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ORIGINAL PAPER

Efficacy of larvicidal botanical extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract The present study explored the effects of crude leaf acetone, chloroform, hot water, methanol, petroleum ether (60–80°C), and water extracts of *Calotropis procera* (Ait) R. Br., *Canna indica* L., *Hibiscus rosa-sinensis* Linn., *Ipomoea carnea* Jacq. spp. *fistulosa* Choisy, and *Sarcostemma brevistigma* Wight that were selected for investigating larvicidal potential against second and fourth instar larvae of the laboratory-reared mosquito species, *Culex quinquefasciatus* Say, in which the major lymphatic filariasis was used. All plant extracts showed moderate larvicidal effects after 24 h of exposure at 1,000 ppm; however, the highest larval mortality was found in leaf acetone, chloroform, methanol, and petroleum ether of *C. indica* (LC_{50}=29.62, 59.18, 40.77, and 44.38 ppm; LC_{90}= 148.55, 267.87, 165.00, and 171.91 ppm) against second instar larvae (LC_{50}=121.88, 118.25, 69.76, and 56.31 ppm; LC_{90}=624.35, 573.93, 304.27, and 248.24 ppm) and against fourth instar larvae and acetone, hot water, methanol, and petroleum ether extracts of *I. carnea* (LC_{50}= 61.17, 41.07, 41.82, and 39.32 ppm; LC_{90}=252.91, 142.67, 423.76, and 176.39 ppm) against second instar larvae (LC_{50}= 145.37, 58.00, 163.81, and 41.75 ppm; LC_{90}=573.30, 181.10, 627.38, and 162.63 ppm) and against fourth instar larvae of *C. quinquefasciatus*, respectively. These results suggest that the acetone, methanol extracts of *C. indica* and hot water, petroleum ether extracts of *I. carnea* have the potential to be used as an ideal eco-friendly approach for the control of the major lymphatic filariasis vector, *C. quinquefasciatus*.

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Introduction

Culex quinquefasciatus is the most widely distributed mosquito in India, mainly found in urban and suburban areas. The most efficient approach to control the vector is to target the immature stages of the life cycle. Synthetic chemical larvicides continue to be applied for controlling mosquitoes in most parts of the world. But many of these chemicals are toxic to human, plant, and animal life, and resistance can be problematic in maintaining control, especially with organophosphate and pyrethroid larvicides. There has been serious concern about the use of chemical-based mosquito larvicides and repellents in recent past. As a result, researchers are currently investigating natural substances to use as insecticides for controlling larval mosquitoes. Plants may be a source of alternative agents for control of vectors because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable. Phytochemical insecticides have received much attention, in this regard, as they are considered to be more environmentally biodegradable and considered safer than synthetic insecticides (Moretti et al. 2002; Cetin et al. 2004). These problems have highlighted the need for the development of new strategies for selective mosquito larval control. Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the effectiveness of plant

extracts or essential oils against mosquito larvae (Rahuman et al. 2008a, b, c; Amer and Mehlhorn 2006a, b).

Calotropis procera, commonly known as 'Vellai erukku' in India, is a popular medicinal plant found throughout the tropics of Asia and Africa. Various parts of this plant have been widely used in traditional systems of medicine. The water, ethanol, acetone, and some other organic solvent extracts have insecticidal activity against *Sarcophaga haemorrhoidalis* (Moursy 1997) as well as anti-bacterial and anti-parasitic (Larhsini et al. 1999) activities. An ethanolic extract of the flower was reported to have anti-microbial, anti-inflammatory, antipyretic, analgesic (Mascolo et al. 1988), and anti-malarial activities (Sharma and Sharma 1999; 2001). The crude latex produced by the green parts of the plant was evaluated for its toxic effects upon egg hatching and larval development (Ramos et al. 2006); the methanol and fresh leaf extract showed larvicidal properties (Singh et al. 2005) and the latex aqueous phase showed activity on third/early fourth instar larvae (Markouk et al. 2000) against *Aedes aegypti*, *Anopheles stephensi*, *C. quinquefasciatus*, and *Anopheles labranchiae*. A cardiac glycosidal (cardenolide) extract isolated from *C. procera* was tested for their effects against larvae and adult stages of *Hyalomma dromedarii* (Al-Rajhy et al. 2003); the latex was used against the third stage larvae of *Musca domestica* (Morsy et al. 2001); decoction provided significant protection against termites *Diorhabda lusca* and *Piesmopoda obliquifasciella* (Bajwa and Rajpar 2001). The leaf extract of *Calotropis gigantea* used at various dose levels was potent in delaying development and in reducing adult emergence activity against *Sitophilus zeamais* (Haque et al. 2000); the petroleum ether–acetone extract showed larvicidal and adulticidal activity against *C. quinquefasciatus* (Neraliya and Srivastava 1996).

