

Larvicidal activity of leaves extract of *Manilkara zapota*

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Abstract

The present study was conducted to evaluate the larvicidal potential of the ethanolic leaf extract of *Manilkara zapota* against mosquito larvae, which are responsible for transmitting various vector-borne diseases. The leaves were shade-dried, powdered, and extracted using ethanol through maceration. The obtained extract was tested for larvicidal activity against the early third and fourth instar larvae of *Aedes aegypti* under laboratory conditions. Different concentrations of the extract were evaluated, and mortality rates were recorded after 24 and 48 hours of exposure. The results revealed a concentration-dependent increase in larval mortality, indicating potent larvicidal efficacy. The LC₅₀ and LC₉₀ values were determined to assess the effectiveness of the extract. The findings suggest that *Manilkara zapota* leaf extract possesses significant larvicidal activity, likely attributed to the presence of bioactive phytochemicals such as alkaloids, flavonoids, and terpenoids. Therefore, the plant could serve as a natural alternative for eco-friendly mosquito control strategies.

Keywords: *Manilkara zapota*, larvicidal activity, ethanolic leaf extract, mosquito control, phytochemicals

Introduction

Mosquito-borne diseases such as dengue, malaria, chikungunya, and filariasis continue to pose serious global public health challenges, particularly in tropical and subtropical regions. The primary method of controlling these diseases is by reducing mosquito populations, especially during their larval stages. However, the widespread use of synthetic insecticides has led to several environmental and health issues, including the development of insecticide resistance, ecological imbalance, and harmful effects on non-target organisms. Consequently, there is growing interest in exploring plant-based larvicides as safe, biodegradable, and eco-friendly alternatives.

Manilkara zapota (commonly known as sapodilla or chikoo) is a member of the family Sapotaceae, traditionally used in folk medicine for its various pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, and wound-healing activities. Phytochemical investigations have revealed the presence of bioactive compounds including alkaloids, saponins, tannins, flavonoids, and terpenoids, which are known to possess insecticidal potential. Despite its wide medicinal use, limited studies have explored its larvicidal efficacy.

Therefore, the present study aims to evaluate the larvicidal activity of ethanolic leaf extract of *Manilkara zapota* against mosquito larvae. This investigation seeks to identify the plant's potential as a natural source of larvicidal agents, contributing to the development of safer and more sustainable vector control strategies.

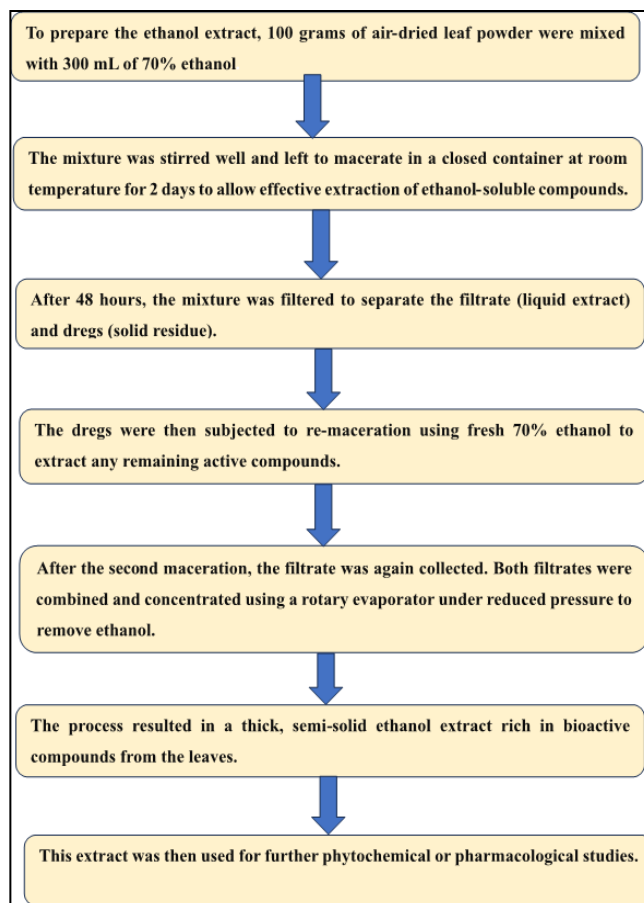
Methodology

Plant collection and Authentication

Manilkara zapota, leaves were collected from Dharmapuri district, Tamil nadu during the month of May 2023. The species for the proposed study was identified and authenticated by

DR. Stephen, Professor, Department of Botany American College, Madurai - 625002.

