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Research Article

COMPARATIVE EVALUATION OF LARVICIDAL CAPABILITY OF *CARICA PAPAYA* METHANOLIC EXTRACTS: LEAF EXHIBITS SUPERIOR THAN ROOT AND LATEX AGAINST *AEDES AEGYPTI*

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ABSTRACT
Mosquito borne disease particularly those which transmitted by *Aedes aegypti (Ae. aegypti)* are leading to a rising incidence of dengue and chikungunya among tropical regions, including India. Carica *papaya (C. papaya)* is a traditional plant known for its therapeutic uses in dengue fever, malaria, menstrual disorders, cancer, arthritis and diabetes. In addition to these benefits, it showed larvicidal potential for mosquito vector control. Therefore, we investigated *C. papaya* botanicals for their effectiveness in inducing mortality in *Ae. aegypti* larvae. Fresh leaves, root and latex part of *C. papaya* were collected from fields of Gorakhpur district, and their methanolic extracts were prepared using soxhlet apparatus. Various concentrations, specifically 100, 200, 300, 400 and 500; ppm were used to treat *Ae. aegypti* larvae at two-time intervals: 24 and 48 hours (h), with five replicates for each treatment. All plant extracts showed significant larvicidal activity compared to the deionised water control. The LC_{50} values of the leaf, latex, and root extracts of *C. papaya* after 24 h were 383.68, 493.42, 667.50; ppm, while the LC_{90} values were 1538.45, 1817.69, 2354.48; ppm respectively. After 48 h, the LC_{50} values for the leaf, latex, and root extracts were 180.58, 266.48, and 383.10 ppm, and the LC_{90} values were 615.76, 949.94, and 1809.95 ppm, respectively. For the synergistic combinations (leaf + latex, leaf + root, and latex + root), the LC_{50} values after 24 h were 235.00, 305.82, and 396.20; ppm, while the LC_{90} values were 799.69, 1383.12, and 1766.32; ppm, respectively. After 48 h, the LC_{50} values for the same combinations were 116.91, 163.34, and 218.25; ppm, and the LC_{90} values were 323.87, 562.14, and 759.44; ppm, respectively. Methanolic extracts of C. papaya were found to exhibit larvicidal activity against Ae. aegypti larvae, the leaf extract showed the strongest larvicidal activity, followed by the latex and root extracts.

Keywords: *Larvicidal activity, Toxicity assessment, Aedes aegypti, Dengue vector, Latex of papaya*

INTRODUCTION
Mosquito-borne diseases, particularly those transmitted by *Aedes aegypti* (*Ae. aegypti*) such as dengue, and chikungunya, remain a significant threat to global public health including India (Yang *et al*., 2009). Dengue fever is transmitted through the bite of a female *Ae. Aegypti* mosquito that becomes infected after feeding on a person that already carrying the virus (Goyal and Shinde, 2020). Countries with tropical climates face a heightened risk of infectious diseases such as India due to the impacts of climate change (Mohankumar *et al.*, 2016) Moreover, it has the potential to trigger epidemics in communities when a new serotype emerges (Moreno-Sanchez *et al.*, 2006; Benelli and Mehlhorn, 2016). *Ae. aegypti* has transmitted over 7.6 million dengue cases, among them 3.4 million confirmed cases, 16380 severe cases, and over 3000 deaths, were reported to WHO by April 30, 2024 from around the world (Dengue - Global Situation, 2024). In India, 4,75,802 dengue cases found and 645 deaths reported from January 2023 to October 2024. Though, dengue related 35743 illness and 37 deaths were reported from Uttar Pradesh alone (*Dengue Situation In India*, 2024). Larvicides are the better option for mosquito control because they either kill the larvae or act as a growth inhibitor upto adults (Konno, 2011).

