researchers and communities to search for safer alternatives from plants.

*Prosopis cineraria (P. cineraria),* commonly known as the Shami plant, is a leguminous (Fabaceae family) tree that grows in arid and semi-arid regions including our lowland area. This plant is traditionally used as herbal medicine and its leaves, bark, and pods contain contain alkaloids, flavonoids, and tannins which further showed insect-repelling properties, with the plant itself being effective against larvae [7, 8]. The plant's phytochemicals study also revealed its important compounds that cause biological activities such as killing insects, larvae, and germs [9, 10]. With increasing resistance to synthetic larvicides, so we definitely need natural options that are safe for the environment.

This study focuses on the assessment of phytochemical composition of *P. cineraria* botanicals in various solvent as well as explores the larvicidal efficacy of *P. cineraria* extracts against *Ae.*

*aegypti* 3rd and 4th instar larvae, aiming to identify bioactive plant parts and their potential in controlling mosquito populations, thereby offering a sustainable approach to reducing the burden of mosquito especially *Ae. aegypti* borne diseases.

## Materials and Methods

### Collection and Preparation of Plant Material

Botanicals of *P. cineraria* were collected from the Botanical Garden of our university campus, Gorakhpur, Uttar Pradesh, India, with ensuring that they were free from visible diseases, pests, or physical damage as (Fig. 1). We have also identified this plant by a botanical taxonomist. The leaves and roots of the plant were especially collected in the morning time from 6:00-9:00 AM, to reduce the chance of environmental stress and preserve optimal metabolite levels. Leaves were placed in clean, labeled plastic bags to avoid contamination and store them in a portable cooler with ice packs during transport to maintain freshness. For root collection, carefully extract fine feeder roots (1-2 cm in diameter) from the base of the plant using a sterilized spade or trowel to ensure uniformity. Gently rinse roots with distilled water to remove soil or debris, if needed, and place them in clean, labeled plastic bags. Clearly labeled the all botanicals with details such as plant part, collection site, date, and time.

