

Phytochemical profiling and mosquitocidal potential of *Carica papaya* bark extract

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Abstract

This research examines the phytochemical constituents and mosquitocidal efficacy of *Carica papaya* bark extract against *Aedes aegypti*, the principal vector of dengue fever. Phytochemical analysis identified bioactive substances including flavonoids, alkaloids, tannins, and saponins, recognised for their insecticidal activities. The extract exhibited considerable larvicidal, pupicidal, ovicidal, and adulticidal effects in a dose-dependent manner. Larvicidal experiments demonstrated elevated death rates at minimal dosages, whilst ovicidal activity revealed suppression of egg hatching. Adulticidal experiments demonstrated reduced survival and reproductive capacity of exposed mosquitoes. The results indicate that *Carica papaya* bark extract is a viable natural option for regulating *Aedes aegypti* populations, providing a sustainable and ecologically safe method to mitigate dengue transmission. Additional study is advised to identify and delineate the active components accountable for its mosquitocidal properties.

Keywords: *Carica papaya*, *Aedes aegypti*, phytochemicals, larvicidal, ovicidal, adulticidal, dengue vector control

Introduction

Climate change is intensifying the effects of mosquito-borne illnesses by broadening the geographic distribution of mosquito vectors. Elevated temperatures, altered precipitation patterns, and heightened humidity foster optimal circumstances for mosquito proliferation and disease dissemination. Consequently, areas hitherto unexposed to mosquito-borne illnesses are now encountering new risks, hence exacerbating worldwide efforts to manage these ailments. Mosquito-borne illnesses represent a considerable risk to global health security, having the capacity to induce extensive epidemics and pandemics. The rapid spread of Zika virus across the Americas in 2015–2016 highlighted the vulnerability of even well-resourced countries to emerging mosquito-borne diseases. Globalization, urbanization, and climate change are increasing the risk of disease transmission, making it imperative for countries to collaborate on surveillance, prevention, and control efforts (Ryan *et al.*, 2019) [1].

Dengue fever, principally carried by the *Aedes aegypti* mosquito, has become a significant public health issue, especially in tropical and subtropical areas. The World Health Organisation (WHO) estimates that there are 390 million dengue infections each year, with about 96 million presenting clinically (Bhatt *et al.*, 2013) [2]. The lack of targeted antiviral therapies and the restricted effectiveness of current vaccinations underscore the urgent need for efficient vector control measures to mitigate the transmission of dengue.

Plants have historically been acknowledged as a prolific source of bioactive chemicals exhibiting various pharmacological and insecticidal activities. *Carica papaya* (papaya), a tropical plant extensively farmed for its nutritional and therapeutic properties, has shown potential as a source of natural pesticides. Different components of the papaya plant, including leaves, seeds, and bark, have been historically utilised in traditional medicine and are recognised for their diverse phytochemical content, such as flavonoids, alkaloids, tannins, and saponins, which demonstrate antimicrobial, antioxidant, and insecticidal

properties (Francis *et al.*, 2017) [3]. The bark of *Carica papaya* is an underutilised resource, with few researches investigating its potential for mosquito control.

The *Carica papaya* plant is a member of the Caricaceae family and is popularly referred to as papaya. It is also referred to as papaw, paw-paw, kapaya, lapaya, tapaya, papayao, papaya, papaia, papita, lechosa, fruta bomba, mamon, mamona, mamao, and tree melon in various regions globally. Notably, the distinct plant *Asimina triloba*, belonging to the Annonaceae family, is sometimes referred to as pawpaw. Papaya originates from the Caribbean Coast of Central America and is cultivated in tropical and subtropical regions globally (Babalola, 2019) [4]. Of the 31 species under the botanical family Caricaceae and the genus *Carica*, the papaya species is the most commercially important and commonly cultivated (Biswal *et al.*, 2022) [5]. This research evaluates the phytochemicals and mosquitocidal abilities of *Carica papaya* bark extract against the dengue vector, *Aedes aegypti*.

Materials and Methods

Collection of *Carica Papaya* Bark

Fresh *Carica papaya* bark was obtained from agricultural areas in Thanjavur district, Tamil Nadu, India.

