Carica papaya (Papaya) latex: a new paradigm to combat against dengue and filariasis vectors *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae)

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Received: 8 May 2017 / Accepted: 7 January 2018 / Published online: 16 January 2018
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Abstract

This study manifests the larvicidal efficacy of *Carica papaya* latex extract and silver nanoparticles (CPAgNPs) synthesized using latex, against developing immature juveniles of *Aedes aegypti* and *Culex quinquefasciatus*. Briefly, the latex was collected and fractioned with different solvents such as chloroform, methanol and aqueously. The obtained crude extracts were subjected to larvicidal activity in the dose-dependent method. After 24 h, the mortality rate was calculated and statistically analyzed. From the results, it was demonstrated that the chloroform extract displayed prominent activity in IIrd and IIIrd instar larvae of *A. aegypti* and *C. quinquefasciatus* with better LC₅₀ values followed by methanol and aqueous extract. Subsequently, we profiled the qualitative analysis of a chloroform extract through biochemical tests; Fourier transform infrared spectroscopy and gas chromatography-mass spectrometry. Moreover, we authenticated the major secondary metabolites and activated larvicidal compound present in the extract. Further, we synthesized CPAgNPs using aqueous latex extract and challenged with IIrd and IIIrd instar larvae of *A. aegypti* and *C. quinquefasciatus*. Noticeably, the synthesized nanoproducts were showed 100% mortality in a 24-h treatment with significant LC₅₀ values. Hence, this study has opened up new vistas in the field of parasitological research to develop *Carica papaya* latex as a new stratagem in the insect vector management program.

Keywords *Aedes aegypti* · *Culex quinquefasciatus* · Latex · *Carica papaya* · Silver nanoparticles

Introduction

Plants and its extracts were practiced in traditional and ethnic medicine for the treatment of many diseases such as malaria, dengue fever, wound healing, viral and bacterial diseases, cancer, arthritis, diabetes, menstrual disorders and as supplementary food for normal metabolism and growth of humans (Shalan et al. 2005). India has a rich floral diversity and bioprospecting of plants in the view of pharmaceutical perspective was still going on. Until now, numerous plants

have been identified as a starting material as lead compounds for the development of next-generation drugs (Ghosh et al. 2012).

Tropical country like India was highly prone to infectious disease due to climate change and anthropogenic activities. Such tropical areas were the hot spots of mosquito (*Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus*) prevalence (Mohankumar et al. 2016). Mosquitoes act as vectors for parasites, pathogens causing malaria, dengue fever, and lymphatic filariasis. These dreadful diseases cost huge sum of people's death worldwide. Moreover, it also affects the government and individual economy every year (Benelli and Mehlhorn 2016; Benelli et al. 2016a). In Indian scenario, the incidence of these dreadful diseases were high in all parts of states, causing high mortality and morbidity rate (Chitra et al. 2014; Ravindran et al. 2015).

Conventionally, synthetic insecticides organochlorines, carbamates, organophosphates, and pyrethroids temephos, fenthion, malathion, and dichlorodiphenyltrichloroethane (DDT) were expensive, leaving a residual effect, adapting

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resistance, non-biodegradable, toxicity to non-target organisms (Anyaele and Amusan 2003). These problems urged the researchers for an expeditious search for new alternatives. Botanical-based insecticides were currently one of the most promising approaches, much research currently being devoted to plant extracts for the development of sustainable botanical insecticides (Liang et al. 2015). Plant extracts contain a mixture of several chemical active ingredients and thus may be able to effectively kill the mosquito through a different mechanism (Pavela 2015). For the past two decades, numerous researches have been conducted on the biological activity of plant extracts against larvae of mosquitoes and insects, in that few plant extracts were commercialized. This shows that plant extracts were environmentally safe, non-toxicity against humans and other organisms (Govindarajan et al. 2016b).