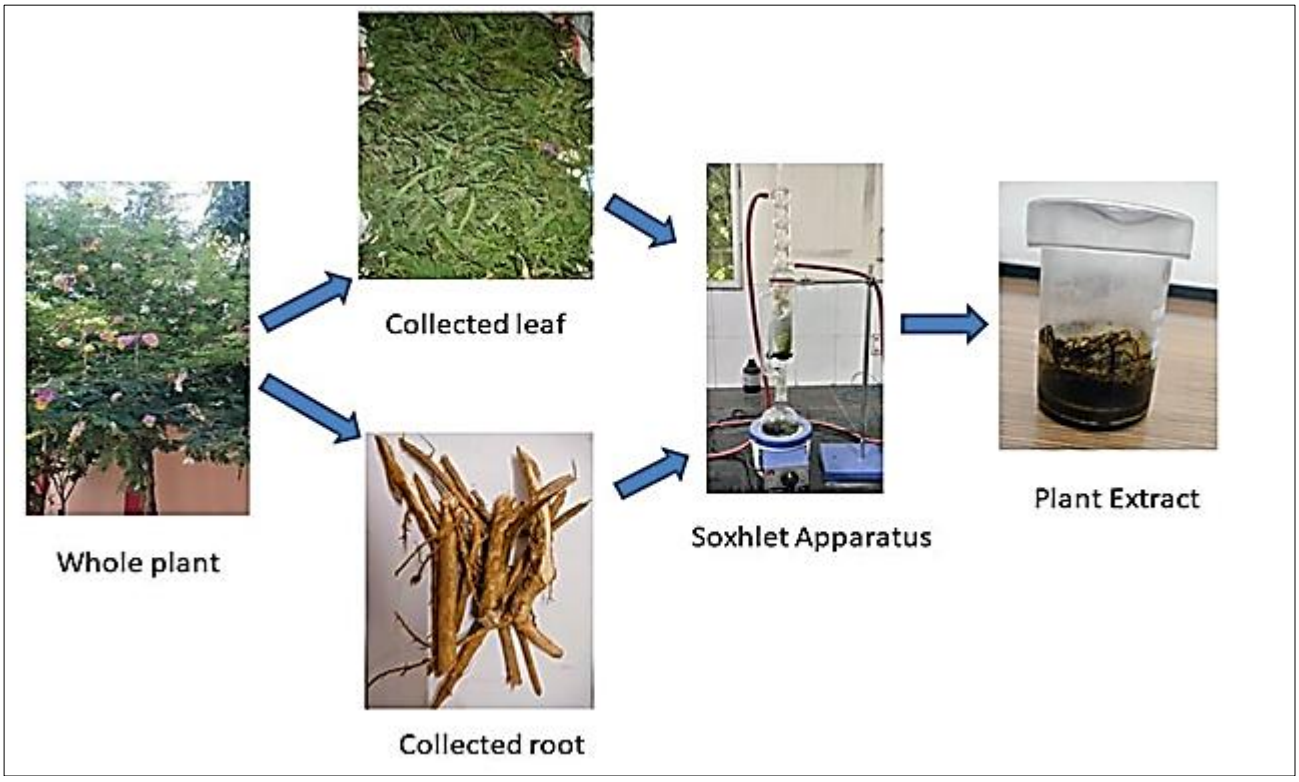


**Fig 1:** Sequential process of plant material collection and extraction of *P. cineraria*: Whole plant → Collected leaf and root → Drying and preparation → Soxhlet extraction with different solvents → Concentrated crude extract.

### Preparation of Extraction (Soxhlet Method)

For extraction, 20 g of dried powdered material of *P. cineraria* (leaf and root separately) was used for each run of Soxhlet extraction. The dry powdered of botanicals were extracted with 300 ml of different solvents (water, methanol, acetone, and hexane) and subjected to continuous soxhlation for about 8 hours and using Whatman filter paper for the thimble. After completion of the extraction process, the solvent fractions were concentrated using a rotary vacuum evaporator operated at 40-45 °C under reduced pressure for removing excess solvent and obtained a semi-solid residue. Then, they were air-dried to constant weight and preserved in containers at 4 °C until further use.

### Preparation and Storage of Stock Solutions

After soxhlation taken the evaporated crude extract of leaf and root each 10 g each that dissolved in 100 ml of distilled water which served as the 10% stock solution. For working solutions, dilution series between 100 to 500 parts per million (ppm) were prepared for bioassay investigation. To prepared 100 mL of 100 ppm working solution, taken 0.1 mL of 10% stock solution was diluted with 99.9 mL of distilled water. Similarly, 0.2 mL stock solution was used for 200 ppm, 0.3 mL for 300 ppm, 0.4 mL for 400 ppm, and 0.5 mL for 500 ppm in 100 mL total volume. All solutions were thoroughly mixed to ensure homogeneity.

### Collection and identification of *Ae. aegypti* larvae

Larvae were collected and properly transported on a transparent closed container with proper precautions with standard recommendations [11]. Fully fed larvae were collected from various stagnant water sources at multiple town area of Gorakhpur and Maharajganj as mentioned in (Fig. 2). The larvae were examined and identified based on standard morphological keys [12, 13, 14]. Special attention was given to 3rd and 4th instar larvae, which was most suitable for larvicidal bioassay studies due to their consistent size and resilience. For the rearing procedure, the larvae were kept in 250 ml plastic containers with tick gauze cover to avoid contamination and the escape of the adult mosquitoes. The larvae were housed in ambient settings at room temperature. To promote healthy larval growth, fish meal pellets were supplied as a nutritional supply and fed once every two days.

