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ORIGINAL PAPER

Adulticidal, repellent, and ovicidal properties of indigenous plant extracts against the malarial vector, *Anopheles stephensi* (Diptera: Culicidae)

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Abstract Mosquito-borne diseases with an economic impact create loss in commercial and labor outputs, particularly in countries with tropical and subtropical climates. Mosquito control is facing a threat because of the emergence of resistance to synthetic insecticides. Extracts from plants may be alternative sources of mosquito control agents because they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use to control mosquitoes. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the adulticidal, repellent, and ovicidal potential of the crude hexane, ethyl acetate, benzene, aqueous, and methanol solvent extracts from the medicinal plants *Andrographis paniculata*, *Cassia occidentalis*, and *Euphorbia hirta* against the medically important mosquito vector, *Anopheles stephensi* (Diptera: Culicidae).The adult mortality was observed after 24 h of exposure. All extracts showed moderate adulticide effects; however, the highest adult mortality was found in methanol extract of *A. paniculata* followed by *C. occidentalis* and *E. hirta* against the adults of *A. stephensi* with LC_{50} and LC_{90} values of 210.30, 225.91, and 263.91 ppm and 527.31, 586.36, and 621.91 ppm, respectively. The results of the repellent activity of hexane, ethyl acetate, benzene, aqueous, and methanol extract of *A. paniculata*, *C. occidentalis*, and *E. hirta* plants at three different concentrations of 1.0, 3.0, and 6.0 mg/cm^2 were applied on skin of forearm in man and exposed against adult female mosquitoes. In this observation, these three plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity is dependent on the strength of the plant extracts. 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. Mortality of 100 % with methanol extract of *A. paniculata* exerted at 150 ppm and aqueous, methanol extract of *C. occidentalis* and *E. hirta* were exerted at 300 ppm. These results suggest that the leaf extracts of *A. paniculata*, *C. occidentalis*, and *E. hirta* have the potential to be used as an ideal eco-friendly approach for the control of the *A. stephensi*. Further detailed research is needed to identify the active ingredient in the extracts and implement the effective mosquito management program.

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Introduction

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 1,000 deaths are reported every year (Lal et al. 2010). Currently, resistant variety of the malarial parasite is commonly observed in almost all parts of the world where malaria is endemic (Cooper et al. 2005). The increased drug resistance continues to be a major issue, with ongoing problems related to drug quality, availability, and cost to treat the disease (Garcia 2010). So, the transmission of malaria is best reduced by the control of vector mosquito.

Anopheles stephensi is an important vector of urban malaria in several countries of the Middle East and Indian subcontinent (Gayathri et al. 2006). Vector control remains the most effective measure to prevent malaria transmission and is, therefore, one of the four basic technical elements of the global malaria control strategy. The advantage of targeting the larval stages are that mosquitoes are killed before they disperse to human habitations and that larvae, unlike adults, cannot change their behavior to avoid control activities (Killeen et al. 2002). Nowadays, the control of vector-borne diseases is more difficult due to the increased resistance of mosquito populations to synthetic insecticides and even to microbial control agents and because of the resistance of malaria parasites to chemotherapic drugs and some economic issues (Hargreaves et al. 2000; Ranson et al. 2001; Gericke et al. 2002; Shelton et al. 2007). Moreover, continuous application of insecticides poses serious threats to the environment in killing nontarget species such as larval predators, bioaccumulation, hampering biodiversity, and environmental pollution (Maurya et al. 2007).

Phytochemicals have a major role in mosquito control programs. The bioactive plant ingredient(s) can be obtained from the whole plant or from a specific part by extraction with different types of polar and nonpolar solvents, such as petroleum ether, benzene, chloroform, methanol, absolute alcohol, acetone, etc. Thus, the search has been directed extensively to the plant kingdom as many plant chemicals have larvicidal, pupicidal, and adulticidal activities, most being repellants, ovipositional deterrents, and antifeedants against both agricultural pests and medically important insect species. It is in this context the present bioassay was carried out in the Entomology Research Lab of Zoology Department at Bharathiar University, Coimbatore.

The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as insect growth regulators, repellents, and ovipositional attractants, having deterrent activities observed by different researchers (Murugan et al. 1996; Muthukrishnan et al. 1997; Babu and Murugan 1998; Venkatachalam and Jebanesan 2001; Choochote et al. 2004; Amer and Mehlhorn 2006a, b; Bagavan et al. 2008). Elango et al. (2009) have reported that the leaf acetone, chloroform, ethyl acetate, hexane, and methanol extracts of *Aegle marmelos*, *Andrographis lineata*, *Andrographis paniculata*, *Cocculus hirsutus*, *Eclipta prostrate*, and *Tagetes erecta* were tested against fourth-instar larvae of *Anopheles subpictus* and *Culex tritaeniorhynchus* and as an insect repellent (Prakash and Rao 1997). The methanol extracts of *Pelargonium citrosa* leaf were tested for their biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity; repellency; and biting deterrency against *A. stephensi* (Jeyabalan et al. 2003). Prajapati et al. (2005) have revealed the oviposition deterrent, ovicidal, and repellent activities of the essential oils of *Cinnamomum zeylanicum*, *Zingiber officinale*, and *Rosemarinus officinalis* against *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*. The biolarvicidal and pupicidal potential of silver nanoparticles were synthesized using *Euphorbia hirta* against *A. stephensi* (Agalya Priyadarshini et al. 2012), as well as the potential of various extracts of plant origin and efficacy of *Amaranthus oleracea* and *E. hirta* natural potential larvicidal agents against the urban Indian malaria vector, *A. stephensi* (Sharma et al. 2009). Furthermore, studies revealed that *E. hirta* posses galactogenic, anti-anaphylactic, antimicrobial, antioxidant, anticancer, antifeedant, antiplatelet aggregation, and anti-inflammatory, aflatoxin inhibition, antifertility, anthelmintic, antiplasmodial, antiamebic, antimalarial, larvicidal, repellent, and antifeedant activities against *Plutella xylostella* (Anonymous 2008). Govindarajan (2009) reported that the leaf methanol, benzene, and acetone extracts of *Cassia fistula* were studied for the larvicidal, ovicidal, and repellent activities against *A. aegypti*. *A. paniculata* is a traditional medicinal plant that has been used for pest control (Kuppusamy and Murugan 2006). *A. paniculata* leaves or roots decoction are used as a vermifuge (Sugati et al. 1999) against *Brugia malayi* (Zaridah et al. 2001), 100 % mortality obtained against the microfilaria of *Dipetalonema reconditum* (Dutta and Sukul 1982); the methanol and ethyl acetate extracts were tested on cowpea weevil, *Callosobruchus chinensis* (Bright et al. 2001), and relieve itchy skin and insect bites (Burkill 1966).

