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ORIGINAL PAPER

Larvicidal activity of *Morinda citrifolia* L. (Noni) (Family: Rubiaceae) leaf extract against *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*

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Abstract *Morinda citrifolia* leaf extract was tested for larvicidal activity against three medically important mosquito vectors such as malarial vector *Anopheles stephensi*, dengue vector *Aedes aegypti*, and filarial vector *Culex quinquefasciatus*. The plant material was shade dried at room temperature and powdered coarsely. From the leaf, 1-kg powder was macerated with 3.0 L of hexane, chloroform, acetone, methanol, and water sequentially for a period of 72 h each and filtered. The yield of extracts was hexane (13.56 g), chloroform (15.21 g), acetone (12.85 g), methanol (14.76 g), and water (12.92 g), respectively. The extracts were concentrated at reduced temperature on a rotary vacuum evaporator and stored at a temperature of 4°C. The *M. citrifolia* leaf extract at 200, 300, 400, 500, and 600 ppm caused a significant mortality of three mosquito species. Hexane, chloroform, acetone, and water caused moderate considerable mortality; however, the highest larval mortality was methanolic extract, observed in three mosquito vectors. The larval mortality was observed after 24-h exposure. No mortality was observed in the control. The third larvae of *Anopheles stephensi* had values of LC_{50}= 345.10, 324.26, 299.97, 261.96, and 284.59 ppm and LC_{90}= 653.00, 626.58, 571.89, 505.06, and 549.51 ppm, respectively. The *Aedes aegypti* had values of LC_{50}=361.75, 343.22, 315.40, 277.92, and 306.98 ppm and LC_{90}=687.39, 659.02, 611.35, 568.18, and 613.25 ppm, respectively. The *Culex quinquefasciatus* had values of LC_{50}=382.96, 369.85, 344.34, 330.42, and 324.64 ppm and LC_{90}=726.18, 706.57, 669.28, 619.63, and 644.47 ppm, respectively. The results of the leaf extract of *M. citrifolia* are promising as good larvicidal activity against the mosquito vector *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. This is a new eco-friendly approach for the control of vector control programs. Therefore, this study provides first report on the larvicidal activities against three species of mosquito vectors of this plant extracts from India.

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Introduction

Mosquitoes are the principal vector of many vector-borne diseases affecting human beings and animals, in addition to nuisance. Vector-borne diseases in India, e.g., malaria, dengue, chikungunya, filariasis, Japanese encephalitis, and leishmaniasis, cause thousands of deaths per year. India reports 1.48 million malarial cases and about 1,173 deaths, 1.4 million suspected and 1,985 confirmed chikungunya cases, 5,000 Japanese encephalitis cases and approximately 1,000 deaths, and 383 dengue cases and 6 deaths during 2006 and 2007 (Kumar et al. 2007; WHO 2007; Gopalan and Das 2009; Dhiman et al. 2010).

Anopheles stephensi is the primary vector of malaria in India and other West Asian countries, and improved methods of control are urgently needed (Burfield and Reekie 2005; Mittal et al. 2005). Malaria infects more than 500 million humans each year, killing approximately 1.2 to 2.7 million per year. About 90% of all malaria cases occur in Africa, as does approximately 90% of the world's malaria-related deaths (Breman et al. 2004). Malaria, caused by *Plasmodium falciparum*, is one of the leading causes of human morbidity and mortality from infectious diseases, predominantly in tropical and subtropical countries (Snow et al. 2005). Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticaria and angioedema (Peng et al. 2004). Botanical and microbial insecticides have been increasingly used for mosquito control because of their efficacy and documented non-toxic effects on non-target organisms (Ascher et al. 1995). The highest number of malaria, *P. falciparum* cases, and malaria-related deaths is recorded from the state of Orissa located in the eastern part of India (Sharma et al. 2010).

