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ORIGINAL ARTICLE

Larvicidal and ovicidal activity of *Terminalia chebula* Retz. (Family: Combretaceae) medicinal plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*

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Abstract Insect-borne diseases remain to this day a major source of illness and death worldwide. The resistance to chemical insecticides among mosquito species has been considered as a setback in vector control. Mosquito control programs, botanical origin may have the potential to eliminate eggs and larvae. So, the larvicidal and ovicidal activities of crude benzene, hexane, ethyl acetate, chloroform and methanol extracts of *Terminalia chebula* were assayed for their toxicity against three important vector mosquitoes, viz., *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus.* The larval mortality was observed after 24 h of exposure. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found in the methanol extract of *T. chebula* against the larvae of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* with the LC_{50} values were 87.13, 93.24 and 111.98 ppm, respectively. Mean percent hatchability of the ovicidal activity was observed 48 h post treatment. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. All the five solvent extracts showed moderate ovicidal activity; however, the maximum egg mortality (zero hatchability) was observed in the methanol extract of *T. chebula* at 200 and 250 ppm against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* showed 100% mortality at 300 ppm. No mortality was recorded in the control. The finding of the present investigation revealed that the leaf extract of *Terminalia chebula* possesses remarkable larvicidal and ovicidal activity against medically important vector mosquitoes and make this plant product promising as an alternative to synthetic insecticide in mosquito control programs.

Keywords *Terminalia chebula* · Crude extract · Larvicidal · Ovicidal · *A. stephensi* · *A. aegypti* · *C. quinquefasciatus*

Introduction

Mosquitoes represent a significant threat because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide (WHO 2010). Every year, more than one billion people are infected and more than one million people die from vector borne diseases, including malaria, dengue, schistosomiasis, leishmaniasis, Chagas disease, yellow fever, lymphatic filariasis and onchocerciasis (WHO 2014). Among the 3492 species of mosquitoes recorded worldwide, more than a hundred species are capable of transmitting various diseases in human and other vertebrate. Among all subgenera mosquitos, *Aedes aegypti* is a very important disease transmitting vector, causing dengue haemorrhagic fever (DHF) and Chikungunya in human (Ghosh et al. 2012).

Recently, the dengue fever virus is found in the patients of Tamil Nadu, Andhra Pradesh, Karnataka, Kerala and Maharashtra states severely. Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti*, and at present, there are no effective vaccines available for dengue virus control, and *Culex quinquefasciatus* is the vector of lymphatic filariasis in India, which is an endemic disease in South India. India harbours 40% of global disease burden, transmitted by mosquitoes (Ramaiah et al.

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2000). Malaria has been a major killer disease in many countries of Africa and Asia, where it affects approximately 300–500 million people annually, most of them children (Garcia 2010). In India, 2–3 million malaria cases and about 1000 deaths are reported every year (Lal et al. 2010). *Anopheles stephensi* is recognized as a major vector for urban malaria in India. This species prefers to breed in small synthetic water collections and is responsible for frequent outbreaks of malaria, particularly at construction sites in urban areas (Mittal et al. 2005). To prevent mosquito borne diseases and improve public health, it is necessary to control them. In recent years, however, mosquito control programs have been suffering from failures because of the ever increasing insecticide resistance of these vectors (Georghiou and Lagunes-Tejeda 1991; WHO 1992).

The use of conventional pesticides in the water sources, however, introduces many risks to people and the environment. Natural products of plant origin with insecticidal properties have been tried in the recent pest control for a variety of insect pests and vectors. Mosquitoes in the larval stage are attractive targets for pesticides because mosquitoes breed in water, and thus, it is easy to deal with them in this habitat. Many researchers have reported on the effectiveness of plant extract against mosquito larvae (Kalyanasundaram and Das 1985; Prabhu et al. 2011). In recent years, many studies on plant extracts against mosquito larvae have been conducted around the world. The crude hexane extract obtained from the leaf of *Leucas aspera* was tested for the larvicidal activity against *C. quinquefasci at us* and *A. aegypti* (Maheswaran et al. 2008). Govindarajan (2010a) evaluated, the larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC_{50} values ranging between 38 and 48 mg/L. The larvicidal efficacy of the crude leaf extracts of *Ficus benghalensis*, with three different solvents like methanol, benzene, and acetone, was tested against the early second, third, and fourth instar larvae of *C. quinquefasciatus*, *A. aegypti* and *A. stephensi* (Govindarajan 2010b). Rajkumar and Jebanesan (2004) studied ovicidal activity of *Moschosma polystachyum* leaf extract against *C. quinquefasciatus.* Mullai and Jebanesan (2006) reported that the complete ovicidal activity (100% mortality) was attained at 300 ppm for ethanol, benzene, petroleum ether and ethyl acetate extracts of *Citrullus pubescens* against *C. quinquefasciatus.*