Vector control is a viable option for reducing the spread of vector-borne diseases. Traditional vector control strategies, including Synthetic pesticides are effectively work on mosquito killing, but it showed resistant mosquito populations and bio amplification in the food chain including frequently hazardous to people and non-target animals (Malathi and Vasugi, 2015). In response, there is growing interest in exploring plant-based alternatives that are eco-friendly, biodegradable, and potentially less toxic to humans and animals. Alternative options are the need an hour for this significant condition develop by mosquitoes including *Ae. aegypti.*

Larvicidal medicinal phyto extracts especially traditional plants is of the most important factor for reducing the mosquito borne diseases and now preferred in integrated mosquito management (Sanjeev and Sushil, 2022). *Carica papaya (C. papaya)*, a member of the Caricaceae family, can be identified by its feeble and normally an unbranched soft stem. It produces abundant amounts of white latex and is packed by a terminal cluster of enormous, long-stalked leaves (Yogiraj *et al.*, 2014). It is already reported that *C. papaya* leaves and latex possess various pharmacological properties, including antimicrobial, antioxidant, insecticidal activities and also used to treat a wide range of illnesses (Dagne *et al.*, 2021; Srivastava and Singh, 2020; Macalood *et al*., 2013). Botanicals of the *C. papaya* have been reported to be an excellent source of abundant bioactive compounds such as alkaloids, flavonoids, tannins and proteolytic enzymes, which may serve as larvicidal agents against mosquito larvae (Chandrasekaran *et al.*, 2018). Previous studies have shown that extracts from *C. papaya* can affect the survival and development of mosquito larvae (Sesanti *et al.*, 2014). However, a comparative evaluation of the larvicidal potency of its different plant parts has not been extensively studied particularly against *A. aegypti* larvae. Understanding which part of the plant demonstrates the highest efficacy could inform the development of targeted, plant-based larvicidal formulations.

In this perspective, we investigated the presence of secondary metabolites/phytochemicals and relative larvicidal potential of the methanol extracts of leaf, root and latex of *C. papaya* against *Ae. aegypti* larvae. The findings will add to an evidence base in support of botanical insecticides and further establish the most potent extract to be optimized for further development and use in integrated vector management strategies.

MATERIALS AND METHODS

Collection of C. papaya botanicals

The fresh leaves, root and latex of *C. papaya* were collected from the field of Gorakhpur district (as Figure 1. A, B and C). To prevent microbial contamination, the plant samples leaf and root were washed with tap water and then sterilized in 1% sodium hypochlorite. Following that, the plant component latex was dried in the presence of sunlight at low intensity taken 5-6 days. Then air-dried these parts were ground using an electric blender. Fresh latex was collected early morning from healthy, mature plants growing in pesticide-free areas to ensure the purity and integrity of the sample. The unripe green fruits were carefully selected, and small incisions were made on their surface using a sterile stainless-steel blade. The exuding white milky latex was collected in clean, dry glass containers directly beneath the incisions, taking care to avoid contamination with plant debris or external materials. In the laboratory, the latex was filtered through a double layer of muslin cloth to remove any particulate matter or impurities and then stored at 4°C in airtight containers until further use for methanolic extraction and larvicidal bioassays.

Preparation of Extracts from Soxhlet apparatus

Each time of soxhlation we took 100 g of plant extract and 300 ml organic solvent methanol, over the course of eight hours (Figure 1). Using whatman number 1 filter paper for thimble, that was filtered plant components. After that the extracted plant material rotating in a vacuum evaporator and then plant extracts were dried off by evaporation. For latex extract, 10 g of latex was weighed, added to 100 ml of methanol and allowed to stand in an orbital shaker for 24 h at 37°C. Latex mixtures were then filtered and latex mixtures prepared were concentrated using rotary vacuum evaporator to evaporate out solvents.

Stock and working solutions preparation

Phytochemical Analysis

Phytochemical analysis is an essential step in identifying bioactive compounds responsible for biological activities such as insecticidal, larvicidal, and antimicrobial effects. The prepared extract of all three parts of botanicals were utilized to test various phytoconstituents present in them. Various chemical reagents were created, and tests were conducted for particular phytochemicals screening. All chemicals and solvents were commercially available from Rankem Chemicals, India, and were utilized without additional purification. The qualitative methods of phytochemical detection were carried out following the standard protocol (Table 1). In the present study, qualitative screening of *C. papaya* leaf, latex, and root methanolic extracts revealed the presence of multiple secondary metabolites, though their concentration and diversity varied among plant parts.