Extraction of Plant material Plant Extract



Pharmacological Studies

In vitro assay of Larvicidal activity and antioxidant activity

- In vitro*- study of Larvicidal activity
- Antioxidant Activity - DPPH radical scavenging activity

a. *In vitro* Larvicidal Activity

Larvicidal Assay

Hatching of eggs: *Aedes aegypti* eggs collected from ICMR-Vector Control Research Centre (VCRC)'s Madurai. The cards with mosquito eggs were placed in a container containing 1 L of dechlorinated water to obtain the larvae. After hatching, the larvae were fed 1 g of finely ground dog food and yeast (2:1) deposited directly in the water. The container was kept at room temperature (23–26 °C). Then, the larvae were screened, and those that were in the final 3rd or initial 4th instar was separated.

Principle

The larvicidal assay is based on the principle of assessing the mortality rate of mosquito larvae when exposed to different concentrations of a plant extract or chemical compound under controlled laboratory conditions. The test evaluates the toxic or inhibitory effect of bioactive phytochemicals present in the extract on the growth and survival of mosquito larvae.

When *Aedes aegypti* larvae are exposed to increasing concentrations of the test extract, the active compounds penetrate the larval cuticle or are ingested, disrupting vital physiological processes such as respiration, nervous system function, or enzymatic activity. This leads to larval immobility and death in a concentration-dependent manner.

The mortality rate obtained at various concentrations is compared with that of a negative control (dechlorinated water) and a standard chemical larvicide to determine the efficacy and potency of the plant extract. The results are typically expressed as percentage mortality or LC₅₀/LC₉₀ values, indicating the concentration required to kill 50% or 90% of the larvae, respectively.

Negative control: Dechlorinated water 30ml was taken for the activity.

Preparation of test sample: The stock solution of the plant extract was prepared at a concentration of 1000 ppm. From this stock, serial dilutions were prepared to obtain final concentrations of 1000, 500, 250, 100, 50, and 10 ppm. For each dilution, the appropriate volume of stock solution was mixed with water to make a final volume of 30 mL per container. The negative control consisted of 30 mL of dechlorinated water without any extract. These prepared solutions were used 10 larvae for exposure in the experiment, ensuring accurate and reproducible concentrations for testing.

Preparation of standard sample: The standard stock solution was prepared by accurately weighing the required amount of the compound to obtain a 10ppm solution, which was then dissolved completely in dechlorinated water to ensure a homogeneous mixture. From this stock solution, serial dilutions were performed to achieve the desired test

concentrations of 8, 7, 5, 2.5, and 1 ppm. For each dilution, the appropriate volume of stock solution was mixed with seawater to make a final volume of 30 mL per container, which was then used for exposing the *Ae. aegypti* larvae. This procedure ensured precise and reproducible concentrations for evaluating larvicidal activity. Three trials were performed.

Results and Discussion

The fresh leaves of *Manilkara zapota* are subjected to organoleptic and macroscopical studies and result were presented in the table:

Table 1: Organoleptic studies of *Manilkara zapota* Leaf

S.no	Organoleptic characters	observation
1.	Colour	Dark green
2.	Odour	Slightly aromatic
3.	Taste	Slightly bitter or astringent

Discussion

The leaves of sapodilla are simple, alternate and elliptical in shape. They are glossy, dark green and have a leathery texture. The size of the leaves ranges from 5 to 15 cm in length and they are arranged spirally around the branches. The leaves are pinkish brown when they are newly emerged and it turns to light green to dark green when they are matured. The organoleptic and macroscopic characteristics laid down can serve as diagnostic parameters for identifying the plant and serve as a tool for preventing accidental or intentional substitution or adulteration.

Table 2: Morphological studies of *Manilkara zapota* Leaf

Si.no	Morphological characters	Observation
1.	Shape	Oblong-lanceolate or elliptic
2.	Size	7–15 cm in length, 3–5 cm in width
3.	Colour	Dark green (upper surface), pale green (lower surface)
4.	Outer Surface	Smooth, shiny, glabrous (non-hair)
5.	Texture	Leathery and firm

Discussion

Macroscopically, the leaf was green, oblong, leathery, with entire margin, and acute apex and base; petiole length was 4.5 cm. Microscopic examination revealed upper and lower epidermis, mesophyll (differentiated into palisade and spongy parenchyma), vascular bundles, xylem, phloem, trichomes, and collenchyma. Palisade cells were elongated and chlorophyll-rich, while spongy parenchyma contained irregular cells. Vascular bundles were arc-shaped with lignified xylem and non-lignified phloem. Powder microscopy showed thick-walled epidermal cells, unicellular trichomes, calcium oxalate crystals, pitted vessels, actinocytic stomata, and xylem with spiral thickening.