Latex was a fluid secreted by laticifer cells in plants. The biological role of latex secretion in plants remains unknown, but several types of research proved that it has insecticidal property (Rajkuberan et al. 2015). Latex contains highly active chemicals such as aromatics, terpenoids, alkaloids, phenols, glycosides, carbohydrates and proteins (Konno 2011). Further, in ancient time, latex was practiced in traditional and folk medicine for treating ailments and diseases. With this rationale, our research intends to evaluate the efficacy of *Carica papaya* latex against larvae of *Aedes aegypti* and *Culex quinquefasciatus*. The study was carried out in two different approaches. First, latex extract fractionated using solvent was evaluated against mosquito larvae. Second, silver nanoparticles were synthesized using latex and evaluated against larvae.

Materials and methods

Latex collection and processing

The plant *C. papaya* was located in and around of Bharathidasan University campus (10.6747°N, 78.7442°E) and taxonomically identified in the Botanical Survey of India, South circle, TNAU campus, Coimbatore, India (Fig. 1a). For the latex collection, Macalood et al. (2013) methodology was adopted with minor modification. Briefly, the latex was collected early morning at 6.00–7.00a.m. In unripe mature fruit surface, 6–8 longitudinal incisions at 3 mm deep were made of the fruit stalk using a stainless steel knife. As a result, latex exuded out and collected in a sterile bottle (Fig. 1b). After collection, the latex was poured on a Petri plate and kept in hot-air oven at 45 °C for 24 h for drying. Then, the dried latex was triturated using a mortar and pestle. Finally, the obtained powder form of latex was used for the study.

Latex extract preparation

To prepare latex extracts, 5 g of latex extract was weighed and mixed with 100 ml of chloroform; methanol; double-distilled water and kept in an orbital shaker for 24 h at 37 °C. After then, latex mixtures were filtered through Whatman filter paper no: 1 and the resulting latex mixtures were concentrated using rotary vacuum evaporator for removal of solvents. Further, the obtained crude extracts were stored at – 4 °C for further use.

Carica papaya latex silver nanoparticles (CPAgNPs)

The synthesis of *C. papaya* latex-mediated CPAgNPs was achieved using our previously validated method reported elsewhere (Rajkuberan et al. 2016b).

Fig. 1 a *Carica papaya* tree and b latex collection in sterile bottle from unripe mature fruit

Mosquitocidal activity

Collection of eggs and maintenance of larvae

The eggs were obtained from Indian Council of Medical Research (ICMR), Madurai, Tamil Nadu, India. Further, the eggs were maintained [27 ± 2 °C, 75–85% R.H., 14:10 (L:D) photoperiod] and placed in $18 \times 13 \times 4$ cm plastic containers containing 500 ml of tap water, waiting for hatching. Larvae were fed with a mixture of dog biscuits and yeast (3:1) ratio.

Larvicidal activity of latex extracts and CPAgNPs

According to the standard, World Health Organization larval susceptibility test method (WHO 2005) was conducted. Briefly, 1 g of latex extracts were dissolved in 100 ml of acetone considered as 1% stock solution. From the stock solution, different concentrations 100, 200, 300, 400 and 500 ppm/l and CPAgNPs 10, 20, 30, 40 and 50 ppm/L were prepared. IInd and IIIrd instar larvae of *A. aegypti* and *C. quinquefasciatus* were introduced into the 500 ml glass beaker containing 249 ml of dechlorinated tap water and 1 ml of desired concentrations of *C. papaya* latex extracts (100, 200, 300, 400 and 500 ppm/l) and CPAgNPs (10, 20, 30, 40 and 50 ppm/l) was added separately and kept in an environmental chamber at 25 °C with a 16:8 light/dark cycle. Three replicates were set up for each concentration and larvae placed in dechlorinated water without acetone considered as control. Papaya latex extracts were subjected to dose response assay against IInd and IIIrd instar larvae of *A. aegypti* and *C. quinquefasciatus*. Different concentrations of Papaya latex extracts (100–500 ppm) and CPAgNPs (10–50 ppm) were assayed for larvicidal activity. After 24 h, the numbers of dead larvae were counted and mean percentage of mortality rate was calculated from the average of three replicates.

Mortality was corrected using Abbott (1925) formula and percentage mortality was calculated as follows:

$$\text{Percentage mortality (\%)} = \frac{(X - Y)}{X} \times 100$$

Here *Y*—number of live larvae treated; *X*—number of live larvae introduced; Mortality was assessed after 24 h of exposure to ascertain the acute toxicity of IInd and IIIrd instar larvae of *A. aegypti* and *C. quinquefasciatus*.