Dengue is a vector-borne disease of tropical and subtropical human populations, which occurs predominantly in urban areas. The global increase in urbanization, such that the world's urban population of 1.7 billion in 1980 is expected to be 6.9 billion by the United States Census Bureau 2010, is likely to lead to an increase in dengue in the future. Dengue is transmitted by Aedes mosquitoes that breed in container habitats. The main vector, *Aedes aegypti*, is a cosmotropical species that proliferates in water containers in and around houses. Secondary vectors include *Aedes albopictus*, an important vector in South-East Asia that has spread to the Americas, western Africa, and the Mediterranean rim; *Aedes mediovittatus* in the Caribbean; and *Aedes polynesiensis* and *Aedes scutellaris* in the western Pacific region. *Aedes aegypti* breeds in many types of household containers, such as water storage jars, drums, tanks, and plant or flower containers (Muir and Kay 1998; Honório et al. 2003; Harrington et al. 2005).

Culex quinquefasciatus is a predominant house-resting mosquito in many tropical countries. It is important as a vector of filariasis in some countries as well as a nuisance mosquito. Mosquitoes breed in polluted waters such as blocked drains, damaged septic tanks, or soak age pools close to human habitations. Lymphatic filariasis is probably the fastest spreading insect-borne disease of man in the tropics, affecting about 146 million people (WHO, 1992). *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. Lymphatic filariasis is a mosquito-borne disease caused by mosquito-transmitted filarial nematodes, including *Wuchereria bancrofti* and *Brugia malayi*. The infected people carry the nocturnally periodic *W. bancrofti*, which has *Culex quinquefasciatus* as the main mosquito vector. *Culex quinquefasciatus* is a vector of lymphatic filariasis, which is a widely distributed tropical disease with around 120 million people infected worldwide, and 44 million people have common chronic manifestation (Bernhard et al. 2003). According to WHO, about 90 million people worldwide are infected with *W. bancrofti*, the lymphatic dwelling parasite, and ten times more people are at the risk of being infected. In India alone, 25 million people harbor microfilaria (mf), and 19 million people suffer from filarial disease manifestations (NICD, 1990; Maheswaran et al. 2008; Kovendan et al. 2009).

Morinda citrifolia L. (Noni) is also known as Indian mulberry and belongs to family Rubiaceae (Fig. 1). *M. citrifolia* fruit has a long history of use as a food in tropical regions throughout the world. Written documentation of the consumption of this fruit as a food source precedes the twentieth century. Captain James Cook of the British Navy noted in the late 1700s that the fruit was eaten in Tahiti (Cheeseman 1903). It mainly contains saponins, tannins, triterpenes, alkaloids, and flavonoids. It is mainly used for the bowel disorders, including arthritis, atherosclerosis, bladder infections, boils, burns, cancer, chronic fatigue syndrome, circulatory weakness, cold, congestion, constipation, diabetes, eye inflammations, fever, fractures, gastric ulcers, gingivitis, headaches, heart diseases, hypertension, immune weakness, indigestion, intestinal parasites, kidney disease, malaria, menstrual cramps, mouth sores, respiratory disorders, ringworms, sinusitis, sprains, stroke, skin inflammation, and wounds (Elkins 1997).

Taxonomy
Kingdom: Plantae
Subkingdom: Viridaeplantae
Phylum: Tracheophyta
Subphylum: Euphyllophytina
Class: Magnoliopsida
Subclass: Asteridae
Order: Gentianales

Fig. 1 *Morinda citrifolia* (Noni)

Family: Rubiaceae
Subfamily: Rubioideae
Genus: *Morinda*
Species: *citrifolia*
Botanical Name: *Morinda citrifolia* L. (Zipcode zoo 2012)

A number of major components have been identified in the Noni plant, such as scopoletin, octoanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin- 1-methyl ether, anthraquinone glycoside), b-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, Alizarin, amino acids, acubin, *L*-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin, and a putative proxeronine (Simonsen 1920; Balakrishna et al. 1961; Moorthy and Reddy 1970; Singh and Tiwari 1976; Levand and Larson 1979; Heinicke 1985; Budavari et al. 1989; Daulatabad et al. 1989; Peerzada et al. 1990; Higa and Fuyama 1993; Legal et al. 1994; Farine et al. 1996).