Table 1: Qualitative methods of phytochemical detection in C. papaya extracts

Phytochemicals	Test Method	Observation	Reference
Alkaloids	Wagner's / Dragendorff's test	Precipitate formation (white, brown, or orange)	(Harborne, 1998)
Flavonoids	Alkaline reagent test	Yellow coloration that disappears on acidification	(Kokate, 2014)
Tannins	Ferric chloride test	Blue-black or green coloration	(Sofowora, 1993)
Saponins	Foam test	Persistent froth formation	(Trease and Evans, 1989)
Phenols	Ferric chloride test	Deep blue or black coloration	(Harborne, 1998)
Terpenoids	Salkowski test	Bluish-green coloration	(Harborne, 1998)
Steroids	Liebermann-Burchard test	Reddish-brown ring at the interface	(Sofowora, 1993)
Anthraquinones	Borntrager's test	Formation of a pink to red color in the ammoniacal layer	(Harborne, 1998)
Resins	Alcohol test	Appearance of turbidity or precipitate upon addition of alcohol	(Khandelwal, 2008)

Collection and identification of Ae. aegypti larvae

Fully fed larvae were collected from various stagnant water sources at multiple location of Gorakhpur using a transparent closed container with proper precautions. The larvae were examined and identified based on standard morphological keys (Kokate, 1999; Sofowora, 1993; Trease and Evans, 1989). Special attention was given to 3rd and 4th instar larvae, which are most suitable for larvicidal bioassay studies due to their consistent size and resilience. The presence of comb scales on the eighth abdominal segment with a single row of large median and stout submedian spines, which distinguish them from other mosquito species was key points for *Ae. aegypti* larvae as compared to *Culex* and *Anopheles* laravae (as Figure 1. D and E). 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 kept at room temperature under ambient conditions. Fish food pellets were provided as a nutritional source, fed once every two days to support healthy larval development.