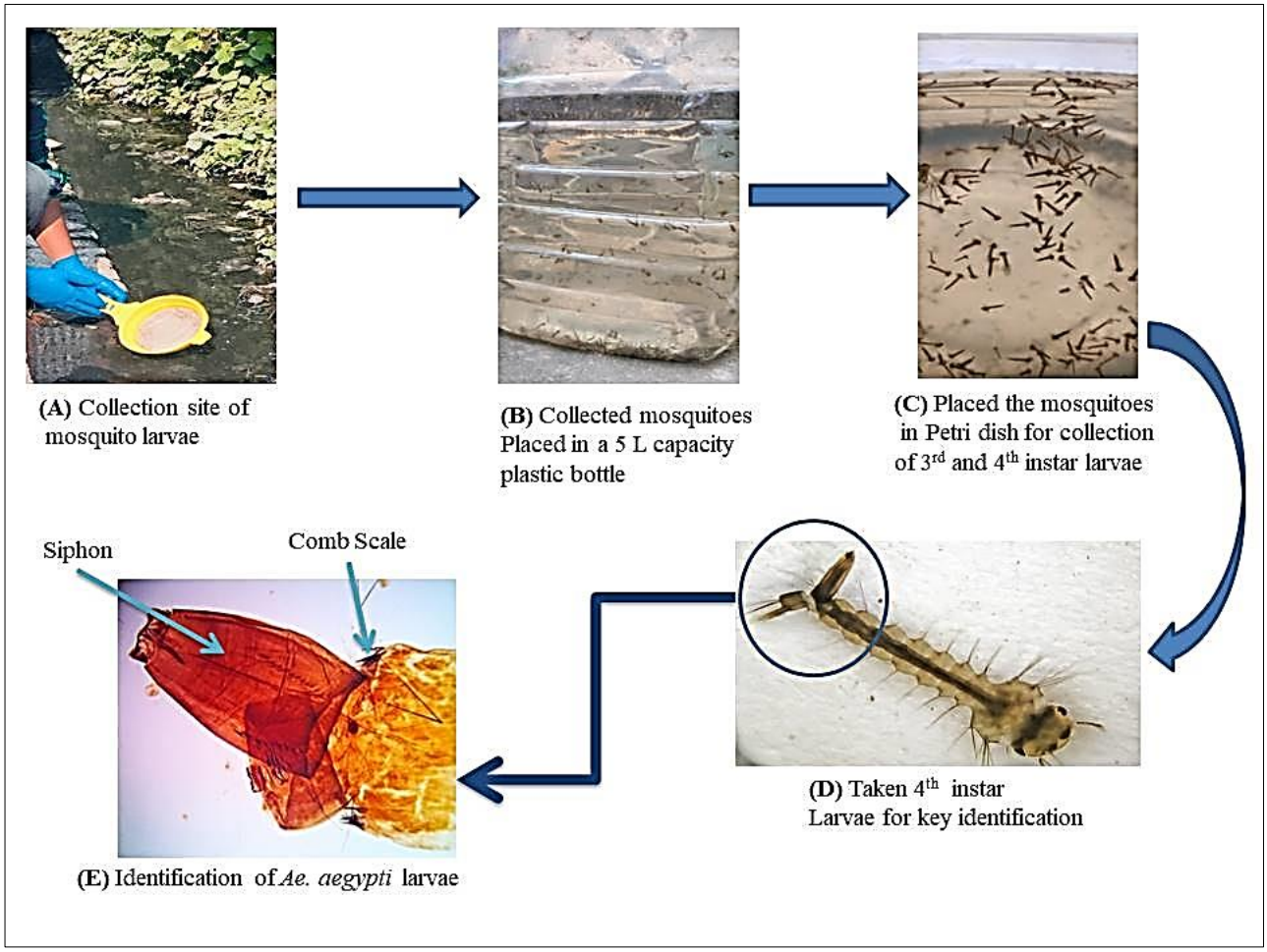


**Fig 2:** Collection and identification of mosquito larvae; **(A)** Larvae collected from drains of colony, **(B)** Larvae transferred to 5 L Plastic bottle, **(C)** Larvae placed in Petri dishes for morphological identification, **(D)** 3rd & 4th instar larvae taken for key identification, **(E)** Identified as *Aedes* larvae with the presence of comb scales on the eighth abdominal segment with a single row of large median and stout submedian spines and small and wide siphon, which distinguish them from other mosquito species

**Phytochemical Analysis**

The prepared extract of all two parts of botanicals was utilized to test various phytoconstituents present in them as per standard methods in Table I. All chemicals and solvents were commercially available from Rankem Chemicals, India, and various chemical reagents were also prepared and then used for particular phytochemicals screening. In this present study, qualitative phytochemical screening of *P. cineraria* leaves and root extracts were prepared in distilled water, methanol, acetone and hexane solvents revealed the presence of multiple phytochemical/secondary metabolites, though their concentration and diversity varied among plant parts.