Andrographis paniculata (Burm. f) Nees is a genus of herbs and shrubs, with a distribution mostly in the tropical and moist regions. It comprises about 19 plant species found in India and Sri Lanka, and certain parts of Thailand and Bangladesh. In India, it is grown in Assam, Bihar, Karnataka, Karla, Madhya Pradesh Andhra Pradesh, and West Bengal. Kalmegh, also known as "King of bitter", is one among the prioritized medicinal plants in India, and this herb is being used mainly for treating fever, liver disease, diabetes, snake bite, etc. The leaf and the whole herb contain medicinal properties (Kapur 1999). The plant contains andrographolide, neo-andrographolide, deoxy-andrographolide, and andrographiside. The leaves contain active principle like andrographolide, homo-andrographolide andrographesterol, and andrographone. Andrographolide is the major constituent in leaves which is a bitter substance (Gorter 1911). Malaria is still a prevalent disease in many tropical and subtropical countries. *A. paniculata* was found to considerably inhibit the multiplication of *Plasmodium berghei* (Misra et al. 1992). The protective action of *A. paniculata* is proposed to be due to reactivation of the key antioxidant enzyme superoxide dismutase (Chander et al. 1995).

Cassia occidentalis Linn. (Caesalpiniaceae) is an erect, annual herb or undershrub. The leaves are lanceolate or

ovate-lanceolate; the leaflets, three-paired, membranous, glaucous, ovate, or lanceolate; the flowers, yellow, in short racemes; the pods, recurved, glabrous, and compressed; and the seeds, dark olive green, ovoid, compressed, hard, smooth, and shiny. It is extensively used in the indigenous and folklore medicine systems to treat hepatotoxicity. In unani medicine, it is used as an antidote of poisons, as a blood purifier, expectorant, anti-inflammatory agent, and a remedy for the treatment of liver diseases (Kabiruddin 1951). It is also an important ingredient of several polyherbal formulations marketed for liver diseases. Its root, flowers, seeds, and leaves have been employed in herbal medicine around the world (Kirtikar and Basu 1933; Chopra et al. 1956; Nadkami 1976) for a variety of purposes such as laxative, expectorant, antimalarial (Tona et al. 2001), analgesic, vermifuge, and febrifuge. The main plant chemicals in *C. occidentalis* include achrosine, emodin, anthraquinones, anthrones, apigenin, sitosterols, tannins, and xanthones. Toxicity studies on the aerial parts, leaves, and roots of *C. occidentalis* reported that various leaf and root extracts given to mice (administered orally and injected at up to 500 mg/kg) cause mortality (Bin-Hafeez and Hussaini 2001; Chidambara et al. 2003). The *C. occidentalis* ethanol extract showed larvicidal activity against the malarial vector *A. stephensi* at a dose equivalent to LC_{50} ranging between 60.69 %, 64.76 %, 67.78 %, 70.56 %, and 92.21 % for I, II, III, and IV instar larvae and pupa, respectively. The smoke toxicity was more effective against *A. stephensi*. Smoke-exposed gravid females oviposited fewer eggs when compared to those that were not exposed (Abirami and Murugan 2011).

Euphorbia hirta belongs to the family Euphorbiaceae. It is a small annual herb common to tropical countries (Soforowa 1982). It can grow to a height of 40 cm. The stem is slender and often reddish in color, covered with yellowish bristly hairs especially in the younger parts. The leaves are oppositely arranged, lanceolate, and are usually greenish or reddish underneath measuring about 5-cm long. In the axils appear very small dense round clusters of flowers. The small green flowers constitute the inflorescence characteristic of the euphorbias. The stem and leaves produce white or milky juice when cut (Lind and Tallantire 1971). The aerial parts of the plant are qualitatively well investigated for presence diterpenoids, triterpenoids, flavonoids, phenolics, tannins, carbohydrates, hydrocarbons (Chen 1991; Mallavadhani and Narasimhany 2009), and scopoletin (**1**), scoparone (**2**), isoscopoletin (**3**), quercetin (**4**), isorhamnetin (**5**), pinocembrin (**6**), kaempferol (**7**), luteolin (**8**), and gallic acid (**9**) (Yi et al. 2012) etc. from this species.

