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Larvicidal, ovicidal and repellent activity of selected indigenous medicinal plants against malarial vector *Anopheles stephensi* (Liston.), dengue vector *Aedes aegypti* (Linn.) and Japanese encephalitis vector, *Culex tritaeniorynchus* (Giles.) (Diptera : Culicidae)

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Abstract The present investigation was undertaken to assess the larvicidal, ovicidal, and repellent potential of the ethanolic crude extracts from the medicinal plants *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis* and *Spermacoce hispida* against the medically important mosquito vectors, *Anopheles stephensi*, *Aedes aegypti* and *Culex tritaeniorhynchus*. Among the plant extract tested against *An. stephensi*, *Ae.aegypti* and *Cx. tritaeniorhynchus* the pronounced lethal activity was recorded against *G. ula* extract in the experimental larvae of *An. stephensi* (LC_{50}=82.86ppm), followed by *S. hispida* (LC_{50}=89.45ppm). Similarly, *S. amplexicaulis* showed the LC_{50} value of 109.37ppm against the larvae of *Cx. tritaeniorhynchus* followed by *A. cadamba* with *An. stephensi* larvae (LC_{50}=109.87ppm). *C. argentea* showed more toxicity to the larvae of *Ae. aegypti*. Ovicidal activity revealed that, *S. hispida* showed more than 50% activity against the eggs of selected mosquitoes at 100 ppm concentration itself. Whereas, 150 ppm concentration of all the plants extracts repeats the trend of same. Notably, at 200ppm concentration of all the plants showed 100% ovicidal activity against *An. stephensi*, *Ae.aegypti* and *Cx. tritaeniorhynchus* (*i.e*., no hatchablity was recorded). The selected five plant extract offers 100% protection against *An. stephensi*, *Ae.aegypti* and *Cx. tritaeniorhynchus* adult female mosquitoes in terms of repellency up to 120 minutes of exposure periods. From these results, it was concluded that among the five plant extract, *G. ula* and *S. hispida* have significantly higher larvicidal, ovicidal and repellent activity against selected human vector mosquitoes *An. stephensi*, *Ae. aegypti* and *Cx. tritaeniorhynchus.*

Key words: *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis, Spermacoce hispida, Anopheles stephensi, Aedes aegypti, Culex tritaeniorhynchus,* larvicidal activity, ovicidal activity, repellent activity.

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Introduction

Mosquitoes are nuisance pests and a major vector for the transmission of several life threatening diseases such as malaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis, etc., causing millions of deaths every year (WHO, 2010). Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (Peng *et al*., 1999). *Ae. aegypti* is generally known as a vector for an arbovirus responsible for dengue fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever, and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Pancharoen *et al*., 2002). *An. stephensi* is the primary vector of malaria in India and other West Asian countries, Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths (WHO, 2010). Japanese encephalitis (JE) is a vector-borne viral disease that occurs in large parts of South Asia, Southeast Asia and the Pacific (Solomon, 2006). An estimated 3 billion people are at risk for JE virus reported by Erlanger (2009). JE has been reported that it was highly endemic in some parts of India and Southern India (Reuben and Gajanana, 1997; Kanojia and Geevarghese, 2004; Das *et al*., 2004; Kanojia *et al*., 2010). *Culex tritaeniorhynchus* has been implicated as major vectors of JE in India Reuben *et al*., 1994). Keiser *et al*. (2005) reported that JE is a disease caused by an arbovirus that is spread by marsh birds, amplified by pigs, and mainly transmitted by the bite of infected *Cx. tritaeniorhynchus* mosquitoes. The estimated annual incidence and mortality rates are 30,000 – 50,000 and 10,000, respectively, and the estimated global burden of JE was 709,000 disability-adjusted life years (DALYs) lost in 2003(WHO, 2004). According to Sabesan (2003), JE outbreak occurs frequently in 14 Asian countries with about 3060 million people at the risk of infection. Approximately 2 billion people live in countries where JE presents a significant risk to humans and animals (Arunachalam, 2005), particularly in India and China, with at least 700 million potentially susceptible children Gould, 2008).

Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these

insecticides may enter into the food chain, and thereby, the liver, kidney, etc., may be irreversibly damaged. They even result in mutation of genes and these changes become prominent only after a few generations (Ghosh, 1991). The widespread use of synthetic insecticides has led to many negative consequences[17], resulting in increasing attention to natural products (Pirali-Kheirabadi and da Silva, 2010). In this context, screening of natural products has received the attention of researchers around the world (Kebede *et al*., 2010). Among biopesticides, botanical ones are experiencing a revival due to their eco-toxicological properties (Cosimi *et al*., 2009). Plants play pivotal roles in ecological systems (Garcia *et al*., 2007). They may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals (Qin *et al*., 2010). Many secondary plant metabolites are known for their insecticidal properties (Lopez *et al*., 2008), and in many cases plants have a history of use as home remedies to kill or repel insects (Kim *et al*., 2010). In recent decades, research on the interactions between plants and insects has revealed the potential use of plant metabolites for this purpose (Elumalai *et al*., 2003, 2005, 2008ab, 2010ab; Elangovan *et al*., 2009; Baskaran *et al*., 2010ab).

Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Amer and Mehlhorn, 2006). Phytochemicals are advantageous due to their eco-safety, target-specificity, non-development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Phytochemicals obtained from the whole plant or specific part of the plant by the extraction with different types of solvent such as aqueous, methanol, chloroform, benzene, acetone, etc., depending on the polarity of the phytochemical. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and growth inhibitor or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant (Markouk *et al*., 2001).

Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable. They

are potentially suitable for use in integrated pest management programs (Alkofahi *et al*., 1989): the mosquito larvicidal properties of leaf and seed extract of plant *Agave Americana* (Dharmshaktu *et al*., 1987); the mosquito larvicidal activity in the extract of *Tagetes minuta* flowers against *Ae. aegypti* (Green *et al*., 1991); the methanolic fraction of leaves of *Mentha piperita, Phyllanthus niruri, Leucas aspera*, and *V. negundo* against larvae of *C. quinquefasciatus* (Pandian *et al*., 1994); the methanolic extracts of *Solanum suratense, Azadirachta indica*, and *Hydrocotyle javanica* exhibited larvicidal activity against *C. quinquefasciatus* (Muthukrishnan *et al*., 1997); the benzene and methanol extracts of *Artemisia vulgaris* has been repellent activity against *Aedes aegypti* (Yit *et al*., 1985); the *Zanthoxylum armatus, Zanthoxylum alatum* (Rutaceae), *Azadirachta indica* (Mailiaceae), and *Curcuma aromatica* (Zingiberaceae) were possessing repellent properties against mosquitoes (Das *et al*., 2000); the repellent activity of active compound Octacosane from *Moschosma polystachyum* against the vector *C. quinquefasciatus* (Rajkumar and Jebanesan, 2004); and the essential oil of *Zingiber officinalis* as a mosquito larvicidal and repellent agent against the filarial vector *C. quinquefasciatus* (Pushpanathan *et al.* 2008).The acetone, chloroform, ethyl acetate, hexane, and methanol leaf extracts of *Acalypha indica, Achyranthes aspera, Laucas aspera, Morinda tinctoria,* and *Ocimum sanctum* were studied against the early fourth instar larvae of *Aedes aegypti* and *C. quinquefasciatus* (Bagavan *et al*., 2008a, b). Larvicidal activity of crude hexane, ethyl acetate, petroleum ether, acetone, and methanol extracts of the leaf of five species of cucurbitaceous plants, *Citrullus colocynthis, Coccinia indica, Cucumis sativus, Momordica charantia,* and *Trichosanthes anguina,* were tested against the early fourth instar larvae of *Aedes aegypti* L. and *C. quinquefasciatus* (Rahuman *et al*., 2008). Mullai *et al.* (2008) have reported that the leaf extract of *Citrullus vulgaris* with different solvents, viz., benzene, petroleum ether, ethyl acetate, and methanol, were tested for larvicidal, ovicidal, repellent, and insect growth regulatory activities against *An. stephensi*. Ovicidal effects of the seed extract of *Atriplex canescens* was reported against *C. quinquefasciatus* (Ouda *et al*., 1998). Su and Mulla (1998) reported the ovicidal activity of the Neem product azadirachtin against the mosquitoes *C. tarsalis* and *C. quinquefasciatus*. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal, ovicidal, and repellent potential of the ethanolic crude extracts from the medicinal plants *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis* and *Spermacoce hispida* against the medically important mosquito vectors, *An. stephensi*, *Ae.aegypti* and *Cx. tritaeniorhynchus*.

Materials and methods

Collection of plants

Fully developed leaves of the plants listed in Table1 were collected from during the flowering season (From January 2010-April 2011) in and around Nagapattinam District of Tamil Nadu, India. The collected plants were authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at Department of Zoology, Annamalai University, Annamali Nagar, Tamilnadu, India.

Extraction

The leaves were washed with tap water, shade-dried, and finely ground with the help of electrical blender. The finely ground plant leaf powder (1.0 kg) was loaded in Soxhlet apparatus and was extracted with ethanol by adapting a standard protocol (Vogel, 1978). The solvents from the extracts were removed using a rotary vacuum evaporator (Rota vapour, Systronics India Ltd., Chennai, India) to collect the crude extract. 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 larvicidal, ovicidal, and repellent bioassays.