Govindarajan and Karuppannan (2011) investigated the larvicidal and ovicidal activities of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Eclipta alba* against the dengue vector, *A. aegypti.* Murugan et al. (2012) evaluated the larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus.* The larvicidal activity of crude petroleum ether, ethyl acetate and methanol extracts of the whole plants of *Phryma leptostachya* was assayed for its toxicity against the early fourth instar larvae of *C. pipiens* pallens (Xiao et al. 2012). Kovendan et al. (2011) investigated the larvicidal activity of methanol solvent extracts from leaves of *Jatropha curcas* and *Bacillus thuringiensis* var. *israelensis* against the lymphatic filarial vector *C. quinquefasciatus.*

Terminalia chebula Retz. belongs to the family Combretaceae and commonly called as King of medicine and the active ingredient of the well known herbal preparation Triphala. The main phyto constituents reported are tannins such as chebulic acid, chebulinic acid, chebulagic acid, gallic acid, corilagin, ellagic acid and flavonoids, sterols, amino acids, fructose, resin, fixed oils etc., (Kumar 2006). The methanolic extract of the fruits of the plant is reported for antiulcer activity (Raju et al. 2009). Reported pharmacological activities of the plant are antibacterial, antifungal, antiviral, antiamoebic, immunomodulatory, anti plasm odial, antidiabetic, retinoprotective, antianaphylactic, adaptogeni c, anti nociceptive, cardioprotective, hepatoprotective, chemopreventive, hypolipidemic, hypocholesterolem ic, antispermatogenic, mollusicidal, anthelm intic, anti m utagenic, anticarcinogenic, antioxidant, antiarthritic, wound healing, cytoprotective, antiaging, radioprotective (Gupta 2012). In view of the recent increased interest in developing plant origin insecticides as an alternative to chemical insecticides so, this study was undertaken to assess the larvicidal and the ovicidal potential of the different solvent extracts from the medicinal plant *T. chebula* against the medically important vectors, *A. stephensi*, *A. aegypti* and *C. quinquefasciatus.*

Material and methods

Collection of plant materials

The healthy leaves of *T. chebula* Retz. (Combretaceae), were collected from Eastern Ghats of Tamil Nadu, India and the taxonomic identification were made by Dr. V. Chelladurai, Retired Research Officer-Botany, Central Council for Research in Ayurvedha and Sida, Tirunelveli, Government of India.

Extraction

The dried leaves (800 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with benzene, hexane, ethyl acetate, chloroform and methanol in a Soxhlet apparatus (Vogel 1978) (boiling point range 60–80 °C for 8 h). The extract was concentrated under reduced pressure 22–26 mm/Hg at 45 °C and the residue obtained was stored at 4 °C. The extracts

filtered through a Buchner funnel with Whatman number 1 filter paper. The yield of extracts was benzene (9.36 g), hexane (10.47 g), ethyl acetate (8.64 g), chloroform (10.12 g), and methanol (13.81 g). One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1% stock solution. From the stock solution, 50–250 ppm various concentrations was prepared with decholorinated tap water respectively and these solutions were used for larvicidal and ovicidal bioassay.