Purification of a n-BuOH-soluble partition of the MeOH extract of *Morinda citrifolia* (Noni) fruits led to the isolation of two new iridoid glucosides, 6alpha-hydroxyadoxoside (1) and 6beta,7beta-epoxy-8-epi-splendoside (2), as well as 17 known compounds, americanin A (3), narcissoside (4), asperuloside, asperulosidic acid, borreriagenin, citrifolinin B epimer a, citrifolinin B epimer b, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, epi-dihydrocornin, D-glucose, D-mannitol, methyl alpha-D-fructofuranoside, methyl beta-D-fructofuranoside, nicotifloroside, and beta-sitosterol 3-O-beta-D-glucopyranoside. The structures of the new compounds were determined by spectroscopic data interpretation. Compound 4, borreriagenin, cytidine, deacetylasperuloside, dehydromethoxygaertneroside, epi-dihydrocornin, methyl alpha-D-fructofuranoside, and methyl beta-D-fructofuranoside were isolated for the first time from *M. citrifolia* (Su et al. 2005).

The present study would be useful in promoting research aiming at the development of new agent for mosquito control based on plant source of natural products. In view of the recent increased interest in developing plant-based insecticides as an alternative to chemical insecticides, this study was undertaken to assess the mosquitocidal properties of *M. citrifolia* leaf extracts against the medically important mosquito vectors *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi* as target species.

Materials and methods

Collection of eggs and maintenance of larvae

The eggs of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* were collected from National Centre for Disease Control field station of Mettupalayam, Tamil Nadu, India, using an "O"-type brush. These eggs were brought to the laboratory and transferred to 18×13×4 cm enamel trays containing 500 mL of water for hatching. The mosquito larvae were pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

Maintenance of pupae and adults

The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500 mL of water with the help of a dipper. The plastic jars were kept in a 90×90×90 cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27+2°C, 75–85% relative humidity, under a photoperiod of 14:10 (light/dark). A 10% sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult mosquito vectors

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Collection of plant and preparation of extract

The *M. citrifolia* plants were collected from in and around Alleppy (sea sources) districts in Kerala, India. The plants were identified by the Taxanomist, Department of Botany, University of Madras, Chennai, Tamil Nadu. *M. citrifolia* leaves were washed with tap water and shade dried at room temperature (28±2°C) for 10 to 20 days. The air-dried plant materials (leaves) were powdered by an electrical blender. From the leaf, 1-kg powder was macerated with 3.0 L of hexane, chloroform, acetone, methanol, and water sequentially for a period of 72 h each and filtered. The yield of extracts was hexane (13.56 g), chloroform (15.21 g), acetone (12.85 g), methanol (14.76 g), and water (12.92 g), respectively. The extracts were concentrated at reduced temperature on a rotary vacuum evaporator and stored at a temperature of 4°C. One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) considered as 1% stock solution. From this stock solution, concentrations were prepared ranging from 200, 300, 400, 500 and 600 ppm, respectively.

Larval toxicity test

A laboratory colony of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* of mosquito larvae was used for the larvicidal activity. Twenty-five numbers of third instar larvae were introduce into 500-mL glass beaker containing

249 mL of dechlorinated water, and 1 mL of desired concentrations of plant leaf extract was added. Larval food was given for the test larvae. Five replicates were set up for each concentration (200, 300, 400, 500, and 600 ppm) and mixing of acetone and Triton-80 (mixing solution). The control was set up by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae were exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula (Abbott's 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis (Finney 1971).

Results

The crude hexane, chloroform, acetone, methanol, and water extracts of the leaves of the plant *M. citrifolia* were studied for use as eco-friendly insecticides instead. Results on the larvicidal activities of leaf extracts obtained in this study (Tables 1, 2, and 3) confirm their potential for the control of larval population of mosquito vectors. Hexane, chloroform, acetone, and water resulted in moderate mortality; however, the highest larval mortality was methanolic extract observed in three mosquito vectors. The third instar larvae of *Anopheles stephensi* had values of LC_{50}=345.10, 324.26, 299.97, 261.96, and 284.59 ppm and LC_{90}=653.00, 626.58, 571.89, 505.06, and 549.51 ppm, respectively. *Aedes aegypti* had values of LC_{50}=361.75, 343.22, 315.40, 277.92, and 306.98 ppm and LC_{90}=687.39, 659.02, 611.35, 568.18, and 613.25 ppm, respectively. *Culex quinquefasciatus* had values of LC_{50}=382.96, 369.85, 344.34, 330.42, and 324.64 ppm and LC_{90}=726.18, 706.57, 669.28, 619.63, and 644.47 ppm (Fig. 2), respectively. The χ^2 values are significant at $P<$ 0.05 level. The 95% confidence limits LC_{50} (LFL–UFL) and LC_{90} (LFL–UFL) were also calculated. Larval mortality was observed after 24-h exposure; no mortality was observed in the control group. The results of larvicidal activity clearly indicate that the percentage of mortality is directly proportional to the concentration of the extract. Solvents of the plant extract of *M. citrifolia* were used at different concentrations, ranging from 200 to 600 ppm, respectively.

