Bioefficacy of *Morinda tinctoria* and *Pongamia glabra* plant extracts against the malaria vector *Anopheles stephensi* (Diptera: Culicidae)

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Abstract

Mosquito-borne diseases have an economic impact, including loss in commercial and labour outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases. The aim of the present study was to investigate the larvicidal, adulticidal and ovicidal activity of dried leaf chloroform, ethyl acetate, acetone, aqueous, and methanol extracts of *Morinda tinctoria* and *Pongamia glabra* against larvae of *Anopheles stephensi* (Diptera: Culicidae). Larvae were exposed to varying concentrations of plant extracts for 24 h. All extracts showed moderate larvicidal effects after 24 h of exposure; however, the highest larval mortality was found with the leaf methanol extracts of *M. tinctoria* and *P. glabra* against the larvae of *A. stephensi* lethal concentration $(LC)_{50}$=136.24 and 141.05 ppm; LC_{90}=342.67 and 368.89 ppm, respectively. The results of the adulticidal activity assays of chloroform, ethyl acetate, acetone, aqueous, and methanol extracts of *M. tinctoria* and *P. glabra* showed significant mortality against larvae of *A. stephensi*. The methanol extract showed maximum activity compared with the other extracts. The greatest effect on mean percentage hatch in the ovicidal assays was observed 48 h post-treatment. Percent hatch was inversely proportional to the concentration of extract, and directly proportional to the number of eggs. A mortality of 100% was observed with 100-400 ppm methanol extracts and 200-400 ppm aqueous extracts of *M. tinctoria*, and 200-400 ppm aqueous and methanol extracts of *P. glabra*. This study provides the first report of the larvicidal, adulticidal and ovicidal activities of *M. tinctoria* and *P. glabra* plant extracts against the malaria vector, *A. stephensi*, representing an ideal eco-friendly approach for its control.

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Introduction

The prevalence of mosquito-borne diseases is one of the world's most important health problems. Mosquitoes are responsible for transmitting various infectious diseases; for this reason, the mosquito has been declared *public enemy number one* (World Health Organisation, 1996). Mosquitoes belonging to the genera *Anopheles*, *Culex* and *Aedes* are vectors for the pathogens of different diseases such as malaria, filariasis, Japanese encephalitis, dengue and dengue haemorrhagic fever, yellow fever and chickungunya (Rahuman *et al*., 2009; Borah *et al*., 2010). They cause allergic responses, including local skin and systemic reactions such as angioedema and urticaria (Peng *et al*., 1999). Tropical areas are more vulnerable to parasitic diseases, and the risk of contracting arthropod-borne illnesses has increased due to climate change and intensifying globalisation (Karunamoorthy *et al*., 2010). It is necessary to prevent mosquito-borne diseases and improve public health by controlling mosquitoes.

Malaria is an infectious disease that is prevalent in tropical and some temperate areas of the world. Malaria is caused by a parasite that is transmitted from one human to another by the bite of infected *Anopheles stephensi*. Half of the world's population is at risk from malaria. Each year, almost 250 million cases occur, causing 860,000 deaths (World Health Organisation, 2010). In India, 2-3 million malaria cases and about 1000 deaths are reported every year (Lal *et al.,* 2010). Currently, a resistant variety of the malarial parasite is commonly found in almost all parts of the world where malaria is endemic (Cooper *et al.,* 2005). The increased incidence of drug resistance continues to be a major issue, with ongoing problems related to drug quality, availability, and cost of treating the disease (Garcia, 2010). For this reason, the transmission of malaria is best reduced by the control of the mosquito vector. 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 (*Plasmodium falciparum*) cases and malaria-related deaths is recorded from the state of Orissa, located in the eastern part of India (Sharma *et al*., 2010).

Widely used chemical insecticides to control mosquitoes are often harmful to other beneficial organisms that prey on mosquito larvae, as well as to humans (Amer & Mehlhorn, 2006). Alternative pest control strategies, especially those that are effective and low-cost, are therefore needed. A recent emphasis has been placed on plant materials

that demonstrate larvicidal properties (Kovendan *et al*., 2012b; Mahesh Kumar *et al*., 2012a; Panneerselvam *et al*., 2013b).

Morinda tinctoria, which belongs to the family Rubiaceae, grows wild and is distributed throughout Southeast Asia. Commercially known as Nunaa, it is indigenous to tropical countries and is considered an important traditional medicine, where its leaves and roots are used as an astringent and emmengogue, and to relieve pain caused by gout (Thirupathy *et al*., 2009). There is a greater demand for fruit extracts of *Morinda* species in treatments for arthritis, cancer, gastric ulcers and other heart diseases (Mathivanan *et al*., 2006). Anti-convulsant, analgesic, anti-inflammatory anti-oxidant activities of *M. tinctoria* leaves have been reported (Jeyabalan & Palayan, 2009; Sreena *et al*., 2011). The major components that have been identified in the Nunaa plant include octoanicacid, potassium, vitamin C, terpenoids, scopoletin, flavones, glycosides, linoleic acid, anthraquinones, morindone, rubiadin, and alizarin (Moorthy & Reddy, 1970; Singh & Tiwari, 1976; Levand & Larson, 1979; Farine *et al*., 1996). Deepti *et al*. (2011) studied the *in vitro* free radical-scavenging efficacy of methanolic, chloroform and ethyl acetate extracts of *M. tinctoria*, which can be recommended as potential antioxidants.