Mosquito Rearing

The mosquitoes, *Ae.aegypti*, *An. stephensi* and *Cx. tritaeniorhynchus* were procured from the Centre for Research in Medical Entomology (ICMR), Viruddhachalam, reared in the laboratory, Department of Zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at $(28\pm2)^0$c, 70%-85% relative humidity (RH), with a photo period of 14 h light, 10 h dark.

Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by World Health Organization (2005). From the stock solution, four different test concentrations (50, 100, 150 and 200ppm) were prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of *Ae.aegypti, An. stephensi* and *Cx. tritaeniorhynchus.* The larvae of test species (25) were introduced in 500-ml plastic cups containing 250 ml of

aqueous medium (249 ml of dechlorinated water+1ml of emulsifier; DMSO) and the required amount of plant extract was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time.

Ovicidal activity

The method of Su and Mulla (1998) was slightly modified and used to test the ovicidal activity. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs/eggs raft of *Ae.aegypti*, *An. stephensi* and *Cx. tritaeniorhynchus* were counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment.

Repellent activity

The repellent study was following the methods of World Health Organization (2009). 3–4 days old blood-starved female *Ae.aegypti, An. stephensi* and *Cx. tritaeniorhynchus* mosquitoes (100) was kept in a net cage (45×45× 40cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of the test person were cleaned with isopropanol. After air drying the arm only 25 cm^2 of the dorsal side of the skin on each arm was exposed, the remaining area being covered by rubber gloves. The plant extract was dissolved in isopropanol and this alcohol served as control. The selected medicinal plant leaf extract at 1.5 mg/cm^2 concentration was applied. The control and treated arms were introduced simultaneously into the cage. The numbers of bites were counted over 5 min every40 min andthe experiment was conducted five times. It was observed that there was no skin irritation from the plant extract.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, and other statistics at 95% confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression value, slope, and chi-square values were calculated using the SPSS17.0 (Statistical Package of Social Sciences) software. The $LC_{50}$and LC $_{90}$values were

calculated by using probit analysis (Finney, 1979). Results with $p<0.05$ were considered to be statistically significant.

Results

The larvicidal activity of the selected plants and their perusal of the data are shown in Figure 1. *C. argentea* showed significant activity against *Ae.aegypti* at 50ppm concentration. Similar concentration of *A. cadamba* and *G. ula* showed significant larval toxicity against the larvae of *An. stephensi*. Besides, notable larval toxicity was recorded with *S. amplexicaulis* and *S. hispida* against *An. stephensi* larvae at the same concentration. The 100ppm concentration of *C. argentea* showed larviciadal activity in the following order *Aedes aegypti <An. stephensi < Cx. tritaeniorhynchus*. In the same, 100ppm concentration of *A. cadamba* and *G. ula* showed larvicidal toxicity in the increasing order of *An. stephensi >Ae.aegypti>Cx. tritaeniorhynchus*. The larvae of *Ae.aegypti* showed more susceptibility to the extract of *S. amplexicaulis* and the trend was found reflected in *S. hispida* against the test organissms at 150ppm concentration *Ae. aegypti* larvae showed more susceptibility against *C. argentea*, *A. cadamba* and *G. ula*. *Cx. tritaeniorhynchus* larvae were found susceptible to the leaf extract of *S. amplexicaulis* and *S. hispida* (Figure 1). 200ppm concentration of the leaf extract showed strong larvicidal activity than the other concentrations, as it is evidenced from the Figure 1. Among the plant extract tested against *An. stephensi*, *Ae.aegypti* and *Cx. tritaeniorhynchus* the pronounced lethal activity was recorded against *G. ula* extract in the experimental larvae of *An. stephensi* (LC_{50}=82.86ppm), followed by *S. hispida* (LC_{50}=89.45ppm). Similarly, *S. amplexicaulis* showed the LC_{50} value of 109.37ppm against the larvae of *Cx. tritaeniorhynchus* followed by *A. cadamba* with *An. stephensi* larvae (LC_{50}=109.87ppm) as shown in Figure 2. *C. argentea* showed more toxicity to the larvae of *Ae. aegypti.*