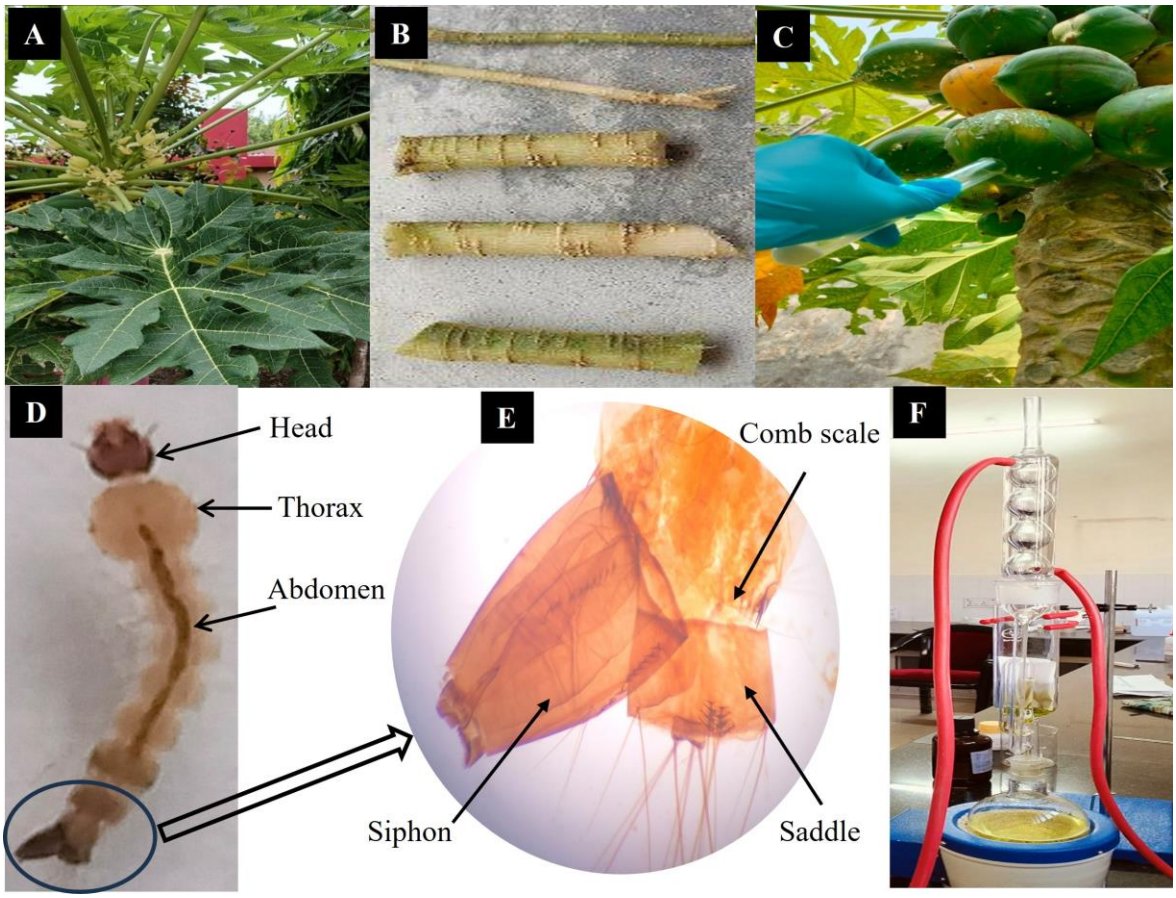

Figure 1. Collection of *Carica papaya* plant parts and indentifcation of *Aedes aegypti* larva; (A). Fresh leaves, (B). Root, (C). Latex in a sterile test tube from unripe mature fruit (D). whole body of *Aedes* larva at 10 X, (E). At 100 X magnification siphon showed the presence of short and stout, with one pair of tufts, saddle and single row comb scale as their key indetification and (F). Soxhlation process for preparation of methnolic extracts

Procedure of Bioassay testing

We used each 10 gm of leaf, root and latex were dissolved in 100 ml of methanol, which served as the 10% (w/v) stock solution. Then, a series of working dilutions ranging from 100 ppm to 500 ppm were prepared for larvicidal bioassay experiments. The larvicidal potential of methanolic extracts were evaluated through a standard mosquito larval bioassay protocol (Khandelwal, 2008). The bioassay was conducted in two experimental sets for 3rd and 4th instar larvae through individual extract of part testing and synergistic combination testing for evaluating the potential synergistic effects, combinations of extracts were prepared in equal ratios for the following pairs: leaf + latex, leaf + root, and latex + root.

For each treatment, a series of working concentrations (100, 200, 300, 400, and 500 ppm) were used against the group of twenty healthy *Ae. aegypti* larvae (III and IV instars) into 100 ml of each test solution in clean petri dishes. Each concentration and treatment were tested in triplicate to ensure reproducibility and statistical reliability. Control groups containing only methanol diluted in water were also maintained under the same conditions. Mortality of larvae was checked on 24 hours (h) and then 48 h on exposure. Larvae which did not respond to a slight touch with a glass rod were considered dead.

Statistical analysis

Larval mortality rate was evaluated for dosage and time dependent for single and combined extracts in term of Mean±standerd deviation (S.D.) and all replicates were combined for statistical analysis. Median

lethal concentration (LC_{50}) and the 90% lethal concentration (LC_{90}) were calculated by probit analysis of log transformed dose–mortality data by (POLO software version 2.0 (LeOra Software; (Khandelwal, 2008). A set of bioassays was considered valid if the CV was ≤25%, or if the 95% confidence intervals of the LC_{50} values overlapped (i.e., if there was significant difference at $P < 0.05$).

RESULTS

The phytochemical screening revealed that all three plant parts contain a wide range of secondary metabolites known for their bioactive properties (Table 2). In methanol extract, phytochemical analysis of *C. papaya* exhibited that the extract of leaves possessed higher flavonoids content, tannins, phenols and terpenoids, whereas latex had highest amount of proteins followed by steroids and resins. In contrast, the root extract was only positive for tannins, steroids and some amount of anthraquinones, but exhibited overall lower phytochemical richness.