Pongamia glabra Vent. (Syn. *Pongamia pinnata*), in the family Fabaceae, commonly known as Karanja, is a tree found all over India that bears imparipinnate leaves and pinkish-white flowers (Kirtikar & Basu, 1984). Roots, bark, leaves, flowers and seeds of this plant also have medicinal properties and are traditionally used as medicinal treatments. All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, wounds and ulcers (Tanaka *et al*., 1992). Seeds contain karanjin, ponga-mol and glabrin. Karanjin is reported to be an effective remedy for all skin diseases such as scabies, eczema, leprosy and ulcers (Sivarajan & Balachandran, 1999). The leaves are spicy, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations. A hot infusion of leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers from gonorrhoea and scrofulous enlargement (Chopra, 1933; Satyavati *et al*., 1987). In addition, phytochemical examinations of this plant have indicated the presence of furanoflaovones, furanoflavonols, chromenoflavones, flavones, furanodiketones and flavonoid glucosides (Rangaswami *et al*., 1942; Murthy & Seshadri, 1944; Sharma *et al*., 1973; Talapatara *et al*., 1980; Pathak, 1983; Tanaka *et al*., 1992; Ahemed *et al*., 2004; Yin *et al*., 2006).

The acetone, chloroform, ethyl acetate, hexane, and methanol leaf extracts of *Acalypha indica*, *Achyranthes aspera*, *Leucas aspera*, *M. tinctoria*, and *Ocimum sanctum* were studied against the early fourth-instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* (Bagavan *et al*., 2008); the chloroform fruit extract of *Morinda pubescens* (*M. tinctoria*) showed wound-healing properties in rats (Mathivanan *et al*., 2006). Larvicidal effects of neem (*Azadirachta indica*) and karanja (*P. glabra*) oil cakes (separately and in combination) were studied against several mosquito species. The combination of neem and karanja oil cakes in equal proportion proved to be more effective than individual treatments against the mosquitoes *C. quinquefasciatus*, *A. aegypti* and *A. stephensi,* with lethal concentration $(LC)_{95}$ values of 0.93, 0.54 and 0.77 ppm, respectively (Shanmugasundaram *et al*., 2008). Oviposition deterrent activity of the ethanolic extract of *Pongamia pinnata*, *Coleus forskohlii*, and *Datura stramonium* leaves against *A. aegypti* and *C. quinquefaciatus* was reported by Swathi *et al*. (2010). The individual and combined efficacy of *Annona squamosa* and *P. glabra* extracts against three mosquito vectors, *C. quinquefasciatus*, *A. stephensi*, and *A. aegypti*, compared with that of *A. indica* was investigated by George & Vincent (2005). Leaf extracts of some common plants such as *Vitex negundo*, *Gliricidia maculata*, *Wedelia chinensis*, *M. tinctoria*, and *P. glabra*, were evaluated for their acaricidal activity against the red spider mite, *Oligonychus coffeae*, in the laboratory using a leaf disc method under controlled conditions. Among them, the aqueous extract of *M. tinctoria* and *P. glabra* showed maximum ovicidal action, ovicidal deterrence and 100% adult mortality (Vasanthakumar *et al*., 2012).

In the present study, we report for the first time on larvicidal, adulticidal and ovicidal activities of different solvent extracts of *M. tinctoria* and *P. glabra* against the malarial mosquito vector, *A. stephensi.*

Materials and methods

Plant collection

Fully developed fresh leaves of *M. tinctoria* and *P. glabra* were collected from the Maruthamalai Hills, near the Bharathiar University campus in Coimbatore. Identification was authenticated by a plant taxonomist from the Department of Botany, Bharathiar University. A voucher specimen is deposited at the herbarium of the Entomology Division, Bharathiar University.

Extraction

The leaves were washed with tap water, shade-dried at room temperature (28±2°C) for 5-10 days. The air-dried materials were powdered individually using a commercial electric blender. The finely ground plant material (1000 g/solvent) was loaded into a Soxhlet apparatus and extracted individually with five different solvents: chloroform, ethyl acetate, acetone, aqueous, and methanol. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of these plants varies with the solvents used. The *M. tinctoria* and *P. glabra* with five different solvents yielded 58.20, 64.09, 54.11, 67.34, 88.05 g and 47.10, 53.31, 41.18, 54.30, 79.16 g of crude residue, respectively. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared, and these solutions were used for the larvicidal, adulticidal and ovicidal bioassays.

Insect rearing

The eggs of *A. stephensi* were collected from different breeding sites (overhead tanks) in Coimbatore District, Tamil Nadu, India. These were taken to the laboratory and transferred (in approximately the same aliquot numbers of eggs) to 18 cm L×13 cm W×4 cm D enamel trays containing 500 mL of water, where they were allowed to hatch.

Mosquito larvae were reared (and adult mosquitoes held) at 27°C±2°C and 75%-85% relative humidity in a 14:10 (L:D) photoperiod. Larvae were fed 5 g of ground dog biscuit and brewer's yeast daily in a 3:1 ratio. Pupae were collected and transferred to plastic containers with 500 mL of water. The container was placed inside a screened cage (90 cm L×90 cm H×90 cm W) to retain emerging adults, for which 10% sucrose in water solution (v/v) was available *ad libitum*. On days 5 post-emergence, the mosquitoes were provided access to a rabbit host for blood feeding. The shaved dorsal side of the rabbit was positioned on the top of the mosquito cage in contact with the cage screen (using a cloth sling to hold the rabbit) and held in this position overnight. Glass Petri dishes lined with filter paper and containing 50 mL of water were subsequently placed inside the cage for oviposition by female mosquitoes.