Test organisms

The eggs/egg raft of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* were collected from the Field station, Centre for Research in Medical Entomology (ICMR-Government of India), Madurai, Tamil Nadu, and India. These eggs were brought to the laboratory and transferred to 18 × 13 × 4 cm, enamel trays containing 500 ml of water for hatching. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. At the time of adult feeding, these mosquitoes were 3–4 days old after emergences (maintained on raisins and water) and were starved for 12 h before feeding. Each time, 500 mosquitoes per cage were fed on blood using a feeding unit fitted with parafilm as membrane for 4 h. *A. aegypti* feeding was done from 12 noon to 4:00 p.m. and *A. stephensi* and *C. quinquefasciatus* were fed during 6:00–10:00 p.m. A membrane feeder with the bottom end fitted with parafilm was placed with 2.0 ml of the blood sample (obtained from a slaughterhouse by collecting in a heparinized vial and stored at 4 °C) and kept over a netted cage of mosquitoes. The blood was stirred continuously using an automated stirring device, and a constant temperature of 37 °C was maintained using a water jacket circulating system. After feeding, the fully engorged females were separated and maintained on raisins. Mosquitoes were held at 28 ± 2 °C, 70–85% relative humidity, with a photoperiod of 12-h light and 12-h dark.

Larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by World Health Organization (2005). Batches of 25 third instar larvae were transferred to small disposable paper cups, each containing 200 ml of water. The appropriate volume of dilution was added to 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration (50–250 ppm). Four replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. To this, 1 ml of acetone was added. The LC_{50} (lethal concentration that kills 50% of the exposed larvae) and LC_{90} (lethal concentration that kills 90% of the exposed larvae) values were calculated after 24 h by probit analysis (Finney 1971).

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae in treatment}} \times 100.$$

Ovicidal bioassay

For ovicidal activity, the method of Su and Mulla (1998) was performed. A hundred freshly laid eggs/egg raft of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*, were exposed to six different concentrations (50, 100, 150, 200, 250 and 300 ppm) of leaf extracts. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated five times along with appropriate control. The hatch rates were assessed 48 h post treatment by the following formula.

$$\% \text{ of hatchablity} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \times 100.$$

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) values and Chi square test was calculated using the SPSS 20.0 (Statistical Package of Social Sciences Inc., USA) software.

Results

The results of the larvicidal activity of crude benzene, hexane, ethyl acetate, chloroform and methanol solvent extracts of leaf of *T. chebula* against third instar larvae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* are presented in the Tables 1, 2 and 3. All crude extracts showed moderate larvicidal activity; however, the maximum efficacy was observed in the methanol extracts of *T. chebula* against the larvae of *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* with the LC_{50} and LC_{90} values were 87.13, 93.24, 111.98 and 173.01, 186.76, 218.95 ppm, respectively. The Chi square values are significant at $p < 0.05$ level. The Chi square values in the bioassays indicated probably the heterogeneity of the test population. The 95% confidence limits LC_{50} and LC_{90} were also calculated. The mean percent egg hatchability of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* were tested with five different solvents at different concentrations of *T. chebula* leaf extracts, and the results are presented in Table 4. The

Table 1 Larvicidal activity of different solvent extract of *T. chebula* against *Anopheles stephensi*

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50}, ppm (LFL–UFL)	LC_{90}, ppm (LFL–UFL)	χ^2
Benzene	Control	0.0 ± 0.0			
	50	29.2 ± 1.10			
	100	41.0 ± 1.37	126.54	242.66	15.803
	150	63.1 ± 1.45	(94.01–158.64)	(201.00–328.08)	
	200	76.4 ± 1.16			
	250	89.4 ± 1.50			
Hexane	Control	0.0 ± 0.0			
	50	31.4 ± 1.45			
	100	45.3 ± 1.21	118.07	228.38	17.031
	150	66.2 ± 1.52	(84.56–149.63)	(188.51–310.14)	
	200	79.1 ± 1.66			
	250	92.8 ± 1.48			
Ethyl acetate	Control	0.0 ± 0.0			
	50	37.0 ± 1.86			
	100	50.5 ± 1.54	102.74	199.78	19.120
	150	72.0 ± 1.81	(68.60–132.98)	(163.59–273.74)	
	200	88.0 ± 1.49			
	250	95.0 ± 1.71			
Chloroform	Control	0.0 ± 0.0			
	50	41.4 ± 1.49			
	100	53.4 ± 1.13	93.92	187.88	24.030
	150	76.0 ± 1.10	(53.57–127.48)	(149.77–272.96)	
	200	92.1 ± 1.82			
	250	97.6 ± 1.84			
Methanol	Control	0.0 ± 0.0			
	50	43.4 ± 1.48			
	100	59.6 ± 1.65	87.13	173.01	22.343
	150	81.0 ± 1.30	(50.49–117.66)	(138.46–247.81)	
	200	93.1 ± 1.11			
	250	99.1 ± 1.81			