Preparation of *Carica Papaya* Bark Extract

The bark of *Carica papaya* was rinsed with tap water, air-dried in the shade at room temperature ($28 \pm 2^\circ\text{C}$) for 10-15 days, and then ground into powder using a blender. Twenty grammes of the powder were put on Whatman filter paper and extracted with methanol (99% purity) in a Soxhlet apparatus for seventy-two hours at $30-40^\circ\text{C}$. The resultant 100g extract was evaporated to dryness via a rotating vacuum evaporator and thereafter kept in hermetic containers in a refrigerator for future use.

Preliminary Phytochemical Analysis

The *Carica papaya* bark extract underwent chemical analysis to identify several phytoconstituents, using the standard protocols established by Harborne (1984) and

Trease and Evans (1979). The following procedures were used for the qualitative chemical analysis of several phytochemicals to provide a broad understanding of the phyto-constituents found in *Carica papaya* bark extract.

Gas Chromatography—Mass Spectroscopy Analysis

The methanolic extract of *Carica papaya* bark was analyzed using GC-MS utilizing a SHIMADZU QP2010 equipped with an Elite-DB-5M column and GC-MS Solution software (v2.53). The oven temperature was first adjusted at 70°C for 2 minutes, then incrementally raised to 300°C over a duration of 35 minutes. A 4µl sample was injected, using helium gas (99.995% purity) as the carrier gas at a flow rate of 1.5 ml/min. The injector temperature was maintained at 260°C with a 20:1 split ratio. Ionisation occurred at 70 eV, and mass spectra were acquired during a 35-minute duration over the 40-1000 m/z range. Compound identification relied on mass spectrum comparisons with the NIST 2008 and WILEY8 databases.

Larval/Pupal Toxicity Test

Laboratory colonies of mosquito larvae and pupae were used for assessing larvicidal and pupicidal activities. Twenty-five specimens of I to IV instar larvae and pupae were introduced into a 500ml glass beaker containing 249ml of dechlorinated water and 1ml of the specified doses of *Carica papaya* bark extract. Larval nourishment was provided for the test larvae. For each tested concentration, 2 to 5 trials were conducted, with each trial including three duplicates. The larvae/pupae subjected to de-chlorinated water without *Carica papaya* bark extract acted as the control group. The control mortalities were adjusted using Abbott's formula (Abbott's, 1925).

Ovicidal Bioassay

To assess ovicidal activity, newly deposited eggs were collected via ovitraps positioned in mosquito enclosures two days post-feeding of the female mosquitoes. Eggs deposited on filter paper inside the ovitrap were counted, and 100 gravid mosquitoes were introduced into a screened enclosure containing ten oviposition cups. Nine cups contained test solutions, whereas one cup included a control (water and Polysorbate 80). A minimum of 100 eggs were used for each treatment, and the experiment was conducted five times. Following treatment, eggs were cleaned, immersed in dechlorinated water, and observed for hatching after 98 hours. Egg mortality was determined by assessing non-hatchability (unopened opercula).

Adulticidal Bioassay

Adult female mosquitoes, aged 5 to 6 days and nourished with sugar, were subjected to different doses of *Carica papaya* bark extract, which was diluted with acetone and applied on filter sheets measuring 140×120 mm. A blank sheet of paper treated with ethanol served as a control. The documents were air-dried overnight at ambient temperature. The bioassay was performed using two cylindrical tubes measuring 125×44 mm, in accordance with WHO (1981) guidelines. One tube subjected mosquito to the extract, while the other contained them before to and after exposure. The treated paper was positioned in the exposure tube, and 20 mosquitoes (fed on sugar and deprived of blood) were released. Mortality was monitored every 10 minutes for a duration of 3 hours. Following exposure, mosquitoes were

confined in a holding tube containing cotton pads saturated with a 10% sugar solution for a duration of 24 hours. Mortality was documented, and lethal concentrations (LC₅₀, LC₉₀) were determined by probit analysis (Finney, 1971).

