



RESEARCH ARTICLE

MOSQUITOCIDAL ACTIVITIES OF INDIAN MEDICINAL PLANT PAVONIA ODORATA WILLD
(MALVACEAE) AGAINST SELECTED VECTOR MOSQUITOES (DIPTERA: CULICIDAE)

Balu Selvakumar, * Gokulakrishnan J., Elanchezhyan K., Deepa J.,

Department of Zoology, Poompuhar College (Autonomous), Poompuhar, Melaiyur-609 107, Tamilnadu, India

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ABSTRACT

To determine the larvicidal and repellent activities of benzene and methanol extract of Pavonia odorata against Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus Twenty five 3rd instar larvae of selected mosquitoes species were exposed to various concentrations (60-300ppm) and were assayed in the laboratory by using the protocol of WHO 2005; the 24 h LC50 values of the P. odorata leaf extract was determined following Probit analysis. The repellent efficacy was determined against selected mosquitoes at three concentrations viz., 1.0, 2.0 and 3.0 mg/cm² under the laboratory conditions. The LC50 and LC90 values of benzene and methanol extract of P. odorata against Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus larvae in 24 h were 78.52, 82.58, 88.32, 64.14, 58.22, 52.35 and 254.53, 261.82, 273.44, 242.46, 239.82 and 230.56ppm, respectively. In repellent activity, among two extracts tested P. odorata methanol extract had strong repellent action against selected mosquitoes as it provided 100% protection against Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus for 280min. From the results it can be concluded the P. odorata extract was an excellent potential for controlling Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus mosquitoes.

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INTRODUCTION

Despite centuries of control efforts, mosquito-borne diseases are flourishing worldwide. With a disproportionate effect on children and adolescents, these conditions are responsible for substantial global morbidity and mortality. Vector-borne diseases continue to inflict high morbidity and mortality in the tropical and sub-tropical countries particularly in the resource constrained developing countries (Karunamoorthi *et al.*, 2010). Mosquitoes are the principle and well-known vectors of several disease causing pathogens, which affect several millions of people world-wide in terms of morbidity and mortality (Hotez *et al.*, 2004). Vector-borne diseases are infectious diseases that are transmitted by organisms that include insects, snails and rodents. These diseases represent a heavy burden on people, their families and communities in developing countries. Some of the most debilitating of these diseases are malaria, dengue, lymphatic filariasis, Japanese encephalitis, leishmaniasis, onchocerciasis, schistosomiasis and trypanosomiasis. For example, lymphatic filariasis can cause morbidity for life, while malaria causes the highest mortality, especially among young children and pregnant women. Vector-borne diseases also result in school absenteeism, loss of productivity, aggravation of poverty, high costs for health care and a burden on public health services (WHO, 2012). Anopheles mosquitoes are potentially capable of transmitting malaria in throughout world. Malaria is one of the most common mosquito-borne diseases in the tropical and subtropical countries, particularly in the sub-Saharan Africa (Karunamoorthy and Illango, 2010). The recent WHO Malaria Report estimates that 3.3 billion people were at the risk of

malaria in 2010, although of all geographical regions, populations living in sub-Saharan Africa have the highest risk of acquiring malaria; among 216 million episodes of malaria in 2010, of which approximately 81%, or 174 million cases, were observed from the African Region. There were an estimated 655,000 of malaria deaths in 2010, of which 91% were from Africa. Resurgent vector-borne diseases result in a high burden of disease, estimated as about 56 million disability-adjusted life years (TDR, 2009). An. stephensi Listen is the primary vector of malaria in India and other Western 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). C. quinquefasciatus, a vector of lymphatic filariasis, is widely distributed in tropical and subtropical countries, with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard *et al.*, 2003).

