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ORIGINAL PAPER

Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad

A. Abdul Rahuman · P. Venkatesan · Geetha Gopalakrishnan

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Abstract In mosquito control programs, botanical origin may have the potential to be used successfully as larvicides. The larvicidal activity of crude acetone, hexane, ethyl acetate, methanol, and petroleum ether extracts of the leaf of *Centella asiatica* Linn., *Datura metal* Linn., *Mukia scabrella* Arn., *Toddalia asiatica* (Linn.) Lam, extracts of whole plant of *Citrullus colocynthis* (Linn.) Schrad, and *Sphaeranthus indicus* Linn. were assayed for their toxicity against the early fourth instar larvae of *Culex quinquefasciatus* (Diptera: Culicidae). The larval mortality was observed after 24 h exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in whole plant petroleum ether extract of *C. colocynthis*. In the present study, bioassay-guided fractionation of petroleum ether extract led to the separation and identification of fatty acids; oleic acid and linoleic acid were isolated and identified as mosquito larvicidal compounds. Oleic and Linoleic acids were quite potent against fourth instar larvae of *Aedes aegypti* L. (LC_{50} 8.80, 18.20 and LC_{90} 35.39, 96.33 ppm), *Anopheles stephensi* Liston (LC_{50} 9.79, 11.49 and LC_{90} 37.42, 47.35 ppm), and *Culex quinquefasciatus* Say (LC_{50} 7.66, 27.24 and LC_{90} 30.71, 70.38 ppm). The structure was elucidated from infrared, ultraviolet, ^{1}H-nuclear magnetic resonance, ^{13}C-NMR, and mass spectral data. This is the first report on the mosquito larvicidal activity of the reported isolated compounds from *C. colocynthis*.

A. A. Rahuman (✉)
Unit of Bioactive Natural Products,
P.G & Research Department of Zoology,
C. Abdul Hakeem College,
Melvisharam 632 509 Vellore Dist. Tamil Nadu, India
e-mail: abdulrahuman6@hotmail.com

P. Venkatesan
Department of Zoology, Loyola College,
Chennai 600 032, India
e-mail: p_venkatesan@yahoo.com

G. Gopalakrishnan
Center for Natural Products, SPIC Science Foundation,
64, Mount Road,
Chennai 600 032, India
e-mail: geethagopal@hotmail.com

Introduction

Mosquito borne diseases have an economic impact, including loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases (Fradin and Day 2002). Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year. *Aedes aegypti,* a vector of dengue is widely distributed in the tropical and subtropical zones. Dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50% (Hales et al. 2002). *Anopheles stephensi* is the major malaria vectors in India. With an annual incidence of 300–500 million clinically manifest cases and a death toll of 1.1–2.7 million; malaria is still one of the most important communicable diseases. Currently about 40% of the world's population lives in areas where malaria is endemic (Wernsdorfer and Wernsdorfer 2003). *Culex quinquefasciatus,* a vector of lymphatic filariasis and its widely distributed tropical diseases with around 120 million people infected

worldwide and 44 million people have common chronic manifestation (Bernhard et al. 2003). Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance (Brown 1986), undesirable effects on non-target organisms, and fostered environmental and human health concern. Chemical control methods using synthetic insecticides are in practice due to their speedy action and ease of application. Use of chemical agents however results in environmental degradation in addition to accumulation of toxicants as residual deposits in non-target species.

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Plants are considered as a rich source of bioactive chemicals (Wink 1993) and they may be an alternative source of mosquito control agents. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to their innate biodegradability.