Statistical Analysis

All data were subjected to analysis of variance (ANOVA). The LC₅₀ and LC₉₀ values, together with their 95% confidence intervals, were calculated using a probit regression model to examine the relationship between larval mortality percentage and the logarithmic concentration of the chemical. The model's fit was assessed with the Chi-square test. An AP value below 0.05 was deemed a substantial deviation of the model from the data. In cases of significant variation, a heterogeneity factor was used to calculate the 90% confidence limits for LC₅₀ and LC₉₀. Data analysis was performed using SPSS Software version 16.0.

Results and Discussion

Preliminary Phytochemicals Analysis of *Carica Papaya* Methanolic Bark Extract

The first phytochemical analysis of the methanolic extract of *Carica papaya* bark indicated the existence of many bioactive components. The findings are contained in Table 1. The first phytochemical investigation of the methanolic extract of *Carica papaya* bark demonstrated the existence of many bioactive chemicals, as shown by numerous assays. These chemicals are often linked to pharmacological actions, including antibacterial, antioxidant, and anti-inflammatory properties.

Table 1. Preliminary phytochemicals analysis of *Carica papaya* bark methanolic extract

Phytochemicals	<i>Carica papaya</i>
Alkaloids	++
Flavonoids	+
Phenolics	++
Tannins	+
Saponin	+
Triterpenoids	+
Steroids	+
Quinones	+

- Absent, + moderate, ++ high

The findings of this first phytochemical investigation indicate that *Carica papaya* bark encompasses a diverse array of bioactive chemicals with possible therapeutic advantages. The presence of alkaloids, flavonoids, and tannins indicates that the bark may possess considerable antibacterial and anti-inflammatory capabilities (Rani *et al.*, 2023).

GCMS Mass Spectroscopy Analysis of *Carica Papaya* Bark Methanolic Extract

The Gas Chromatography-Mass Spectrometry (GC-MS) examination of the methanolic extract of *Carica papaya* bark identified many bioactive chemicals, as shown by the retention periods and mass spectrum data of the distinct peaks. The chromatogram showed many unique peaks, each representing a separate chemical. The mass spectra were analysed against established databases, including the NIST library, resulting in the identification of many chemicals, which are detailed in Table 2 and shown in Fig. 1. A total of

58 chemicals were identified in the methanolic extract of *Carica papaya* bark, including squalene, phenol, phytol, and gamma. Beta-sitosterol. Amyrin, 11-octadecenoic acid, phthalic acid, n-hexadecanoic acid, cyclopropyl carbinol, and catechol are mostly found

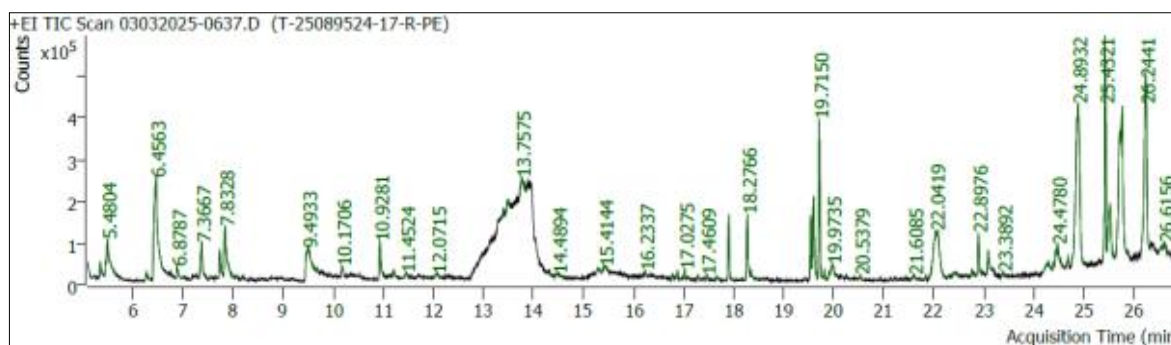


Fig 1: GCMS spectrum of *Carica papaya* bark methanolic extra

Table 2: Chemical composition of *Carica papaya* bark methanolic extract present in GCMS analysis