LFL lower fiducial limits, *UFL* upper fiducial limits, χ^2 chi square value, Significant at $P < 0.05$ level

percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Among the five solvent extracts tested for ovicidal bioassay, the methanol extracts of leaf of *T. chebula* observed 100% mortality (zero hatchability) at 200 and 250 ppm against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* exerted 100% mortality at 300 ppm.

Discussion

The search for new strategies or natural products to control vectors of diseases is desirable due to the prevalent occurrence of vector resistance to synthetic insecticides, and the problem of toxic, non-biodegradable residues contaminating the environment and the undesirable effects on non-target organisms (Jantan et al. 2005). Development of resistance to commercial insecticides of vectors has stimulated the search for new control strategies. In recent times, many botanicals have been used traditionally by human communities in many parts of the world against pest species of insects. Many plants produce secondary metabolites that inhibit the growth of insects. Though several plants from different families have been reported for mosquitocidal activity, only very few botanicals have moved from the laboratory to field use. Simple crude extracts from plants have been used as insecticides in many countries for centuries (Govindarajan 2011a).

Crude plant extracts often consist of complex mixtures of active compounds. Advances by using a complete

Table 2 Larvicidal activity of different solvent extract of *T. chebula* against *Aedes aegypti*

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50}, ppm (LFL-UFL)	LC_{90}, ppm (LFL-UFL)	χ^2
Benzene	Control	0.0 ± 0.0			
	50	26.2 ± 1.49			
	100	37.2 ± 1.12	136.23	256.40	14.315
	150	59.4 ± 1.51	(105.47–168.40)	(213.56–343.36)	
	200	71.3 ± 1.15			
	250	87.4 ± 1.56			
Hexane	Control	0.0 ± 0.0			
	50	27.5 ± 1.58			
	100	41.1 ± 1.21	128.52	244.35	14.716
	150	62.4 ± 1.52	(97.56–159.40)	(203.72–325.40)	
	200	75.1 ± 1.14			
	250	89.4 ± 1.11			
Ethyl acetate	Control	0.0 ± 0.0			
	50	33.4 ± 1.12			
	100	47.0 ± 1.15	113.17	222.23	18.301
	150	68.6 ± 1.48	(77.78–145.45)	(182.19–305.81)	
	200	83.2 ± 1.51			
	250	92.1 ± 1.09			
Chloroform	Control	0.0 ± 0.0			
	50	36.8 ± 1.45			
	100	51.8 ± 1.11	101.90	200.52	19.620
	150	73.0 ± 1.13	(66.49–132.84)	(163.63–276.59)	
	200	89.6 ± 1.60			
	250	95.2 ± 1.10			
Methanol	Control	0.0 ± 0.0			
	50	41.2 ± 1.12			
	100	56.4 ± 1.50	93.24	186.76	21.898
	150	77.6 ± 1.42	(55.41–124.94)	(150.17–265.32)	
	200	91.1 ± 1.36			
	250	97.3 ± 1.62			

LFL Lower fiducial limits, *UFL* upper fiducial limits, χ^2 chi square value, significant at $P < 0.05$ level

mixture may act synergistically, they may show greater overall bioactivity compared to the individual constituents (Sumroiphon et al. 2006) and insect resistance is much less likely to develop with mixtures. These reasons support the use of crude chemically unrefined plant extracts, containing mixtures of bioactive plant compounds rather than the use of pure individuals. The mode of action of these leaf extracts on mosquito larvae is not known, but previous studies demonstrated that phytochemicals interfered with the proper functioning of mitochondria more specifically at the proton transferring sites (Usta et al. 2002) and other studies by Rey et al. (1999) and 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.