Extraction was done by maceration technique using ethanol as a solvent

The extract of the *Manilkara zapota* leaf was prepared by maceration method using Ethanol as a solvent. The physical characters of the obtained extract were given in the table

Table 3: Organoleptic Characters of leaf extract of *Manilkara zapota*

S.no	Organoleptic Characters	Leaf extract of <i>Manilkara zapota</i>
1	Colour	Dark green
2	Odour	Characteristic aromatic odour
3	Taste	Bitter and astringent
4	Consistency	Thick and sticky

Pharmacological Activity

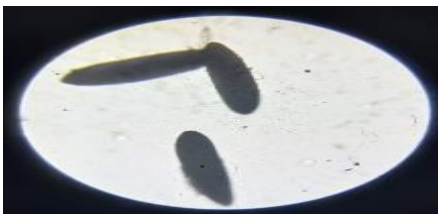
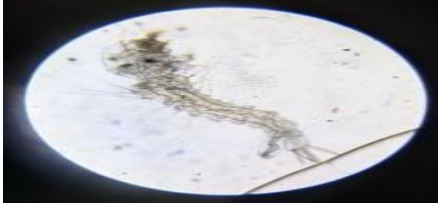
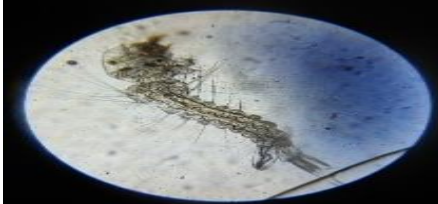
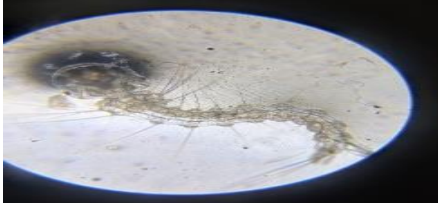

a. *In vitro* Larvicidal Activity

Hatching of Larvae

Mosquito eggs successfully hatched in

dechlorinated/artificial water under controlled laboratory conditions. The hatching rate was high, and healthy, active larvae were obtained within 24–48 hours. These larvae were later used for the larvicidal bioassay.

Table 4: Stages of *aedes aegypti* and morphology

Stages	Morphology	Observation
Egg	Small, elongated, dark coloration	
Instar I	-Transparent. -Head and thorax are slightly darker. -Thin, delicate body. Few setae (bristle-like structures).	
Instar II	-Head becomes more defined. -The thorax enlarges. -Bristles and hairs begin developing.	
Instar III	-Body thickens and segments are more clearly visible. -Bristles and fine hair increase, aiding in swimming and sensing the environment. -Siphon are well-developed and longer.	
Instar IV	-Thick, opaque, and robust body. -Segmentation, spines, and hairs are fully developed. -Head, thorax, and abdomen clearly distinguished. -Siphon are fully developed for effective breathing.	

The successful hatching of mosquito eggs confirms that the culture conditions (light, aeration, and water quality) were suitable for larval growth. The larvae obtained were active and uniform, which ensured reliability in the larvicidal experiments. The absence of mortality in the control group also indicated that the rearing conditions were non-toxic and appropriate. Proper hatching and maintenance of larvae are essential for evaluating the effectiveness of plant extracts or chemical compounds in mosquito control studies.

Morphology of instar III

In this study, fourth instar (*L4*) larvae of *Aedes aegypti* were used due to their advanced development and importance in the mosquito life cycle. The *L4* larvae, about 8 mm long, showed well-defined segmentation, a prominent siphon, and active feeding behavior. This final larval stage is critical before pupation, making it ideal for evaluating larvicidal effects and developing effective mosquito control strategies against vector-borne diseases.