S. No	Retention Time	Compound Name	Formula	Area%-T
1	5.3347	Butyrolactone	C ₄ H ₆ O ₂	0.41
2	5.4804	1,2-Cyclopentanedione	C ₅ H ₆ O ₂	2.56
3	6.2669	Phenol	C ₆ H ₆ O	0.27
4	6.4563	2-Hydroxy-gamma-butyrolactone	C ₄ H ₆ O ₃	8.68
5	6.8787	1,2-Cyclopentanedione	C ₆ H ₈ O ₂	0.43
6	7.3667	2,5-Dimethylfuran-3,4(2H,5H)-dione,	C ₆ H ₈ O ₃	1.36
7	7.7308	Phenol,	C ₇ H ₈ O ₂	0.95
8	7.8328	Cyclopropyl carbinol	C ₄ H ₈ O	2.58
9	9.4933	Catechol	C ₆ H ₆ O ₂	3.54
10	10.1706	Bis(trimethylsilyl) methylphosphonate	C ₇ H ₂₁ O ₃ PSi ₂	0.34
11	10.9281	2-Methoxy-4-vinylphenol,	C ₉ H ₁₀ O ₂	1.25
12	11.2121	D-Alanine	C ₂ H ₄ NO ₂	0.21
13	11.4524	Phenol	C ₈ H ₁₀ O ₃	0.19
14	12.0715	Undecanal,	C ₁₁ H ₂₂ O	0.22
15	13.4079	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	0.13
16	13.4880	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	0.42
17	13.7575	3-Hydroxy-3-methylvaleric acid	C ₆ H ₁₂ O ₃	1.00
18	14.4894	6-Hydroxycyclodecanone	C ₁₀ H ₁₈ O ₂	0.39
19	15.2869	3-Deoxy-d-mannoic lactone	C ₆ H ₁₀ O ₅	0.35
20	15.4144	Melezitose,	C ₁₈ H ₃₂ O ₁₆	0.49
21	16.2337	d-Glucitol,	C ₁₄ H ₂₈ O ₅	0.12
22	16.7799	2-Ethylhexyl salicylate	C ₁₅ H ₂₂ O ₃	0.11
23	16.8782	Isopropyl myristate	C ₁₇ H ₃₄ O ₂	0.21
24	17.0275	9-Eicosyne	C ₂₀ H ₃₈	0.22
25	17.2824	N-(1-Cyano-3-methyl-but-2-enyl)-acetamide	C ₈ H ₁₂ N ₂ O	0.11
26	17.4609	13-Oxabicyclo [10.1.0] tridecane	C ₁₂ H ₂₂ O	0.12
27	17.6575	Homosalate	C ₁₆ H ₂₂ O ₃	0.19
28	17.9051	Hexadecanoic acid,	C ₁₇ H ₃₄ O ₂	1.60
29	18.2766	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	2.03
30	18.3458	Phthalic acid	C ₂₅ H ₃₆ O ₄	0.26
31	19.5438	9,11-Octadecadienoic acid	C ₁₉ H ₃₄ O ₂	1.51
32	19.6021	11-Octadecenoic acid,	C ₁₉ H ₃₆ O ₂	2.95
33	19.7150	Phytol	C ₂₀ H ₄₀ O	3.90
34	19.8169	Methyl stearate	C ₁₉ H ₃₈ O ₂	0.18
35	19.9735	9,12,15-Octadecatrienoic acid,	C ₁₈ H ₃₀ O ₂	0.92
36	20.5379	Benzoic acid	C ₁₇ H ₂₆ O ₂	0.12
37	21.6085	2-Propenoic acid,	C ₁₈ H ₂₆ O ₃	0.17
39	21.8525	1-Isobutyl-3-methylbutyl methylphosphonofluoridate	C ₁₀ H ₂₂ FO ₂ P	0.07
40	22.0419	Vitamin E	C ₂₉ H ₅₀ O ₂	6.16
41	22.3951	3-(2,4-Dimethoxyphenyl) butan-2-one	C ₁₂ H ₁₆ O ₃	0.29
42	22.7738	Octanoic acid,	C ₂₄ H ₄₈ O ₂	0.20
43	22.8976	Behenic alcohol	C ₂₂ H ₄₆ O	1.21
44	23.0906	Hexadecanoic acid,	C ₁₉ H ₃₈ O ₄	0.71
45	23.3892	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	0.13
46	24.2923	Campesterol	C ₂₈ H ₄₈ O	0.70
47	24.4088	Octocrylene	C ₂₄ H ₂₇ NO ₂	0.18
49	24.4416	n-Tetracosanol-1	C ₂₄ H ₅₀ O	0.43