India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 crores (Hotez *et al.*, 2004). Ae. aegypti is the most important vector of dengue viruses world-wide, yellow fever virus in urban settings, and is a competent vector of chikungunya virus. Dengue causes more human morbidity and mortality than any other vector-borne viral infection. Ae. aegypti is uniquely adapted to a close association with humans, which facilitates efficient virus transmission (Morrison *et al.*, 2008). This mosquito is more widely dispersed now than any time in the past, placing billions of humans at risk of infection. It enjoys greater geographical distribution and is established virtually in

all tropical countries (Halstead, 2008; Gilles *et al.*, 2007; Goutham Chandra *et al.*, 2010). In the absence of an effective vaccine/antiviral therapy, vector control is at present the only way to limit these mosquito-borne diseases (Mariappan, 2007). Conventional pesticides such as malathion, DDT and pyrethroids that are generally used for mosquito control are known to cause the problems such as environmental pollution, residual effects and resistance of mosquito species. Development of resistance in *C. quinquefasciatus* and *Ae. aegypti* has been noted by World Health Organization and by other studies (Polson *et al.*, 2011). DDT, hexachlorocyclohexane and malathion are used to control malaria throughout India, especially in rural areas. However, the development of insecticide resistance threatens to halt these once effective methods of control and prevention. In particular, growing insecticide resistance in the predominant malaria vectors such as *An. culicifacies* and *An. stephensi* is a major concern (Singh *et al.*, 2009). Resistance to insecticides is an increasing problem in vector control because of the reliance on chemical control and expanding operations, particularly for malaria and dengue control. Furthermore, the chemical insecticides used can have adverse effects on health and the environment. Vector control is often not sufficiently adapted to local or changing circumstances because many countries lack capacity in decision-making for vector control. Such decisions should be based on evidence about the characteristics of local vectors and human behaviour and on the effectiveness of vector control methods. Furthermore, aspects of global change, such as climate change, environmental degradation, water scarcity and urbanization, are affecting the distribution of vector-borne diseases. Vector control must be adapted locally to these diverse and changing conditions and also to community preferences and needs (WHO, 2012). These problems forced to search for new, alternative and safer control measures especially from plant source. Because, plant derived molecules are eco-friendly, biodegradable and target specific. Moreover, the development of resistance by vectors against plant derived molecules has not been reported so far (Nathan and Kalivani, 2005).

This has necessitated the need for search and development of environmentally safer, low cost, indigenous methods for vector control. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides (Elumalai *et al.*, 2012a, b; Elumalai *et al.*, 2013a, b; Elangovan *et al.*, 2012a, b). In addition to application as general toxicant against mosquito larvae, botanical insecticides also have potential uses as growth and reproduction inhibitors, repellents, ovicidal and oviposition deterrents (Prajapati *et al.*, 2005). A huge number of botanical derivatives exhibited mosquitocidal activity (Krishnappa and Elumalai, 2012; Dhanasekaran *et al.*, 2013; Balu Selvakumar *et al.*, 2012; Gokulakrishnan *et al.*, 2012; Krishnappa and Elumalai, 2014). The bioactive constituents of these plants could be either a single substance or a mixture of substances. The separation of the mixture is neither practical nor advantageous in the insect economic control strategies. The aim of the current study is to investigate the chemical composition and mosquitocidal activity of *Pavonia odorata* willd (Malvaceae) against the larvae and adults of *Ae. aegypti*, *An. stephensi*, and *C. quinquefasciatus* (Diptera : Culicidae)

MATERIALS AND METHODS

Vector rearing

The selected mosquitoes larvae were collected from nearby water bodies in and around Poompuhar Village, Melaiyur, Nagapattinam District, Tamilnadu, India and maintained in cages of dimension, 40 X 60 X 40 cm³ at ambient conditions (27±1°C, 75±2% RH and 12 h light and 12 h dark photoperiod) in the laboratory. Yeast suspension (10% w/v) was served as food source for larval stages. Adult females were fed with chick blood and males with sucrose solution (10% w/v) soaked in cotton pads. The eggs collected from the field conditions were washed with 0.01% formaldehyde solution for 30 - 40 minutes as recommended by Al - Masghadani *et al.* (1980). This is necessary as a precaution against possible microsporidian infections which might interfere with the normal development of the immature stages of mosquitoes (Anosike and Onwuliri, 1992) and soaked in water to facilitate hatching. After hatching, first instar larvae were distributed in bowls 30cm in diameter and 12.5cm in depth. Care was taken to prevent overcrowding until development to early 4th instar larvae required for the study. The larvae were kept in the plastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

Plant extract

Fresh leaves of *Pavonia odorata* were collected from in and around Poompuhar Village, Nagapattinam District, Tamil Nadu, India. The collected plants were authenticated by a plant taxonomist in the Department of Botany. The leaves were washed with tap water, shade-dried for 15 days at room temperature (28 ±2 C), and then 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 sequentially with benzene and methanol by adapting a standard protocol. The solvents from the extracts were removed using a rotary vacuum evaporator (Rota vapour, Systronics India Ltd., Chennai, India) to collect the crude extract.