Discussion

Mosquitoes are responsible for the transmission of more diseases than any other group of arthropods and play an important role as etiologic agents of malaria, filariasis, dengue, yellow fever, Japanese encephalitis, and other viral diseases (James 1992). In 2001, resistance to insecticides concerned 540 species of arthropod, of which 198 were of medical and veterinary importance (Bills 2001).

David et al. (2000) found that phytochemicals primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae. Muthukrishnan and Pushpalatha (2001) studied the effect of plant extracts on fecundity and fertility against *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti*. Crude extract of leaves of *Solanum nigram* in water showed larvicidal activity against *Anopheles culcifacies*, *Culex quinquefasciatus*, and *Aedes aegypti* at a doe's equivalent to LC_{90} ranging between 0.18% and 0.21% (Singh et al. 2002). Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors (Maurya et al. 2007).

Earlier authors reported that the methanol extract of *Cassia fistula* exhibited LC_{50} values of 17.97 and 20.57 mg/L, *Anopheles stephensi* and *Culex quinquefasciatus*, respectively (Govindarajan et al. 2008). The neem formulation, Neem Azal, produced an overall mortality or inhibition of emergence of 90% (EI_{90}, when third instar larvae were treated) at 0.046, 0.208, and 0.866 ppm in *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti*, respectively (Gunasekaran et al. 2009). In the present results, *M. citrifolia* against *Anopheles stephensi* had values of LC_{50}=345.10, 324.26, 299.97, 261.96, and 284.59 ppm and LC_{90}=653.00, 626.58, 571.89, 505.06, and 549.51 ppm, respectively.

Yadav et al. (2002) have reported the methanol, chloroform, and ether extracts of *E. tirucalli* latex, and stem bark was evaluated for larvicidal activity against laboratory-reared larvae of *Culex quinquefasciatus*. Dua et al. (2006) have reported that the mean median lethal concentration values of the aqueous extract from the roots of *H. abelmoschus* against the larvae of *Anopheles culicifacies*, *Anopheles stephensi*, and *Culex quinquefasciatus* were 52.3, 52.6, and 43.8 ppm, respectively. Sharma et al. (2005) reported that the acetone extract of *Nerium indicum* and *T. orientelis*

Table 1 Larvicidal activity of different solvent extracts of *M. citrifolia* against *Anopheles stephensi*

Name of the extract	Concentration (ppm)	% Mortality ±SD	LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL–UFL)	χ^2 (df=4)
Hexane	Control	0.0±0.0			
	200	27.3±1.72			
	300	43.8±1.85	345.10	653.00	
	400	57.6±1.67	(312.84–373.51)	(599.99–730.65)	0.25*
	500	73.2±1.41			
	600	86.5±1.62			
Chloroform	Control	0.0±0.0			
	200	29.2±1.89			
	300	48.5±1.72	324.26	626.58	
	400	61.8±1.32	(290.36–352.90)	(576.86–698.91)	1.14*
	500	74.2±1.85			
	600	89.7±1.93			
Acetone	Control	0.0±0.0			
	200	32.4±1.72			
	300	52.2±1.01	299.97	571.89	
	400	64.6±1.85	(266.92–327.26)	(530.66–629.83)	1.38*
	500	81.4±1.62			
	600	94.0±1.41			
Methanol	Control	0.0±0.0			
	200	39.4±1.16			
	300	59.2±1.85	261.96	505.06	
	400	73.0±1.01	(149.29–322.43)	(433.52–669.56)	6.63*
	500	85.5±1.72			
	600	100.0±0.00			
Water	Control	0.0±0.0			
	200	35.2±1.41			
	300	54.7±1.35	284.59	549.51	
	400	68.0±1.72	(250.42–312.22)	(510.50–603.98)	2.39*
	500	82.5±1.74			
	600	96.3±1.85			