Table 2 shows the ovicidal activity of selected plants extract against the three important vector mosquitoes such as *An. stephensi, Ae.aegypti and Cx. tritaeniorhynchus.* Perusal of the data clearly indicates that the plant extracts showed spectrum of ovicidal activity against the different species of selected mosquitoes. It seems that, *S. hispida* showed more than 50% activity against the eggs of selected mosquitoes at 100 ppm concentration itself. Whereas, 150 ppm concentration of all the plants extracts repeats the trend of same. Notably, at 200ppm concentration of all the plants showed 100% ovicidal activity against *An. stephensi,Ae.aegypti* and *Cx. tritaeniorhynchus* (*i.e*., no hatchablity was recorded; Table 2). The selected five plant extract offers 100% protection against *An. stephensi, Ae.aegypti*and *Cx. tritaeniorhynchus* adult female

mosquitoes in terms of repellency up to 120 minutes of exposure periods. Even after that, *G. ula* and *S. hispida* holds their repellency up to 160 minutes as it is evidenced from the Table 3. Thereafter, the repellent activity followed the declined trend.

Table 1. List of indigenous medicinal plants used (Specimen Voucher Number) and their medicinal values

Sl. No.	Name of the plant (Voucher Number)	Family	Parts used	Medicinal values
1	*Celosia argentea* Linn. (ZD/PC/C04)	Amaranthaceae	Leaves	The whole plant has been used for dysentary, coughs, spitting up blood, excessive menstruation, amenorrhea, intestinal bleeding, bleeding from the lungs, this plant possesses anti-inflammatory, antipyretic, anti-diabetic and antibacterial (Bhujbal *et al*., 2008).
2	*Anthocephalus cadamba* Roxb.(ZD/PC/A10)	Rubiaceae	Leaves	The plant is used in the treatment fever, uterine complaints, diarrhoea and as an anti bacterial agent, antihatotoxic, antimalarial, analgesic, anti-inflammatory (Acharyya et al 2011).
3	*Gnetum ula* (ZD/PC/G01)	Gnetaeceae	Leaves	Aerial parts of this plant reported the various pharmacological activities like hepatoprotective, antifertility, anti-diabetic, analgesic, antipyretic and anti-inflammatory. The plant was found to contain various triterpenes and steroidal compounds.
4	*Solena amplexicaulis* Lam. (ZD/PC/S04)	Cucurbitaceae	Leaves	Plant pacifies vitiated kapha vata, anorexia, dyspepsia, colic, asthma, cough, renal calculi, urinary retention, hemorrhoids, splenomegaly and constipation.
5	*Spermacoce hispida* Linn. (ZD/PC/S07)	Rubiaceae	Leaves	The choornam of the samoolam of this plant is helpful in reducing the over weight or obesity. The leghyam prepared from this seeds is given twice daily for bloody diarrhoea. The roots are dried and powdered and given along with cows milk daily twice for conditions like urinary infections, oligurea, etc. The choornam of the roots is taken daily for reducing the internal heat, venereal diseases etc. A decoction of the samoolam is helpful in treating head ache.

Table 2. Ovicidal activity of the ethanol extract of the selected plants against important vector mosquito species

Plants tested	Test organisms	Concentrations used (ppm)					
		50	100	150	200	250	300
Celosia argentea	*Anopheles stephensi*	16.45	31.33	59.34	91.15	100.00	100.00
	Aedes aegypti	22.56	41.26	69.55	98.46	100.00	100.00
	Culex tritaeniorynchus	12.72	31.50	64.62	96.73	100.00	100.00
Anthocephalus cadamba	*Anopheles stephensi*	19.26	31.66	42.68	54.82	65.71	100.00
	Aedes aegypti	16.58	32.14	62.43	72.65	82.42	100.00
	Culex tritaeniorynchus	13.62	34.55	61.95	75.88	82.47	100.00
Gnetum ula	*Anopheles stephensi*	14.54	28.41	56.96	88.62	100.00	100.00
	Aedes aegypti	18.82	26.63	62.42	92.48	100.00	100.00
	Culex tritaeniorynchus	22.48	36.59	73.51	95.71	100.00	100.00
Solena amplexicaulis	*Anopheles stephensi*	31.53	58.73	84.55	96.57	100.00	100.00
	Aedes aegypti	18.71	34.85	73.52	92.52	100.00	100.00
	Culex tritaeniorynchus	22.59	3843	83.64	95.95	100.00	100.00
Spermacoce hispida	*Anopheles stephensi*	34.72	69.23	93.42	100.00	100.00	100.00
	Aedes aegypti	42.61	75.52	96.43	100.00	100.00	100.00
	Culex tritaeniorynchus	38.83	71.78	95.98	100.00	100.00	100.00

Values represents mean of five replications.