Larvicidal bioassays

A laboratory colony of *A. stephensi* larvae was used for the larvicidal activity. Twenty-five individuals of early fourth-instar larvae were kept in a 500-mL glass beaker containing 249 mL of dechlorinated water, and 1 mL of the desired concentration of plant extracts were added. Larval food was provided for the test larvae. At each tested concentration, two to five trials were made and each trial consisted of five replicates. The control was set up by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae exposed to dechlorinated water without

acetone served as a control. The control mortalities were corrected using Abbott's formula (Abbott, 1925). LC_{50} and LC_{90} were calculated from toxicity data using probit analysis (Finney, 1971).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100 \quad (1)$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (2)$$

Adulticidal bioassays

Sugar-fed adult female mosquitoes (5-6 days old) were used. The *M. tinctoria* and *P. glabra* leaf extracts were diluted with acetone to make different concentrations. The diluted plant extracts were impregnated on filter papers (140×120 mm). A blank paper consisting of only ethanol was used as a control. The papers were left to dry overnight at room temperature to let the ethanol evaporate. Impregnated papers were prepared fresh prior to testing. The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes, both measuring 125×44 mm, following the method of World Health Organisation (1981). One tube served to expose the mosquitoes to the plant extract and the other tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Each tube was closed at one end with a 16-mesh wire screen. Sucrose-fed and blood-starved mosquitoes (20) were released into the tube, and the mortality effects of the extracts were observed every 10 min for a 3-h exposure period. At the end of 1-, 2-, and 3-h exposure periods, the mosquitoes were placed in the holding tube. Cotton pads soaked in 10% sugar solution with vitamin B complex were placed in the tube during the holding period for 24 h. Mortality of the mosquitoes was recorded after 24 h. The above procedure was replicated three times using plant extracts of each concentration.

Ovicidal activity assays

Freshly laid eggs were collected by providing ovitraps in mosquito cages. Ovitraps were kept in the cages 2 days after the female mosquitoes were given a blood meal. The eggs were laid on filter paper lining provided in the ovitrap. After scoring, 100 gravid females were placed in a screen cage where 10 oviposition cups were introduced for oviposition 30 min before the start of the dusk period. Of these 10 cups, each nine were filled with test solution of 12.5, 25.0, 50.0, 100.0, 200.0, 400.0 ppm, respectively and one was filled with 100 mL of the water and Polysorbate 80 that served as a control. The experiment was repeated trice with three replicates. A minimum of 100 eggs was used for each treatment, and the experiment was replicated five times. After treatment, the eggs were sieved through muslin cloth, thoroughly rinsed with tap water, and left in plastic cups filled with dechlorinated water for hatching assessment after counting the eggs under microscope (Su & Mulla, 1998). The percentage of egg mortality was calculated on the basis of non-hatch of eggs with unopened opercula (Chenniappan & Kadarkarai, 2008). The hatching rate of eggs was assessed after 98 h post-treatment, as per the method of Rajkumar & Jebanesan (2009).

Statistical analysis

The average adult mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% upper and lower fiducial limits, and Chi-square values were calculated by using the SPSS Statistical software package, ver. 16.0. Results with $P<0.05$ were considered to be statistically significant.

Results

The present study explored the potential mosquitocidal properties of two plants, using different solvents for the crude extracts (Table 1). Chloroform, ethyl acetate, acetone, aqueous, and methanol leaf extracts of the plants *M. tinctoria* and *P. glabra* was studied for use as eco-friendly insecticides, as alternatives to potentially harmful synthetic insecticides. Results of larvicidal and adulticidal assays with these leaf extracts (Tables 2-5) confirm their potential ability to control adult and larval populations of the mosquito *A. stephensi*. All extracts showed moderate larvicidal effects; however, the highest larval mortality was found with the methanol extract of *M. tinctoria* and *P. glabra* against the fourth-instar larvae of *A. stephensi* (LC_{50}=136.24 and 141.05 ppm; LC_{90}=342.67 and 368.89 ppm, respectively) (Figure 1). The chi-square values are significant at the $P<0.05$ level. The high chi-square values in the bioassays possibly indicate the heterogeneity of the test population. The 95% confidence limits for the LC_{50} lower/upper fiducial limits (LCL-UCL) and LC_{90} (LCL-UCL) were also calculated. No mortality was recorded in the control. The results of the larvicidal assay clearly indicate that the percentage of mortality was directly proportional to concentration of the extract. After exposure to the test concentrations, the treated larvae exhibited restlessness, sluggishness, tremors, and convulsions, followed by paralysis. Five different solvents were tested, and the highest adulticidal activity was observed with the methanol extract of *M. tinctoria* followed by *P. glabra*, with LC_{50} values of 194.78 and 198.65 ppm and LC_{90} values of 336.27 and 357.92 ppm, respectively (Figure 2). At higher concentrations, the adults showed restless movement for some time, accompanied by abnormal wiggling movements,

Table 1. List of medicinal plants tested for bioactivity against eggs, larvae and adults of *Anopheles stephensi*.

Botanical name	Common name (Tamil)	Family	Medicinal property	Plant parts tested
Morinda tinctoria Roxb.	Mannanunai	Rubiaceae (coffee family)	Leaves are useful as tonic, febrifuge and emmenagogue. It is also used for curing dyspepsia, diarrhoea, ulceration, stomatitis, digestion, wound and fever. The poultice or the paste of its leaves is applied on the wounds and swellings for relief. The green fruit and leaves are used to treat menstrual cramps, bowel irregularities and urinary tract infections	Leaves
Pongamia glabra Vent.	Pungai	Fabaceae or Leguminosae	Leaves of *P. glabra* have been known as a remedy for diarrhoea. It is also used as a digestive and laxative and to treat inflammation and wounds. Leaf juice aids in treatment of leprosy, gonorrhoea, flatulence, coughs, and colds. The leaf infusions and extracts alleviate rheumatism and itches, respectively	Leaves

and death. The mean percentage egg hatch of *A. stephensi* was also tested against these solvents and leaf extracts; results are shown in Table 6. The percentage hatch was inversely proportional to the concentration of extract, and directly proportional to the no. of eggs. Of the extracts tested for ovicidal activity, the leaf methanol extract of *M. tinctoria* resulted in 100% mortality (no hatch) at both 100 and 400 ppm. The leaf extract of *M. tinctoria* was more effective than *P. glabra* against larvae and eggs of this mosquito vector. Eggs in the control treatment had 100% hatch.