Novel findings reveal that *A. paniculata* has the potential role in eco-friendly mosquito control programs. *A. paniculata* exerted a mosquitocide influence against the malaria vector, *A. stephensi*. The larvicidal, pupicidal, adulticidal, and ovicidal properties of whole plant ethanolic extract were evaluated under laboratory conditions, and the most effective results were obtained for larvicidal and pupicidal activities (Kuppusamy and Murgan 2009). Yang et al. (2004) studied the repellent activity of methanol extracts from 23 aromatic medicinal plant species and a steam distillate against female blood-starved *A. aegypti*. At a dose of 0.1 mg/cm^2, the repellency extracts of *Cinnamomum cassia* root bark (91 %), *Nardostachys chinensis* rhizome (81 %), *Paeonia suffruticosa* root bark (80 %), and *Cinnamomum camphora* steam distillate (94 %) were compared to DEET (*N*,*N*-diethyl-*m*-toluamide) and lasted for 1h. Relatively short duration of repellency was observed in *P. suffruticosa* root bark extract and *C. camphora* steam distillate. *Cassia* species have been of medical interest due to their good therapeutic value in folk medicine; the crude ethanol extracts of *Cassia alata* and *C. occidentalis* have effective in vitro antimalarial activity using the microdilution test against *P. falciparum* (Kayembe et al. 2010).

In the present study, we describe the effect of *A. paniculata*, *C. occidentalis*, and *E. hirta* leaf extracts against the malarial vector, *A. stephensi*. The aim of this study was to investigate the mosquito adulticidal, repellent, and ovicidal activities of different solvent extracts of three plant species from Coimbatore District, Tamil Nadu, India.

Materials and methods

Plant collection

The fully developed fresh leaves of *A. paniculata*, *C. occidentalis*, and *E. hirta* were collected from the Maruthamalai Hills, near the Bharathiar University campus in Coimbatore. It 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 and shade-dried at room temperature (28±2 °C) for 5 to 10 days. The air-dried materials were powdered separately using a commercial electrical blender. The finely ground plant material (1,000 g/solvent) was loaded in Soxhlet apparatus and was extracted with five different solvents, namely, hexane, ethyl acetate, benzene, aqueous, and methanol individually. 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 *A. paniculata*, *C.*

occidentalis, and *E. hirta* with five different solvents yielded 65.15, 74.50, 61.10, 73.40, 91.28 g; 56.12, 63.22, 51.20, 67.80, 89.30 g; and 46.14, 55.20, 40.50, 52.60, 77.23 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 adulticidal, repellent and ovicidal bioassays.

Insect rearing

The eggs of *Anopheles stephensi* were collected from different breeding sites (overhead tanks) in Coimbatore District, Tamil Nadu, India. These were returned to the laboratory and transferred (in approximately the same aliquot numbers of eggs) to 18 cmL×13 cmW×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±2 °C and 75–85 % RH in a 14:10 (L/D) photoperiod. Larvae were fed 5 g 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 W) to retain emerging adults, for which 10 % sucrose in water solution (v/v) was available ad libitum. On day 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.

Repellent bioassay

The stock solutions of the extracts were diluted with acetone, polysorbate 80, and distilled water to obtain test solutions of 1.0, 3.0, and 6.0 mg/cm^2 (hexane, ethyl acetate, benzene, aqueous, and methanol) prepared separately. For repellent experiment, 50 laboratory reared blood-starved adult female mosquitoes that were between 3 and 10 days old were placed into separate laboratory cages (45×45×40 cm). Before each test, the forearm and hand of a human subject were washed with unscented neutral soap, thoroughly rinsed, and allowed to dry for 10 min before extract application. The different plant extracts being tested were applied from the elbow to the fingertips. The arm was left undisturbed. An arm treated with acetone and polysorbate 80 served as control. The control and treated arms were introduced simultaneously into the cage. The number of bites was counted over 15 min, every 30 min, and from 1800 h to 0600 h. Protection time was recorded as the time elapsed between repellent application and the observation period immediately preceding that in which a confirmed bite was obtained. If no bites were confirmed at 210 min, tests were discontinued and protection time was recorded as 210 min. An attempt of the mosquito to insert its stylets was considered a bite. No mosquito attempted to bite the control arm during the observation period; that trial was discarded, and the test was repeated with a new batch of mosquitoes to ensure that lack of bites was due to repellence and not to mosquitoes not being predisposed to get a blood meal at the time. The experiments were conducted five times in separate cages, and in each replicate different volunteers were used to nullify any effect of skin differences on repellency. It was observed that there was no skin irritation from the plant extract. The percentage protection was calculated by using the following formula (Fradin and Day 2002; Venkatachalam and Jebanesan 2001).

$$\text{Protection} = \frac{(\{\text{No. of bites received by control arm}\} - \{\text{No. of bites received by treated arm}\})}{(\text{No. of bites received by control arm})} \times 100$$

Adulticidal bioassay

Sugar-fed adult female mosquitoes (5 to 6 days old) were used. The *A. paniculata*, *C. occidentalis*, and *E. hirta* leaf extract 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 control. The papers were left to dry at room temperature to let the ethanol evaporate overnight. 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 WHO (1981). One tube served to expose the mosquitoes to the plant extract and another 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 was placed in the tube during the holding period for 24 h. Mortality of the mosquitoes was recorded after 24 h. The above procedure was carried out in triplicate for plant extract of each concentration.

Ovicidal activity assay

For ovicidal activity, the 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 gravids 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 ten cups, nine were each filled with test solution of 18.75, 37.5, 75.0, 150.0, 300.0, and 600.0 ppm, and one was filled with 100 ml of respective solvent containing water and Polysorbate 80 that served as a control. 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 cubs filled with dechlorinated water for hatching assessment after counting the eggs under microscope (Su and Mulla 1998). The percent egg mortality was calculated on the basis of nonhatchability of eggs with unopened opercula (Chenniappan and Kadarkarai 2008). The hatching rate of eggs was assessed after 98 h post-treatment as per the method of Rajkumar and 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 % fiducidal limits of upper fiducidal limit and lower fiducidal limit, and chi-square values were calculated by using the SPSS Statistical software package 16.0 version. Results with $P < 0.05$ were considered to be statistically significant.