Many studies on plant extracts against mosquito larvae have been conducted around the world. The rhizomes hexane, ethyl acetate, and butanol extracts of *Centella asiatica* were used for antifilarial, antifeedant, and antibacterial control and stigmasterol beta-D-glucopyranoside compound isolated from the hexane extract showed antifeedant activity against the larvae of *Spilosoma obliqua* (Srivastava et al. 1997a, b); ethyl acetate extract showed antihelmintic properties and antifilarial effects (Chakraborty et al. 1996). *Citrullus colocynthis* is an annual herb found in wild as well as cultivated throughout India in the warm areas. The petroleum ether and ethyl acetate seeds extracts showed antioviposition, F1 adult emergence, ovicidal, and repellent activity against the pulse beetle *Callosobruchus maculatus* (Seenivasan et al. 2004); the crude extracts (70% ethanol) were tested for their mortality, repellency, and the number of eggs laid against the carmine spider mite *Tetranychus cinnabarinus* (Mansour et al. 2004); the petroleum ether extract of *C. colocynthis* and methanol extract of *Momordica charantia* were found toxic to the larvae of *A. aegypti* and *C. quinquefasciatus* (Rahuman and Venkatesan 2008); the leaf benzene, ethyl acetate, petroleum ether, and methanol extract of *C. colocynthis* were tested for larvicidal, ovicidal, and repellent activities against the mosquito *C. quinquefasciatus* (Mullai and Jebanesan 2007); the isolated compound cucurbitacins showed activity against rootworm beetle, Diabrotica spp., Aulacophora spp (Metcalf 1986); cucurbitacin B isolated compound showed antifeedant and oviposition on insects (Tallamy et al. 1997). The leaf extract of *Datura metal* was reported to be toxic to *Spotoptera litura* (Murugan et al. 1999); the aqueous extract of leaves inhibited the larval hatching of *Meloidogyne incognita* (Goswami and Vijayalakshmi 1987). The methanol extract of *Sphaeranthus indicus* showed macrofilaricidal activity by worm motility and subsequent mortality was observed (Nisha et al. 2007); the petroleum ether extract showed ovicidal and growth disrupting activities against *C. quinquefasciatus* (Sharma and Saxena 1996). Essential oil extracted from the leaves of *Toddalia asiatica* showed microbial and insecticidal activity (Bandara et al. 1990); nitidine compound was isolated from the root and tested for antimalarial activity (Gokunju et al. 1996); the aqueous extract showed antifeedant activity against the sixth instar larvae of *Helicoverpa armigera* (Sundararajan and Kumuthakalavalli 2001). Recently the researchers have reported the bioactivity of essential oils from various plants against the larvae of mosquitoes. (Amer and Mehlhorn 2006a, b).

The acetone crude extract of *Ocimum canum*, *Ocimum sanctum*, and *Rhinacanthus nasutus* (Kamaraj et al. 2008); *Fagonia indica* and *Arachis hypogaea* (Chaubal et al. 2005); *Nerium indicum* and *Thuja orientelis* (Sharma et al. 2005) were tested against mosquito larvae. The ethyl acetate extract of leaf extract of *Acalypha indica* (Govindarajan et al. 2008); extract of fruit mesocarp of *Balanites aegyptiaca* (Wiesman and Chapagain 2006); the crude hexane extracts obtained from flower heads of *Spilanthes acmella*, *Spilanthes calva*, and *Spilanthes paniculata* (Pandey et al. 2007); seeds extract of *Sterculia guttata* (Katade et al. 2006); the methanol extracts of *Cryptomeria japonica* (Cheng et al. 2008); *R. nasutus* (Rongsriyam et al. 2006); *Chamaecyparis obtusa* (Jang et al. 2005); leaf extract of *Citrullus vulgaris* (Mullai et al. 2008); the petroleum ether extract of *Solanum xanthocarpum* (Mohan et al. 2007); leaves extracts of *Artemisia annua* and *Azadirachta indica* (Tonk et al. 2006); *Ajuga remota* (Sharma et al. 2004); *Abutilon indicum* (Rahuman et al. 2008a) were tested against the larvae of *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus*.