Each value (mean±SD) represents mean value of five replicates

Control Nil mortality, *LFL* lower fiducial limit, *UFL* upper fiducial limit, χ^2 chi-square value, *df* degrees of freedom

*Significant at $P<0.05$ level

have been studied with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against III instar larvae of *Anopheles stephensi* and *Culex quinquefasciatus*, respectively. Halim (2008) has reported the insecticidal activity of *Z. officinale* against the larval maturation, and adult emergency of *Anopheles pharoensis* third stage was evaluated. Concentrations of 100%, 70%, 50%, 25%, 5%, 2%, 1%, 0.9%, 0.7%, 0.5%, and 0.3% showed 100% larval mortality rate and, at 0.2% and 0.1%, caused mortality of 66.7%, respectively. In previous study, the oils of 41 plants were evaluated for their effects against third instar larvae of *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. At first, the oils were surveyed against *Aedes aegypti* using a 50-ppm solution.

Thirteen oils from 41 plants (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum, and sandalwood) induced 100% mortality after 24 h, or even after shorter periods. The pest oils were tested against third instar larvae of the three mosquito species in concentrations of 1, 10, 50, 100, and 500 ppm. The lethal concentration 50 values of three oils ranged between 1 and 101.3 ppm against *Aedes aegypti*, between 9.7 and 101.4 ppm for *Anopheles stephensi*, and between 1 and 50.2 ppm for *Culex quinquefasciatus* Amer and Mehlhorn (2006). Mathew et al. (2009) reported that leaf chloroform extracts of *Nyctanthes arbortristis* showed lethal values (LC_{50}=526.3 and 780.6 ppm (24 h) and LC_{50}= 303.2 and 518.2 ppm (48 h)) against *Aedes aegypti* and *Anopheles stephensi*, respectively. Flower methanol extracts of the aforementioned plants showed lethal values (LC_{50}= 679.4 and 244.4 ppm; LC_{90}=1071.3 and 433.7 ppm) against *Anopheles stephensi* after 24 and 48 h, respectively. The LC_{50} values of hexane, chloroform, ethyl acetate, acetone, and methanol extract of *O. thymiflorus* third instar larvae of

Table 2 Larvicidal activity of different solvent extracts of *M. citrifolia* against *Culex quinquefasciatus*

Name of the extract	Concentration (ppm)	% Mortality ±SD	LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL–UFL)	χ^2 (df=4)
Hexane	Control	0.0±0.0			
	200	23.5±1.32			
	300	39.7±1.41	382.96	726.18	
	400	52.0±1.85	(350.26–414.13)	(659.10–829.04)	0.27*
	500	67.5±1.78			
	600	78.5±1.16			
Chloroform	Control	0.0±0.0			
	200	25.5±1.89			
	300	41.0±1.85	369.85	706.57	
	400	52.9±1.72	(336.83–400.33)	(643.02–803.14)	0.22*
	500	69.5±1.49			
	600	81.0±1.41			
Acetone	Control	0.0±0.0			
	200	27.0±1.60			
	300	46.5±1.41	344.34	669.28	
	400	57.0±1.85	(310.10–374.14)	(611.94–755.07)	0.76*
	500	72.2±1.89			
	600	84.9±1.72			
Methanol	Control	0.0±0.0			
	200	34.5±1.72			
	300	52.7±1.85	330.42	619.63	
	400	63.0±1.62	(295.48–330.55)	(567.83–696.67)	0.98*
	500	77.4±1.93			
	600	90.2±1.41			
Water	Control	0.0±0.0			
	200	31.5±1.85			
	300	47.4±1.72	324.64	644.47	
	400	59.6±1.62	(288.75–354.69)	(590.36–724.88)	0.84*
	500	74.0±1.41			
	600	88.5±1.32			

Each value (mean±SD) represents mean value of five replicates

Control Nil mortality, *LFL* lower fiducial limit, *UFL* upper fiducial limit, χ^2 chi-square value, *df* degrees of freedom

*Significant at $P<0.05$ level

Anopheles stephensi were LC_{50}=201.39, 178.76, 158.06, 139.22, and 118.74 ppm; LC_{50}=228.13, 209.72, 183.35, 163.55, and 149.96 ppm for *Culex quinquefasciatus*; and LC_{50}=215.65, 197.91, 175.05, 154.80, and 137.26 ppm for *Aedes aegypti*, respectively (Kovendan et al. 2012e).