Results

Table 1 shows the list of medicinal plants tested for the bioactivity against vector mosquito. The results obtained from the present study confirm the adulticidal activity of hexane, ethyl acetate, benzene, aqueous, and methanol extract of *A. paniculata*, *C. occidentalis*, and *E. hirta* against the adult of malarial vector, *A. stephensi*, and are presented in Tables 2, 3, and 4 (Fig. 1). Among three plants tested, the highest adulticidal activity was observed in methanol extract of *A. paniculata* then *C. occidentalis* and *E. hirta* against *A. stephensi*. At higher concentrations, the adult showed restless movement for some time with abnormal wagging and then died. The rates of mortality were directly proportional to concentration. The LC_{50} and LC_{90} values were 210.30, 225.91, 263.91 ppm and 527.31, 586.36, 621.916 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} (LCL–UCL) and LC_{90} (LCL–

Table 1 List of medicinal plants tested for the bioactivity against *A. stephensi*

Botanical name	Common name (Tamil)	Family	Medicinal property	Plant parts tested
Andrographis paniculata (Burm. f.) Wall. ex Nees	Nilavembu	Acanthaceae	Efficacious against malaria, antihepatotoxic, antibiotic, antihepatitic, antithrombogenic, anti-inflammatory, anti-snake venom, and antipyretic properties	Leaves
Cassia occidentalis Linn.	Peyavirai	Caesalpiniaceae	The whole plant is generally considered as an anti-inflammatory, antipyretic, febrifuge, sudorific, diaphoretic, and immune stimulant. The leaves and seeds are considered expectorant and is used to treat cough, whooping cough, and bronchitis. It is also used as a diuretic, liver detoxifier, and as a hepato-tonic (balances and strengthens the liver)	Leaves
Euphorbia hirta Linn.	Amman paccarici	Euphorbiaceae	The leaves are mixed with those of *Datura metel* L. in preparing "asthma cigarettes". Its use in the treatment of gastrointestinal disorders, including intestinal parasites, diarrhea, peptic ulcers, heartburn, vomiting and amebic dysentery, jaundice, hypertension, edema, anemia and malaria, as an aphrodisiac, and to facilitate childbirth has also been reported	Leaves

Table 2 Adulticidal activity of different solvent extract of *A. paniculata* against *Anopheles stephensi*

Name of the extract	Concentration (ppm)	% Mortality ±SD	LC_{50}, ppm (LFL–UFL)	LC_{90}, ppm (LFL–UFL)	χ^2
Hexane	Control	0.0±0.0	344.66 (302.42–382.30)	749.54 (681.76–846.53)	0.675*
	140	27.22±1.45			
	280	39.01±1.21			
	420	61.41±1.52			
	560	74.21±1.48			
	700	87.42±1.66			
Ethyl acetate	Control	0.0±0.0	314.91 (271.37–352.33)	706.05 (643.80–794.12)	0.508*
	140	29.44±1.10			
	280	44.03±1.37			
	420	64.24±1.45			
	560	77.11±1.76			
	700	90.82±1.50			
Benzene	Control	0.0±0.0	267.79 (222.04–305.38)	633.17 (579.26–707.85)	0.723*
	140	35.06±1.77			
	280	48.45±1.12			
	420	70.20±1.48			
	560	85.03±1.37			
	700	94.00±1.87			
Aqueous	Control	0.0±0.0	235.40 (189.68–272.24)	569.33 (522.16–33.40)	1.870*
	180	39.42±1.81			
	260	51.63±1.78			
	340	76.25±1.39			
	420	89.41±1.67			
	500	97.08±1.79			
Methanol	Control	0.0±0.0	210.30 (163.16–247.45)	527.31 (483.29–586.79)	2.232*
	180	42.44±1.76			
	260	57.40±1.13			
	340	79.00±1.87			
	420	91.42±1.10			
	500	99.10±0.74			

LFL lower fiducial limits, *UFL* upper fiducial limits, χ^2 chi-square value

*Significant at P <0.05 level

UCL) were also calculated. No mortality was recorded in the control. The hexane, ethyl acetate, benzene, aqueous, and methanol extract of *A. paniculata* then *C. occidentalis* and *E. hirta* show significant repellency against *A. stephensi* (Tables 5, 6, and 7). In this observation, these three plant crude extracts gave protection against mosquito bites without any allergic reaction to the test person, and also, the repellent activity is dependent on the strength of the plant extracts. The highest repellency of 150, 180, and 210 min was observed in methanol extract of *A. paniculata* followed by *C. occidentalis* and *E. hirta* against *A. stephensi*. The mean percent of egg hatchability of *A. stephensi* were tested with five different solvents at different concentrations of *A. paniculata*, *C. occidentalis*, and *E. hirta* leaf extracts, and the results are listed in Table 8. The percent hatchability was inversely proportional to the concentration of extract and directly proportional to the eggs. Among the extracts tested for ovicidal activity against *A. stephensi*, the leaf methanol extract of *A. paniculata* exerted 100 % mortality (zero hatchability) at 150 and 300 ppm, respectively. The leaf extract of *A. paniculata* was found to be most effective than *C. occidentalis* and *E. hirta* against larvae and eggs of vector mosquitoes. Control eggs showed 100 % hatchability.