Larvicidal bioassay

The larvicidal activity of methanol extract was evaluated as per the protocol previously described WHO (2005) Based on the wide range and narrow range tests, essential oil major chemical compounds tested ranging from 60-300ppm were prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of selected mosquito species. The extract was dissolved in 1 ml DMSO and then diluted in 249 ml of dechlorinated tap water to obtain each of the desired concentrations. The control was prepared using 1ml of DMSO in 249 ml of dechlorinated water. 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 DMSO) and the required amount of chemical compositions 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. The LC₅₀ value was calculated by using probit analysis (Finney, 1971).

Repellent Activity

The repellent study will be made by following the method of WHO (2005). Three-day-old blood-starved female mosquitoes (100) were kept in a net cage (45 cm × 30 cm × 45 cm). The volunteer had no contact with lotions, perfumes or perfumed soaps on the day of the assay. The arms of volunteer, only 25 cm² dorsal side of the skin on each arms were exposed and the remaining area covered by rubber gloves. The crude extract was applied at 1.0, 2.0 and 3.0 mg/cm² separately in the exposed area of the fore arm. Only ethanol served as control. The time of the test dependent on whether the target mosquitoes day-or night biters. *Ae. aegypti* will be tested during the day time from 07.00 to 17.00h, while *Cx. quinquefasciatus* and *An. stephensi* will be tested during the night from 19.00 to 05.00h. The control and treated arm will be introduced simultaneously in to the mosquito cage, and gently tapping the sides on the experimental cages, the mosquitoes will be activated. Each test concentration was repeated six times. The volunteer conducted their test of each concentrations were inserted their treated and control arm in to the same cage for one full minute for every five minutes. The mosquitoes that landed on the hand will be recorded and then shaken off before imbibing any blood; making out a 5 minutes protection. The percentage of repellency will be calculated by the following formula.

$$\% \text{ Repellency} = [(T_a - T_b) / T_a] \times 100$$

Where *T_a* is the number of mosquitoes in the control group and *T_b* is the number of mosquitoes in the treated group.

RESULTS

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide must not cause high mortality in target organisms in order to be acceptable many researchers. The results of the present study clearly have shown in table 1&2. Data of the larvicidal activity of the of crude methanolic leaf extract of *Pavonia odorata* against selected species of mosquitoes are presented in Table 1. The LC₅₀ and LC₉₀ values of benzene and methanol extract of *Pavonia odorata* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* larvae in 24 h were 78.52, 82.58, 88.32, 64.14, 58.22, 52.35 and 254.53, 261.82, 273.44, 242.46, 239.82 and 230.56ppm, respectively. In repelent activity, among two extracts tested *Pavonia odorata* methanol extract had strong repellent action against

selected mosquitoes as it provided 100% protection against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* for 280min (Table 2). It showed that repellency depends on the strength of the extract concentration. From the results it can be concluded the crude extract of *Pavonia odorata* was an excellent potential for controlling *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* mosquitoes.