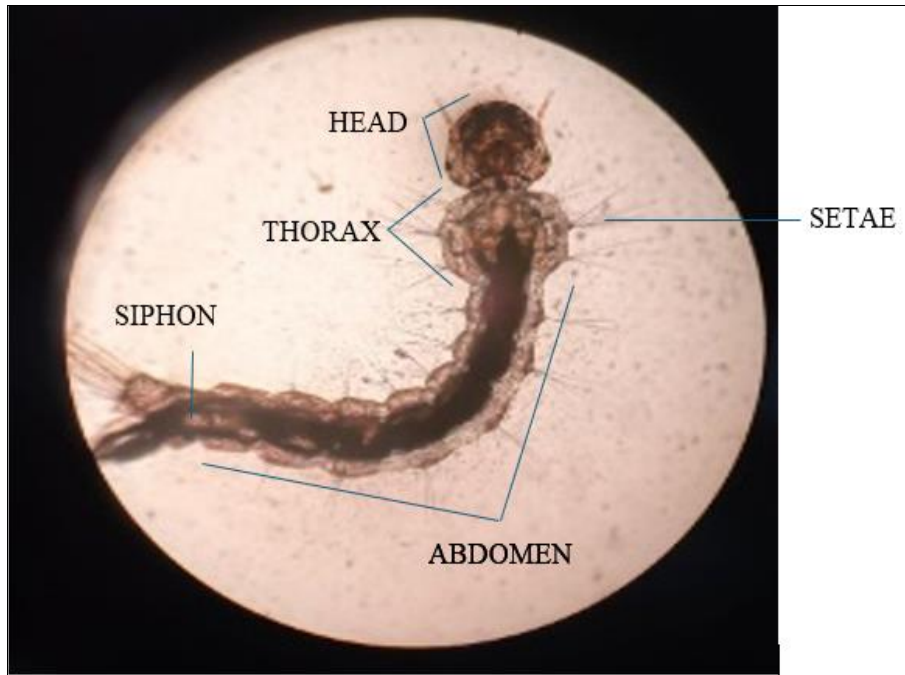


Fig 1: Morphology of 3rd instar larvae

Effect of calcium hypochlorite on larval mortality

Table 5: Effect of mosquito larvae mortality rate in different concentrations and LC50 value of calcium hypochlorite

S.no	Compound	Concentration	% Mortality	LC 50 µG/ML	LC90 µG/ML
1	Negative control (dechlorinated water)	----	----	----	---
2	Positive control (calcium hypochlorite)	1	20	2.57 µg/mL	8 µg/mL
		2.5	50		
		5	70		
		7	80		
		8	90		
		10	100		

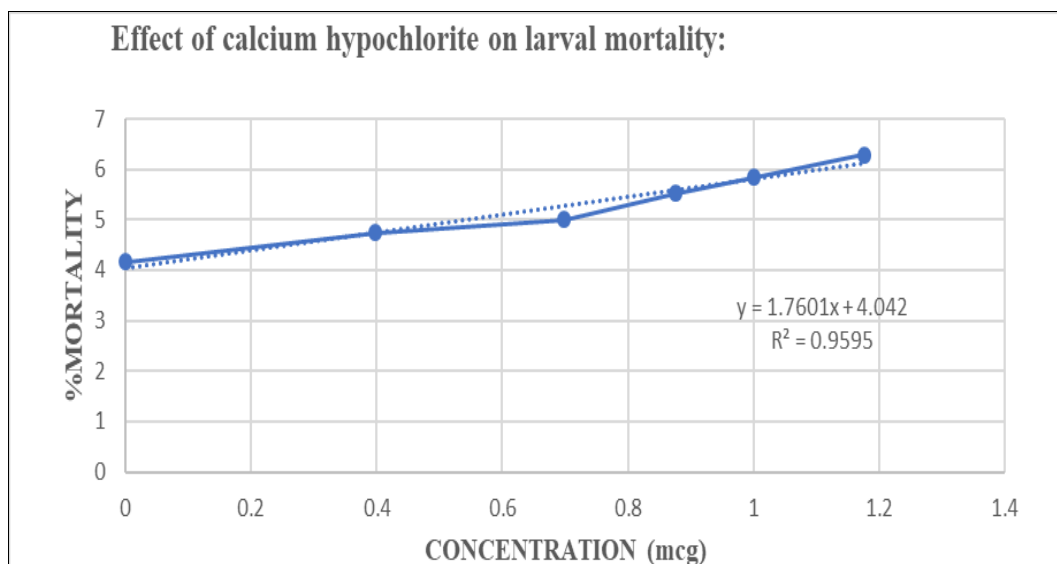


Fig 2: Mortality curve of mosquito larvae mortality rate on calcium hypochlorite.

Calcium hypochlorite showed strong dose-dependent larvicidal activity against mosquito larvae, with 20% mortality at 1 µg/mL and 100% at 10 µg/mL. The LC₅₀ and LC₉₀ values were 2.57 µg/mL and 8 µg/mL, respectively, indicating high potency at low concentrations. These

findings suggest its effectiveness for mosquito control with minimal chemical use and environmental impact.