Statistical analysis

The mortality data were subjected to probit regression analysis. LC_{50} , LC_{90} , 95% confidence interval (upper confidence limit) and (lower confidence limit), Chi-squares Intercept,

Chi-square test, linear regression, *F* and *R* value were determined using Statistical Package for the Social Sciences (SPSS) software (version 16).

Qualitative analysis of *C. papaya* latex extract

According to Harborne (1998), the phytochemical analysis was performed to determine the presence of bioactive secondary metabolites such as alkaloids, terpenoids, tannins, flavonoids, phenols, proteins and carbohydrates.

Fourier transform infrared spectroscopy analysis (FTIR)

To identify the presence of functional groups, a thin film of sample (latex extract) was applied to the glass plate and IR Perkin Elmer spectrophotometer, Spectrum Instrument, Germany.

Gas chromatography–mass spectrometry (GC–MS) analysis

The analysis was performed to know the active ingredients present in *C. papaya* latex extract. For analysis, GC–MS QP 2010 (Shimadzu) instrument was used. For analysis, the following conditions were performed: the column was fused with silica capillary column, 30×0.25 mm ID; coated with DB-I, 0.25 µm film thickness. Subsequently, the column temperature was programmed to 70–250 °C at the rate of 10 °C/min increase, injection port temperature at 250 °C. Helium was used as the carrier gas at a constant pressure of 100 kPa and flow rate of 20 ml/min. Samples which dissolved in chloroform were run fully at a scan range of 60–550 amu and the results were compared using National Institute of Standards and Technology (NIST) Spectral library search program.

Results and discussion

Carica papaya was an important commercial crop throughout the world. The ethnobotanical value of *C. papaya* fruit was also well known. Even today, *C. papaya* leaves are being used for treating viral infectious diseases chikungunya, dengue fever (Aravind et al. 2013). Such kind of therapeutic properties the plant bestows.

In this study, we have evaluated the efficacy of *C. papaya* latex extracts against IInd and IIIrd instar larvae of *A. aegypti* and *C. quinquefasciatus*. The analyses result exhibited as Tables 1, 2, 3, 4 and 5, *C. papaya* exhibited effective larvicidal activity in a dose-dependent manner. Among the latex extracts tested, chloroform extract had the most pronounced larvicidal activity (*A. aegypti* IInd instar 90.33%; IIIrd instar

Table 1 Mortality rate (percentage) of *C. papaya* latex extracts against *A. aegypti* in 24 h

<i>C. papaya</i> latex extract	Concentrations (ppm/l)	Percent mortality (IInd larvae)	Percent mortality (IIIrd larvae)
Chloroform	100	46.66 ± 2.88	53.33 ± 2.88
	200	60.00 ± 5.00	65.00 ± 5.00
	300	73.33 ± 2.88	78.33 ± 2.88
	400	81.66 ± 2.88	88.33 ± 2.88
	500	90.00 ± 5.00	98.33 ± 2.88
Methanol	100	33.33 ± 2.88	33.33 ± 2.88
	200	45.00 ± 5.00	46.66 ± 2.88
	300	58.33 ± 2.88	61.66 ± 2.88
	400	68.33 ± 2.88	75.00 ± 5.00
	500	80.00 ± 0.00	85.00 ± 5.00
Aqueous	100	16.66 ± 2.88	20.00 ± 0.00
	200	35.00 ± 5.00	31.66 ± 5.00
	300	43.33 ± 2.88	38.33 ± 2.88
	400	55.00 ± 5.00	51.66 ± 5.00
	500	61.66 ± 2.88	60.00 ± 0.00

Values are mean ± SD of three replicates

Table 2 Mortality rate (percentage) of *C. papaya* latex extracts against *C. quinquefasciatus* in 24 h