Table 2. Qualitative analysis of Carica papaya botanical's extract in methanolic solvent

Phytochemicals/ Secondary metabolites	Leaf	Latex	Root
Alkaloids	+	+	+
Flavonoids	+++	++	+
Tannins	++	+	++
Saponins	++	+	+
Phenols	++	+	+
Terpenoids	++	++	+
Steroids	+	++	++
Anthraquinones	-	-	+
Resins	+	++	+

***Abbreviations**: Means +++ strongly present, ++ moderately present, + present, - Absent.*

Larvicidal effect of botanicals of C. papaya against Ae. aegypti larvae

The number of dead larvae is mentioned in mean and standard deviation in a table 3. The leaf extracts exhibited varying mortality rates of 15%, 25%, 40%, 50%, and 65% after 24 h. Similarly, the latex extract showed mortality percentages of 10%, 15%, 35%, 45%, and 55% within the same timeframe. The root extract demonstrated mortality rates of 5%, 15%, 25%, 30%, and 40% after 24 h. The corresponding LC_{50} values of leaf, latex and root extract of *C. papaya* after 24 h were 383.68, 493.42, 667.50; ppm and the LC_{90} were 1538.45, 1817.69, 2354.48; ppm; respectively (Table 4).

We extended the experiment duration to 48 h at the same concentration levels. The leaf extract's mortality rate increased to 30%, 45%, 70%, 80%, and 90%. The latex's larvicidal effect was slightly lower, reaching 25%, 35%, 50%, 60%, and 80% at identical concentrations after 48 h. Similarly, the root extract of *C. papaya* demonstrated effective mortality rates of 15%, 30%, 40%, 50%, and 60% at the same concentrations, which were lower than both leaf and latex extracts after 48 h. The LC_{50} values for leaf, latex, and root extracts of *C. papaya* after 48 h were 180.58, 266.48, and 383.10; ppm, respectively. The corresponding LC_{90} values were 615.76, 949.94, and 1809.95; ppm, respectively (Table 4).

Table 3. Larvicidal effect of extracts of leaf, latex and root of C. papaya in 24 and 48 h
Data are expressed as the mean ± S.D. Twenty larvae groups were exposed to different (ppm)

S. no.	Stock solution (in ppm)	Leaf		Latex		Root	
		24 h	48 h	24 h	48 h	24 h	48 h
1	control	0	0	0	0	0	0
2	100	2.6±1.01	6.2±1.16	1.6±1.01	4.4±1.35	0.8±.40	2.8±0.74
3	200	4.8±1.16	9.2±0.74	2.6±1.01	6.2±0.74	1.6±.80	5.8±0.74
4	300	7.8±0.74	14.4±1.01	6.6±1.01	9.8±0.74	4.4±1.01	8.2±0.74
5	400	10.2±1.16	15.6±1.2	8.6±1.01	12.6±0.8	5.6±1.01	10.2±0.74
6	500	12.6±1.01	17.8±0.74	10.2±1.16	16.6±1.01	8.2±.74	12±0.63

concentrations in containers. All experiments were repeated five times and mortalities were observed every 24–48h.

Table 4: Toxicity values of LC50 and LC90 of of *C. papaya* extracts of leaf, latex and root against *Ae. aegypti* larvae

Parts of *C. papaya*		LC_{50} (ppm)	95% confidence limits		LC_{90} (ppm)	95% confidence limits		Slop value	't' ratio	Hetero-geneity
			LCL	UCL		LCL	UCL			
Leaf	24 h	383.68	335.93	455.06	1538.45	1082.77	2716.19	2.13±0.27	7.95	0.40
	48 h	180.58	155.64	203.83	615.76	509.71	807.07	2.41±0.25	9.50	0.51
Latex	24 h	493.42	424.32	614.03	1817.69	241.89	3297.45	2.26±0.29	7.66	0.58
	48 h	266.48	236.72	299.81	949.94	741.60	1376.11	2.32±0.26	9.10	0.77
Root	24 h	667.50	546.63	930.62	2354.48	1486.08	5366.01	2.34±0.35	6.76	0.38
	48 h	383.10	30.85	465.01	1809.95	1199.08	3639.10	1.90±0.26	7.32	0.16

Statistical evaluation was performed (significant p-value below 0.05; including confidence limits (LCL = lower and UCL = upper limit); slope values (standard error); and t-ratio calculation for comparable differences to controls). The LC50 and LC90 indicate the concentration that kills 50 and 90% of Ae. aegypti larvae, respectively.