Phytochemicals may serve as suitable alternatives to synthetic insecticides in the future as they are relatively safe, inexpensive and are readily available in many areas of the world. According to Bowers et al. (1995), the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products, and stimulate local efforts to enhance public health. Different parts of plants contain a complex of chemicals with unique biological activity (Govindarajan et al. 2008a, b, c), which is thought to be due to toxins and secondary metabolites which act as mosquitocidal agent (Niraimathi et al. 2010). In general, extracts of the plants derived from specific solvents can influence the bioactivity, probably because of the active components are present in large quantities (Oliveira et al. 2010). In our results showed that the crude benzene, hexane, ethyl acetate, chloroform and methanol extracts of leaf of *T. chebula* have significant larvicidal as well as ovicidal properties against three important vector mosquitoes, viz., *A.*

Table 3 Larvicidal activity of different solvent extract of *T. chebula* against *Culex quinquefasciatus*

Name of the extract	Concentration (ppm)	% of mortality ± SD	LC_{50}, ppm (LFL-UFL)	LC_{90}, ppm (LFL-UFL)	χ^2
Benzene	Control	0.0 ± 0.0			
	50	20.0 ± 1.56			
	100	29.4 ± 1.15	155.71	280.71	10.414
	150	52.1 ± 1.12	(129.77–185.67)	(238.43–360.51)	
	200	64.4 ± 1.33			
	250	81.6 ± 1.49			
Hexane	Control	0.0 ± 0.0			
	50	23.4 ± 1.85			
	100	34.0 ± 1.47	144.76	268.88	12.622
	150	56.6 ± 1.52	(115.83–176.49)	(225.44–355.07)	
	200	69.4 ± 1.11			
	250	83.4 ± 1.11			
Ethyl acetate	Control	0.0 ± 0.0			
	50	26.8 ± 1.54			
	100	39.6 ± 1.13	132.20	251.97	14.805
	150	61.6 ± 1.43	(100.57–164.40)	(209.35–338.88)	
	200	74.2 ± 1.60			
	250	86.7 ± 1.71			
Chloroform	Control	0.0 ± 0.0			
	50	29.5 ± 1.12			
	100	41.4 ± 1.46	122.32	233.92	15.948
	150	65.8 ± 1.09	(90.23–153.21)	(194.32–313.26)	
	200	80.6 ± 1.15			
	250	89.9 ± 1.51			
Methanol	Control	0.0 ± 0.0			
	50	33.4 ± 1.62			
	100	46.6 ± 1.36	111.98	218.95	18.377
	150	69.7 ± 1.50	(76.94–143.82)	(179.70–300.13)	
	200	84.8 ± 1.42			
	250	92.2 ± 1.21			

LFL Lower fiducial limits, *UFL* upper fiducial limits, χ^2 Chi square value, significant at $P < 0.05$ level

stephensi, *A. aegypti*, and *C. quinquefasciatus.* This result is also comparable to earlier reports of Rahuman et al. (2008) who, observed the larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbittaceous plants, *C. colocynthis*, *C. indica*, *C. sativus*, *M. charantia*, and *T. anguina*, were tested against the early fourth instar larvae of *A. aegypti* and *C. quinquefasciatus.* Pushpanathan et al. (2008) studied the larvicidal activity of *Z. officinalis* oil against filarial vector *C. quinquefasciatus.* Kannathasan et al. (2007) reported that the methanol leaf extracts of *V. negundo*, *V. trifolia*, *V. peduncularis*, and *V. altissima* were used for larvicidal assay with LC_{50} value of 212.57, 41.41, 76.28, and 128.04 ppm, respectively, against the early fourth instar larvae of *C. quinquefasciatus.*

Mullai et al. (2008) reported that the leaf extract of *C. vulgaris* with different solvents viz, benzene, petroleum ether, ethyl acetate and methanol were tested for larvicidial, ovicidal, repellent and insect growth regulatory activities against *A. stephensi.* The larval mortality was observed after 24 h exposure. The LC_{50} values are 18.56, 48.51, 49.57 and 50.32 ppm, respectively. The mean percent hatchability of the egg of *A. stephensi* was observed after 48 h. Hundred percent mortality was exerted at 250 ppm with benzene extract and the other extracts exerted 100% mortality at 300 ppm. Elango et al. (2009) reported that ethyl acetate extract from the leaves of *Aegle marmelos* exhibited high larvicidal properties against *Anopheles subpictus* and *Culex tritaeniorhynchus*, having LC_{50} values of 167.00 and 99.03 ppm, respectively.