Discussion and conclusions

Insect pest control is often a complex, expensive task, frequently requiring the cooperative efforts of communities as well as such groups as industry, agriculture, state, and local governments. We must be concerned with the harmful effects of synthetic pesticides on the environment and people, and reports have emerged on the resurgence of several mosquito-borne diseases in the world as a consequence of the increasing resistance of mosquitoes to commercial insecticides (Becker *et al*., 2003). This has necessitated the need for research and development of an environmentally safe, biodegradable and indigenous material for vector control. Many herbal products were used as natural insecticides before the discovery of synthetic organic insecticides (Mittal & Subbarao, 2003). Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents, because plants produce a large variety of compounds that increase their resistance to insect attack (Berenbaum, 1988; Murugan *et al*., 1996; Senthil Nathan *et al*., 2005). In this study, good larvicidal activity against *A. stephensi* was achieved with different solvent extracts of *M. tinctoria* and *P. glabra*. The mode of action of these leaf extracts on mosquito larvae is not known, but previous studies have demonstrated that phytochemicals interfere with the proper functioning of mitochondria, more specifically at the proton transferring sites (Usta *et al*., 2002). Other studies by Rey *et al*. (1999) and David *et al*. (2000) found that phytochemicals primarily affect the midgut epithe-

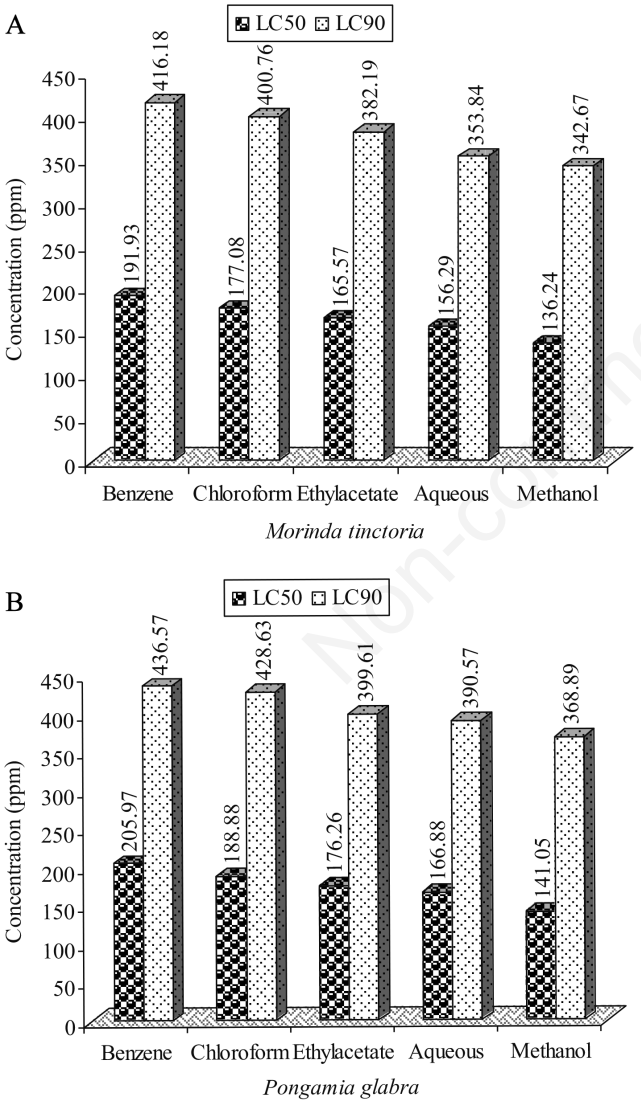

Figure 1. Larvicidal activity of A) *Morinda tinctoria* and B) *Pongamia glabra* leaf extracts against *Anopheles stephensi* with lethal concentration $(LC)_{50}$ and LC_{90} values.