The effect of dried, coarsely powdered leaf, flower, rhizome, and seed benzene and methanol extracts of *Canna indica* showed significant central, peripheral analgesic activity in mice and anthelmintic activity against *Pheritima posthuma* (Nirmal et al. 2007); the water extracts of *Ipomoea carnea* subsp. *fistulosa* (aerial parts), *Vitex glabrata* (branch), *Vitex trifolia* (aerial part), *Vitex negundo* (aerial part), *C. indica* (rhizome), and *Justicia gendarussa* (aerial part) showed HIV-1 RT inhibition (Woradulayapinij et al. 2005); the active fractions of *Punica granatum* bark or *C. indica* root or in combination with other plant-derived molluscicides significantly inhibited the activity of acetylcholinesterase, acid/alkaline phosphatase, Na(+)K(+) ATPase, and lactic dehydrogenase in the nervous tissue of *Lymnaea acuminate* (Tripathi et al. 2004); the toxic effects of *P. granatum* and *C. indica* were tested against *L. acuminate* (Tripathi and Singh 2000). The molluscicidal activity potency of *C. indica* studied against *Bilinus truncate* increased according to its prolonged exposure to sub-lethal concentrations, and it was shown that the plant extracts produce an immediate effect against the fecundity and embryonic survival of the snail (Farag and Khalil 1990); an increase in molluscicide concentration at sub-lethal levels resulted in a significant reduction in embryo growth rate and embryo hatchability (Cheung and Lam 1998).

The herb *Hibiscus rosa-sinensis* is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colors of flowers. The larvicidal activity of methanol extract was evaluated against the *Aedes*(*s*) *albopictus* and *C. quinquefasciatus* (Nath et al. 2006), and the aqueous fraction exhibited a combination of weak spasmogenic and spasmolytic effects (Gilani et al. 2005). Dua et al. (2006) have reported that the aqueous extract from the roots of *Hibiscus abelmoschus* was evaluated against the larvae of *Anopheles culicifacies*, *A. stephensi*, and *C. quinquefasciatus*.

Alkaloids 3, 4, and 6 isolated from the leaves, flowers, and seeds of *I. carnea* showed a potent inhibitory activity toward rat lysosomal beta-glucosidase (Haraguchi et al. 2003). Saxena and Sumithra (1985) confirmed that the insecticidal effects of acetone leaf extract of *I. fistulosa* caused 70% larval mortality; 15% in pupae and 10% in adult emergence against *A. stephensi*. Poor farmers have developed a novel technique to use the extracts of *I. fistulosa* for controlling insect pests, such as the "pod-borer" in pigeon pea, and for the control of "boil-worms" in cotton in the Vidharbha region of Maharastra, India (Sinha 1998). The essential oil extracted by steam distillation from leaves of *Ipomoea cairica* was evaluated for their topical repellency effects against malarial vector *A. stephensi* (Rajkumar and Jebanesan 2007); it is most highly toxic to the larvae of *C. tritaeniorhynchus* followed by *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* (Thomas et al. 2004), and the steroidal alkaloid toxin isolated from *Ipomoea* species was reported poisonous to animals (Molyneux et al. 2007). *Ipomoea aquatica* extract was tested for their activity against fibroblast cell lysis after *Heterometrus laoticus* scorpion venom treatment (Uawonggul et al. 2006); the hot water extract of *Ipomoea sepiaria* was tested against *Dicladispa armiger* (Haque 2002). Hebling et al. (2003) reported that the laboratory colonies of the leaf-cutting ants *Atta sexdens* fed daily with leaves of *I. batatas* showed ant mortality and a significant decrease in the size of the fungal garden after the second week, with complete depletion of nests after 5 weeks of treatment.