DISCUSSION

Though there are no reports available regarding the potential of *Pavonia odorata* as mosquito larvicide, several reports are available on other plant extracts and volatile oils which reveal their efficacy against mosquito larvae. The result of the present study was also comparable to the earlier reports on the larvicidal activities of the four major compounds, -terpinene, linalool, borneol and germacrene D. The LC₅₀ values of terpinene were 30.7 and 29.8.g/mL against the larvae of *An. aegypti* and *An. albopictus* (Prabhu *et al.*, 2011; Nikkon *et al.*, 2011;). Liang Zhu and Ying Juan Tian (2011) analysed the chemical composition of *Blumea martiniana* and assayed them for their larvicidal activity against *An. stephensi*. Cheng *et al.*, (2004) compared the essential oils from the leaves of *Cinnamomum osmophloeum* had an excellent inhibitory effect against the fourth instar larvae of *Ae. aegypti*. The larvicidal activity of cinnamon and other oils were recorded by Zhu *et al.*, (2008) against 4th instars of *Ae. albopictus*, *Ae. aegypti*, and *C. pipiens pallens*. Senthilkumar *et al.*, (2008) reported larvicidal effect of *Blumea mollis* essential oil against *C. quinquefasciatus*, with LC₅₀ and LC₉₀ values of 52.2 and 108.7 mg/L, respectively. According to Gleiser and Zygadlo (2007), the essential oils of *Lippia turbinata* and *Lippia polystachya* exhibit LC₅₀ values of 74.9 and 121 mg/L, respectively against *C. quinquefasciatus*. The essential oil of *Zanthoxylum armatum* was tested against three species of mosquitoes by Tiwari *et al.*, (2007). He found that among all the three species *C. quinquefasciatus* was the most sensitive with LC₅₀ and LC₉₅ values of 49 and 146 ppm, respectively followed by *Ae. aegypti* and *An. stephensi* with LC₅₀ values in the range of 54-58 ppm. Pushpalatha and Muthukrishnan (1995) reported that the petroleum ether : ethyl acetate (3:1) fraction of *V. negundo* leaf extract showed LC₅₀ value of 8.21 ppm against the 2nd instar larvae of *C. quinquefasciatus*. But the 2nd instar larvae are more susceptible to larvicidal principles than the 4th instar larvae. A saponin isolated from *Achyranthus aspera* recorded the LC₅₀ value of 18.20 and 27.24 ppm against *A. aegypti* and *C. quinquefasciatus*, respectively (Bagavan *et al.*, 2008). Two other study reported the LC₅₀ values of linalool at

Table 1 Larvicidal activity of benzene and methanol extracts of *Pavonia odorata* tested against freshly molted 3rd instar larvae of selected mosquitoes

Solvent tested	Mosquitoes	LC ₅₀ (ppm)	95% Fiducial Limit (ppm)		LC ₉₀ (ppm)	95% Fiducial Limit (ppm)		Slope	Chi-square
			LCL	UCL		LCL	UCL		
Benzene extract	<i>Aedes aegypti</i>	78.52	52.67	106.18	254.53	210.01	322.71	4.2930726	15.629
	<i>Anopheles stephensi</i>	82.58	57.21	112.37	261.82	216.39	338.22	4.0638280	14.038
	<i>Culex quinquefasciatus</i>	88.32	62.19	118.55	273.44	225.46	345.93	4.5219377	15.372
Methanol extract	<i>Aedes aegypti</i>	64.14	47.83	95.39	242.46	205.46	316.38	4.4928019	14.748
	<i>Anopheles stephensi</i>	58.22	43.60	88.36	239.82	192.18	308.81	3.0489208	15.894
	<i>Culex quinquefasciatus</i>	52.35	41.86	85.27	230.56	189.22	301.44	3.2436914	13.273

LC50=Lethal Concentration brings out 50% mortality and LC90 = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Slope; Chi-square.

of germacrene D were 63.6 and 59.5.g/mL against the larvae of *An. aegypti* and *An. Tephensi* Kiran *et al.*, (2006). Several researchers reported, phytochemical based experiments for exploring the insecticidal activity on mosquito vectors (Siddique *et al.*, 2008; Rasheed *et al.*, 2005). Different parts of the Citrus plant *i.e.* fruits, seeds, roots and leaves have been tested for their use as mosquitocidal components (Akram *et al.*, 2010). A number of studies have also been carried out on the larvicidal potential of essential oil extracted from the Citrus leaves and peels (Melliou *et al.*, 2009).

plants are a promising tool especially for targeting mosquitoes in the larval stage (Amer and Mehlhorn, 2006).