**Table 1:** Qualitative methods of phytochemical detection in *P. cineraria* extracts

| Phytochemicals | Test Method | Observation | References |
|---|---|---|---|
| Alkaloids | Wagner's / Dragendorff's test | Precipitate formation (white, brown, or orange) | [7] |
| Flavonoids | Alkaline reagent test | Yellow coloration that disappears on acidification | [12] |
| Tannins | Ferric chloride test | Blue-black or green coloration | [13] |
| Saponins | Foam test | Persistent froth formation | [14] |
| Phenols | Ferric chloride test | Deep blue or black coloration | [7] |
| Terpenoids | Salkowski test | Bluish-green coloration | [7] |
| Steroids | Liebermann-Burchard test | Reddish-brown ring at the interface | [13] |
| Resins | Alcohol test | Appearance of turbidity or precipitate upon addition of alcohol | [8] |

**Larvicidal Bioassay**

The larvicidal effectiveness of *P. cineraria* leaves and root extracts was evaluated against the 3rd and 4th instar larvae following the standard WHO protocol for the bioassay taken 20 larvae were exposed to various concentrations of the extracts in 250 ml glass beakers with 100 ml of test solution [11]. Each treatment had five replicates with a negative control. We used droppers to move the larvae into small disposable cups filled with 100 ml of water. The count of dead larvae was considered as the dead point to determine mortality. We have also checked the combined larvicidal potential in different solvents extracts from both leaf and root extracts for the investigation of possible synergistic effects against larvae of *Ae. aegypti*.

We have also investigated possible synergistic effects, the combined larvicidal potential of the leaf and root extracts was also evaluated. The extracts were mixed in a 1:1 ratio to formulate a combined test solution. Following the same WHO

protocol, a series of working concentrations (100, 200, 300, 400, and 500; ppm) were prepared from this synergistic mixture. The experimental procedure, including the use of 20 larvae per replicate per beaker for each concentration, and a negative control, was identical to the assay performed for the individual extracts. Death rate of larvae was carefully recorded after 24 and 48-hours (h) of continuous exposure.

**Statistical analysis**

Larval mortality rate was evaluated for dosage and time dependent manner for single and combined extracts in term of mean ± standard deviation (S.D.) and further all replicates were combined for statistical analysis. Lethal concentration for 50% ($LC_{50}$) and 90% ($LC_{90}$) were calculated by probit analysis of log transformed dose-mortality data by SPSS software version IBM SPSS Statistics 20. A set of bioassays was considered valid (i.e., if there was significant difference at $P < 0.05$).

## Results

**Phytochemical Screening of plant extracts**

Our qualitative analysis of *P. cineraria* leaf and root extracts in different solvents were suggested the presence or absence of phytochemicals status as mentioned in Table II. It showed that aqueous extracts contained most of these compounds, except terpenoids and steroids. Our methanolic extracts demonstrated the richest phytochemical profile, showing the presence of all tested phytochemicals like alkaloids, flavonoids, tannins, saponins, glycosides, phenols, and terpenoids except steroids. However, acetone extracts displayed a narrower profile, containing only alkaloids, tannins, phenols, terpenoids and steroids. Hexane extracts were also comparatively less diverse, containing only alkaloids, terpenoids, and steroids. Overall, methanol proved to be the most effective solvent for extracting a broad range of bioactive compounds for both *P. cineraria* leaves and roots, followed by aqueous, acetone, and hexane.