<i>C. papaya</i> latex extract	Concentrations (ppm/l)	Percent mortality (IInd larvae)	Percent mortality (IIIrd larvae)
Chloroform	100	41.66 ± 2.88	43.33 ± 2.88
	200	55.00 ± 5.00	51.66 ± 2.88
	300	66.66 ± 2.88	63.33 ± 2.88
	400	73.33 ± 2.88	73.33 ± 2.88
	500	81.00 ± 2.88	83.33 ± 2.88
Methanol	100	31.66 ± 2.88	31.66 ± 2.88
	200	50.00 ± 5.00	41.66 ± 2.88
	300	60.00 ± 0.00	56.66 ± 2.88
	400	66.66 ± 2.88	63.33 ± 5.00
	500	78.33 ± 2.88	68.33 ± 5.00
Aqueous	100	20.00 ± 5.00	20.00 ± 0.00
	200	35.00 ± 5.00	31.66 ± 5.00
	300	43.33 ± 2.88	38.33 ± 2.88
	400	55.00 ± 5.00	51.66 ± 5.00
	500	61.66 ± 2.88	60.00 ± 0.00

Values are mean ± SD of three replicates

98.33%; *C. quinquefasciatus* IInd instar 81.00% IIIrd instar; 83.33%) followed by methanol extract (*A. aegypti* IInd instar 80.00%; IIIrd instar 85.00%; *C. quinquefasciatus* IInd instar 78.33% IIIrd instar; 68.33%) and aqueous extract exhibited the least activity (*A. aegypti* IInd instar 61.66%; IIIrd instar 60.00%; *C. quinquefasciatus* IInd instar 61.66% IIIrd instar; 60.00%). The corresponding LC₅₀ values of chloroform,

Table 3 Mortality rate (percentage) of *C. papaya* latex silver nanoparticles against *A. aegypti* and *C. quinquefasciatus* in 24 h

Mosquito	CPAgNPs concentration (ppm)	(% mortality (24 h))	(% mortality (24 h))
<i>A. aegypti</i>	10	66.66 ± 2.88	58.33 ± 2.88
	20	78.33 ± 2.88	75.00 ± 5.00
	30	88.33 ± 2.88	81.66 ± 2.88
	40	95.00 ± 0.00	90.00 ± 5.00
	50	100.00 ± 0.00	100.00 ± 0.00
<i>C. quinquefasciatus</i>	10	60.00 ± 5.00	46.66 ± 7.63
	20	78.33 ± 2.88	61.66 ± 2.88
	30	85.00 ± 0.00	78.33 ± 2.88
	40	90.00 ± 0.00	88.33 ± 2.88
	50	98.33 ± 2.88	96.66 ± 2.88

Values are mean ± SD of three replicates

methanol, aqueous extract of *C. papaya* latex in IInd and IIIrd instar larvae of *A. aegypti* were 124.43, 107.30; 207.11, 187.81; 346.02, 387.04 ppm and in IInd and IIIrd instar larvae of *C. quinquefasciatus* LC₅₀ was 149.00, 152.79; 199.48, 236.94; 349.11, 387.04 ppm. The descending order of toxicity is a chloroform extract followed by methanol extract and aqueous extract. The distinct larvicidal activity between extracts suggesting the existence of a qualitative/quantitative differences in metabolite content of extracts (Meyer and Paul 1992). Moreover, other factors such as solvent used, susceptibility differences of mosquito vectors used might also contribute to the variation in efficiency of latex extracts.

In a previous study, we determined that the chloroform extract of *Euphorbia antiquorum* latex had an effective action against IInd, IIIrd and IVth instar larvae of *A. aegypti* and *C. quinquefasciatus* (Rajkubera et al. 2017). From this point of view, our study also confirms the results, but, however, the *C. papaya* latex extracts efficacy level was significant in causing mortality of both *A. aegypti* and *C. quinquefasciatus* larvae. Comparing the lethal concentration determined by us with the results of *E. tirucalli* latex (Yadav et al. 2002); *C. procera* latex (Shahi et al. 2010) and *F. benghalensis* latex (Ali and El-Rabaa 2010), we can note that the extract from *C. papaya* latex shows an efficacy in terms of acute toxicity similar to the above-mentioned latex reports.