Ansari *et al.*, (2000) suggested that the peppermint oil *Mentha piperita* showed strong repellent activity against adult mosquitoes when applied on the human skin. The protection obtained against *An. annularis*, *An. culicifacies*, and *C. quinquefasciatus* was 100.0%, 92.3%, and 84.5%, respectively. Nathan *et al.*, (2005) considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity against *An. stephensi* and the larval mortality was dose-dependent with the highest

Table 2 Repellent activity of benzene and methanol extracts of *Pavonia odorata* against selected mosquitoes

Mosquitoes	Concentration (mg/cm ²)	Percentage of repellency, Time post application of repellent(min)							
		40 ppm	80 ppm	120 ppm	160 ppm	200 ppm	240 ppm	280ppm	320 ppm
Benzene extract									
<i>Aedes aegypti</i>	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	75.3±1.4	62.4±1.9
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	81.4±1.7	68.3±1.6
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	93.8±1.6	75.2±1.4
<i>Anopheles stephensi</i>	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	73.5±1.3	59.3±1.4
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	79.4±1.6	64.8±1.6
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	88.8±1.9	73.6±1.8
<i>Culex quinquefasciatus</i>	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	72.4±1.2	62.4±1.7	48.8±1.5
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	85.6±1.5	72.6±1.9	51.4±1.3
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	91.2±1.6	81.3±1.5	64.1±1.7
Methanol extract									
<i>Aedes aegypti</i>	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	69.2±1.8
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	75.6±1.6
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	84.8±1.5
<i>Anopheles stephensi</i>	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	71.3±1.6
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	78.2±1.7
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	86.7±1.4
<i>Culex quinquefasciatus</i>	1.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	69.2±1.9
	2.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	75.6±1.5
	3.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	100±0.0	82.4±1.3

Each value mean± S.D represents average of five values.

Nour *et al.*, (2009) reported that the essential oils from four basil accessions, *Ocimum basilicum*, conferred complete repellency against *Anopheles* mosquito lasting for 1.5 to 2.5 h per one application of 0.1 mL to a human volunteer's arm. *Trachyspermum ammi* seed oil could achieve a repellency of 45.0% with repellent dose (RD₅₀) observed as 25.02 mg/mat against *An. stephensi* adults (Pandey *et al.*, 2009). Redwane *et al.*, (2002) reported that gallotannins isolated from *Quercus lusitania* var.*infectoria* galls had the LC₅₀ value of 373 ppm against *C. pipiens*. Earlier, Tawatsin *et al.*, (2008) have reported that plant essential oils were more effective against *An. dirus*, *An. albopictus* and *Culex*. Essential oil of *Cinnamomum zeylanicum*, *Zingiber officinale*, and *Rosmarinus officinalis* also showed repellent activities against *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* (Gillij *et al.*, 2008). Mathu *et al.*, (2010) reported that the 9-oxoneoprocumenol from *Curcuma aromatica* and neoprocumenol from *Curcuma aromatica* against vector mosquito. Komalasmira *et al.*, (2005) who have been reported the ethanol extracts of *P. beetle* has successfully killed the larvae of 4 mosquito vectors *Ae. aegypti*, *C. quinquefasciatus*, *An. dirus* and *Monsonia uniformis*. Mosquito control is vital for many countries and is still in a state of evolution. During the last decades, it depended upon synthetic organic insecticides, many of which have been removed from the arsenal of weapons (Floore, 2006) and botanicals are the new weapons of mosquito control under exploration. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. Natural pesticides derived from

affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *An. stephensi*. Similarly, the aqueous and hydro-alcoholic extracts of *Melia azedarach* leaves and seeds were tested to explore the in vitro ovicidal and larvicidal activity against *Haemonchus contortus* (Sharma *et al.*, 2006). Karunamoorthi and Ilango (2010) have reported that the LC₅₀ and LC₉₀ values of methanol leaf extracts of *Croton macrostachyus* were 89.25 and 224.98 ppm, respectively against late third instar larvae of malaria vector, *An. arabiensis*. The screening of *Artemisia annua* plants against larvicidal activity of *Anopheles* mosquito, it produced maximum activity and LC₅₀ values were 16.85 ppm and 11.45 ppm after 24 and 48 h of exposure, respectively (Singh *et al.*, 2006).