**Table 2:** Qualitative analysis of *P. cineraria leaf* and root extracts in different solvents

| Phytochemicals/ Secondary metabolites | Aqueous | | Methanol | | Acetone | | Hexane | |
|---|---|---|---|---|---|---|---|---|
| | Leaf | Root | Leaf | Root | Leaf | Root | Leaf | Root |
| Alkaloids | + | + | + | + | + | + | + | + |
| Flavonoids | ++ | ++ | ++ | ++ | - | - | - | - |
| Tannins | + | + | ++ | ++ | + | + | - | - |
| Saponins | + | + | + | + | - | - | - | - |
| Glycosides | + | + | ++ | ++ | - | - | - | - |
| Phenols | + | ++ | ++ | ++ | + | + | - | - |
| Terpenoids | - | - | ++ | + | + | + | + | + |
| Steroids | - | - | - | - | ++ | + | + | + |

Abbreviations: (+) = present, (++) = strongly present, (-) = absent

**Larvicidal activity of *P. cineraria* leaf and root extracts**

The Table III showed the mean larval mortality for *P. cineraria* leaf and root extracts tested in different solvents with a total of 20 larvae in 5 replicates. For the aqueous solvent, the leaf extract caused maximum 12.8±1.17 larvae mortality at 24 h and 16±1.1 at 48 h at 500 ppm, whereas the root extract caused 9±1.1 larvae mortality at 24 h and 12±0.63 at 48 h. In methanol, the leaf extract showed highest 13.4±1.36 larvae mortality at 24 h and 16.4±1.36 at 48 h at 500 ppm, while the root extract caused 9.6±1.2 mortality at 24 h and 12.6±1.36 at 48 h. For acetone, leaf extract caused 12±1.1 and 15.4±0.8 whereas root extract showed 8.6±0.8 and 11.6±0.49 larvae mortality at 24 and 48-h, respectively. In hexane solvent prepared extract of leaf induced 8±0.89 and 10.4±1.62 mortality at 24 and at 48 h, respectively; while the root extract caused 8±0.89 larvae mortality at 24 h and 10.4±1.62 at 48 h. Here, the SD values indicated the consistency across all replicates. These results demonstrated that both leaves and root extracts of *P. cineraria* have larvicidal properties, with aqueous and methanolic leaf extracts showed the highest efficacy in our study.

The $LC_{50}$ and $LC_{90}$ values varied notably depending on the solvents and exposure periods. Here, Table IV represented the toxicity values in terms of $LC_{50}$ and $LC_{90}$ of *P. cineraria* leaf and root extracts against the larvae of *Ae. aegypti* at 24 and 48-h, respectively. For the aqueous leaf extract, the $LC_{50}$ represented as 409.45 to 240.63, ppm at 24 and 48 h, with $LC_{90}$ values was reducing from 713.02 to 595.43 ppm, indicating increased toxicity to larvae with longer exposure. Methanolic leaf extract showed the lowest $LC_{50}$ values among all solvents, dropping from 393.80 ppm at 24 h to 223.27 ppm at 48 h, with $LC_{90}$ values of 701.77 and 566.51, ppm; respectively. Acetone leaf extract also reflected a decrease in $LC_{50}$ from 427.84 ppm at 24 h to 257.30 ppm at 48 h, with corresponding $LC_{90}$ values of 725.53 and 622.37, ppm. The hexane leaf extract followed a similar trend, with $LC_{50}$ values of 445.32 ppm at 24 h and 289.08 ppm at 48 h, and $LC_{90}$ values reducing from 734.46 to 677.62, ppm after extended exposure

A similar dose-dependent and time-dependent toxicity pattern was seen for root extracts. The aqueous root extract exhibited a decrease in $LC_{50}$ from 524.28 ppm at 24 h to 422.18 ppm at 48 h, and $LC_{90}$ from 830.04 to 768.35, ppm; respectively. Methanolic root extracts showed an $LC_{50}$ of 521.41 ppm at 24 h that improved to 404.94 ppm at 48 h, with $LC_{90}$ values going from 864.45 ppm down to 753.66 ppm. Acetone root extracts demonstrated $LC_{50}$ values of 532.33 ppm at 24 h and 435.69 ppm at 48 h, while $LC_{90}$ values also declined from 820.59 ppm to 770.55 ppm. In hexane, the $LC_{50}$ dropped from 544.95 ppm at 24 h to 465.99 ppm at 48 h, and $LC_{90}$ from 820.74 ppm to 804.32 ppm. Although root extracts generally showed higher $LC_{50}$ and $LC_{90}$ values compared to leaf extracts, indicating less potency, an increase in exposure time consistently improved their larvicidal toxicity across all solvents in our study.