Table 3. Repellent activity of ethanol extract of selected plants against important vector mosquitoes

Plants tested	Test organisms	Duration (min.)				
		40	80	120	160	200
Celosia argentea	*Anopheles stephensi*	100±0.0	100±0.0	100±0.0	82.34±1.22	56.34±1.17
	Aedes aegypti	100±0.0	100±0.0	100±0.0	85.64±2.15	48.82±1.99
	Culex tritaeniorynchus	100±0.0	100±0.0	100±0.0	78.72±1.90	45.33±2.52
Anthocephalus cadamba	*Anopheles stephensi*	100±0.0	100±0.0	100±0.0	89.64±6.99	69.00±1.67
	Aedes aegypti	100±0.0	100±0.0	100±0.0	84.22±2.41	66.32±1.21
	Culex tritaeniorynchus	100±0.0	100±0.0	100±0.0	80.56±1.55	68.00±1.87
Gnetum ula	*Anopheles stephensi*	100±0.0	100±0.0	100±0.0	100±0.0	88.44±3.99
	Aedes aegypti	100±0.0	100±0.0	100±0.0	100±0.0	85.63±1.49
	Culex tritaeniorynchus	100±0.0	100±0.0	100±0.0	100±0.0	86.22±1.37
Solena amplexicaulis	*Anopheles stephensi*	100±0.0	100±0.0	100±0.0	89.52±1.47	67.38±2.88
	Aedes aegypti	100±0.0	100±0.0	100±0.0	86.85±3.17	64.44±2.00
	Culex tritaeniorynchus	100±0.0	100±0.0	100±0.0	80.62±2.88	59.37±1.82
Spermacoce hispida	*Anopheles stephensi*	100±0.0	100±0.0	100±0.0	100±0.0	89.40±1.72
	Aedes aegypti	100±0.0	100±0.0	100±0.0	100±0.0	85.84±2.41
	Culex tritaeniorynchus	100±0.0	100±0.0	100±0.0	100±0.0	93.36±2.25

Mean ± SD value of five replications.

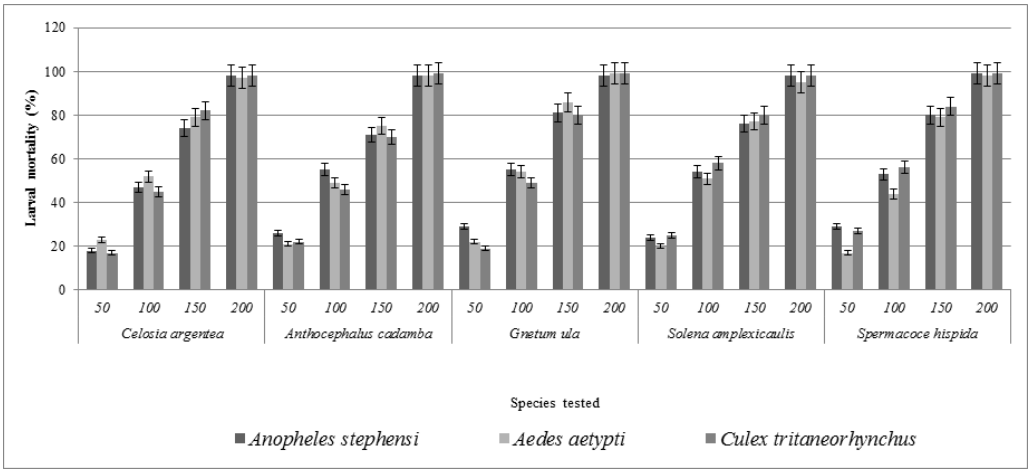

Fig. 1. Mean (%) larval mortality of the selected plants against three human vector mosquitoes. Rectangular bars bears with S.D. bar.