Liang Zhu and Ying Juan Tian (2011) analysed the chemical composition of *Blumea martiniana* and assayed them for their larvicidal activity against *An. stephensi*. Cheng *et al.* (2004) compared the essential oils from the leaves of *Cinnamomum osmophloeum* had an excellent inhibitory effect against the fourth instar larvae of *Ae. aegypti*. The larvicidal activity of cinnamon and other oils were recorded by Zhu *et al.* (2006 & 2008) against 4th instars of *Ae. albopictus*, *Ae. aegypti*, and *C. pipiens pallens*. Senthilkumar *et al.* (2008) reported larvicidal effect of *Blumea mollis* essential oil against *C. quinquefasciatus*, with LC₅₀ and LC₉₀ values of 52.2 and 108.7 mg/L, respectively. According to Gleiser and Zygadlo (2007), the essential oils of *Lippia turbinata* and *Lippia polystachya* exhibit LC₅₀ values of 74.9

and 121 mg/L, respectively against *C. quinquefasciatus*. The essential oil of *Zanthoxylum armatum* was tested against three species of mosquitoes by Tiwari *et al.* (2007). He found that among all the three species *C. quinquefasciatus* was the most sensitive with LC₅₀ and LC₉₅ values of 49 and 146 ppm, respectively followed by *Ae. aegypti* and *An. stephensi* with LC₅₀ values in the range of 54-58 ppm. Pushpalatha and Muthukrishnan (1995) reported that the petroleum ether : ethyl acetate (3:1) fraction of *V. negundo* leaf extract showed LC₅₀ value of 8.21 ppm against the 2nd instar larvae of *C. quinquefasciatus*. But the 2nd instar larvae are more susceptible to larvicidal principles than the 4th instar larvae. A saponin isolated from *Achyranthus aspera* recorded the LC₅₀ value of 18.20 and 27.24 ppm against *A. aegypti* and *C. quinquefasciatus*, respectively (Bagavan *et al.*, 2008). Two other study reported the LC₅₀ values of linalool at 24 h were 155.73.g/mL against fourth instar larvae of *Ochlerotatus caspius* Knio *et al.* (2008) and the LC₅₀ values of germacrene D were 63.6 and 59.5.g/mL against the larvae of *An. aegypti* and *An. Tephensi* Kiran *et al.* (2006). The LC₅₀ values of Borneol were 43.5 mg/L against the larvae of *An. Aegypti* (Rajkumar and Jebanesan, 2010). Several researchers reported, phytochemical based experiments for exploring the insecticidal activity on mosquito vectors (Vasudevan *et al.*, 2009; Siddique *et al.*, 2005; Siddique *et al.*, 2008; Rasheed *et al.*, 2005). Different parts of the Citrus plant *i.e.* fruits, seeds, roots and leaves have been tested for their use as mosquitocidal components (Traboulsi *et al.*, 2005; Akram *et al.*, 2010). A number of studies have also been carried out on the larvicidal potential of essential oil extracted from the Citrus leaves and peels (Lee, 2006; Melliou *et al.*, 2009).

Nour *et al.* (2009) reported that the essential oils from four basil accessions, *Ocimum basilicum*, conferred complete repellency against *Anopheles* mosquito lasting for 1.5 to 2.5 h per one application of 0.1 mL to a human volunteer's arm. *Trachyspermum ammi* seed oil could achieve a repellency of 45.0% with repellent dose (RD₅₀) observed as 25.02 mg/mat against *An. stephensi* adults (Pandey *et al.*, 2009). Redwane *et al.* (2002) reported that gallotannins isolated from *Quercus lusitania* var. *infectoria* galls had the LC₅₀ value of 373 ppm against *C. pipiens*. Earlier, Tawatsin *et al.* (2008) have reported that plant essential oils were more effective against *An. dirus*, *An. albopictus* and *Culex*. Essential oil of *Cinnamomum zeylanicum*, *Zingiber officinale*, and *Rosmarinus officinalis* also showed repellent activities against *An. stephensi*, *Ae. aegypti*, and *C. quinquefasciatus* (Gillij *et al.*, 2008). The bioassay-guided fractionation of *Abutilon indicum* led to the separation and identification of a -sitosterol with LC₅₀ value of 11.49 and 26.67 ppm against *Ae. aegypti* and *C. quinquefasciatus* (Rahuman *et al.*, 2008). There are reports available where essential oils have shown repellent properties in the fields. The essential oils extracted from some Verbenaceae plants have shown repellent and also insecticidal effects against mosquitoes (Karunamoorthy *et al.*, 2008 a & b). Mathu *et al.* (2010) reported that the 9-oxoneoprocumeneol from *Curcuma aromatica* and neoprocumeneol from *Curcuma aromatica* against vector mosquito.

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