The oleic acid and linoleic acid were isolated from the leaves of *Helichrysum pedunculatum* inhibited the growth of five Gram-positive bacterial species (Dilika et al. 2000); the roots of *Salvia miltiorrhiza* showed amidolytic activity (Wang et al. 1998); the seed hexane extract of *Dirca palustris* showed insecticidal against fourth instar *A. aegypti* larvae and exhibited potent feeding deterrent activity against neonate larvae of *Helicoverpa zea*, *Lymantria dispar*, *Orgyia leucostigma*, and *Malacosoma disstria* (Ramsewak et al. 2001); the petroleum ether and EtOAc-soluble extracts of the seeds of *Ziziphus jujuba* were evaluated for their inhibitory effects against both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Su et al. 2002); isolated from the n-hexane extract of rhizomes of *Atractylodes lancea* showed inhibitory effects on 5-lipoxygenase (5-LOX) and COX-1 (Resch et al. 2001). Oleic and linoleic acid inhibited the parasitemic development in mice infected with *Plasmodium vinckei* or

with *Plasmodium yoelii* in a 4-day suppressive test (Krugliak et al. 1995).

In the light of earlier literature, it is known that larvicides play a vital role in controlling mosquitoes in their breeding sites, but still vectors resistance to them remains unanswered. In addition they show a negative impact in areas of beneficial and non-target organisms. Though various biocontrol measures are in vogue, their effective control of larval mosquitoes has not been hitherto highlighted, whereas possibilities of plant extract and isolation of active components have been fragmentally documented. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant source. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the extracts from the medicinal plant against three medically important species of mosquito vectors, *A. aegypti, A. stephensi*, and *C. quinquefasciatus*.

Materials and methods

Plant materials

The leaf of *C. asiatica* Linn. (Gentianaceae), *D. metal* Linn. (Solanaceae), *Mukia scabrella* Arn. (Cucurbitaceae), *T. asiatica* (Linn.) Lam (Rutaceae), whole plant of *C. colocynthis* (Linn.) Schrad (Cucurbitaceae) and *S. indicus* Linn. (Compositae) were collected from Chitheri Hills, Dharmapuri district (11° 53′ 28″ N, 78° 30′ 26″ E, altitude 959), Tamil Nadu, India in January 2007 and was authenticated by Dr. B. Annadurai, Department of Plant Biology and Biotechnology, C. Abdul Hakeem College, Melvisharam, India. Voucher specimen has been deposited in the laboratory of Zoology, C. Abdul Hakeem College, Melvisharam.

Mosquito culture

A.aegypti, *A. stephensi*, and *C.quinquefasciatus* eggs were obtained from Zonal Entomological Research Centre, Vellore (12° 55′ 48″ N, 79° 7′ 48″ E) to start the colony and larvae were reared in plastic and enamel trays containing tap water. They were maintained, and all the experiments were carried out, at 27±2°C and 75–85% relative humidity under a 14:10 light and dark cycles. Larvae were fed a diet of Brewers yeast, dog biscuits, and algae collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained in our insectary (45×45×40 cm) where adults emerged. Adults were maintained in glass cages and were continuously provided with 10% sucrose solution in a jar with a cotton wick. On day 5 the adults were given a blood meal from a pigeon placed in resting cages overnight for blood feeding by females. Glass petridishes with 50 ml of tap water lined with filter paper was kept inside the cage for oviposition.

Preparation of plant extracts

The dried leaf (750 g) and whole plant (1,200 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with acetone (1,200 ml, Qualigens), ethyl acetate (1,500 ml, Qualigens), hexane (1,000 ml, Fine), methanol (2,000 ml, Qualigens) and petroleum ether (1,700 ml, Qualigens, 60–80°C) in a soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22–26 mm Hg at 45°C and the residue obtained was stored at 4°C.