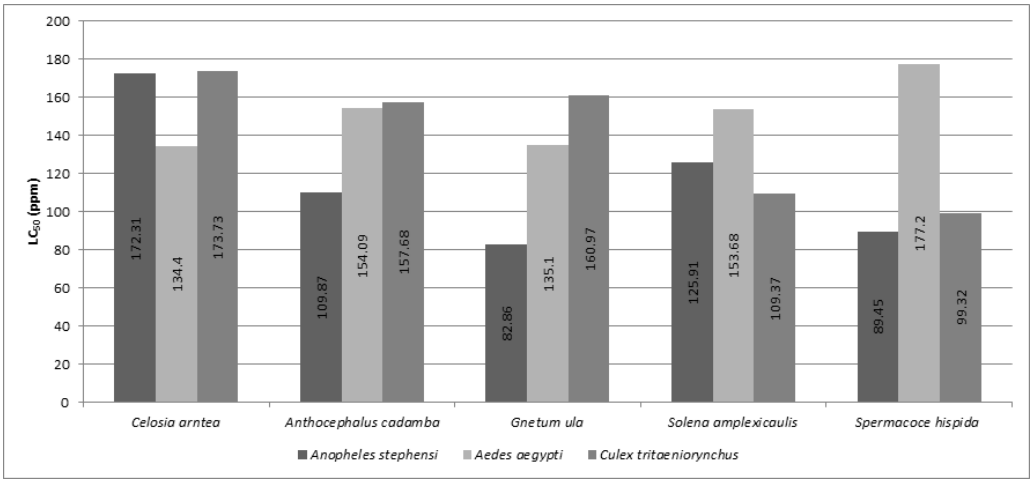

Fig. 2. Lethal concentration LC_{50} (ppm) of ethanol extract of selected plants against important vector mosquitoes.

Discussion

Our results showed that, the crude ethanol extract of *G. ula* and *S. hispida* have significant larvicidal, ovicidal and repellent activity against selected human vector mosquitoes *An. stephensi, Ae. aegypti* and *Cx. tritaeniorhynchus.* The results are in comparable with an earlier report by Karunamoorthi *et al.* (2008) were evaluated the petroleum ether extracts of the leaves of *Vitex negundo* for larvicidal activity against larval stages of *C.tritaeniorhynchus* in the laboratory with LC_{50} and LC_{90} values of 2.4883 and 5.1883 mg/l, respectively. The methanol leaves extracts of *V. negundo, V. trifolia, V. peduncularis* and *V. altissima* possessed varying levels of larvicidal activity on C. quinquefasciatus and An. stephensi and were found with LC_{50} value of 212.57, 41.41, 76.28, and 128.04 ppm respectively (Pushpalatha and Muthukrishnan, 1995).

Singh *et al.* (2003) who observed the larvicidal activity of *Ocimum canum* oil against vector mosquitoes, namely, *Ae.aegypti* and *C. quinquefasciatus* (LC_{50}=301 ppm) and *An. stephensi* (234 ppm). Larvicidal efficacy of leaf methanol extracts of *Pavonia zeylanica* and *Acacia ferruginea* were tested against the late third instar larvae of *C. quinquefasciatus* with LC_{50} values of 2,214.7 and 5,362.6 ppm, respectively (Vahitha *et al*., 2002). Earlier authors 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* (Kannathasan *et al*., 2007). The same extracts of *Euphorbia tirucalli* latex and stem bark were evaluated for larvicidal

activity against laboratory-reared larvae of *C. quinquefasciatus* with LC_{50} values of 177.14 and 513.387 mg/l, respectively (Yadav *et al*., 2002). Sharma *et al.* (2005) reported that the acetone extract of *Nerium indicum* and *Thuja orientelis* have been studied with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against third instar larvae of *An. stephensi* and *C. quinquefasciatus*. Larvicidal activity of the same extracts of *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafoetida*, and *Trigonella foenum* was tested out using different concentrations of each plant (range, 25-900 ppm) against *Ae.aegypti* larvae (Harve and Kamath, 2004); Rahuman and Venkatesan (2008) reported that the petroleum ether extract of *Citrullus colocynthis; methanol extracts of Cannabis indica, Cannabis sativus,* and *Momordica charantia*; and acetone extract of *Trichosanthes anguina* against the larvae of *Ae.aegypti* (LC_{50}=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *C. quinquefasciatus* (LC_{50} = 88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively. Larvicidal efficacies of methanol extracts of *Momordica charantia, Trichosanthes anguina, Luffa acutangula, Benincasa cerifera,* and *Citrullus vulgaris* tested with LC_{50} values were 465.85, 567.81, 839.81, 1,189.30 and 1,636.04 ppm, respectively, against the late third larval age group of *C. quinquefasciatus* (Prabakar and Jebanesan, 2004).The findings of our results is in corroboration with important findings of Sumroiphon *et al.* (2006) who have reported that the effect of water extract of citrus seed extract showed LC_{50} values of 135,319.40 and 127,411.88 ppm against the larvae of *Ae.aegypti* and *C. quinquefasciatus*. Amer and Mehlhorn (2006a) have reported that the five most effective oils were those of Litsea, Cajeput, Niaouli, Violet, and Catnip which induced a protection time of 8 h at the maximum and 100% repellency against *Ae.aegypti*, *An. stephensi*, and *C. quinquefasciatus*. The essential oil of *T. minuta* providing a repellency of 90% protection for 2 h against *An. stephensi, C. quinquefasciatus*, and *Ae. aegypti* was observed by Tyagi *et al.* (1994).