Clitoria ternatea leaf methanol extract showed dose-dependent larvicidal activity against *Anopheles stephensi* with LC_{50} values of 555.6 (24 h) and 867.3 (48 h) ppm, also the root extracts with LC_{50} value of 340 ppm (48 h). Seed extract showed larvicidal activity (LC_{50}=116.8 and 195 ppm) after 24 h and (LC_{50}= 65.2 and 154.5 ppm) after 48 h treatment against *Anopheles stephensi* and *Aedes aegypti*, respectively. Larvicidal activity of flower methanol extract showed LC_{50} values of 233 and 302.5 ppm against *Anopheles stephensi* and *Aedes aegypti*, respectively, after 48-h treatment. Methanol extract showed lowest LD values against several instar of larvae and 50 adult (121.59, 142.73, 146.84, 202.98, 290.65, 358.42, and 300.03 μg/cm^2, respectively) which indicates highest toxicity or insecticidal activity (Ashraful Alam et al. 2009). In the present results, *M. citrifolia* against *Aedes aegypti* had values of LC_{50}=361.75, 343.22, 315.40, 277.92, and 306.98 ppm and LC_{90}=687.39, 659.02, 611.35, 568.18, and 613.25 ppm, respectively.

Singhi et al. (2006) have reported that the latex of *Calotropis procera* has shown larvicidal efficacy against all three important vector species: *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* in India. Prophiro et al. (2012) reported that the susceptibility of the larvae was determined under three different temperatures of 15°C, 20°C, and 30°C with lethal concentrations for Copaifera sp. ranged from LC_{50} 47 to LC_{90} 91 (milligrams per liter), and for *Carapa*

Table 3 Larvicidal activity of different solvent extracts of *M. citrifolia* against *Aedes aegypti*

Name of the extract	Concentration (ppm)	% Mortality ±SD	LC_{50} (ppm) (LFL–UFL)	LC_{90} (ppm) (LFL–UFL)	χ^2 (df=4)
Hexane	Control	0.0±0.0			
	200	25.7±1.85			
	300	41.2±1.72	361.75	687.39	
	400	55.7±1.41	(329.14–391.34)	(627.99–776.35)	0.05*
	500	71.0±1.85			
	600	82.3±1.32			
Chloroform	Control	0.0±0.0			
	200	26.5±1.35			
	300	45.7±1.72	343.22	659.02	
	400	58.5±1.67	(309.85–372.32)	(604.27–739.96)	0.42*
	500	73.5±1.78			
	600	85.0±1.85			
Acetone	Control	0.0±0.0			
	200	30.7±1.49			
	300	49.5±1.16	315.40	611.35	
	400	61.6±1.85	(281.22–343.92)	(563.92–679.77)	0.59*
	500	78.5±1.72			
	600	90.0±1.62			
Methanol	Control	0.0±0.0			
	200	36.7±1.41			
	300	55.4±1.85	277.92	568.18	
	400	69.5±1.72	(239.05–308.37)	(524.82–630.39)	1.23*
	500	80.9±1.49			
	600	94.2±1.16			
Water	Control	0.0±0.0			
	200	33.4±1.60			
	300	50.6±1.93	306.98	613.25	
	400	62.0±1.41	(270.40–336.80)	(564.07–685.17)	1.13*
	500	77.5±1.85			
	600	91.0±1.72			

Each value (mean±SD) represents mean value of five replicates

Control Nil mortality, *LFL* lower fiducidal limit, *UFL* upper fiducidal limit, χ^2 chi-square value, *df* degrees of freedom