During preliminary screening the early fourth instar larvae of *C. quinquefasciatus* were used for our bioassay test. Experiments were conducted for 24 h at room temperature (28±2°C). The experimental media, in which 100% mortality of larvae occurs alone, were selected for isolation and purification of crude extracts. Among the crude extracts tested for larvicidal activity, petroleum ether whole plant extract of *C. colocynthis* showed maximum activity and it was selected for the purpose of isolation and purification of compounds by column chromatographic method.

Isolation and purification of active principle

The active petroleum ether whole plant crude extract (24.714 g) of *C. colocynthis* was subjected to a column chromatography (50×5 cm, gravity, 1:2 charcoal, si gel 60–120 mesh, 340 g) to obtain three fractions A, B, and C by increasing polarity of eluents n-hexane and ethyl acetate 100:0 (11×200 ml), 50: 50 (24×200 ml), and 0:100 (16×200 ml) respectively. Further elution of the column with different proportions of chloroform and methanol yielded three more fractions namely D, E, and F with the elutions of 100:0 (10×200 ml), 50:50 (12×200 ml), and 0:100 (15×200 ml) respectively. Each fraction (A–E) obtained was tested against fourth instar larvae of *C. quinquefasciatus* at the concentration of 1,000 ppm and those fractions showing 100% mortality in 24 h alone were selected for further separation by column chromatography.

Fractions A (5.472 g), A1 (3.025 g), A1B (1.944 g), and A1B3 (0.947 g) and fractions C (6.842 g), C4 (4.428 g), and C4E (2.964 g) were subjected to a subsequent repeated column chromatography (gravity) separately used different si gel mesh (70–320 mesh 140 g, 220 g and 230–400 mesh 100 g, 80 g) with varying proportions of n-hexane and ethyl acetate as eluents to collect different subfractions. Bioassay

guided fractionation was carried out and the pure compounds A1B3D (0.514 g) and C4E6 (1.046 g) were obtained from V and IV column with the elutions of 72:28 (26×10 ml) and 58:42 (60×10 ml) respectively. The fractions collected were combined based on thin layer chromatography (TLC) results. After 24 h of exposure, the percentage mortality of larvae is reported from the average of five replicates. All fractions were monitored by TLC (precoated plate, 0.02 mm thick, E. merck, Germany 60 F_{254}) until a single spot was obtained. The pure fractions were carefully evaporated to dryness and subsequently characterized by spectral analysis.

Gas chromatography-mass spectrometry analysis

The pure compounds were subjected to infrared (IR), ultraviolet (UV), 1H-nuclear magnetic resonance (NMR) and 13C NMR and mass spectral analysis. IR spectra were recorded on a Bruker FT-IR instrument and UV spectra were recorded on a Shimadzu instrument. The 1H and 13C NMR was recorded in Bruker 200 MHz DPX instrument using CDCI3 with tetramethylsilane as internal standard. The mass spectra were recorded in SHIMADZU QP 5000 gas chromatography-mass spectrometry instrument using a temperature program 60–250°C over a period of 15 min. The injection volume was 2 μl, as hexane solution 1H–1H COSY, 13C–1H HETCOR and 13C–1H COLOC were performed using Bruker standard microprograms.

Larvicidal bioassay

During preliminary screening with the laboratory trial, the larvae of *C. quinquefasciatus* were collected from the stagnant water in and around the College Campus, Melvisharam and identified in Vector Control Research Centre, Puducherry. One gram of crude extract was first dissolved in 100 ml of respective solvent (stock solution). From the stock solution, 1,000 ppm was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. Early fourth instar larvae were used for bioassay test. A total of 100 larvae were exposed in five replicates of 20 larvae each. Experiments were conducted for 24 h at room temperature (28±2°C). The control was set up with solvent and polysorbate 80. The experimental media, in which 100% mortality of larvae occurs alone, were selected for isolation and purification.