Sarcostemma brevistigma grows throughout India and other tropical regions of the world. It is found to be active as an anti-rheumatic, anti-allergy, anti-emetic, and bronchodilator (Kirtikar and Basu 1993); the effect of chloroform-soluble fraction (F-A) of twigs of *S. brevistigma* on

contractions induced by KCl, histamine, and acetylcholine in the isolated guinea pig ileum and taenia coli smooth muscles has been evaluated (Kumar et al. 2007). Sacidum-lignan A (1) isolated from the ethanolic extract of the whole plant of *Sarcostemma acidum* showed moderate antimicrobial activities against two Gram-positive bacteria in an in vitro study (Gan et al. 2005); stem extract arrests spermatogenesis in male rats (Venma et al. 2002). Sethuraman et al. (2003) have reported decreased activities of GOT and GPT and increased level of ALP in CCl4-intoxicated rats fed with a crude extract of *S. brevistigma*.

Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of *Abutilon indicum*, *Aegle marmelos*, *Euphorbia thymifolia*, *Jatropha gossypifolia*, and *Solanum torvum* was assayed (Rahuman et al. 2008a); the extracts of peel and leaf extracts of *Citrus sinensis*, *Ocimum canum*, *Ocimum sanctum*, and *Rhinacanthus nasutus* (Bagavan et al. 2009); the water, hot water leaf, stem-bark, and flower extracts of *Acacia arabica*, *Cedrus deodara*, *Hibiscus rosa-sinensis*, *Mangifera indica*, *Nerium indicum*, *Nicotiana tabacum*, *Pongamia pinnata*, and *Solanum nigrum* (Rahuman et al. 2009); the extracts of the leaf of *Centella asiatica*, *Datura metal*, *Mukia scabrella*, *Toddalia asiatica*, and extracts of whole plant of *Citrullus colocynthis* and *Sphaeranthus indicus* (Rahuman et al. 2008b); the same solvent extracts of *C. sinensis*, *O. canum*, *O. sanctum*, and *Rhinacanthus nasutus* (Kamaraj et al. 2008a,b); extracts of the leaf and bark of *Ficus racemosa* (Rahuman et al. 2008d); the leaf extracts of *Acalypha indica*, *Achyranthes aspera*, *Leucas aspera*, *Morinda tinctoria*, and *O. sanctum* (Bagavan et al. 2008); and extracts of *Citrullus colocynthis*, *Coccinia indica*, *Cucumis sativus*, *Momordica charantia*, and *Trichosanthes anguina* (Rahuman and Venkatesan 2008) were assayed for their toxicity against the early fourth instar larvae of *C. quinquefasciatus*, *A. subpictus*, *C. tritaeniorhynchus, A. stephensi*, and *A. aegypti*. Chaubal et al. (2005) have reported that the alicyclic polyalcohol, which was found to be D-pinitol (=3-*O*-methyl-D-chiro-inositol; 1), isolated from the aerial parts of acetone extract of *Acacia nilotica* showed chronic toxicity against *A. aegypti* and *C. quinquefasciatus* fourth instar larvae. Aqueous extracts of *Piper retrofractum* showed the highest level of larvicidal activity against *C. quinquefasciatus* and *A. aegypti* (Chansang et al. 2005); the ethanolic and acetone extracts of *Nerium indicum* and *Thuja orientalis* (Sharma et al. 2005), the acetone and petroleum ether extracts of *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafetida*, and *Trigonella foenum graceum* (Harve and Kamath 2004), and the acetone fraction of the petroleum ether extract of seeds from *Argemone mexicana* (Sakthivadivel and Thilagavathy 2003) exhibited larvicidal and growth-inhibiting activity *A. stephensi*, *C. quinquefasciatus*, and *A. aegypti*.

The purpose of the present investigation was to explore the larvicidal activity of different solvent extracts from leaves of five plant species, *C. procera*, *C. indica*, *H. rosa-sinensis*, *I. carnea* spp. *fistulosa*, and *S. brevistigma*, against the lymphatic filariasis vector *C. quinquefasciatus*.