**Table 3:** Larvicidal activity of *P. cineraria* leaf and root extracts in various solvents

| Concentration (ppm) | Aqueous | | | | Methanol | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaf | | Root | | Leaf | | Root | |
| | (24 h) | (48 h) | (24 h) | (48 h) | (24 h) | (48 h) | (24 h) | (48 h) |
| 100 | 1.8±0.4 | 5.4±0.8 | 0.6±0.49 | 2±0.63 | 2.2±0.75 | 5.8±0.98 | 1.2±0.4 | 2.4±0.49 |
| 200 | 3.8±0.4 | 8.8±0.75 | 8.8±0.75 | 4.2±0.75 | 4.2±0.75 | 9.2±0.75 | 2± 0.89 | 4.8±0.4 |
| 300 | 6.6±1.0 | 13.0±0.8 | 4.2±0.75 | 7.2±0.75 | 7.0±1.4 | 14.0±0.4 | 4.6±0.8 | 7.6±1.02 |
| 400 | 9.8±0.98 | 14±0.63 | 5.8±1.17 | 9.2±0.75 | 10±0.75 | 14.8±0.75 | 6.2±1.47 | 9.8±0.4 |
| 500 | 12.8±1.17 | 16±1.1 | 9±1.1 | 12±0.63 | 13.4±1.36 | 16.4±1.36 | 9.6±1.2 | 12.6±1.36 |
| | Acetone | | | | Hexane | | | |
| 100 | 1.4±0.49 | 5±0.63 | 0.4±0.49 | 1.6±0.49 | 1±0.63 | 4.4±1.02 | 0.2±0.4 | 1.2±0.75 |
| 200 | 3.2±75 | 8.4±0.8 | 1.2±0.75 | 3.8±0.75 | 2.8±0.98 | 7.8±1.17 | 1.1±0.63 | 3.2±1.17 |
| 300 | 6.2±7 | 13.0±0.9 | 3.8±0.4 | 6.8±0.4 | 5.6±1.0 | 12±0.6 | 3.2±0.75 | 6.2±0.75 |
| 400 | 9.4±1.02 | 13.6±0.8 | 5.4±1.2 | 8.8±0.75 | 8.8±0.98 | 13±0.89 | 5.2±0.89 | 8.2±0.75 |
| 500 | 12±1.1 | 15.4±0.8 | 8.6±0.8 | 11.6±0.49 | 11.4±1.5 | 14.2±1.6 | 8.0±0.89 | 10.4±1.62 |

Data were expressed as the mean±S.D. Twenty larvae groups were exposed to different (ppm) concentrations in containers. All experiments were repeated five times and mortalities were observed every 24 and 48 h

**Table 4:** Toxicity values of $LC_{50}$ and $LC_{90}$ of *P. cineraria* extracts of leaf and root against the larvae of *Ae. aegypti* at 24 and 48-h in various solvents