The peel methanol extract of *Citrus sinensis* and the leaf and flower ethyl acetate extracts of *O. canum* were tested against the larvae of *An. stephensi* (LC_{50}=95.74, 101.53, and 28.96 ppm; LC_{90}=303.20, 492.43 and 168.05 ppm), respectively (Kamaraj *et al.* 2008a, b). Rahuman *et al.* (2008) have reported that the LC_{50} value of petroleum ether extracts of *Jatropha curcas, Pedilanthus tithymaloides, Phyllanthus amarus, Euphorbia hirta,* and *Euphorbia tirucalli* were 8.79, 55.26, 90.92, 272.36, and 4.25 ppm, respectively, against *Ae.aegypti* and 11.34, 76.61, 113.40, 424.94, and 5.52 ppm, respectively, against *C. quinquefasciatus,* and the larvicidal effect of ten plants corresponding to different botanical families on *An. stephensi* and *C.quinquefasciatus*. The highest larval mortality was found in leaf acetone and methanol of *Canna*

indica (LC_{50}=29.62 and 40.77 ppm; LC_{90}=148.55 and 165.00 ppm) against second instar larvae (LC_{50} = 121.88 and 69.76, ppm; LC_{90} = 624.35and 304.27 ppm) and against fourth instar larvae of *An. stephensi* and in methanol and petroleum ether extracts of *Ipomoea carnea* (LC_{50}=41.82 and 39.32 ppm; LC_{90}= 423.76 and 176.39 ppm) against second instar larvae (LC_{50}=163.81 and 41.75 ppm; LC_{90}=627.38 and 162.63 ppm) and against fourth instar larvae of *C.quinquefasciatus*, respectively (Rahuman *et al*., 2009). The benzene extracts of *Citrullus vulgaris* exerted 100% mortality (zero hatchability) at 250 ppm, a very low hatchability (11.8%) at 200 ppm, and complete ovicidal activity at 300 ppm. The fraction I at 80 ppm exerted a very low hatchability rate of 3.2% followed by fraction II (6.9%), and fractions III and IV afforded 4.9% and 5.3% hatchability recorded against *An. stephensi* and *Ae. aegypti*, respectively (Mullai *et al*., 2008). The ovicidal effect of *Solenostemma argel* was low; however, concentrations of 0.05% and 0.1% exhibited significant effects (p<0.05), producing 65% and 75% and 62.9% and 62.9%, respectively, on the 1st and 2nd day after treatment, respectively. The 0.1% concentration reduced egg hatch by 33.7%, compared with the control, and 100% mortality values were evident in concentrations as low as 0.025% at 2 days post hatching against *Culex pipiens* (Al-Doghairi *et al*., 2004).

The ethanolic leaf extract of *Cassia obtusifolia*had significant larvicidal effect against *An. stephensi* with LC_{50} and LC_{90} values of 52.2 and 108.7 mg/l, respectively (Rajkumar and Jebanesan, 2009); the emodin compound was isolated from seeds and showed the LC_{50} values of 1.4, 1.9, and 2.2 mg/L against *C. pipiens*, *Ae.aegypti*, and *Ae. togoi*, respectively (Yang *et al*., 2003). Mullai and Jebanesan (2006) reported the complete ovicidal activity (100% mortality) was attained at 428 300 ppm for methanol, benzene, petroleum ether, and ethyl acetate extracts of *Citrullus pubescens* against *C. quinquefasciatus*. The leaf extract of *Solanum 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). Bagavan *et al.* (2008a, b) have reported that peel chloroform extract of *Citrus sinensis*, leaf ethyl acetate extracts of *O. canum and O. sanctum*, and leaf chloroform extract of *Rhinacanthus nasutus* against the larvae of *An. subpictus* (LC_{50}=58.25, 88.15, 21.67, and 40.46 ppm; LC_{90}=298.31, 528.70, 98.34, and 267.20 ppm) and peel methanol extract of *Citrus sinensis*, leaf methanol extract of *O. canum*, and ethyl acetate extracts of *O. sanctum* and *R. nasutus* against the larvae of *C. tritaeniorhynchus* (LC_{50}=38.15, 72.40, 109.12, and 39.32 ppm; LC_{90}= 184.67, 268.93, 646.62, and 176.39 ppm), respectively.

Cavalcanti *et al.* (2004) reported that the larvicidal activity of essential oils of Brazilian plants against *Ae. aegypti* and observed the LC_{50} to range

from465 60 to 533 ppm. Prajapati *et al.* (2005) reported that the larvicidal activity of different plants essential oil showedvaried LC_{95} values against *C. quinquefasciatus*. They were *Pimpinella anism* (149 µg/ml), *Z. officinalis* (202 µg/ml), *Junipers macropoda* (204 µg/ml), *Cinnamomum zeylanicum* (277 µg/ml), *Curcuma longa* (292 µg/ml), *Cyperus scariosus* (408 µg/ml), *Ocimum basilicum* (315 µg/ml), *Cuminum cyminum* (344 µg/ml), and *Nigella sativa* (365 µg/ml). Mullai and Jebanesan (2007) have reported that ethyl acetate, petroleum ether, and methanol leaf extracts of *Citrullus colocynthis* and *Cucurbita 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.