As reported, the phytochemicals exert their mechanism of action in mosquitoes by targeting various sites such as inhibition of acetylcholinesterase, GABA-gated chloride ion channel, sodium and potassium ion exchange disruption, cytochrome P450, hormonal disruption, osmosis and ionic imbalance, mitotic poisoning, inhibition of cholinergic system, neuromuscular coordination (Benelli 2015). Hence, we speculate that the above mode of mechanism might put

Table 4 Larvicidal activity of *C. papaya* latex extract and silver nanoparticles against *A. aegypti* *I*nd and *III*rd instar larvae

Latex extract	LC ₅₀ (ppm)	Confidence interval (95%)		LC ₉₀ (ppm)	Confidence interval (95%)		Intercept	Chi-square test	Linear regression	F value	R
		Lower	Upper		Lower	Upper					
<i>A. aegypti</i> II nd instar larvae											
Aqueous	346.028	298.029	417.033	1812.32	1180.82	3801.10	- 4.525	0.322	9.33X + 0.110	96.088	0.985
Methanol	203.119	167.896	236.781	1115.52	792.299	1985.63	- 3.998	3.237	22.00X + 0.117	1.83	0.999
Chloroform	124.435	92.664	151.231	630.58	492.50	937.902	- 3.809	0.322	37.833X + 0.108	201.190	0.993
CPAgN PS	1.461	0.188	2.450	6.452	4.425	17.237	- 0.327	6.131	59.16X + 4.08	13.168	0.902
<i>A. aegypti</i> III rd instar larvae											
Aqueous	387.047	324.271	497.590	2594.470	1489.17	7359.42	- 4.014	1.695	10.33X + 0.100	385.714	0.996
Methanol	187.818	157.995	215.582	810.838	628.113	1204.91	- 4.587	4.249	20.83X + 0.132	693.444	0.998
Chloroform	107.300	12.201	170.253	423.817	271.557	3064.78	- 4.362	10.454	42.66X + 0.113	867.000	0.998
CPAgNPS	1.767	0.109	2.947	7.094	4.602	40.462	- 0.525	9.712	51.496X + 4.917	114.723	0.987

The control has nil mortality. Values were significant at the $P < 0.05$ level

LC₅₀ lethal concentration that kills 50% of the exposed larvae, LC₉₀ lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit (95% fiducial limit), LCL lower confidence limit (95% fiducial limit), χ^2 Chi square, linear regression, F and R value

Table 5 Larvicidal activity of *C. papaya* latex extract and silver nanoparticles against *C. quinquefasciatus* *I*nd and *III*rd instar larvae

Latex extract	LC ₅₀ (ppm)	Confidence interval (95%)		LC ₉₀ (ppm)	Confidence interval (95%)		Intercept	Chi-square test	Linear regression	F value	R
		Lower	Upper		Lower	Upper					
<i>C. quinquefasciatus</i> <i>I</i> nd instar larvae											
Aqueous	344.117	292.432	423.595	2125.54	1299.14	5191.89	- 4.111	0.455	12.00X + 0.103	180.188	0.992
Methanol	199.482	163.105	233.742	1154.73	808.348	2128.50	- 3.865	1.158	24.33X + 0.110	096.088	0.985
Chloroform	149.000	109.753	181.828	1028.00	713.231	1985.66	- 3.320	1.075	34.16X + 0.098	155.866	0.991
CPAgNPS	1.583	1.047	2.042	6.861	5.751	8.828	- 0.401	4.244	55.83X + 4.417	41.512	0.966
<i>C. quinquefasciatus</i> <i>III</i> rd instar larvae											
Aqueous	387.04	324.27	497.59	2594.47	1489.1	7359.42	- 4.014	1.695	10.333X + 0.100	385.714	0.996
Methanol	236.94	192.44	284.90	1893.32	1134.6	5061.53	- 3.372	0.946	23.833X + 0.095	079.244	0.982
Chloroform	152.79	113.34	185.94	1063.30	731.76	2090.15	- 3.322	4.155	32.500X + 0.102	1.59	0.999
CPAgNPS	2.476	1.068	3.476	8.725	6.124	21.915	- 0.923	6.926	36.333X + 6.333	149.380	0.990