The different fractions isolated were tested against the early fourth instar larvae of mosquitoes by the procedure of WHO (1996) with some modification and as per the method of Rahuman et al. (2000). For Bioassay test, larvae were taken in five batches of 20 in 249 ml of water and 1.0 ml of plant extract concentration. From the stock solution, different concentrations ranging from 2.5 to 80 ppm were prepared. The numbers of dead larvae were counted after 24 h of exposure, and the percentage mortality was reported from the average of five replicates.

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

A literature survey of the plant-isolated compound revealed that the compound under investigation could be oleic and linoleic acid. The physical and spectral data of the present compound were in agreement with those of the values reported in the literature (Roberts et al. 2006; León et al. 2004; Sun et al. 1994; Dilika et al. 2000). However a TLC analysis with the standard oleic and linoleic acid (Sigma-Aldrich) confirmed the identity of compounds.

All extracts showed moderate larvicidal effects however the highest larval mortality was found in petroleum ether whole plant extract of *C. colocynthis* (Table 1 and Fig. 1a). Among the crude extracts tested, the petroleum ether extract of whole plant of *C. colocynthis* showed 100% larval mortality at 1,000 ppm. The petroleum ether extracts of *C. colocynthis*, *Coccinia indica*, *Cucumis sativus*, *M. charantia*, and *Trichosanthes anguina* (Rahuman and Venkatesan 2008); *C. vulgaris* (Mullai et al. 2008); *Jatropha curcas*, *Pedilanthus tithymaloides*, *Phyllanthus amarus*, *Euphorbia hirta*, and *Euphorbia tirucalli* (Rahuman et al. 2008b) were reported with less activity compare with the *C. colocynthis* extract against the larvae of *A. aegypti, A. stephensi*, and *C. quinquefasciatus*.

With regard to the present findings, the LC_{50} values of pure compounds oleic acid were 8.80, 9.79, and 7.66, and linoleic acid were 18.20, 11.49, and 27.24 ppm on *A. aegypti, A. stephensi*, and *C. quinquefasciatus* respectively (Tables 2 and 3, Fig. 1b). Higher percentages of mortality were observed at higher concentrations of the isolated compound. Similar study conducted by Zani et al. (1997) and reported the larvicidal activity of isolated compound—thiophene derivatives from the ethanol extract of *Tagetes minuta* showed LC_{50} value of 1.0 mg/l on *Aedes fluviatilis*. The toxic effect of neolignans compound separated from *Piper decurrens* showed maximum activity on mosquito (Chauret et al. 1996). Perich et al. (1995) reported the isolated compounds thiophenes-5- (but-3-

Table 1 Larvicidal activity of crude extracts against early fourth instar larvae of *C. quinquefasciatus* at 1,000 ppm

Botanical name/family (herbarium numbers) parts used/vernacular names	% mortality[a] (ppm)±SD				
	1	2	3	4	5
Centella asiatica (Linn.) Gentianaceae [LC/ZB/016–2007] leaf/Vallarai	36±4.6042	24±3.6330	28±3.2862	06±1.7888	46±3.8986
Citrullus colocynthis (Linn.) Schrad Cucurbitaceae [LC/ZB/019–2007] whole plant/Verittumatti	92±1.6733	96±1.0954	58±2.9664	68±2.6076	100±0.0000
Datura metal (Linn.) Solanaceae [LC/ZB/018–2006] leaf/Ummatta	88±3.2863	68±2.6076	20±3.7416	76±3.0331	92±2.1908
Mukia scabrella Arn. Cucurbitaceae [LC/ZB/026–2007] leaf/Musumu sukkai	00±0.00	00±0.00	08±1.6733	18±2.9664	00±0.00
Sphaeranthus indicus (Linn.) Compositae [LC/ZB/005–2007] whole plant/Kottakaranthai	96±1.0954	52±2.6076	24±2.2803	38±1.6733	88±1.6733
Toddalia asiatica (Linn.) Lam Rutaceae [LC/ZB/006–2006] leaf/Milagarnai	40±2.2803	00±0.00	14±2.2803	00±0.00	78±4.0987

Control—Nil mortality; 1=acetone, 2=ethyl acetate, 3=hexane, 4=methanol, 5=petroleum ether (60–80°C)
[a] Mean value of five replicates

ene-1-ynyl)-2,2 bithiophene and 5 (but-3-ene-1-ylyl)-5methyl-2, 2 bithiophene showed toxic effect on *A. aegypti* and *A. stephensi* larvae. Compounds oleic and linoleic acid were more active compare with the other plant compounds. No mortality was observed in the controls.