Discussion

In view of residue problems in the environment and the development of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to explore plants to obtain extracts that are safe for nontarget animals and do not pose any residue problem but are still able to suppress pest populations. Though several compounds of plant origin have been reported as insecticides–larvicides, there is a wide scope for the discovery of more effective plant products. Further research undoubtedly

Table 3 Adulticidal activity of different solvent extract of *C. occidentalis* against *Anopheles stephensi*

Name of the extract	Concentration (ppm)	% Mortality± SD	LC_{50}, ppm (LFL–UFL)	LC_{90}, ppm (LFL–UFL)	χ^2
Hexane	Control	0.0±0.0	377.82 (337.53–415.52)	787.79 (715.60–891.79)	0.956*
	140	24.12±1.12			
	280	35.43±1.15			
	420	57.63±1.50			
	560	69.23±1.45			
	700	85.42±1.16			
Ethyl acetate	Control	0.0±0.0	347.74 (307.36–384.11)	737.42 (672.85–828.75)	0.842*
	140	26.21±1.49			
	280	39.22±1.44			
	420	60.43±1.14			
	560	73.41±1.16			
	700	89.22±1.50			
Benzene	Control	0.0±0.0	297.63 (253.10–335.21)	681.47 (622.14–764.79)	0.374*
	140	31.43±1.17			
	280	45.22±1.49			
	420	66.03±1.54			
	560	81.61±1.81			
	700	91.02±1.86			
Aqueous	Control	0.0±0.0	266.34 (221.50–303.31)	623.41 (571.24–695.12)	1.175*
	140	34.41±1.13			
	280	48.42±1.10			
	420	71.06±1.84			
	560	87.61±1.82			
	700	93.20±1.49			
Methanol	Control	0.0±0.0	225.91 (174.70–266.11)	586.36 (535.58–656.49)	0.601*
	140	39.81±1.65			
	280	54.80±1.30			
	420	75.41±1.11			
	560	89.40±1.81			
	700	95.21±1.48			

LFL lower fiducial limits, *UFL* upper fiducial limits, χ^2 chi-square value

*Significant at P <0.05 level

will lead the improved formulations with enhanced activity, which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control.

The mode of action of these leaf extracts on mosquito larvae are 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. 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). In our result, it showed that crude hexane, ethyl acetate, benzene, and aqueous and methanol extracts of the leaf of the plants *A. paniculata* then *C. occidentalis* and *E. hirta* have significant adulticidal, repellent as well as ovicidal activity. This result is also comparable to earlier reports of other authors; the LC_{50} and LC_{90} values of *Cassia tora* leaf extracts against adulticidal activity of hexane, chloroform benzene, acetone, and methanol (*C. quinquefasciatus*, *A. aegypti*, and *A. stephensi*) were 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, 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 (Amerasan et al. 2012). The potential of three plants *A. paniculata*, *C. occidentalis*, and *E. hirta* against *A. stephensi*, as observed at 24 h following treatment in this

Table 4 Adulticidal activity of different solvent extract of *E. hirta* against *Anopheles stephensi*

Name of the extract	Concentration (ppm)	% Mortality ±SD	LC_{50}, ppm (LFL–UFL)	LC_{90}, ppm (LFL–UFL)	χ^2
Hexane	Control	0.0±0.0	402.10 (363.30–439.87)	809.90 (735.80–916.64)	1.235*
	140	22.46±1.12			
	280	31.65±1.50			
	420	54.27±1.42			
	560	66.92±1.36			
	700	83.86±1.62			
Ethyl acetate	Control	0.0±0.0	365.26 (324.81–402.48)	768.48 (699.20–867.60)	0.714*
	140	25.27±1.45			
	280	36.51±1.11			
	420	58.49±1.13			
	560	71.88±1.60			
	700	86.46±1.10			
Benzene	Control	0.0±0.0	327.91 (284.43–365.77)	729.89 (664.26–823.50)	0.538*
	140	28.45±1.12			
	280	41.47±1.15			
	420	63.64±1.48			
	560	76.09±1.51			
	700	88.49±1.09			
Aqueous	Control	0.0±0.0	292.89 (248.36–330.35)	672.73 (614.54–754.17)	1.257*
	140	32.89±1.58			
	280	43.67±1.47			
	420	67.64±1.52			
	560	82.44±1.14			
	700	91.47±1.11			
Methanol	Control	0.0±0.0	263.91 (218.66–301.10)	621.916 (569.61–693.91)	0.933*
	140	35.24±1.49			
	280	48.41±1.12			
	420	71.61±1.51			
	560	86.43±1.15			
	700	94.06±1.56			

LFL lower fiducial limits, *UFL* upper fiducial limits, χ^2 chi-square value

*Significant at P <0.05 level

present investigation, was strong and found to have various degrees of adulticides. The malarial vector *A. stephensi* adults were most susceptible to methanol extract of *A. paniculata* followed by *C. occidentalis* and *E. hirta* (LC_{50} and LC_{90}=210.30, 225.91, 263.91 ppm and 527.31, 586.36, and 621.91 ppm female, respectively). However, the adulticidal activity of the two former species, *Piper sarmentosum* and *Piper ribesoides*, showed no statistically significant difference and was considered to be approximately equal and higher than that of *Piper longum*. The variety in adulticidal activity of these extracts is probably due to variation in the types and levels of active ingredients that depend on not only the genetic characteristics of the plant species but also the conditions under which they were grown and harvested (Tawatsin et al. 2001; Vieira and Imon 2000). Adulticidal activity of the essential oil isolated from *Mentha longifolia* was screened by fumigant toxicity assay against the house mosquito, *Culex pipiens* L. (Diptera: Culicidae), by Oz et al. (2007). The adult mortality was found in ethanol extract of *Citrus sinensis* with the LC_{50} and LC_{90} values of 272.19 and 457.14 ppm, *A. stephensi* of 289.62 and 494.88 ppm, and *A. aegypti* of 320.38 and 524.57 ppm, respectively (Murugan et al. 2012). Biosurfactant surfactin, produced by *Bacillus subtilis* subsp. *subtilis* (VCRC B471), is a potential bioadulticide for ULV spray against malaria-transmitting *A. stephensi* mosquitoes (Geetha et al. 2011).