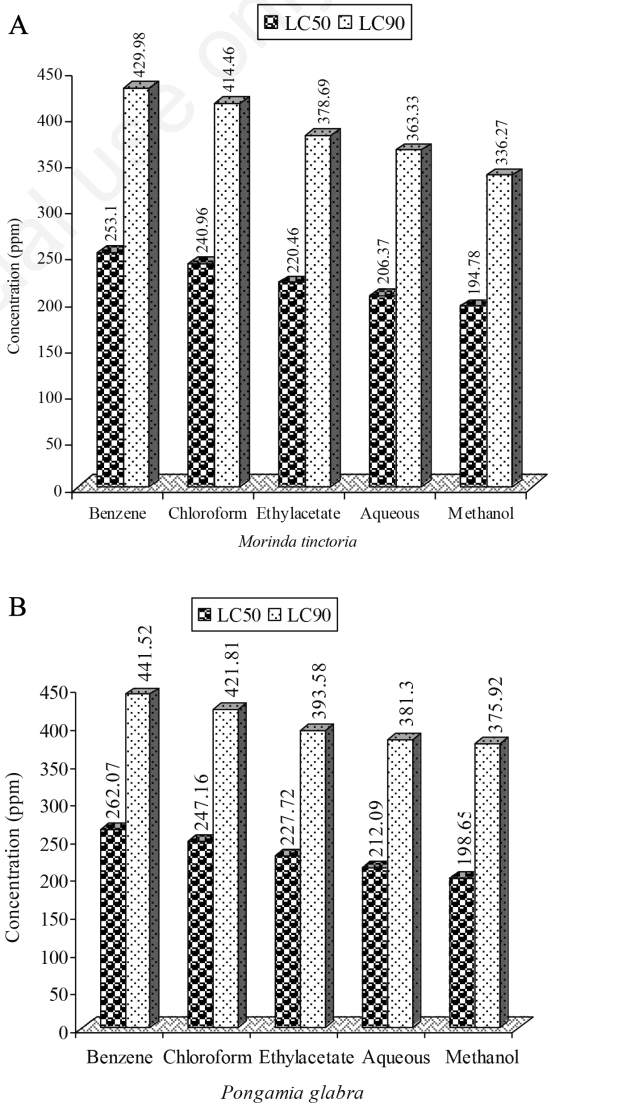

Figure 2. Adulticidal activity of A) *Morinda tinctoria* and B) *Pongamia glabra* leaf extracts against *Anopheles stephensi* with lethal concentration $(LC)_{50}$ and LC_{90} values.

Table 2. Larvicidal activity of different solvent extracts of *Morinda tinctoria* against fourth instar larvar of *Anopheles stephensi*.

Solvent extracts	Concentration (ppm)	% Mortality±SD	LC_{50}, ppm (LFL-UFL)	LC_{90}, ppm (LFL-UFL)	χ^2
Chloroform	Control	0.0±0.0	191.938 (168.13-212.98)	416.188 (379.32-468.55)	3.751*
	80	26.36±1.62			
	160	45.44±1.43			
	240	59.38±1.40			
	320	71.02±1.96			
	400	92.22±1.57			
Ethyl acetate	Control	0.0±0.0	177.080 (151.89-198.57)	400.763 (365.20-451.21)	3.295*
	80	29.48±1.48			
	160	48.34±1.59			
	240	62.24±1.52			
	320	74.42±1.61			
	400	93.54±1.46			
Acetone	Control	0.0±0.0	165.573 (139.98-186.98)	382.190 (348.85-429.06)	4.702*
	80	32.82±1.04			
	160	49.52±1.51			
	240	64.04±0.99			
	320	77.06±1.07			
	400	96.02±1.18			
Aqueous	Control	0.0±0.0	156.298 (91.6-198.70)	353.845 (297.79-470.72)	5.452*
	80	34.06±1.02			
	160	51.14±1.12			
	240	66.44±1.77			
	320	81.84±1.23			
	400	98.34±1.31			
Methanol	Control	0.0±0.0	136.242 (48.50-184.70)	342.678 (282.75-480.98)	6.210*
	80	39.32±1.62			
	160	56.22±1.78			
	240	70.06±1.20			
	320	83.24±1.64			
	400	99.04±1.16			

SD, standard deviation; LC, lethal concentration; LFL, lower fiducidal limits; UFL, upper fiducidal limits; χ^2 Chi square value. *Significant at P<0.05 level.

Table 3. Larvicidal activity of different solvent extracts of *Pongamia glabra* against fourth instar larvar of *Anopheles stephensi*.

Solvent extracts	Concentration (ppm)	% Mortality±SD	LC_{50}, ppm (LFL-UFL)	LC_{90}, ppm (LFL-UFL)	χ^2
Chloroform	Control	0.0±0.0	205.973 (182.63-227.268)	436.574 (397.02-493.32)	3.103*
	80	23.34±1.47			
	160	44.36±1.64			
	240	55.06±1.13			
	320	69.04±1.18			
	400	89.22±1.35			
Ethyl acetate	Control	0.0±0.0	188.888 (163.09-211.26)	428.639 (388.43-487.00)	2.174*
	80	28.42±1.62			
	160	45.12±1.69			
	240	60.62±1.78			
	320	71.02±1.26			
	400	90.12±1.10			
Acetone	Control	0.0±0.0	176.264 (151.03-197.77)	399.610 (364.25-449.68)	1.206*
	80	30.06±1.10			
	160	47.08±1.11			
	240	62.32±1.53			
	320	77.06±1.07			
	400	92.34±1.59			
Aqueous	Control	0.0±0.0	166.882 (140.53-188.85)	390.575 (355.82-439.81)	1.264*
	80	32.12±1.13			
	160	49.52±1.35			
	240	63.08±1.22			
	320	79.42±1.54			
	400	93.04±1.06			
Methanol	Control	0.0±0.0	141.058 (110.40-165.10)	368.890 (335.10-417.11)	1.965*
	80	37.06±1.24			
	160	56.46±1.67			
	240	68.38±1.54			
	320	81.66±1.83			
	400	95.16±1.42			

SD, standard deviation; LC, lethal concentration; LFL, lower fiducidal limits; UFL, upper fiducidal limits; χ^2 Chi square value. *Significant at P<0.05 level.