The control has nil mortality. Values were significant at the $P < 0.05$ level

LC₅₀ lethal concentration that kills 50% of the exposed larvae, LC₉₀ lethal concentration that kills 90% of the exposed larvae, UCL upper confidence limit (95% fiducial limit), LCL lower confidence limit (95% fiducial limit), χ^2 Chi square, linear regression, F and R value

Table 6 Phytochemical analysis of *C. papaya* latex extract

S. No.	Functional groups	Indications
1	Alkaloids	+++
2	Tannins	–
3	Phenols	–
4	Alcohols	+
5	Protein	+
6	Terpenoids	+
7	Lipids	+
8	Terpenoids	+

Here + presence of metabolites; – absence of metabolites; +++ metabolite present in high amount

forth by *C. papaya* latex extracts against larvae of *A. aegypti* and *C. quinquefasciatus*.

Another important assay we conducted was the larvicidal efficacy of silver nanoparticles synthesized using latex *C. papaya*. As reported earlier, the synthesis of highly stable, crystalline CPAgNPs with improved surface chemistry was achieved using the aqueous latex extract of *C. papaya* (Rajkuberan et al. 2016b). Tables 3, 4 and 5, showed that *C. papaya* latex synthesized silver nanoparticles revealed 100% mortality in IIInd and IIIrd instar larvae of *A. aegypti* (LC₅₀ 1.46; 1.76 ppm), while 97% mortality observed in IIInd and IIIrd instar larvae of *C. quinquefasciatus* (LC₅₀ 1.58; 2.47 ppm). Hence, our findings corroborate with other reports, the larvicidal activity of AgNPs synthesized from the latex of *Plumeria rubra* (Patil et al. 2012a), *Perularia daemia* (Patil et al. 2012b) and *Euphorbia antiquorum* (Rajkuberan et al. 2016a). Concerning the toxicity, *C.*

papaya latex was a safe therapeutic agent, evidenced by the reported toxicity studies of *C. papaya* in catfish *Clarias gariepinus* (Ayotunde et al. 2011) and Wistar albino rats (Oduola et al. 2007).

In this study, we have synthesized spherical-shaped nanoparticles with an average size of 12 ± 6 nm. Size and shape was an important determining factor for any biological assay to evaluate (Sathishkumar et al. 2017). Herein, the particles with a smaller size and the coating of natural products (protein) in nanoparticles accelerate the activity thereby resulting in 100% mortality. Little precise mechanisms have been hypothesized, the toxicity leading to death in mosquito larvae exposed to green-synthesized nanoparticles. Penetration of AgNPs through the exoskeleton and binds to the sulphur from proteins or phosphorous from DNA resulting in blockage of translation/transcription, failure of respiratory chain reaction, further the decrease in membrane permeability and disturbance in proton motive force may cause loss of cellular function and finally leads to death (Benelli et al. 2016b).

In recent years, numerous studies reported the green-synthesized AgNPs was a potent larvicidal and pupicidal agent against *Aedes aegypti*; *Culex quinquefasciatus*, *Ae. albopictus* and *Culex tritaeniorhynchus*. Silver nanoparticles synthesized using seaweed *Hypnea musciformis* have shown a considerable toxicity against *A. aegypti* and cabbage pest *P. xylostella* (Roni et al. 2015). Moreover, AgNPs synthesized using *Artemisia vulgaris* performed an effective toxicity against *A. aegypti* and did not negatively impact predation rates of the Asian bullfrog tadpoles, *Hoplobatrachus tigerinus* (Murugan et al. 2015). Govindarajan et al. (2016a, c) reported the biosynthesis of AgNPs from *Nicandra physalodes*, *Zornia diphylla* and demonstrated that the AgNPs

Fig. 2 FTIR spectroscopic analysis of *C. papaya* latex extract

Fig. 3 GC–MS chromatogram of *C. papaya* chloroform extract

were found safer to non-target organisms *Chironomus circumdatus*, *Anisops bouvieri*, *Gambusia affinis* and *Diplo-nychus indicus*. From the above-mentioned reports, it was fairly proved that AgNPs synthesized using biological entities hold a strong antagonism against *Aedes aegypti*; *Culex*

quinquefasciatus, *Ae. albopictus* and *Culex tritaeniorhynchus* and safer to non-target organism inhabiting along with mosquito larvae. This shows the possibility of *C. papaya* latex and CPaAgNPs could be developed as a new generation of pesticides in vector-management program.