Kamaraj *et al.* (2008 a,b) reported that the highest larval mortality was found in leaf petroleum ether, flower methanol extracts of *Cryptocoryne auriculata*, flower methanol extracts of *L. aspera* and *R. nasutus*, leaf and seed methanol extracts of *Solanum torvum*, and leaf hexane extract of *V. negundo* against the larvae of *An. subpictus* (LC_{50}=44.21, 44.69, 53.16, 41.07, 35.32, 28.90, and 44.40 ppm; LC_{90}=187.31, 188.29, 233.18, 142.66, 151.60, 121.05, and 192.11 ppm, respectively) and against the larvae of *C. tritaeniorhynchus* (LC_{50}=69.83, 51.29, 81.24, 71.79, 44.42, 84.47, and 65.35 ppm; LC_{90}=335.26, 245.63, 300.45, 361.83, 185.09, 351.41, and 302.42 ppm, respectively). Ansari *et al.* (2005) observed the larvicidal activity of *Pinus longifolia* oil against three vector mosquitoes namely *Ae. aegypti* (LC_{50}, 82.1 ppm), *C. quinquefasciatus* (LC_{50}, 85.7 ppm), and *An. stephensi* (LC_{50}, 112.6 ppm).

Rajkumar and Jebanesan (2004) studied ovicidal activity of *M. polystachyum* leaf extract against *C. quinquefasciatus* and observed 100% egg mortality at 100 ml/l. The seed extract of *Atriplex canescens* showed complete ovicidal activity at 1,000 ppm concentration in eggs of *C. quinquefasciatus* (Ouda *et al*., 1998). Komalamisra *et al.* (2005) have reported that the petroleum ether and methanol extracts of *R. nasutus* and *Derris elliptica* exhibited larvicidal effects against *Ae. aegypti*, *C. quinquefasciatus*, *An. dirus*, and *Mansonia uniformis* with LC_{50} values between 3.9 and 11.5 mg/L, while the MeOH extract gave LC_{50} values of between 8.1 and 14.7 mg/L. *D. elliptica* petroleum ether extract showed LC_{50} values of between 11.2 and 18.84 mg/L, and the MeOH extract exhibited LC_{50} values between 13.2 and 45.2 mg/L. Earlier authors reported that the n-hexane, ethyl acetate, and methanol extracts of *Cassia nigricans* showed 100% larval mortality against *Ochlerotatus triseriatus* (Georges *et al*., 2008). Jang *et al.* (2002) have reported that the methanol extracts of *Cassia obtusifolia, Cassia tora,* and *Vicia tetrasperma* exhibited more than 90% larval mortality at 200 ppm on *A. aegypti* and *C. pipiens*. The larvicidal activity of petroleum ether, ethanolic, aqueous extracts

of dried leaves, and fixed oil from the seeds of *Caesalpinia bonduc* showed 100% mortality in 1% concentration of petroleum ether and ethanolic extract of leaf, whereas it was 55% in 2.5% concentration of aqueous extract and 92.6% in 2.5% concentration of fixed oil against the fourth instar larvae of *C. quinquefasciatus* (Saravanan *et al*., 2007); the petroleum ether extract of *Solanum xanthocarpum* was observed to be the most toxic with LC_{50} of 1.41 and 0.93 ppm and LC_{90} of 16.94 and 8.48 ppm at 24 and 48 h after application, respectively, against *An. stephensi* (Mohan *et al*., 2007). Venketachalam and Jebanesan (2001) have also reported that the repellent activity of methanol extract of *Ferronia elephantum* leaves against *Ae. 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/cm^2 for 10 h. Compared with earlier authors' reports, our results revealed that the experimental plant extracts were effective to control *An. stephensi*, *Ae.aegypti*, and *C. quinquefasciatus*. From these results, it was concluded that the plants *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis* and *Spermacoce hispida* exhibit larvicidal, ovicidal, and repellent activities against three important vector mosquitoes. Further analysis to isolate the active compound for larval control is under way in our laboratory. More studies are needed to elucidate the ovicidal activity against a wide range of mosquito species, and the active compound responsible for repellent activity should be identified, which could be used to control different mosquito species in the future. 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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