*Significant at $P<0.05$ level

guianensis, they were LC_{50} 136 to LC_{90} 551 (milligrams per liter), respectively. Recent studies on the larval and pupal mortality of *Anopheles stephensi* after the treatment of methanolic extract of *Clerodendrone inerme* leaf extract showed 22% mortality at I instar larvae as a result of treatment at 20 ppm; in contrast, it was increased to 81% at 100 ppm of *Clerodendrone inerme* leaf extract of larval and pupal mortality of *Anopheles stephensi* (I to IV instars) after the treatment of methanolic extract of *Acanthus ilicifolius* at different concentrations (20 to 100 ppm). A 23% mortality was noted at I instar larvae by the treatment of *Acanthus ilicifolius* at 20 ppm, whereas it was increased to 89% at 100 ppm of *Acanthus ilicifolius* leaf extract treatment (Kovendan and Murugan 2011). Kovendan et al. (2011, 2012a) have reported the leaf extract of methanol *Jatropha curcas* against *Culex quinquefasciatus* and *L. aspera* leaf extract against *Anopheles stephensi*, respectively.

Khanna et al. (2011) have reported that the larvicidal crude leaf extract of *Gymnema sylvestre* showed the highest mortality in the concentration of 1,000 ppm against the larvae of *Anopheles subpictus* (LC_{50}= 166.28 ppm) and against the larvae of *Culex quinquefasciatus* (LC_{50}=186.55 ppm), and the maximum efficacy was observed in gymnemagenol compound isolated from petroleum ether leaf extract of *G. sylvestre* with LC_{50} values against the larvae of *Anopheles subpictus* at 22.99 ppm and against *Culex quinquefasciatus* at 15.92 ppm. Santhoshkumar et al. (2011) reported that the maximum efficacy was observed in crude methanol and aqueous leaf extracts of *Nelumbo nucifera* against the larvae of *Anopheles subpictus* (LC_{50}=8.89 and 11.82 ppm, and LC_{90}=28.65 and 36.06 ppm), respectively,