49	24.4780	9-Octadecenoic acid (Z)-,	C21H40O4	1.19
50	24.6892	4-tert-Butyl-2-methylthiophenol,	C15H15F7OS	0.24
51	24.8932	2'-(Trimethylsilyl) oxy-3,	C22H28O6Si	13.71
52	25.4321	Squalene	C30H50	6.05
53	25.5195	Osajin	C25H24O5	3.10
54	25.7343	Phenol,	C21H23F5O	6.37
55	25.7708	. gamma. -Sitosterol	C29H50O	6.36
56	26.2441	. beta. -Amyrin	C30H50O	9.47
57	26.6156	Osajin	C25H24O5	0.70
58	26.8960	. alpha. -Amyrin	C30H50O	2.81

The GC-MS study of the methanolic extract of *Carica papaya* bark identifies a variety of bioactive chemicals that may enhance its medicinal efficacy. The discovery of various phenolic compounds is crucial, since these molecules are well-known for their antioxidant properties. This discovery indicates that the bark of *Carica papaya* may be used in the formulation of natural antioxidants, beneficial for alleviating oxidative stress-related ailments, including cardiovascular disorders and certain malignancies (Dwivedi *et al.*, 2020) [6]. The presence of chemicals, particularly squalene, is significant (Liu *et al.*, 2025) [7]. Octadecenoic acid has anti-inflammatory, analgesic, and antibacterial effects. This component, along with other identified terpenoids, may have a role in the traditional use of *Carica papaya* bark for the treatment of inflammation, wounds, and infections.

larvicidal/pupicidal activity of *Carica Papaya* bark methanolic extract against dengue vector *aedes aegypti*

The methanolic extract of *Carica papaya* bark was assessed for its larvicidal and pupicidal efficacy against the dengue

vector *Aedes aegypti*, a significant mosquito species implicated in the transmission of illnesses such as dengue, chikungunya, and Zika virus. The larvicidal and pupicidal effects of *C. papaya* bark methanolic extract at several doses (100, 200, 300, 400, and 500 µg/mL) on the larval instars and pupae of the dengue vector, *Ae. aegypti*, are shown in Table 3. The death rate is 100% in I and II instar larvae, decreasing to 95.4%, 85.2%, and 75.2% in III and IV instar larvae and pupae, respectively, at a dose of 500 µg/mL. The determined LC₅₀ values are 231.204, 240.694, 263.747, and 293.745 µg/mL for instars I to IV larvae, respectively, whereas the value increases to 329.941 µg/mL for pupae. The calculated chi-square values are 4.928, 6.362, 2.023, 0.083, and 0.646 for instars I, II, III, IV, and pupae, respectively. The calculated chi-square values indicate little disparity between the predicted and actual death rates. The larvicidal and pupicidal effects demonstrated a dose-dependent escalation in mortality, with the maximum dosage (500 µg/mL) yielding the most pronounced effect on both larvae and pupae.

Table 3: Larval and pupal toxicity effect of *Carica papaya* bark methanolic extract on dengue vector, *Aedes aegypti*

Mosquito life stages	LC ₅₀ (LC ₉₀) (µg/mL)	95% confidence Limit		Regression equation	χ ² (df=4)
		LC ₅₀ (LC ₉₀)			
		LCL	UCL		
1 st Instar	231.204 (414.763)	210.875 (387.007)	250.082 (450.639)	y = -1.614 + 0.007 x	4.928 n.s.
2 st Instar	240.694 (426.728)	187.537 (367.640)	286.126 (538.728)	y = -1.658 + 0.007 x	6.362 n.s.
3 st Instar	263.747 (470.079)	242.575 (437.711)	283.993 (512.540)	y = -1.638 + 0.006 x	2.023 n.s.
4 st Instar	293.745 (544.505)	269.680 (501.224)	317.544 (604.034)	y = -1.501 + 0.005 x	0.083 n.s.
Pupa	329.941 (622.209)	303.050 (564.376)	358.627 (706.214)	y = -1.447 + 0.004 x	0.646 n.s.