Table 4 Ovicidal activity of *T. chebula* leaf extracts against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*

Mosquito species	Name of the solvent	Percentage of egg hatch ability						
		Concentration (ppm)						
		Control	50	100	150	200	250	300
A. stephensi	Benzene	100.0 ± 0.0	60.6 ± 1.8	52.3 ± 0.2	40.2 ± 1.8	32.5 ± 1.8	21.0 ± 1.5	11.5 ± 1.9
	Hexane	100.0 ± 0.0	52.2 ± 1.5	43.1 ± 1.5	35.5 ± 1.5	29.2 ± 1.5	12.0 ± 1.3	NH
	Ethyl acetate	100.0 ± 0.0	43.1 ± 2.0	36.4 ± 1.8	24.2 ± 2.0	19.1 ± 1.7	NH	NH
	Chloroform	100.0 ± 0.0	39.4 ± 1.3	32.1 ± 1.2	23.6 ± 1.3	14.5 ± 1.2	NH	NH
	Methanol	100.0 ± 0.0	32.3 ± 1.9	28.5 ± 1.4	20.0 ± 1.7	NH	NH	NH
A. aegypti	Benzene	100.0 ± 0.0	66.3 ± 1.0	55.2 ± 1.0	45.3 ± 1.5	38.3 ± 1.9	26.0 ± 1.8	16.2 ± 1.5
	Hexane	100.0 ± 0.0	59.4 ± 1.6	46.5 ± 1.1	38.7 ± 1.2	32.7 ± 2.0	19.0 ± 1.0	14.3 ± 1.2
	Ethyl acetate	100.0 ± 0.0	50.6 ± 1.2	42.3 ± 1.5	32.8 ± 1.9	21.4 ± 1.1	13.6 ± 1.9	NH
	Chloroform	100.0 ± 0.0	45.1 ± 1.7	37.1 ± 2.0	29.5 ± 1.8	19.3 ± 1.6	NH	NH
	Methanol	100.0 ± 0.0	39.5 ± 1.4	36.9 ± 1.2	28.1 ± 1.6	16.9 ± 1.4	NH	NH
C. quinquefasciatus	Benzene	100.0 ± 0.0	70.1 ± 1.3	58.1 ± 1.8	52.3 ± 1.8	46.5 ± 1.0	29.9 ± 1.2	21.8 ± 1.3
	Hexane	100.0 ± 0.0	61.2 ± 1.0	51.0 ± 1.9	40.3 ± 1.2	36.2 ± 1.2	24.5 ± 1.6	19.3 ± 2.0
	Ethyl acetate	100.0 ± 0.0	55.4 ± 2.0	48.2 ± 1.4	36.0 ± 1.5	25.3 ± 1.5	18.0 ± 0.0	12.6 ± 1.6
	Chloroform	100.0 ± 0.0	50.2 ± 1.9	42.4 ± 1.0	33.2 ± 1.7	18.5 ± 1.4	12.3 ± 1.9	NH
	Methanol	100.0 ± 0.0	43.9 ± 1.3	39.4 ± 1.5	33.4 ± 1.1	25.1 ± 1.0	10.5 ± 0.9	NH

NH No hatchability

Pushpanathan et al. (2006) studied larvicidal, ovicidal and repellent activities of *C. citrates* against the second, third and fourth filarial larval instar was 144.54, 165.70 and 184.18 ppm, respectively and the 100% ovicidal activities were observed at 300 ppm. The benzene, hexane, ethyl acetate, methanol, and chloroform leaf extract of *A. paniculata* was found to be more effective against *C. quinquefasciatus* than *A. aegypti*. The LC_{50} values were 112.19, 137.48, 118.67, 102.05, 91.20 ppm and 119.58, 146.34, 124.24, 110.12, 99.54 ppm, respectively (Govindarajan 2011b). The methanolic extracts of *S. suratence*, *A. indica* and *H. javanica* exhibited larvicidal activity against *C. quinquefasciatus* (Venkatachalam and Jebanesan 2001). Mullai and Jebanesan (2007) have reported that ethyl acetate, petroleum ether and methanol leaf extracts of *C. colocynthis* and *C. 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.