Govindarajan and Sivakumar (2012) reported 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 effect on the adult mosquitoes after 24 h of exposure period. However, compared to other solvents, the highest mortality was found in methanol extract of *C. halicacabum* against all the three mosquitoes. Among them *A. stephensi* produce the

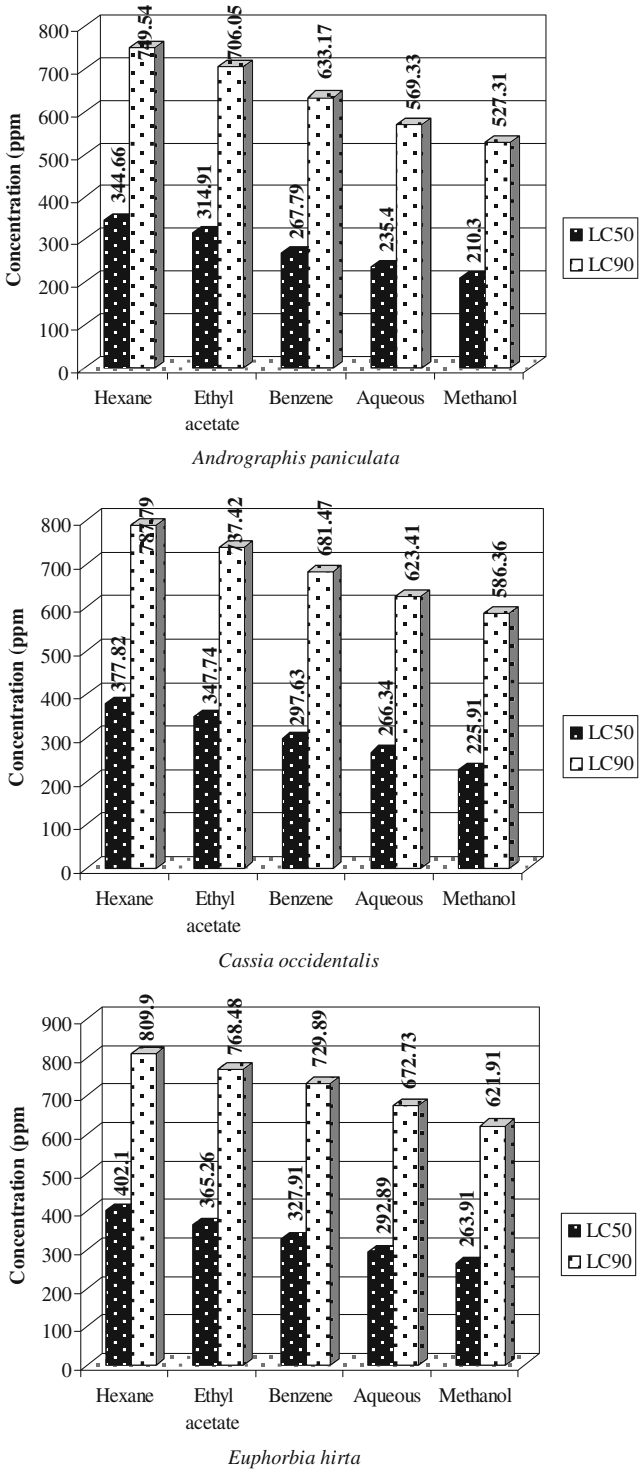

Fig. 1 Graph showing the LC_{50} and LC_{90} values of *A. stephensi*

highest LC_{50} and LC_{90} (186.00 and 346.06 ppm) values. Nathan et al. (2005) considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity against *A. stephensi*, and the larval mortality was dose dependent with the highest dose of 1 ppm azadirachtin evoking almost 100 % mortality, affecting pupicidal and adulticidal activity and significantly decreasing fecundity and longevity of *A. stephensi*.

A large number of synthetic chemicals have been tested for their repellent activity against mosquitoes. However, the prohibitive retail cost of proprietary formulations of chemicals like DEET (*N*, *N*-diethyl-*m*-toluamide) restricts their usage by the poor in countries such as India. Hence, the search for a safer, better, and cheaper repellent is an ongoing effort. Since cost is an important factor, investigation on the use of local plants as repellents is strongly recommended (Curtis 1990). Repellents of plant origin should be nontoxic, nonirritating, and long lasting. Plants of terrestrial origin have been reported to be a source of mosquito repellents (Hwang et al. 1985). Repellents are used as personal protection methods against biting arthropods with the major aim of avoiding nuisance (Trigg 1996). Insect repellents are considered useful alternatives where other control measures are neither practical nor possible. Repellent properly utilized are an inexpensive means of reducing or preventing a wide range of vectors (Gupta and Rutledge 1994). Certain natural products have been investigated for repellent activity against mosquitoes. *Zanthoxylum armatum*, DC. syn. *Zanthoxylum alatum* Roxb. (Rutaceae); *Azadirachta indica* (Maliaceae); and *Curcuma aromatica* (Zingiberaceae) were among them and have been reported to possess repellent properties against mosquitoes (Das et al. 2000). The skin repellent activities of *Solanum trilobatum* leaf extract against *A. stephensi* with higher concentration provided over 100 min of protection against mosquito bites. Lower concentrations provided 70 to 90 min of protection (Rajkumar and Jebanesan 2005). *Cymbopogan citratus* had repellency activity against the adult mosquito *Culex quinquefasciatus*. Maximum of 100% protection time was obtained at the concentration of 5.0 mg/cm^2 (Pushpanathan et al. 2006). Mullai et al. (2008) have also reported that the skin repellent test at 1.0, 2.5, and 5.0 mg cm^2 concentration gave the mean complete protection time that ranged from 119.17 to 387.83 min against *A. stephensi* with the benzene, petroleum ether, ethyl acetate, and methanol extracts of *Citrullus vulgaris* tested. In the present study, we observed that the methanol extract of *A. paniculata* was found to be more repellent than *C. occidentalis* and *E. hirta* extract. A higher concentration of 6.0 mg/cm^2 provided 100 % production up to 150, 180, and 210 min against *A. stephensi* (Tables 4, 5, and 6).