Synergistic effect of C. Papaya effects against *Ae. aegypti*

In addition to our initial experiments, we conducted synergistic tests combining different parts of *C. papaya* (leaf + latex, leaf + root, and latex + root), which yielded varying results. The combined larvicidal effect of leaf and latex showed mortality rates of 25%, 35%, 50%, 65%, and 90% after 24 h. The leaf and root combination were less effective, with mortality rates of 20%, 30%, 45%, 55%, and 70% in the same timeframe. Furthermore, the latex and root combination resulted in mortality rates of 15%, 25%, 35%, 45%, and 65% at identical concentrations after 24 h (Table 5). The LC_{50} values for these synergistic extracts were 235.00, 305.82, and 396.20; ppm, while the LC_{90} values were 799.69, 1383.12, and 1766.32;

ppm, respectively (Table 6). These synergistic experiments demonstrated that the leaf and latex combination was more effective than the other combinations after 24 h.

When we extended the experiment duration to 48 h at the same concentrations, the mortality rates for the leaf and latex combination increased to 50%, 65%, 80%, 100%, and 100%. The leaf and root combination showed lower mortality rates of 35%, 50%, 70%, 85%, and 100% after 48 h. The latex and root extract exhibited mortality rates of 25%, 40%, 55%, 70%, and 90%, which were lower than both other synergistic combinations at 48 h (**Table 5**). The LC_{50} values for the synergistic extracts after 48 h were 116.91, 163.34, and 218.25; ppm, while the LC_{90} values were 323.87, 562.14, and 759.44; ppm, respectively (**Table 6**). The corresponding LC_{50} values of synergistic extract of *C. papaya* after 48 h experiments (leaf+latex, leaf+root, and latex+root) were 116.91, 163.34, 218.25 and the LC_{90} were 323.87, 562.14, 759.44 respectively (**Table no.5**).

Table 5: Synergistic larvicidal effect of leaf, latex and root of *C. papaya* in 24 and 48 h

S. no.	Stock solution (in ppm)	Leaf + latex		Leaf + root		Latex + root	
		24 h	48 h	24 h	48 h	24 h	48 h
1	control	0	0	0	0	0	0
2	100	5.2±0.4	9.8±0.74	4.4±0.48	6.8±0.74	3.4±0.74	5.2±0.40
3	200	7±0.89	13.4±0.48	5.6±1.01	10.4±0.48	4.8±0.74	8.2±0.74
4	300	10.2±0.74	16.2±0.74	9.4±1.01	14.4±0.48	6.8±0.97	11.4±0.48
5	400	13.4±0.48	19.6±0.48	11.6±0.48	17.2±0.74	9.4±0.48	14.2±0.74
6	500	18.2±0.74	20±0	14.4±0.48	19.4±0.48	13.4±0.48	17.6±0.48

Table 6: Synergistic toxicity values of LC_{50} and LC_{90} of *C. papaya* extracts against *Ae. aegypti* larvae

Synergistic extracts of *C. papaya*		LC_{50} (ppm)	95% confidence limits		LC_{90} (ppm)	95% confidence limits		Slop value	't' ratio	Hetero-geneity
			LCL	UCL		LCL	UCL			
Leaf + latex	24 h	235.00	208.22	262.84	799.69	642.87	1099.97	2.41±0.25	9.47	0.99
	48 h	116.91	97.30	134.18	323.87	284.38	383.66	2.89±0.31	9.78	0.87
Leaf + Root	24 h	305.82	267.16	355.58	1383.12	977.13	2433.80	1.96±0.25	7.82	0.40
	48 h	163.34	128.11	194.19	562.14	442.67	832.41	2.39±0.25	9.39	1.71
Latex + Root	24 h	396.20	342.80	480.62	1766.32	1188.53	3419.05	1.97±0.26	7.55	0.50
	48 h	218.25	191.71	244.76	759.44	612.63	1039.84	2.37±0.25	9.35	0.45