Table 7 Tentative identified compounds present in the chloroform extract from latex of *C. papaya*

Peak#	R. time	Area	Area%	M.W	Mol formula	Name
1	7.723	258,973	0.26	159	C ₈ H ₁₇ NO ₂	L-Leucine, ethyl ester
2	9.124	148,307	0.15	168	C ₁₂ H ₂₄	Nonyl cyclopropane
3	9.241	157,059	0.16	170	C ₁₂ H ₂₆	Dodecane
4	10.411	2,486,533	2.50	184	C ₁₁ H ₂₀ O ₂	6-Octen-1-ol, 3,7-dimethyl-, formate
5	11.973	499,287	0.50	200	C ₁₃ H ₂₈ O	1-Tridecanol
6	12.071	235,208	0.24	226	C ₁₆ H ₃₄	Hexadecane
7	13.664	6,903,715	6.93	206	C ₁₇ H ₃₀ O-Si	Phenol, 2,4-bis(1,1-dimethylethyl)-
8	14.493	2,570,329	2.58	214	C ₁₄ H ₃₀ O	1-Tetradecanol
9	14.572	634,928	0.64	256	C ₁₇ H ₃₆	Heptadecane
10	14.696	326,249	0.33	222	C ₁₂ H ₁₄ O ₄	1,2-Benzenedicarboxylic acid, diethyl ester
11	16.324	367,770	0.37	282	C ₂₀ H ₄₂	2,6,10,14-Tetramethylhexadecane
12	16.485	657,173	0.66	228	C ₁₄ H ₂₈ O ₂	Tetradecanoic acid
13	16.751	4,160,472	4.18	284	C ₁₉ H ₄₀ O	1-Nonadecanol
14	16.815	620,836	0.62	352	C ₂₅ H ₅₂	Pentacosane
15	17.974	720,637	0.72	180	C ₁₂ H ₂₀ O	2-Octylfuran
16	18.265	510,131	0.51	276	C ₁₇ H ₂₄ O ₃	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
17	18.576	4,798,388	4.82	652	C ₃₈ H ₆₈ O ₈	l-(+)-Ascorbic acid 2,6-dihexadecanoate
18	18.799	4,694,000	4.71	284	C ₁₉ H ₄₀ O	1-Nonadecanol
19	18.982	229,851	0.23	212	C ₁₃ H ₂₄ O ₂	3,7-Dimethyl-6-octenyl propionate
20	19.342	519,623	0.52	552	C ₃₆ H ₇₂ O ₃	Palmitic acid, 2-(octadecyloxy)ethyl ester
21	19.534	1,415,563	1.42	270	C ₁₇ H ₃₄ O ₂	Heptadecanoic acid
22	19.820	208,718	0.21			
23	19.868	691,969	0.69	296	C ₁₉ H ₃₆ O ₂	9-Octadecenoic acid, methyl ester
24	20.327	8,904,199	8.94	268	C ₁₈ H ₃₆ O	(Z)-9-Octadecen-1-ol,
25	20.437	483,207	0.49	322	C ₂₁ H ₃₈ O ₂	n-Propyl linoleate
26	20.485	530,174	0.53	310	C ₂₀ H ₃₈ O ₂	Ethyl oleate
27	20.672	5,199,419	5.22	396	C ₂₇ H ₅₆ O	1-Heptacosanol
28	21.846	407,944	0.41	278	C ₂₀ H ₃₈	3,3'-Bi-p-menthane
29	21.883	220,103	0.22	187	C ₁₀ H ₂₁ NO ₂	N-(2-Hydroxyethyl)octanamide
30	21.928	649,949	0.65	299	C ₁₈ H ₃₇ NO ₂	Palmitoylethanolamide
31	22.153	1,169,110	1.17	224	C ₁₆ H ₃₂	Decylcyclohexane
32	22.422	1,102,484	1.11	252	C ₁₇ H ₃₄ O	14-Methyl-8-hexadecyn-1-ol
33	22.611	2,482,265	2.49	396	C ₂₇ H ₅₆ O	1-Heptacosanol
34	23.307	2,344,799	2.36			
35	23.731	6,342,443	6.37			
36	24.833	1,686,538	16.94	390	C ₂₄ H ₃₈ O ₄	Di-isooctyl phthalate
37	25.378	2,404,318	2.41	396	C ₂₇ H ₅₆ O	1-Heptacosanol
38	28.243	2,466,074	2.48	430	C ₂₂ H ₃₉ F ₅ O ₂	Nonadecyl pentafluoropropionate
39	28.701	1,138,283	1.14	410	C ₃₀ H ₅₀	Squalene
40	29.473	539,098	0.54	298	C ₁₉ H ₃₈ O ₂	Ethyl heptadecanoate
41	30.823	542,711	0.55	298	C ₂₀ H ₄₂ O	1-Eicosanol
42	31.262	498,510	0.50	283	C ₁₈ H ₃₇ NO	Octadecanamide
43	31.918	1,338,635	1.34	454	C ₃₁ H ₅₀ O ₂	Stigmasta-5,22-dien-3-ol, acetat, (3-beta, 22-z)
44	32.901	2,889,558	2.90	678	C ₄₇ H ₈₂ O ₂	Stigmast-5-en-3-ol, oleate
45	33.249	7,229,582	7.26	436	C ₂₃ H ₃₃ BrO ₃	16-Bromo-20-oxopregn-5-en-3-yl acetate