lium and secondarily the gastric caeca and the malpighian tubules in mosquito larvae. Furthermore, the crude extracts may be more effective than the individual active compounds, due to a natural synergism that discourages the development of resistance in the vectors (Maurya *et al*., 2007). The present investigation revealed that the crude chloroform, ethyl acetate, acetone, aqueous, and methanol leaf extracts of the plants *M. tinctoria* and *P. glabra* have significant larvicidal, adulticidal as well as ovicidal activity. These results are comparable to earlier reports of Panneerselvam *et al*. (2012), who observed larvicidal activity of *Artemisia nilagirica* against *A. stephensi*. They reported LC_{50} values against the first instar of 272.50 ppm, second instar 311.40 ppm, third instar 361.51 ppm, and fourth instar 442.51 ppm; the corresponding LC_{90} values were: first instar, 590.07 ppm, second instar, 688.81 ppm, third instar, 789.34 ppm, and fourth instar, 901.59 ppm; the LC_{50} and LC_{90} values against the pupae were 477.23 and 959.30 ppm, respectively. The petroleum ether (PE) and methanol (MeOH) extracts of *Rhinacanthus nasutus* and *Derris elliptica* exhibited larvicidal effects against *A. aegypti*, *C. quinquefasciatus*, *Anopheles dirus*, and *Mansonia uniformis*, with LC_{50} values between 3.9 and 11.5 mg/L, while the MeOH extract gave LC_{50} values between 8.1 and 14.7 mg/L. *D. elliptica* PE extract showed LC_{50} values between 11.2 and 18.84 mg/L, and the MeOH extract exhibited LC_{50} values between 13.2 and 45.2 mg/L, respectively (Komalamisra *et al*., 2005); the n-hexane, ethyl acetate, and methanol extracts of *Cassia nigricans* showed 100% larval mortality against *Ochlerotatus triseriatus* (Georges *et al*., 2008). The leaf hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Acalypha alnifolia* were tested for larvicidial activity against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*, with LC_{50} values for *A. stephensi* of 197.37, 178.75, 164.34, 149.90 and 125.73 ppm, respectively; for *A. aegypti*, 202.15, 182.58, 160.35, 146.07 and 128.55 ppm, respectively; and for *C. quinquefasciatus*, 198.79, 172.48, 151.06, 140.69 and 127.98 ppm, respectively (Kovendan *et al*., 2012c). In our results, the larvicidal activity of chloroform, ethyl acetate, acetone, aqueous, and methanol extracts of *M. tinctoria* and *P. glabra* exhibited larvicidal effects against *A. stephensi* with LC_{50} values of (with *M. tinctoria*) 191.93, 177.08, 165.57, 156.29 and 136.24; and (with *P. glabra*) 205.97, 188.88, 176.26, 166.88 and 141.05, respectively. The leaf benzene, petroleum ether, ethyl acetate, and methanol extracts of *Citrullus vulgaris* were previously tested for larvicidial activity against *A. stephensi*, with LC_{50} values of 18.56, 48.51, 49.57, and 50.32 ppm, respectively (Mullai *et al*., 2008b). The insecticidal activity of *Zingiber officinale* against third-instar larval maturation and adult emergence of *Anopheles pharoensis* was evaluated at concentrations of 100, 70, 50, 25, 5, 2, 1, 0.9, 0.7, 0.5 and 0.3%, showing 100% larval mortality and, at 0.2% and 0.1%, mortality of 66.7%.

The effects of the tested extracts on adult emergence and adulticidal activity against the mosquitoes are remarkably greater than those reported for other plant extracts in the literature. For example, at the highest concentration, 50% inhibition of adult emergence was observed from the ethyl acetate fractions of *Calophyllum inophyllum* seed and leaf, *Solanum suratense* and *Samadera indica* leaf extracts, and the petrol ether fraction of *Rhinocanthus nasutus* leaf extract for *C. quinquefasciatus*, *A. stephensi* and *A. aegypti* (Muthukrishnan & Puspalatha, 2001). Similarly, 88% adult mortality was observed from *Pelargonium citrosa* leaf extracts at 2% concentration against *A. stephensi* (Jeyabalan *et al*., 2003). Adult mortality was caused by the ethanol extract of *Citrus sinensis*, with LC_{50} and LC_{90} values of 272.19 and 457.14 ppm, and for *A. stephensi*, 289.62 and 494.88 ppm, and *A. aegypti,* 320.38 and 524.57 ppm, respectively (Murugan *et al*., 2012). These findings correspond with those of Govindarajan & Sivakumar

Table 4. Adulticidal activity of different solvent extracts of *Morinda tinctoria* against *Anopheles stephensi*.

Solvent extracts	Concentration (ppm)	% Mortality±SD	LC_{50}, ppm (LFL-UFL)	LC_{90}, ppm (LFL-UFL)	χ^2
Chloroform	Control	0.0±0.0	253.101 (235.09-269.40)	429.981 (399.62-473.73)	0.864*
	160	26.44±1.65			
	220	37.28±1.62			
	280	60.14±1.99			
	340	72.44±1.46			
	400	86.06±1.00			
Ethyl acetate	Control	0.0±0.0	240.968 (222.22-257.28)	414.465 (386.03-455.13)	0.098*
	160	27.44±1.45			
	220	43.36±1.80			
	280	62.54±1.73			
	340	76.08±1.94			
	400	88.06±1.11			
Acetone	Control	0.0±0.0	220.466 (201.34-236.39)	378.697 (354.98-411.62)	0.594*
	160	33.12±0.97			
	220	47.02±1.28			
	280	68.18±1.11			
	340	84.52±1.39			
	400	92.62±1.52			
Aqueous	Control	0.0±0.0	206.379 (185.55-223.09)	363.338 (340.67-394.75)	1.082*
	160	37.36±1.47			
	220	50.28±1.52			
	280	74.54±1.74			
	340	86.06±1.01			
	400	94.44±1.77			
Methanol	Control	0.0±0.0	194.785 (174.49-210.88)	336.270 (316.49-363.13)	1.563*
	160	41.02±1.29			
	220	55.56±1.57			
	280	76.52±1.40			
	340	90.54±1.75			
	400	98.02±1.27			

SD, standard deviation; LC, lethal concentration; LFL, lower fiducidal limits; UFL, upper fiducidal limits; χ^2 Chi square value. *Significant at P<0.05 level.