Materials and methods

The leaves of *Calotropis gigantean* (L.) W.T. Aiton (Asclepiadaceae), *Canna indica* L. (Cannaceae), *Hibiscus rosa-sinensis* Linn. (Malvaceae), *Sarcostemma brevistigma* Wight (Asclepiadaceae), and *Ipomoea carnea* Jacq. spp. *fistulosa* Choisy (Convolvulaceae) were collected from the Tamil Nadu Medical Plant Farms & Herbal Medicine Corporation Limited medicinal plant farm, Arumbakkam (13°13′ 4 N, 79° 59′ 7 E; altitude 118 ft), Chennai, Tamil Nadu and the taxonomic identification was made by Dr. B. Annadurai, Department of Plant Biology and Biotechnology, C. Abdul Hakeem College, Melvisharam, India.

Insect rearing

C. quinquefasciatus Say (Diptera: Culicidae) larvae were collected from a stagnant water area of Melvisharam (12° 56′ 23″ N, 79° 14′ 23″ E) and identified in Zonal Entomological Research Centre, Vellore (12° 55′ 48″ N, 79° 7′ 48″ E), Tamil Nadu to start the colony, and larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory as per the method of Rahuman et al. (2008d).

Preparation of plant extracts

The dried leaves (750 g) were powdered mechanically using a commercial electrical stainless steel blender and extracted with acetone (1,000 ml; Qualigens), chloroform (1,200 ml; Fine), methanol (2,000 ml; Qualigens), and petroleum ether (60–80°C, 2,200 ml; Qualigens) in a Soxhlet apparatus separately until exhaustion. The aqueous and hot water extracts were prepared as per the procedure of Chowdhury et al. (2008) and Ross and Brian (1977). The extract was concentrated under a reduced pressure of 22–26 mmHg at 45°C and the residue obtained was stored at 4°C.

The *C. quinquefasciatus* larvicidal activity was assessed by the procedure of WHO (1996) with some modification and as per the method of Rahuman et al. (2000, 2008d). From the stock solution, different concentrations ranging from 4.69 to 1,000 ppm were prepared. Based on the preliminary screening results, different solvent crude leaf extracts prepared from *C. indica* and *I. carnea* were subjected to dose–response bioassay for larvicidal activity against *C. quinquefasciatus*. The numbers of dead larvae

were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates. However, at the end of 24 h, the selected test samples turned out to be equal in their toxic potential.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using the software developed by Reddy et al. (1992). Results with $p<0.05$ were considered to be statistically significant.

Results and discussion

The preliminary screening is a good means of evaluating the potential larvicidal activity of plants popularly used for this purpose. Larvicidal activity of different solvent crude extracts of six plants are noted and presented in Table 1 and Fig. 1a. Among the crude extracts tested, the present results showed the highest larval mortality was found in the leaf acetone, chloroform, methanol, and petroleum ether of *C. indica* (LC_{50}=29.62, 59.18, 40.77, and 44.38 ppm; LC_{90}= 148.55, 267.87, 165.00, and 171.91 ppm) against second instar larvae (LC_{50}=121.88, 118.25, 69.76, and 56.31 ppm; LC_{90}=624.35, 573.93, 304.27, and 248.24 ppm) and against fourth instar larvae and acetone, hot water, methanol, and petroleum ether extracts of *I. carnea* (LC_{50}= 61.17, 41.07, 41.82, and 39.32 ppm; LC_{90}=252.91, 142.67, 423.76, and 176.39 ppm) against second instar larvae (LC_{50}= 145.37, 58.00, 163.81, and 41.75 ppm; LC_{90}=573.30, 181.10, 627.38, and 162.63 ppm) and against fourth instar larvae of *C. quinquefasciatus*, respectively. Chi-square value was significant at $p<0.05$ level (Table 2 and Fig. 1b).