Earlier authors reported that the isolated compound neemarin from *A. indica* exhibited LC_{50} and LC_{90} values were 0.35 and 1.81 mg/l for *A. stephensi* and 0.69 and 3.18 mg/l for *C. quinquefasciatus* (Vatandoost and Vaziri 2004); leptostachyol acetate compound isolated from the roots of *Phryma leptostachya* with LC_{50} values of 0.41, 2.1, and 2.3 ppm against third instar larvae of C. *pipiens* pallens, *A. aegypti*, and *Ochlerotatus togoi* (Park et al. 2005); vilasininoid and two havanensinoids were isolated from the chloroform fractions of the methanol extracts of the root barks of *Turraea wakefieldii* and *Turraea floribunda* showed

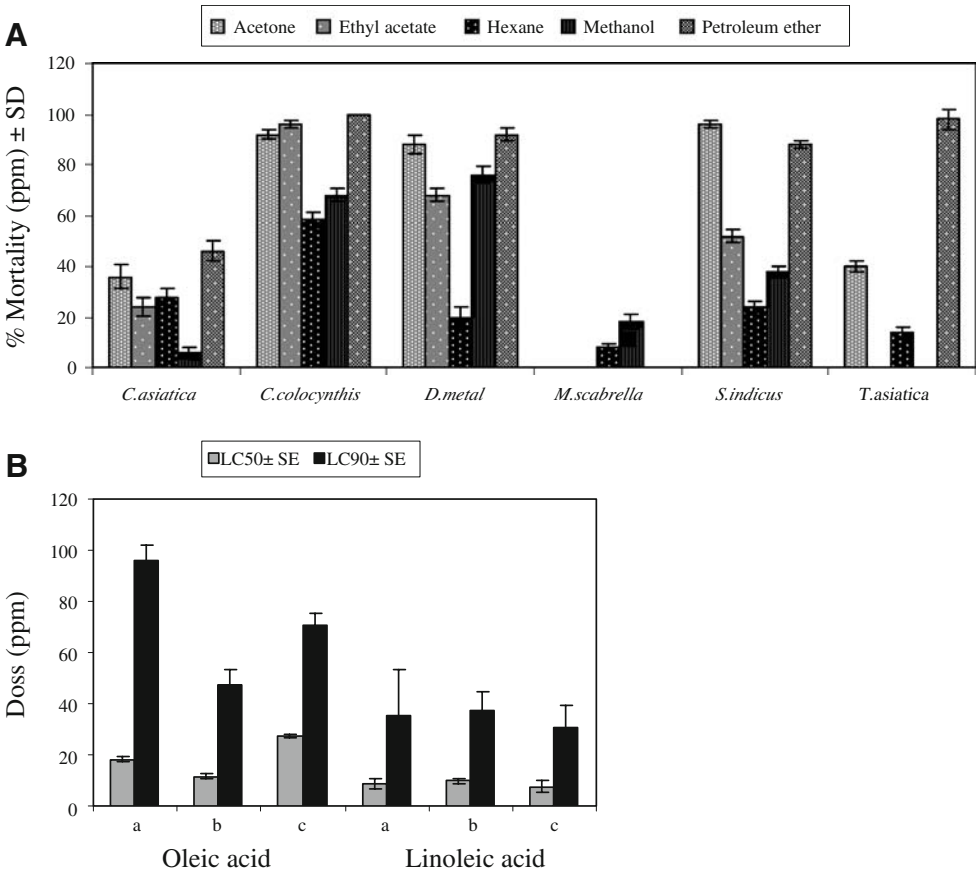

Fig. 1 **a** Larvicidal activity of crude plant extracts against early fourth instar larvae of *C.quinquefasciatus* at 1,000 ppm. **b** Graph showing LC_{50} and LC_{90} values of mosquito vectors *a A.* aegypti, *b A. stephensi*, *c C. quinquefasciatus*