(2012), who reported on the adulticidal activity of hexane, ethyl acetate, benzene, chloroform and methanol leaf extracts of *Cardiospermum halicacabum* against *C. quinquefasciatus*, *A. aegypti* and *A. stephensi*. The plant extracts showed moderate toxic effects on the adult mosquitoes after an exposure period of 24 h. However, when compared with other solvents, the highest mortality was found with a methanol extract of *C. halicacabum* against all three species. Among them, *A. stephensi* had the highest LC_{50} and LC_{90} values (186.00 and 346.06 ppm). Nathan *et al*. (2005) considered pure limonoids from neem seed, testing for biological, larvicidal, pupicidal, adulticidal and antiovipositional activity. Against *A. stephensi*, larval mortality was dose-dependent, with the highest dose of 1-ppm azadirachtin causing almost 100% mortality, exhibiting pupicidal and adulticidal activity and significantly decreased fecundity and longevity. In the present study, we found the methanol extract of *M. tinctoria* to have the highest adulticidal activity compared with *P. glabra*, with LC_{50} and LC_{90} values of 194.78, 198.65 ppm and 336.27, 357.92 ppm, respectively. Similarly, the greatest adulticidal effect was seen from *Piper sarmentosum*, followed by *P. ribesoides* and *P. longum*, with LD_{50} values of 0.14, 0.15 and 0.26 µg/female, respectively (Choochote *et al*., 2006). Adulticidal activity of the essential oil isolated from *Mentha longifolia* was screened using a

Table 5. Adulticidal activity of different solvent extracts of *Pongamia glabra* against *Anopheles stephensi*.

Solvent extracts	Concentration (ppm)	% Mortality±SD	LC_{50}, ppm (LFL-UFL)	LC_{90}, ppm (LFL-UFL)	χ^2
Chloroform	Control	0.0±0.0	262.077 (244.43-278.53)	41.527 (409.68-487.70)	0.677*
	160	23.16±1.15			
	220	36.32±0.81			
	280	58.52±1.54			
	340	70.54±1.45			
	400	83.06±1.14			
Ethyl acetate	Control	0.0±0.0	247.164 (228.86-263.40)	421.814 (392.58-463.72)	0.082*
	160	25.48±1.70			
	220	42.36±1.67			
	280	60.42±1.44			
	340	75.32±1.64			
	400	86.38±1.56			
Acetone	Control	0.0±0.0	227.727 (208.47-243.94)	393.580 (367.89-429.71)	0.378*
	160	30.62±1.77			
	220	46.38±1.48			
	280	65.34±1.73			
	340	82.54±1.71			
	400	90.02±1.01			
Aqueous	Control	0.0±0.0	212.097 (190.37-229.53)	381.306 (356.05-417.02)	1.537*
	160	36.08±1.08			
	220	48.04±1.28			
	280	72.38±1.81			
	340	84.76±1.35			
	400	91.08±1.10			
Methanol	Control	0.0±0.0	198.657 (176.28-216.19)	357.921 (335.27-389.43)	0.586*
	160	39.24±1.70			
	220	54.34±1.76			
	280	74.28±1.64			
	340	88.72±1.10			
	400	94.26±1.78			

SD, standard deviation; LC, lethal concentration; LFL, lower fiducidal limits; UFL, upper fiducidal limits; χ^2 Chi square value. *Significant at P<0.05 level.

Table 6. Ovicidal activity of different plant leaf extracts against eggs of *Anopheles stephensi*.

Plant species	Solvent	Percent egg hatch Concentration (ppm)						
		12.5	25	50	100	200	400	Control
M. tinctoria	Chloroform	88.04±0.95	74.22±1.02	63.52±1.78	53.02±1.20	40.84±1.10	NH	100±0.0
	Ethyl acetate	81.08±0.94	67.12±0.97	55.46±1.73	44.42±1.35	33.54±1.54	NH	100±0.0
	Acetone	76.44±1.43	62.14±1.20	49.48±1.40	36.52±1.57	26.02±0.95	NH	100±0.0
	Aqueous	70.42±1.39	56.32±1.63	40.56±1.72	27.26±1.57	NH	NH	100±0.0
	Methanol	63.36±1.36	51.44±1.77	38.56±1.63	NH	NH	NH	100±0.0
P. glabra	Chloroform	80.14±1.15	71.38±1.89	59.08±1.17	49.18±1.04	34.52±1.77	NH	100±0.0
	Ethyl acetate	75.36±1.57	62.44±1.52	48.98±1.86	38.52±1.07	29.38±1.42	NH	100±0.0
	Acetone	70.26±1.53	57.28±1.47	43.54±1.01	31.24±1.57	22.98±0.95	NH	100±0.0
	Aqueous	63.02±0.63	52.62±1.51	38.82±1.11	23.86±0.97	NH	NH	100±0.0
	Methanol	59.32±1.40	46.04±1.31	32.34±1.65	25.54±1.42	NH	NH	100±0.0

NH, no hatch.