It has been observed earlier that the whole latex of *C. procera* was shown to cause 100% mortality of third instars within 5 min and, when fractionated into water-soluble dialyzable and non-dialyzable fractions, were partially effective in preventing egg hatching; most of the individuals growing under experimental conditions died before reaching second instars or stayed in first instars, and besides, the fractions were very toxic to third instars causing 100% mortality within 24 h against *A. aegypti* (Ramos et al. 2006). The aqueous and ethanolic extracts of flowers and leaves of *C. procera* at 1,000 ppm did not exhibit any activity, and the aqueous phase latex showed activity (with LC_{50}=28 ppm) against *Anopheles labranchiae* (Markouk et al. 2000). Rahuman et al. (2009) have reported that the highest larval mortality was found in stem-bark hot water, acetone, and methanol extracts of *C. deodara* (LC_{50}=133.85, 141.60, and 95.19 ppm; LC_{90}= 583.14, 624.19, and 639.99 ppm) and leaf hot water, acetone, methanol, and chloroform extracts of *N. tabacum* (LC_{50}=76.27, 163.81, 83.38, and 105.85 ppm; LC_{90}= 334.72, 627.38, 709.51, and 524.39 ppm) against the larvae of *C. quinquefasciatus*, respectively. Thomas et al. (2004) reported that the essential oil of *I. cairica* possessed remarkable larvicidal properties as it could produce 100% mortality in the larvae of *Culex tritaeniorhynchus*, *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* mosquitoes at concentrations ranging from 100 to 170 ppm. The median lethal concentration (LC_{50}) values for *Khaya senegalensis* using acetone, ethanol, hexane, and methanol extracts were 20.12, 5.1, 5.08, and 7.62 mg/l, respectively and the LC_{50} values for *Daucus carota* were 236.00, 36.59, 77.19, and 241.8 mg/l, respectively against *Culex annulirostris* (Shaalan et al. 2006). Komalamisra et al. (2005) have reported that the petroleum ether extract of *Rhinacanthus nasutus* possessed larvicidal effects with LC_{50} values between 3.9 and 11.5 mg/l and *Derris elliptica* showed LC_{50} values between 11.2 and 18.84 mg/l against *A.*

Table 1 Larvicidal activity of crude plant leaf extracts against fourth instar larvae of *Culex quinquefasciatus* at 1,000 ppm

Botanical name/Family (herbarium numbers) vernacular names	% Mortality[a] (ppm)±SD					
	1	2	3	4	5	6
Calotropis procera/Asclepiadaceae (ZD/BDC/032-08) Vellai erukku	20±2.983	22±3.346	66±3.033	48±2.302	52±2.092	25±1.934
Canna indica/Cannaceae (ZD/BDC/085-08) Kalvazhai	100±0.000	100±0.000	44±2.092	100±0.000	100±0.000	72±3.286
Hibiscus rosa-sinensis/Malvaceae (ZD/BDC/154-08) Sembaruthi	26±2.916	44±2.834	4±0.834	37±1.817	12±1.924	14±3.309
Ipomoea carnea/Convolvulaceae (ZD/BDC/046-08) Vellaikeerai	100±0.000	94±2.721	100±0.000	100±0.000	100±0.000	88±2.738
Sarcostemma brevistigma/Asclepiadaceae (ZD/BDC/048-08) Kodikkalli	40±2.802	52±2.976	38±1.581	40±1.581	72±2.261	20±1.471

Control—nil mortality

[a] Mean value of five replicates: *1* acetone, *2* chloroform, *3* hot water, *4* methanol, *5* petroleum ether (60–80°C), *6* water