Rahuman et al. (2009a, b) have reported that 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_{50} 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 fourth instar larvae of *C. quinquefasciatus*, respectively. Govindarajan (2011c) reported, that larvicidal and ovicidal activity of crude hexane, ethyl acetate, benzene, chloroform and methanol extracts of the leaf of *C. halcacabum* were tested against the early third instar larvae of *A. stephensi*, the highest larval mortality was found in the methanol extract of *C. halicacabum* against the larvae of *A. stephensi* (LC_{50} 79.68, 112.56 and 133.01 ppm; LC_{90} 154.66, 220.68 and 270.72 ppm), respectively. The percent hatchability was inversely proportional to the concentration of the extract. Mortality of 100% with methanol and benzene extract of *C. halicacabum* exerted at 150 ppm. Besides, larvicidal activities of plant extracts depend on the different parts of the plant, the geographical location, and the application method (Sukumar et al. 1991).

The ovicidal activity of plant extracts has been reported to be affected by different factors, particularly the age of the egg, the concentration and the exposure period. The age of the egg has been found to influence the ovicidal activity of the compounds. Exposure of freshly laid eggs to phytotoxins has been found to cause higher mortality rates. In the present study, freshly laid eggs were exposed to various concentrations of extracts. As reported, the exposure of the eggs to the phytotoxins/extracts at the time of oviposition affects embryogenesis was a likely event cause effective mortality as observed from the results. Rajkumar and Jebanesan (2008) have reported flavonoid compounds from *P. trifoliata* to be effective as an ovicide in the early stage

of egg development of *A. aegypti.* Similarly, Govindarajan et al. (2008a) also observed on the same against the leaf extract of *A. indica* on the eggs of *A. stephensi.* In another study, Govindarajan et al. (2008b) on comparing the ovicidal activity of *C. fistula* methanolic leaf extract against the egg rafts of *C. quinquefasciatus* and eggs of *A. stephensi* showed that younger age group of eggs showed a maximum mortality rate when compared to the older age group. Usta et al. (2002) reported that phytocompounds such as flavonoids acts as an effective ovicide when treated in the early stages of egg development and higher concentration of these compounds cause maximum egg mortality.

Higher concentrations always yielded better mortality rates and this was observed in the present study and similar results were reported by Govindarajan et al. (2008b). Broadbent and Pree (1984) reported that when eggs were directly exposed to higher concentrations of the compounds, more chemicals entered the eggshell, which affected the embryogenesis. Exposure time also has a crucial role in causing toxicity (Miura et al. 1976). Longer exposure periods also facilitate increased penetration of the compounds into the egg shells, thus increasing their effectiveness (Govindarajan 2011d). Shorter duration of treatment was decisively inferior to longer exposure to insecticides at the egg stage (Kuppusamy and Murugan 2008). Smith and Salkeld (1966) reported differences in susceptibility to ovicides to occur due to differential rates of uptake, penetration through the chorion, conversion to active inhibitor, detoxification and failure of the toxicant to reach the target. Grosscurt (1977) observed that the efficiency to act on the embryo inside the egg shell depends on an efficient penetration of the insecticide, which in turn is influenced by the exposure period. The eggs of mosquitoes are found to be much more tolerant to the action of insecticides compared to larval stages. Insect eggs are covered with a shell, which differs biochemically from the integument of the larvae, and the difference in penetration of the insecticide through the egg shell, and the larval integument is reflected in the observed toxicity differences (Kuppusamy and Murugan 2008).

In conclusion, our findings showed that the plant *T. chebula* exhibits larvicidal and ovicidal activity against three important vector mosquitoes. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. In brief, our findings suggested that the crude extracts of *T. chebula* leaves and its effective constituents may be explored as a potential environmental benign larvicide and ovicide. Further investigations for the mode of the constituents' actions, effects on non-target organisms and field evaluation are necessary. These results, obtained are useful in search of more selective, biodegradable and naturally produced larvicidal and ovicidal compounds.

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