fumigant toxicity assay against the house mosquito, *Culex pipiens* L. (Diptera: Culicidae), by Oz *et al*. (2007). The present study corresponds with the findings of Amerasan *et al*. (2012) who reported the LC_{50} and LC_{90} values of *Cassia tora* leaf extracts as adulticidal activity of hexane, chloroform, benzene, acetone, and methanol against *C. quinquefasciatus*, *A. aegypti*, and *A. stephensi* as the following: for *C. quinquefasciatus*, LC_{50} values were 338.81, 315.73, 296.13, 279.23, and 261.03 ppm, and LC_{90} values were 575.77, 539.31, 513.99, 497.06, and 476.03 ppm; for *A. aegypti*, LC_{50} values were 329.82, 307.3, and 252.03 ppm, and LC_{90} values were 563.24, 528.33, 36 496.92, 477.61, and 448.05 ppm; and for *A. stephensi*, LC_{50} values were 317.28, 300.30, 277.51, 263.35, and 251.43 ppm, and LC_{90} values were 538.22, 512.90, 483.78, 461.08, and 430.70 ppm, respectively. The adulticidal activity of the essential oil of *Lantana camara* was evaluated against different mosquito species on 0.208 mg/cm^2 impregnated papers, and the knockdown time $(KDT)_{50}$ and KDT_{90} values of the essential oil were 20, 18, 15, 12 and 14 min and 35, 28, 25, 18 and 23 min against *A. aegypti*, *C. quinquefasciatus*, *A. culicifacies*, *Ancylus fluvialitis* and *A. stephensi*, with corresponding percentage of mortalities of 93.3%, 95.2%, 100%, 100% and 100%, respectively (Dua *et al*., 2010).

The ovicidal efficacy in the current study compared well with an earlier report; the bioactive compound azadirachtin (*A. indica*) showed complete ovicidal activity against eggs of *Culex tarsalis* and *C. quinquefasciatus* exposed to a 10-ppm concentration (Su & Mulla, 1998). The ovicidal activity of 21 hyphomycete fungi species against *A. aegypti* was reported. The tested fungi were *Paecilomyces carneus*, *Paecilomyces marquandii*, *Isaria fumosorosea*, *Metarhizium anisopliae*, *Penicillium* sp., *Paecilomyces lilacinus*, *Beauveria bassiana*, and *Evlachovaea kintrischica*. These are the first results to show the effects of entomopathogenic fungi against eggs of *A. aegypti*, and they suggest their potential as control agents of this vector (Luz *et al*., 2007). Assis *et al*. (2003) reported that egg hatching inhibition of ethyl acetate and methanol extracts of *Spigelia anthelmia* ranged from 97.4-100%, respectively, at 50.0 mg mL^{-1}. The oviposition deterrent properties against *A. stephensi* have been observed for various plant extracts, including the methanol extract of *Pelargonium citrosa*, which exhibited 56% and 92% inhibition of oviposition at 1 and 4 ppm, respectively (Jeyabalan *et al*., 2003). The benzene extracts of *C. vulgaris* caused 100% mortality (zero hatch) at 250 ppm, and at 200 ppm a very low hatch rate (11.8%), with complete ovicidal activity at 300 ppm. Fraction I at 80 ppm caused a very low hatch rate of 3.2%, followed by fraction II (6.9%), fraction III (4.9%), and fraction IV (5.3%) against *A. stephensi* (Mullai *et al*., 2008a). The leaf extracts of *Andrographis paniculata*, *Cassia occidentalis*, and *Euphorbia hirta* with different solvents (*i.e*., hexane, ethyl acetate, benzene, aqueous, and methanol) was studied for adulticidal, repellent and ovicidal activity against *A. stephensi*. Among the extracts tested for ovicidal activity against *A. stephensi*, the leaf methanol extract of *A. paniculata* caused 100% mortality (zero hatch) at 150 and 300 ppm, respectively (Panneerselvam & Murugan, 2013a). The leaf extract of *Cassia fistula* in different solvents (methanol, benzene, and acetone) were studied for larvicidal, ovicidal, and repellent activity against *A. aegypti* (Govindarajan, 2009). In the present work, the crude methanol and aqueous extracts of *M. tinctoria* resulted in zero hatch (100% mortality) at 100 and 200 ppm; followed by crude methanol. The aqueous extract of *P. glabra* caused zero hatch (100% mortality) at 200 ppm for *A. stephensi*. In the case of ovicidal activity, exposure to freshly laid eggs was more effective than with older eggs. It has been shown that the age of the embryos at the time of treatment plays a crucial role with regard to the effectiveness of the chitin synthesis inhibitor, dimilin, to *C. quinquefasciatus* (Miura *et al*., 1976). Malarvanan *et al*. (2009) reported that exposure of *Cipadessa baccifera*, *Melia dubia, Clausena dentata* and *Dodonaea angustifolia* to petroleum ether, hexane, chloroform, acetone and water extracts exhibited ovicidal activity against *Helicoverpa armigera*, and maximum activity was observed with the hexane extract of *Clausena dentate*. The leaf extract of *Solanum trilobatum* reduced egg laying by gravid females of *A. stephensi* from 18% to 99%, compared with ethanol-treated controls at 0.01%, 0.025%, 0.05%, 0.075%, and 0.1% (Rajkumar & Jebanesan, 2005). Recently, ovicidal, repellent, adulticidal and field evaluations of plant extracts were reported against dengue, malaria and filarial vectors (Kovendan *et al*., 2012a). Findings of the present investigation reveal that the leaf extracts of *M. tinctoria* and *P. glabra* possess remarkable larvicidal, adulticidal and ovicidal activity against this malarial vector. The extract could be used directly as a larvicidal, adulticidal and ovicidal agent in small-volume aquatic habitats or breeding sites of limited size around human dwellings. Studies to confirm this hypothesis under field conditions are under way in our laboratory. The results suggest the possible utilisation of cheap and readily available medicinal plants for the possible control of mosquitoes as a part of an integrated vector management program.

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