These findings coincide with the findings of Venketachalam and Jebanesan (2001) who have also reported that the repellent activity of methanol extract of *Ferronia elephantum* leaves against *Aedes aegypti* activity at 1.0 and 2.5 mg/cm^2 concentrations gave 100 % protection up to 2.14± 0.16 h and 4.00±0.24 h, respectively, and the total percentage protection was 45.8 % at 1.0 mg/cm^2 and 59.0 % at 2.5 mg/

Table 5 Repellency of different solvent extracts of *A. paniculata* against *Anopheles stephensi*

Solvents	Concentration, mg/cm^2	% of Repellency±SD							
		Time after application of repellent (min)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	76.9±1.0	67.3±1.8	61.2±0.4
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	84.5±1.6	70.9±1.4	62.4±1.1
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.7±1.0	86.8±1.7	69.3±0.6
Ethyl acetate	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	84±1.2	69.1.±1.8	66.2.±1.3
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	93.8±1.7	77.5±1.4	71.2±1.0
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	82.8±1.5	79.3±2.1
Benzene	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	87.6±0.8	74.4±1.1	70.8±1.3
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.2±1.0	83.4±0.5	78±1.2
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	94.6±0.4	85.8±1.0
Aqueous	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	93.4±1.8	82.4±1.1	76.8±1.3
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.4±1.1	89.6±1.5	80.2±1.0
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	90.6±0.8	89.4 ±1.1
Methanol	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	99.4±0.5	90.8±1.3	80.6±0.8
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.2±1.0	87.6±1.6
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.8±1.6

cm^2 for 10 h. Karunamoorthi et al. (2008b) have also reported that the leaves of *Echinops* sp. (92.47 %), *Ostostegia integrifolia* (90.10 %), and *Olea europaea* (79.78 %) were also effective and efficient to drive away mosquitoes, and the roots of *Silene macroserene* (93.61 %) and leaves of *Echinops* sp. (92.47 %), *O. integrifolia* (90.10 %), and *O. europaea* (79.78 %) exhibited a significant repellency by direct burning. The hexane extract of *A. paniculata* was more effective in exhibiting the repellent action against the mosquito as compared with *Andrographis lineate* extract, and complete protection was observed for 150 min in hexane extract of *A. paniculata* at 500 ppm against *C. tritaeniorhynchus* bites

Table 6 Repellency of different solvent extracts of *C. occidentalis* against *Anopheles stephensi*

Solvents	Concentration (mg/cm^2)	% of Repellency ± SD							
		Time after application of repellent (min)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	72.6±1.5	68.4±1.3	59.8±0.7
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	80.7±1.8	73.4±1.1	64.5±1.1
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	93±1.6	84.2±1.4	71.1±1.5
Ethyl acetate	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	78.6±1.9	71±1.8	63.6.±2.9
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	89.6±1.5	77.4±1.1	69.8±1.9
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.4±1.3	86.2±0.9	75.6±1.0
Benzene	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	83.4±2.1	75.2±2.6	68.7±1.9
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91±1.8	84.8±1.4	75.4±2.1
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	90.6±0.8	80.2±1.0
Aqueous	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	89.4±1.8	78±1.4	69.8±1.3
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	94.2±1.0	86.4±0.8	74.4±1.1
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.2±1.5	87.1 ±1.6
Methanol	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	95.3±1.7	86±1.1	74.7±1.2
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	93.6±1.4	82.8±1.2
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.2±2.1	88.4±1.8

Table 7 Repellency of different solvent extracts of *E. hirta* against *Anopheles stephensi*

Solvents	Concentration (mg/cm^2)	% of Repellency±SD							
		Time after application of repellent (min)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	69.2±1.6	65.8±1.4	57.4±1.1
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	77.9±1.2	68.6±1.9	62.3±1.7
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	90.1±1.5	81.5±1.6	69.2±1.4
Ethyl acetate	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	71.4±1.8	68.8±1.3	60.6±1.9
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	82.2±1.4	73.6±1.5	66.4±1.6
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	94.4±1.1	81.2±1.4	73.7±0.9
Benzene	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	78.3±1.7	70.9±1.2	63.5±1.3
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	86.8±1.0	79.1±1.6	67.5±1.0
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.2±1.4	87.3±0.4	74.1±1.9
Aqueous	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	83.6±1.9	74.2±1.4	65.8±1.6
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	89.1±0.5	81.3±1.7	69.5±1.3
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	98.4±1.6	92.2±1.4	76.6±2.6
Methanol	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	90.2±2.3	80.5±1.3	71.9±1.2
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	96.8±1.6	89.4±1.8	78.6±1.9
	6.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	97.2±1.9	86.8±1.6

(Elango et al. 2010). Amer and Mehlhorn (2006b) have reported that the five most effective oils which induced 100 % repellency against *A. aegypti*, *A. stephensi*, and *C. quinquefasciatus* were those of litsea (*Litsea cubeba*), cajeput (*Melaleuca leucadendron*), niaouli (*Melaleuca quinquenervia*), violet (*Viola odorata*), and catnip (*Nepeta cataria*). The use of the essential oils of *Ocimun basilicum* as promising new natural repellents at 0.1 % concentration against the Anopheles and Aedes mosquitoes has been suggested by Nour et al. (2009). The essential oil of *Tagetes minuta*, providing a repellency of 90 % protection for 2 h against *A. stephensi*, *C. quinquefasciatus*,