Effect of *Manilkara zapota* leaf extract on mosquito larval mortality

Table 6: Effect of mosquito larvae mortality rate at different concentrations and LC50 value of *Manilkara zapota* Leaf Extract

S.no	Compound	Concentration	% MORTALITY	LC50 $\mu\text{g/mL}$	LC90 $\mu\text{g/mL}$
1	Negative control (artificial sea water)	-----	---	----	
2	Plant extract	1000	95	92.04 $\mu\text{g/mL}$	719.5 $\mu\text{g/mL}$
		500	84		
		250	66		
		100	54		
		10	15		

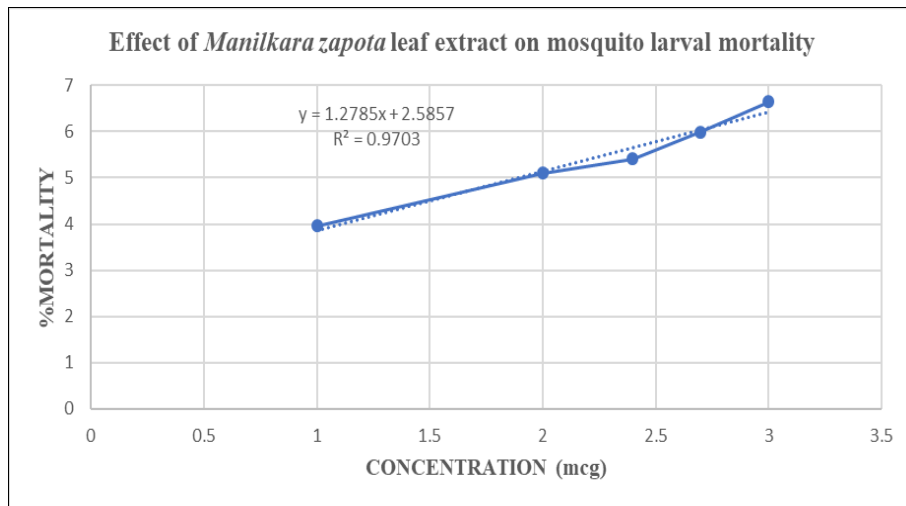


Fig 3: Mortality curve of mosquito larvae mortality rate on Leaf Extract of *Manilkara zapota*

The plant extract showed dose-dependent larvicidal activity, with mortality increasing from 16% at 10 $\mu\text{g/mL}$ to 96% at 1000 $\mu\text{g/mL}$. The LC₅₀ and LC₉₀ values were 92.04 $\mu\text{g/mL}$ and 719.5 $\mu\text{g/mL}$, respectively, indicating moderate

efficacy. Although less potent than calcium hypochlorite, the extract demonstrates promising natural larvicidal potential with lower environmental impact.

Table 7: Comparative Discussion: Larvicidal Activity of Calcium Hypochlorite and *Manilkara zapota* leaf extract

S.no	Compound	LC50 $\mu\text{G/ML}$	LC90 $\mu\text{G/ML}$
1	Negative control (artificial sea water)	----	----
2	Positive control (calcium hypochlorite)	2.57 $\mu\text{g/mL}$	8 $\mu\text{g/mL}$
3	Plant extract	92.05 $\mu\text{g/mL}$	719.5 $\mu\text{g/mL}$

The comparative evaluation of calcium hypochlorite and *Manilkara zapota* leaf extract shows clear differences in larvicidal efficacy and environmental impact. Calcium hypochlorite demonstrated higher potency (LC₅₀ = 2.57 $\mu\text{g/mL}$; LC₉₀ = 8 $\mu\text{g/mL}$) compared to *M. zapota* extract (LC₅₀ = 92.05 $\mu\text{g/mL}$; LC₉₀ = 719.5 $\mu\text{g/mL}$), indicating greater effectiveness at lower concentrations. However, as a synthetic chemical, calcium hypochlorite poses risks such as toxicity to non-target species and environmental hazards. In contrast, *M. zapota* extract, though less potent, is biodegradable, eco-friendly, and safer for long-term use. It represents a sustainable alternative suitable for integrated mosquito control, especially in resource-limited or ecologically sensitive areas. With further refinement and optimization, *M. zapota* could serve as a promising natural bio-larvicide.

Conclusion

The present study demonstrated that the ethanolic leaf extract of *Manilkara zapota* possesses significant larvicidal activity against the larvae of *Aedes aegypti*. The efficacy of the extract increased with concentration, indicating a dose-dependent response. The presence of bioactive

phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolic compounds likely contributes to its larvicidal potential. These findings suggest that *M. zapota* leaf extract can serve as a promising natural alternative to synthetic insecticides for mosquito control, offering an eco-friendly and biodegradable approach to vector management. Further studies involving formulation development, field evaluation, and isolation of active constituents are recommended to enhance its applicability in public health programs.

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