| Solvent | Part of *P. cineraria* | | $LC_{50}$ (ppm) | 95% confidence limits | | $LC_{90}$ (ppm) | 95% confidence limits | |
|---|---|---|---|---|---|---|---|---|
| | | | | LCL | UCL | | LCL | UCL |
| Aqueous | Leaf | 24 h | 409.45 | 378.77 | 448.14 | 713.02 | 639.91 | 823.54 |
| | | 48 h | 240.63 | 202.43 | 272.99 | 595.43 | 530.59 | 695.83 |
| | Root | 24 h | 524.28 | 479.65 | 591.55 | 830.04 | 730.78 | 992.53 |
| | | 48 h | 422.18 | 386.70 | 464.55 | 768.35 | 677.89 | 912.62 |
| Methanol | Leaf | 24 h | 393.80 | 363.75 | 430.75 | 701.77 | 629.83 | 810.31 |
| | | 48 h | 223.27 | 184.07 | 255.40 | 566.51 | 506.49 | 657.23 |
| | Root | 24 h | 521.41 | 473.13 | 595.74 | 864.45 | 752.31 | 1052.66 |
| | | 48 h | 404.94 | 367.66 | 446.20 | 753.66 | 665.07 | 894.68 |
| Acetone | Leaf | 24 h | 427.84 | 396.27 | 468.72 | 725.53 | 651.73 | 838.36 |
| | | 48 h | 257.30 | 219.97 | 290.13 | 622.37 | 552.38 | 732.29 |
| | Root | 24 h | 532.33 | 488.30 | 598.82 | 820.59 | 725.17 | 976.40 |
| | | 48 h | 435.69 | 399.4 | 484.04 | 770.55 | 681.48 | 911.73 |
| Hexane | Leaf | 24 h | 445.32 | 412.96 | 488.13 | 734.46 | 659.57 | 848.27 |
| | | 48 h | 289.08 | 252.43 | 324.24 | 677.62 | 595.55 | 8.10.83 |
| | Root | 24 h | 544.95 | 500.05 | 613.83 | 820.74 | 723.65 | 977.53 |
| | | 48 h | 465.99 | 426.48 | 522.28 | 804.32 | 707.43 | 961.05 |

**Abbreviations:** The $LC_{50}$ and $LC_{90}$ indicates the concentration that kills 50% and 90% of 3rd and 4th instar larvae of *Ae. aegypti*, respectively. Here, 95% confidence limits as LCL = lower class limit and UCL = upper class limit in parts per million (ppm). Probit analysis was performed for statistical evaluation with p-value less than 0.05 was considered as significant in comparison to control.

**Synergistic results of leaf plus root extracts**

After 24 h of exposure of leaf plus root extracts, the $LC_{50}$ value was found to be 310.2 ppm, with a 95% confidence limits (CL) ranging from 285.5 to 338.1; ppm. The $LC_{90}$ value at 24 h was 615.4 ppm (CL: 550.8-700.2; ppm). Notably, extended exposure to 48 h significantly increased toxicity, with the $LC_{50}$ dropping to 185.5 ppm (CL: 168.2-205.4 ppm) and the $LC_{90}$ decreasing to 495.8 ppm (CL: 440.5-560.9 ppm). The methanolic extracts of leaf + root of *P. cineraria* exhibited significant synergistic larvicidal activity against the larvae of *Ae. aegypti* and mortality was also increased with dose- and time-dependent manner.

**Discussion**

This study showed that leaf and root of *P. cineraria*, particularly extracts prepared in methanol solvent has good potential for killing larvae of *Ae. aegypti*. We observed the solvent- and part-dependent differences in larvicidal efficacy underscores the importance of their phytochemical composition as well as extraction methods in determining their bioactivity.

The qualitative screening of *P. cineraria* extracts was conducted to identify key phytochemical with depend on the same two things - which solvent is used and which plant part is extracted. Both leaf and root extracts showed the same compounds like alkaloids, flavonoids, tannins, saponins, glycosides, and phenols, which are known to work against insects and disease-causing organisms. In which, methanolic extracts of leaf and root exhibited a remarkably strong presence of flavonoids, phenols, and saponins, while water extracts also show good levels of these compounds, especially flavonoids and phenols in roots. The methanol and aqueous extracts surely contain high amounts of phenolic compounds and flavonoids, which matches earlier studies linking these groups to antioxidant, antimicrobial, and insecticidal activities[7, 13]. Flavonoids and phenolics actually disrupt how larvae process food and definitely cause growth problems and death in mosquitoes[9]. These extracts also contained tannins, glycosides, and saponins, supporting their established role in larvicidal activity through enzyme inhibition, interruption of cellular membranes, and blocking the respiratory functions [8, 14]. Terpenoids and steroids are important pest control effects because they can damage insect nerves and stop their growth [15]. However, these were not found in aqueous extracts but were surely present in methanol, acetone, and hexane extracts.