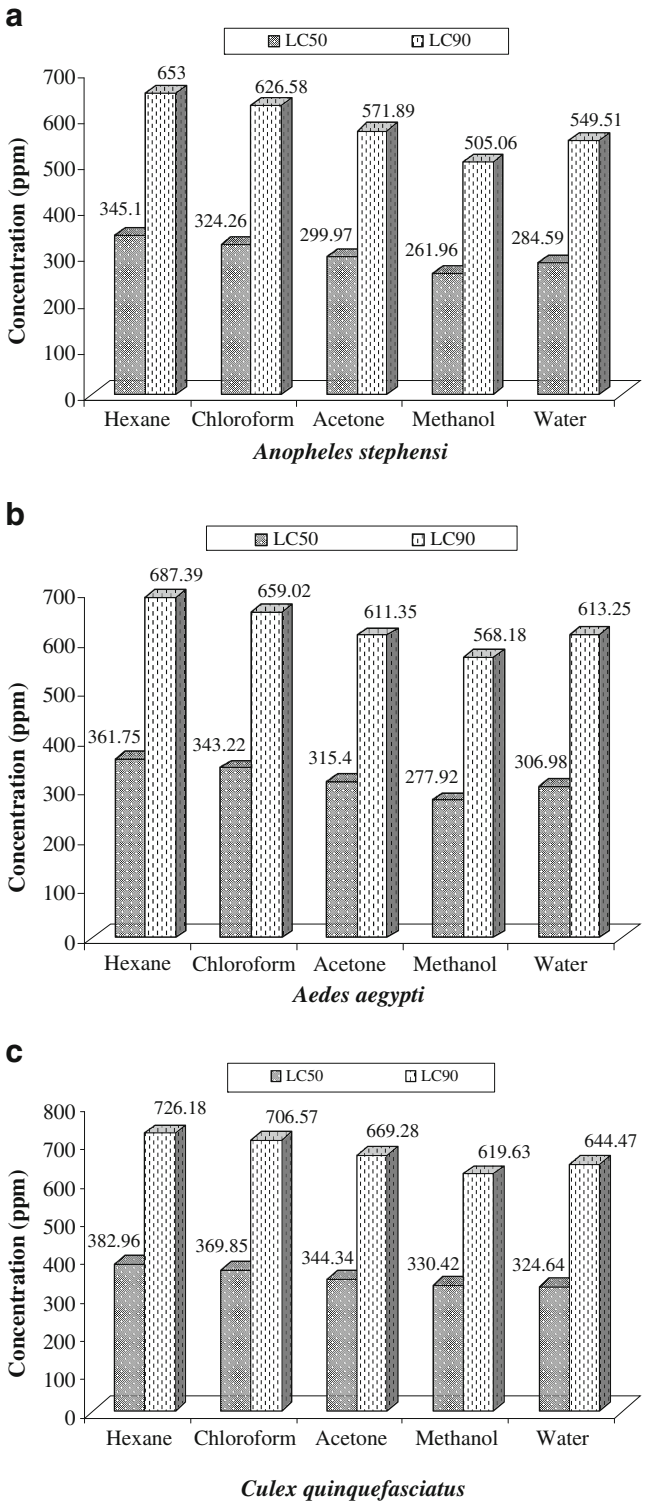

Fig. 2 Graph of the larvicidal activity of crude plant extracts against third instar larvae of mosquito vectors of the LC_{50} and LC_{90} values of *Anopheles stephensi* (**a**), *Aedes aegypti* (**b**), and *Culex quinquefasciatus* (**c**)

and against the larvae of *Culex quinquefasciatus* (LC_{50}= 9.51 and 13.65 ppm, and LC_{90}=28.13 and 35.83 ppm), respectively. The methanol leaf extract of *Calotropis gigantea* against *Culex quinquefasciatus* has LC_{50} values of 104.66, 127.71, 173.75, and 251.65 ppm, respectively. The LC_{90} values are 268.67, 323.50, 432.11, and 581.66 ppm, respectively. The LC_{50} value of pupae was 314.70 ppm, and the LC_{90} value of pupae was 665.04 ppm, respectively (Kovendan et al. 2012d).

In *Calotropis procera* against *Anopheles stephensi*, we observed >95% mortality after 24 h from 256 ppm. Tests with latex showed 99% mortality at 64 ppm for *Anopheles stephensi*, only 44% mortality against *Culex quinquefasciatus*, and a maximum of 67% in 256 ppm, respectively (Shahi et al. 2010). The leaf extract of *Acalypha alnifolia* with different solvents—hexane, chloroform, ethyl acetate, acetone, and methanol—was tested for larvicidal activity against mosquito vectors. The early fourth instar larvae of *Anopheles stephensi* had values of LC_{50}=197.37, 178.75, 164.34, 149.90, and 125.73 ppm and LC_{90}=477.60, 459.21, 435.07, 416.20, and 395.50 ppm, respectively. *Aedes aegypti* had values of LC_{50}=202.15, 182.58, 160.35, 146.07, and 128.55 ppm and LC_{90}=476.57, 460.83, 440.78, 415.38, and 381.67 ppm, respectively. *Culex quinquefasciatus* had values of LC_{50}=198.79, 172.48, 151.06, 140.69, and 127.98 ppm and LC_{90}=458.73, 430.66, 418.78, 408.83, and 386.26 ppm, respectively. The results of the leaf extract of *A. alnifloia* are promising as good larvicidal activity against the mosquito vectors *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* (Kovendan et al. 2012b). The larval and pupal mortality was found in the leaf extract of methanol *Carica papaya* against the first to fourth instar larvae and pupae of values LC_{50} 51.76, 61.87, 74.07, 82.18, and 440.65 ppm, respectively (Kovendan et al. 2012c). In the present results, *M. citrifolia* against *Culex quinquefasciatus* had values of LC_{50}=382.96, 369.85, 344.34, 330.42, and 324.64 ppm and LC_{90}=726.18, 706.57, 669.28, 619.63, and 644.47 ppm, respectively.

In conclusion, the current investigation revealed that the solvents used for extractions also have an impact on the larval mortality. The mortality was maximum in the methanol extract, followed by water, acetone, chloroform, and hexane. This mortality profile shows the extracting properties of different solvents from which the maximum yield was obtained. We observed a functional response by third instar larvae of mosquito vectors *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi* to the natural larvicidal product extracts, the crude extracts of *M. citrifolia*. Therefore, this study provides first report on the larvicidal activities against three species of mosquito vectors of these plant extracts from India. These are new eco-friendly approaches for the control of mosquito vector as target species.

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