From the observation, we noticed that the chloroform extract had performed superior activity than other extracts. Further, we subjected the chloroform extract for its phytochemical profile study in a qualitative approach. From the study, a chloroform extract from the latex of *C. papaya* contains the major groups of secondary metabolites. Table 6 showed the presence/absence of major groups of secondary metabolites. As per the analysis except for tannins and phenols, all other functional groups were present in high amount.

In FTIR analysis (Fig. 2), the functional group of components was separated based on the peak ratio. The absorption band located at 2921.70 and 2851.90 cm^{-1} corresponds to C–H stretch. Vibration 1739.94 cm^{-1} may be attributed to the presence of C=O stretch. The band observed at 1465.58 and 1326.37 cm^{-1} corresponds to C–H, CH_3 C–H bends. Frequency 1027.31 cm^{-1} can be assigned to the C–O stretch, sharp peak at 772.29 cm^{-1} due to the C–Cl stretch. From the FTIR spectrum, it was obvious that alkanes, phenols, alcohols, carboxylic acids, alkenes, aliphatic compounds, carbonyl compounds, esters, phenyls carbonyl compounds, alkyl halides functional groups present in the chloroform extract from the latex of *C. papaya*.

GC–MS analysis of a chloroform extract from the latex of *C. papaya* identified the bioactive compounds and characterized according to their retention time, molecular formula, molecular weight, peak area (%) (Fig. 3). A total of 45 compounds were identified and listed in Table 7. The identified compounds were well-known bioactive molecules having bactericidal, fungicidal, nematocidal, mosquitocidal, and anti-cancer activities (Casuga et al. 2016). From the identified compounds, we speculate that all single-active compounds in the extract synergistically interacted with other compounds and catalyzed the larvicidal activity. But, however, we cannot justify it, the further extensive analysis were needed to harness the potential active constituents exist in chloroform extract of *C. papaya* latex.

Conclusion

Our study highlights the larvicidal efficacy of *C. papaya* latex and CPAgNPs against *A. aegypti* and *C. quinquefasciatus*. The obtained results of latex extracts and CPAgNPs were promising against developmental larval immature stages. Further, the phytochemical profiling studies explored the significant larvicidal compounds. Therefore, we conducted a fundamental study to validate the efficacy of latex and CPAgNPs only, against instar larvae of *A. aegypti* and *C. quinquefasciatus*. As an outcome of the study, *C. papaya* can be suggested to develop as bioinsecticides in vector management after subjecting to the elucidation of active compound structure and function, reference standard,

formulation methods, pilot scale and field trial study, non-target organism study.

Acknowledgements We hereby confirm that funding agencies had no role in the study and preparation of the manuscript.

Compliance with ethical standards

Conflict of interests We authors declare that there is no conflict of interests regarding the paper.

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