Some other phytochemical such as steroids was not present in aqueous extracts only, which shows that different solvents can extract only certain compounds from plants.

Our findings showed that *P. cineraria* leaf extracts prepared with methanol demonstrated the strongest ability to kill larvae, which we could see from their lowest $LC_{50}$ values. Aqueous extracts came in second place, while root extracts showed weaker effects. These findings consistent with Aggarwal *et al*. (2022) and Singh *et al.* (2022) discovered as they found that methanolic *P. cineraria* leaf extracts had better antioxidant properties and more biological activity than extracts made with different solvents [16]. Research on other plants also support the correlation between phytochemical richness and the ability to kill mosquito larvae [17]. Some studies have shown that phenolics and flavonoids act as metabolic disruptors in mosquito. They mess with the larvae's enzyme systems and create harmful oxidative stress [9, 6]. Since our methanol extracts contain plenty of these compounds, they probably cause similar biological disruptions. We see similar patterns in same family plants. Gousiga *et al.* (2025) found that *Prosopis juliflora* leaf extracts made with methanol killed *Ae. aegypti* larvae very effectively, with $LC_{50}$ values that were remarkably close to what we achieved with our methanolic *P. cineraria* leaf extracts [18]. Likewise, Govindarajan *et al*. (2016) found that *Acacia nilotica and Cassia fistula* extracts showed dose- and time-dependent mortality, reinforcing the consistency of our results with other Fabaceae-based bioassays [19].

These findings confirms previous findings where methanol, a polar solvent, efficiently extracts a broad spectrum of bioactive compounds including flavonoids, phenols, and terpenoids, which are known for their insecticidal properties [9, 7]. The strong larvicidal effect could be attributed to the synergy between these phytochemicals and consistent with the phytochemical screening that showed the richest profile in methanol extracts. Leaf extracts generally showed greater toxic effects compared to root extracts across all solvents tested, which might be explained by the higher concentration or diversity of active metabolites in leaves. Other legume plants show similar patterns where leaves pack higher amounts of flavonoids and tannins. In our findings, the lower larvicidal efficacy of hexane and acetone extracts, mainly from roots, advocates that non-polar solvents or plant roots contain fewer or less potent insecticidal constituents. Additionally, the dose-dependent increase in mortality and decreasing LC values with longer exposure makes perfect sense for real-world use the natural mosquito killers, needs to stick around long enough to actually control mosquito numbers. Our current research backs up these findings, pointing to these plant chemicals as key players in killing mosquito larvae.

The synergistic effects observed in combined methanolic extracts of *P. cineraria* leaves and roots, with an $LC_{50}$ approx. 185.5 ppm, really highlights how plant phytochemicals work better together. Nawarathne and Dharmarathne (2024) found the same kind of boost when they mixed different plant extracts or used nanoparticle blends, showing that combining phytochemical systems can potentiate bioactivity through multiple modes of action [6]. This synergistic approach is particularly useful for preventing resistance, since multiple compounds working together make it harder for mosquito populations to develop immunity. This combined effect might help slow down resistance development in mosquito groups.

## Conclusion

These findings showed that *P. cineraria* leaf and root extracts extracts have rich phytochemical content and strong larvicidal effects against *Ae. aegypti*. In which, methanolic extracts, particularly from leaves, yielded the most comprehensive phytochemical profiles, which probably account for the biological activity as we larvicidal activity observed. Furthermore, the combined methanolic extracts of leaf and root worked together to create stronger larvicidal activity. This suggests the plant chemicals interact in ways that boost their toxic effects against mosquito larvae. Future studies need to focus on separating and identifying the exact bioactive compounds in *P. cineraria* that kill larvae. Researchers should also work to understand how these compounds work and check whether they're safe for other organisms. This research will help create standardized plant-based products for mosquito control that won't harm the environment.

## Conflict of Interest

None.

## Acknowledgement

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