Table 2 Lc_{50}, Lc_{90} and regression analysis for the larvicidal activity of oleic acid isolated from *C. colocynthis*

Species (IV instar)	Description	Value (ppm)	V	SE	LL	UL	Other statistics
A.aegypti	log Lc_{50}	0.94	0.001	0.043	0.861	1.028	$\bar{x}$=0.0213
	log Lc_{90}	1.54	0.004	0.067	1.416	1.681	$\bar{y}$=5.1634
	Lc_{50}	8.80	0.749	0.865	7.100	10.492	x^2=1.7562
	Lc_{90}	35.39	30.103	5.486	24.637	46.145	b=2.1198
							b_o=5.1183
A.stephensi	log Lc_{50}	0.99	0.002	0.040	0.912	1.070	$\bar{x}$=0.9771
	log Lc_{90}	1.57	0.005	0.068	1.439	1.707	$\bar{y}$=4.9696
	Lc_{50}	9.79	0.814	0.902	8.024	11.560	x^2=5.1582
	Lc_{90}	37.42	34.81	5.899	25.856	48.982	b=2.2013
							b_o=2.8187
C.quinquefasciatus	log Lc_{50}	0.88	0.002	0.412	0.803	0.965	$\bar{x}$=0.9102
	log Lc_{90}	1.49	0.005	0.069	1.352	1.623	$\bar{y}$=5.0550
	Lc_{50}	7.66	0.529	0.727	6.235	9.087	x^2=3.4756
	Lc_{90}	30.71	23.814	4.880	21.143	40.272	b=2.1256
							b_o=3.1203

V, Variance of estimated log lethal dose, *SE* standard error, *LC_{50}* lethal concentration that kills 50% of the exposed larvae, *LC_{90}* lethal concentration that kills 90% of the exposed larvae, *LL* lower limit (95% confidence limit), *UL* upper limit (95% confidence limit), $\bar{x}$ mean of *x*, $\bar{y}$ mean of y, x^2 Chi-square, *b* regression coefficient, b_o constant of linearity

LD_{50} values of 7.1, 4.0, and 3.6 ppm respectively against third instar larvae of *A. gambiae* (Ndung'u et al. 2004). Similarly, a piperidine alkaloid, pipernonaline isolated from the fruit methanol extract of *Piper longum* showed LD50 value of 0.21 mg/l *Culex pipiens* pallens larvae (Lee 2000). Earlier authors reported that a new tetranortriterpenoid, meliatetraolenone [24,25,26,27-tetranor-apotirucalla-(apoeupha)-6alpha-O-methyl, 7alpha-senecioyl(7-deacetyl)-11alpha, 12alpha, 21,23-tetrahydroxy-21, 23-epoxy-2, 14,20 (22)-trien-1, 16-dione] (1) was isolated from the methanolic extract of fresh leaves of *A. indica* along with the known compound odoratone (3) showed mortality on fourth instar larvae of *A. stephensi* with LC (50) values of 16 and 154 ppm, respectively (Siddiqui et al. 2003); two new triterpenoids, 22,23-dihydronimocinol (1) and desfurano-6alpha-hydroxya-zadiradione (2), were isolated from a methanolic extract of the fresh leaves of *A. indica* along with a known meliacin, 7alpha-senecioyl-(7-deacetyl)-23-O-methylnimocinolide showed mortality for fourth instar larvae of the mosquito (*Anopheles stephensi*), with LC(50) values of 60 and 43 ppm, respectively (Siddiqui et al. 2002); the isolated compound saponin from ethyl acetate extract of *A. aspera* was effective