Table 8 Ovicidal activity of different plant leaf extracts against eggs of *Anopheles stephensi*

Mosquito	Name of the solvent	Percentage of egg hatchability						
		Concentration (ppm)						
		18.75	37.5	75	150	300	600	Control
A. paniculata	Hexane	86.6±1.5	73±1.8	62.2±2.1	51.6±1.9	39.4±0.5	NH	100±0.0
	Ethyl acetate	80.2±1.4	66.4±1.1	53.4±1.6	40.8±1.6	31.6±1.5	NH	100±0.0
	Benzene	74.2±1.0	60.8±1.6	48.6±1.3	35.4±1.1	24.3±1.7	NH	100±0.0
	Aqueous	69.6±1.8	54.1±0.4	38.7±1.2	26.5±0.6	NH	NH	100±0.0
	Methanol	61.7±1.3	49±1.8	34.4±1.8	NH	NH	NH	100±0.0
C. occidentalis	Hexane	80.9±0.6	69.1±2.1	58.3±1.7	48.4±0.5	33.8±1.6	NH	100±0.0
	Ethyl acetate	75.6±1.9	61.2±0.4	47.4±1.1	36.6±1.3	27.6±1.9	NH	100±0.0
	Benzene	69.8±1.6	55.2±2.0	41.2±1.6	29.4±0.8	20.6±2.1	NH	100±0.0
	Aqueous	61.6±1.8	50.8±2.2	36±1.6	21.6±1.5	NH	NH	100±0.0
	Methanol	57.6±1.5	44.3±1.7	30.1±1.6	23.2±1.4	NH	NH	100±0.0
E. hirta	Hexane	72.8±1.9	62.4±0.8	53.8±1.6	41±1.8	28.4±2.1	NH	100±0.0
	Ethyl acetate	68±1.4	54.4±1.6	42.2±1.7	30.8±1.9	21.6±1.8	NH	100±0.0
	Benzene	61.4±1.8	49.6±1.3	37.8±1.3	22.4±1.5	17.6±0.8	NH	100±0.0
	Aqueous	58.8±2.2	46.2±1.0	29.4±1.1	19.8±1.3	15.2±1.7	NH	100±0.0
	Methanol	50.1±0.4	39.6±1.4	26.1±2.1	20.1±1.6	NH	NH	100±0.0

and *A. aegypti*, was reported by Tyagi et al. (1994). It was found that a CO_2 extract of the seeds of the Mediterranean plant *Vitex agnus castus* can be used as a spray to keep away especially *Ixodes ricinus* and *Rhipicephalus sanguineus* ticks from animals and humans for at least 6 h. In addition, mosquitoes, biting flies, and fleas are also repelled for about 6 h (Mehlhorn et al. 2005). The leaf extract of *Artemisia nilagirica* have significant repellent activity against *A. stephensi* and *A. aegypti* mosquitoes. The highest concentrations of 450 ppm provided over 180 and 150 min protection in methanol extracts of *A. nilagirica* and over 90 and 120 min protection in methanol extract of *A. nilagirica* against mosquito bites, respectively (Panneerselvam et al. 2012).

Different solvent extracts of *Andrographis paniculata*, *Cassia occidentalis*, and *Euphorbia hirta* leaf showed ovicidal activity against *A. stephensi*. This result is also comparable to earlier reports of Malarvanan et al. (2009) who reported that *Cipadessa baccifera*, *Melia dubia*, *Clausena dentata*, and *Dodonaea angustifolia* of petroleum ether, hexane, chloroform, acetone, and water extracts exhibited ovicidal activity against *Helicoverpa armigera* and maximum activity was observed in hexane extract of *C. dentata*. Nair and Thomas (2000) reported that methanol extract from *Acorus calamus* exhibited ovicidal activity of 90 % and 96.67 % at 0.06 % and 0.08 % concentrations against *Bactrocera cucurbitae*. Su and Mulla (1998) reported the ovicidal activity of the Neem product azadirachtin against the mosquitoes *Culex tarsalis* and *Culex quinquefasciatus*. Mullai and Jebanesan (2006) reported 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*. The leaf extract of *S. trilobatum* reduced egg laying by gravid females of *Anopheles stephensi* from 18 % to 99 % compared with ethanol-treated controls at 0.01 %, 0.025 %, 0.05 %, 0.075 %, and 0.1 % (Rajkumar and Jebanesan 2005). The leaf extract of *Cassia fistula* with different solvents viz., methanol, benzene, and acetone was studied for the larvicidal, ovicidal, and repellent activity against *A. aegypti* (Govindarajan 2009). The ovicidal activity of 21 hyphomycete fungi species against *A. aegypti* was reported. The reported 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). They reported the ovicidal potential of all the leaf extracts (*A. paniculata*, *C. occidentalis*, and *E. hirta*) against *A. stephensi*, the leaf extract from *E. hirta* being the least effective among the three. The 100 % ovicidal activity of the *Cymbopogon citratus* oil against the adult mosquitoes *C. quinquefasciatus* and *A. aegypti* has been revealed by Pushpanathan et al. (2006) at 300 ppm. In the present work, the crude methanol, aqueous extract of *A. paniculata* exerted zero hatchability (100 % mortality) at 150 and 300 ppm, followed by crude methanol, aqueous extract of *C. occidentalis* and methanol extract of *E. hirta* that exerted zero hatchability (100 % mortality) at 300 ppm for *A. stephensi*, respectively. From the results, it can be concluded that crude extracts of *A. paniculata*, *C. occidentalis*, and *E. hirta* are an excellent potential for controlling malarial vector, *A. stephensi*. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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