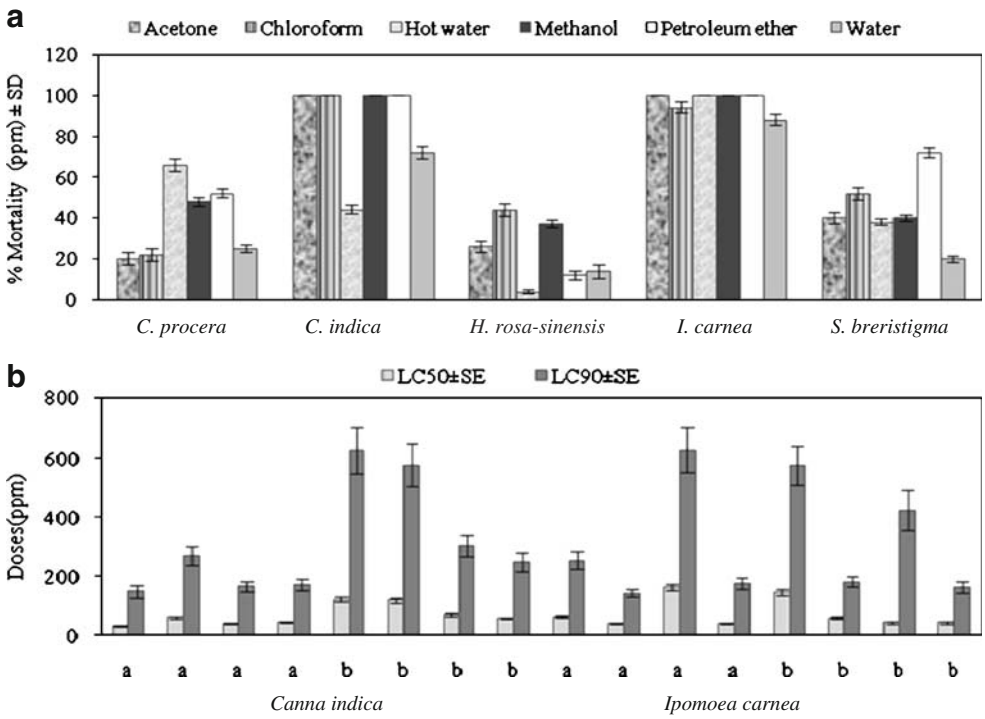

Fig. 1 **a** Graph showing the larvicidal activity of crude extracts against fourth instar larvae of *C. quinquefasciatus* at 1,000 ppm. **b** Graph showing the LC_{50} and LC_{90} values of *C. indica* and *I. carnea* extracts against *C. quinquefasciatus* larvae. (*a*) Second instar larvae; (*b*) fourth instar larvae

aegypti, *C. quinquefasciatus*, *Anopheles dirus*, and *Mansonia uniformis*. Larvicidal efficacies of methanol extracts of *Momordica charantia*, *Trichosanthes anguina*, *Luffa acutangula*, *Benincasa cerifera*, and *Citrullus vulgaris* tested with LC_{50} values were 465.85, 567.81, 839.81, 1,189.30, and 1,636.04 ppm, respectively, against the late third larval age group of *C. quinquefasciatus* (Prabakar and Jebanesan 2004). Sharma et al. (2005) reported that the acetone extract of *Nerium indicum* and *Thuja orientalis* have been studied with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against third instar larvae of *A. stephensi* and *C. quinquefasciatus*. Earlier authors reported that the methanol leaf extracts of *Vitex negundo*, *Vitex trifolia*, *Vitex peduncularis*, and *Vitex altissima* were used

Table 2 Larvicidal activity of different solvent crude extracts against second and fourth instar larvae of *Culex quinquefasciatus*

Plant name	Solvents	Instars	LC_{50}±SE (ppm) (UCL–LCL)	LC_{90}±SE (ppm) (UCL–LCL)	χ^2 (*df*=4)
Canna indica	Acetone	II	29.62±2.24 (34.01–25.24)	148.55±20.06 (187.87–109.23)	9.71
		IV	121.88±8.88 (139.30–104.48)	624.35±77.60 (776.42–472.25)	11.17
	Chloroform	II	59.18±4.17 (67.35–51.01)	267.87±31.95 (330.48–205.26)	6.70
		IV	118.25±8.42 (134.76–101.75)	573.93±69.00 (709.19–438.67)	6.67
	Methanol	II	40.77±2.75 (46.14–35.39)	165.00±18.27 (200.81–129.19)	7.05
		IV	69.76±4.91 (79.39–60.14)	304.27±35.86 (374.56–233.99)	9.75
	Petroleum ether	II	44.38±3.05 (50.35–38.40)	171.91±19.03 (209.21–134.60)	6.14
		IV	56.31±4.07 (64.29–48.33)	248.24±30.35 (307.72–188.76)	7.74
Ipomoea carnea	Acetone	II	61.17±4.27 (69.53–52.81)	252.91±29.81 (311.34–194.47)	6.65
		IV	145.37±9.54 (164.06–126.98)	573.30±63.74 (698.23–448.36)	13.58
	Hot water	II	41.07±2.52 (46.00–36.14)	142.67±13.62 (169.37–115.96)	3.39
		IV	58.00±3.49 (64.93–51.27)	181.10±17.49 (216.27–147.72)	6.80
	Methanol	II	41.82±3.91 (49.50–34.16)	423.76±67.96 (556.97–290.55)	13.60
		IV	163.81±11.55 (186.45–141.17)	627.38±74.40 (773.21–481.55)	8.66
	Petroleum ether	II	39.32±2.69 (44.58–34.05)	176.39±20.16 (215.90–136.88)	7.70
		IV	41.75±2.76 (47.16–36.35)	162.63±17.46 (196.85–128.41)	2.92