Table 3 Lc_{50}, Lc_{90} and regression analysis for the larvicidal activity of linoleic acid isolated from *C. colocynthis*

Species (IV instar)	Description	Value (ppm)	V	SE	LL	UL	Other statistics
A.aegypti	log Lc_{50}	1.26	0.002	0.048	1.167	1.353	$\bar{x}$=0.336
	log Lc_{90}	1.98	0.006	0.081	1.824	2.143	$\bar{y}$=5.1357
	Lc_{50}	18.20	3.986	1.996	14.285	22.110	x^2=9.0496
	Lc_{90}	96.33	32.654	9.070	60.913	131.750	b=1.7708
							b_o=4.5396
A.stephensi	log Lc_{50}	1.06	0.003	0.042	0.979	1.142	$\bar{x}$=0.1044
	log Lc_{90}	1.68	0.005	0.069	1.540	1.810	$\bar{y}$=5.091
	Lc_{50}	11.49	1.224	1.106	9.325	13.661	x^2=3.4220
	Lc_{90}	47.35	56.339	7.506	32.633	62.056	b=2.0845
							b_o=4.8740
C.quinquefasciatus	log Lc_{50}	1.44	0.001	0.033	1.370	1.499	$\bar{x}$=0.4145
	log Lc_{90}	1.85	0.003	0.054	1.742	1.953	$\bar{y}$=4.9358
	Lc_{50}	27.24	4.287	2.070	23.178	31.294	x^2=4.1601
	Lc_{90}	70.38	76.316	8.735	53.254	87.499	b=3.1086
							b_o=3.6474

V Variance of estimated log lethal dose, *SE* standard error, *LC_{50}* lethal concentration that kills 50% of the exposed larvae, *LC_{90}* lethal concentration that kills 90% of the exposed larvae, *LL* lower limit (95% confidence limit), *UL* upper limit (95% confidence limit), $\bar{x}$ mean of *x*, $\bar{y}$ mean of *y*, x^2 Chi-square, *b*=regression coefficient, b_o constant of linearity

against the larvae of *A. aegypti* and *C. quinquefasciatus* with LC_{50} value of 18.20 and 27.24 ppm, respectively (Bagavan et al. 2008).

In the pervious report, showed that the compounds diterpenoid furans 6alpha-hydroxyvouacapan-7beta, 17beta-lactone (1), 6alpha, 7beta-dihydroxyvouacapan-17beta-oic acid (2) and methyl 6alpha, 7beta-dihydroxyvouacapan-17beta-oate (3) isolated from *Pterodon polygalaeflorus* with LC_{50} values of 50.08, 14.69, and 21.76 microg/mL against fourth-instar *A. aegypti* larvae (De Omena et al. 2006); beta-thujaplicin isolated from *Chamaecyparis obtusa* with LC_{50} values of 2.91, 2.60, and 1.33 ppm against *A. aegypti, O. togoi*, and *C. pipiens* pallens larvae (Jang et al. 2005).

The results of this study clearly show that the extract and fraction of *C. colocynthis* that contain oleic and linoleic acid demonstrate a high larval mortality. However it is the first report of isolated active fraction tested for mosquito larvicidal activity. Thus, isolated compounds of the petroleum ether extract of *C. colocynthis* have potential to be developed as natural larvicidal agent. In this context, the highly bioactive compounds of *C. colocynthis*, which is being grown widely in most areas of India, offer an opportunity for developing alternatives to rather expensive and environmentally hazardous organic insecticides. Furthermore, the findings of the high correlation of compound and larval mortality would also open the door for using as oleic and linoleic acid natural larvicidal agents.

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