Mortality rates are means±SD of five replicates,
No mortality was observed in the control
Within each row, means followed by the same letter(s) are not significantly different (P<0.05),

LC₅₀=lethal concentration that kills 50% of the exposed organisms,
LC₉₀=lethal concentration that kills 90 % of the exposed organisms,
χ² = chi-square value, n.s. = not significant (α=0.05).

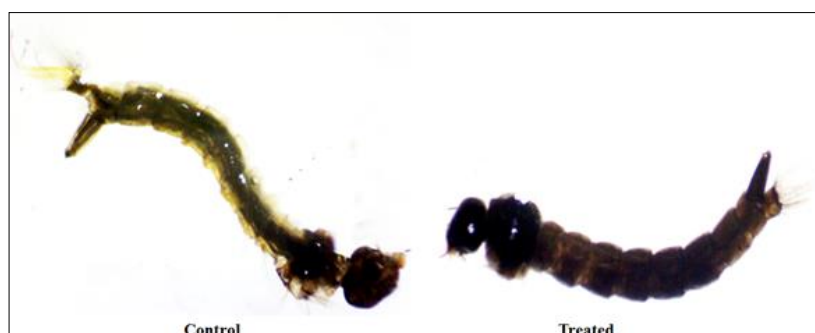


Fig 2: Morphological damage analysis of *Aedes aegypti* mosquito larvae after the treatment of *C. papaya* extract

Ovicidal activity of *Carica papaya* bark methanolic extract against dengue vector *Aedes aegypti*

The methanolic extract of *Carica papaya* bark was evaluated for its ovicidal efficacy against *Aedes aegypti*, primarily to determine its capacity to impede egg hatching and limit larval development. Table 4 presents the ovicidal efficacy of *Ae. aegypti* eggs after treatment with methanolic extract of *Carica papaya* bark. The total number of eggs used for the research was 100 for both the control and test groups, with the control group exhibiting a hatchability rate of 98.2%. The eggs of *Ae. aegypti* subjected to various doses (100, 200, 300, 400, and 500 µg/mL) of *Carica*

papaya bark methanolic extract exhibited ovicidal action, leading to unsuccessful hatching. The hatchability rate was elevated at lower concentrations, but a rise in extract concentrations resulted in a decline in hatchability. The extract induced direct death of the eggs at elevated doses, particularly at 400 µg/mL and 500 µg/mL. These concentrations resulted in the mortality of a substantial number of eggs, inhibiting their advancement to the larval stage. The control group, unexposed to the extract, exhibited around 96% hatching success, so validating that the observed effects were attributable to the *Carica papaya* bark extract.

Table 4: Ovicidal activity of *Carica papaya* bark methanolic extract against *Aedes aegypti*

Treatment	Egg hatchability					
	Concentration (µg/mL)					
<i>Carica papaya</i>	Control	100	200	300	400	500
	96.2±1.92 ^a	80.2±1.30 ^b	42.0±1.58 ^c	5.6±1.34 ^d	NH	NH

Values were means±SD of five replicates.

Different letters indicated significant differences (P<0.05)

NH no hatchability

Conclusion

The methanolic extract of *Carica papaya* bark has significant mosquitocidal efficacy at many stages of the *Aedes aegypti* lifecycle, including larvicidal, pupicidal, ovicidal, and adulticidal activities. The benefits are likely attributable to the presence of bioactive substances, including alkaloids, flavonoids, and phenolic compounds, as shown by phytochemical profiling. The substantial decline in mosquito populations at all stages indicates that *Carica papaya* bark extract may serve as a viable natural option for managing *Aedes aegypti* in regions impacted by dengue illness. The extract's diverse action—affecting larvae, pupae, eggs, and adults—augments its efficacy in integrated vector control programs. In light of growing apprehensions over the detrimental impacts of chemical pesticides on the environment and human health, *Carica papaya* bark extract offers a safer, environmentally sustainable option for mosquito management. It may decrease dependence on synthetic pesticides, alleviating their negative effects on non-target species, such as beneficial insects and aquatic creatures. In conclusion, *Carica papaya* bark extract shows considerable potential as a natural, sustainable method for regulating *Aedes aegypti* populations and perhaps reducing the transmission of dengue illness.

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