Control—nil mortality. Significant at $p<0.05$ level

LC_{50} lethal concentration that kills 50% of the exposed larvae, *LC_{90}* lethal concentration that kills 90% of the exposed larvae, *UCL* upper confidence limit, *LCL* lower confidence limit, χ^2 chi-square, *df* degree of freedom

for larvicidal assay with LC_{50} values of 212.57, 41.41, 76.28, and 128.04 ppm, respectively, against the early fourth instar larvae of *C. quinquefasciatus* (Kannathasan et al. 2007). The larvicidal activity of petroleum ether, ethanolic, and aqueous extracts of dried leaves and fixed oil from the seeds of *Caesalpinia bonduc* showed 100% mortality in 1% concentration of petroleum ether and ethanolic extract of leaves, whereas it was 55% in 2.5% concentration of aqueous extract and 92.6% in 2.5% concentration of fixed oil against the fourth instar larvae of *C. quinquefasciatus* (Saravanan et al. 2007); 100% larval mortality was found at 1,000 ppm in whole plant petroleum ether extract of *C. colocynthis* against the early fourth instar larvae of *C. quinquefasciatus* (Rahuman et al. 2008b); the petroleum ether (60–80°C) extracts of the leaves of *Vitex negundo* were evaluated for larvicidal activity with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/l against larval stages of *C. tritaeniorhynchus*, respectively (Karunamoorthi et al. 2008); the compounds 4-gingerol (1), (6)-dehydrogingerdione (2), and (6)-dihydrogingerdione (3) isolated from petroleum ether extract of *Zingiber officinale* exhibited larvicidal activities against fourth instar larvae of *A. aegypti* (LC_{50}=4.25, 9.80, and 18.20 ppm) and *C. quinquefasciatus* (LC_{50}=5.52, 7.66, and 27.24 ppm), respectively (Rahuman et al. 2008c). The combination of ethanolic water extract (10% concentration) from the seed and leaf parts of *Annona squamosa*, *Artemisia annua*, *Centella asiatica*, *Eucalyptus globulus*, *Myristica fragrans*, and *Cymbopogan citratus* mixed at equal proportions displayed an LC_{50} of 0.80 ppm, making it the most active of all extracts tested to the larvae followed by *E. globules* (1.14 ppm), *C. asiatica* (1.52 ppm), *A. annua* (1.52 ppm), *M. fragrans* (2.22 ppm), *C. citratus* (2.59 ppm), *A. squamosa* (2.65 ppm), *Cassia fistula* (3.30 ppm), *Justicia gendarussa* (4.16 ppm), *I. carnea* (7.36 ppm), *Ricinus communis* (8.56 ppm), and finally by *Datura stramonium* and *Prosopis juliflora* (9.30 ppm) against third instar larvae of *A. stephensi* (Senthilkumar et al. 2009). Rahuman et al. (2008e) have reported that the LC_{50} value of petroleum ether extracts of *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli* were 11.34, 76.61, 113.40, 424.94, and 5.52 ppm, respectively, against the fourth instar larvae of *C. quinquefasciatus*. The methanol extracts of *Euphorbia tirucalli* latex and stem bark were evaluated for larvicidal activity against laboratory-reared larvae of *C. quinquefasciatus* with LC_{50} values of 177.14 and 513.387 mg/l, respectively (Yadav et al. 2002). Mullai and Jebanesan (2007) have reported that the ethyl acetate, petroleum ether, and methanol leaf extracts of *C. colocynthis* and *Cucurbita maxima* showed LC_{50} values of 47.58, 66.92, and 118.74 ppm and 75.91, 117.73, and 171.64 ppm, respectively, against *C. quinquefasciatus* larvae.

In conclusion, an attempt has been made to evaluate the role of plant extract larvicidal bioassay against *C. quinquefasciatus* activity. The results reported here open the possibility of further investigations of efficacy on their larvicidal properties of natural product extracts. The isolation and purification of crude extract of *C. indica* and *I. carnea* are in progress.

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