DISCUSSION

The current research assessed the phytochemical content and larvicidal efficacy of various parts as leaf, latex, and root of *C. papaya* alone as well as the larvae and the synergistic effect among them against the

larvae of *Ae. aegypti*. We have previously found that methanolic extracts of botanicals were more effective against *Ae. aegypti* larvae in comparison to other solvent extracts (Kumar and Kumar 2024). So, we selected as the extracting solvent because of its strong power to extract various bioactive secondary metabolites from various parts of *C. papaya*. The qualitative screening of botanicals revealed that *C. papaya* leaf contained various bioactive compounds, particularly flavonoids, tannins, phenols and terpenoids, all of which have acknowledged pesticidal, antioxidant and antimicrobial activity (Harborne, 1998). Latex contained substantial amounts of flavonoids, terpenoids, steroids and resins, which might play a role in its moderate toxicity via enzymatic and membrane action (Marwah *et al.*, 2007). Meanwhile, the root extract had a limited category of phytochemical test, which tested positive for tannins, steroids, and trace of anthraquinones. Such restricted chemical mixture is probably responsible for its relatively moderate larvicidal activity**.**

Larvicidal assays revealed that all methanolic extracts used dilutions- and time-dependent toxicity against *Ae. aegypti* larvae. The leaf extract was the most potent among the parts investigated, with LC_{50} and LC_{90} values of 180.58 and 615.76; ppm, respectively, at 48 h. These observations are in line with the findings of earlier reports, which demonstrated strong larvicidal potential of the *C. papaya* leaf extracts due to flavonoid mediated oxidative stress and larval developmental inhibition (Govindarajan, 2013; Senthilkumar *et al.*, 2018). The latex and the root extracts were less efficient, as expected from the simpler chemical profiles and larger possibility that the concentration of bioactive compounds is lower.

The synergistic plant extract mixtures showed good larvicidal potential, particularly leaf and latex combination. This combination caused 100% mortality at the highest dose after 48 h and gave the lowest LC_{50} (116.91 ppm) and LC_{90} (323.87 ppm) values. The synergistic cytotoxic effect is probably attributable to the synergistic action by disturbance in gut membrane by the steroids in latex and the neurotoxic effect or metabolic interference by the flavonoids in leaves (Sarker *et al.*, 2021). The leaf + root and latex + root mixtures were less potent, but surpassed that of the pure root extract, indicating an interactive toxicity. The outcomes imply that a mixture of bioactive-enriched plant parts may amplify larvicidal activity over single-extract applications. This approach could provide an alternative to synthetic larvicides, which are currently confronted by concerns regarding, inter-alia, insect resistance and/or environmental toxicity(*Global Vector Control Response 2017-2030*, 2022; Kumar *et al.*, 2016).

The present work, although illustrating the larvicidal potential of *C. papaya* extract, has certain limitations. The bioactive constituents involved in the larvicidal activity were neither isolated nor quantified, and their mechanisms of action have not been elucidated. Furthermore, effects that are post-long observation period, residual and longer-term may have been missed.

CONCLUSIONS

The present study validates the larvicidal activity of *C. papaya* against *Ae. aegypti*, where the leaves extract had highest mortality rate for larvae among the individual parts of the plant. The chemical profile of the leaf, in particular its high flavonoid followed by tennis, saponins, phenols and terpenoids composition, can explain the enhanced larvicidal effect. Furthermore, the synergistic mixtures especially leaf + latex extract, showed significantly increased toxicity and lower LC value suggesting a potential approach against vectors. So, these findings suggest that *C. papaya*-derived botanical products as an environment-friendly, cheap, and bio-degradable larvicide against *Ae. aegypti*. Further studies need to conduct to isolate, identify such active compounds causing larvicidal activity, to find out mode of action, their field applicability/environmental safety in natural habitats.

CONFLICT OF INTEREST

None

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