ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2015; 2 (1): 13-17

Received: 04-10-2014
Accepted: 15-11-2014

K.T. Kamakshi
Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India

R. Raveen
Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India

Samuel Tennyson
Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India

S. Arivoli
Department of Zoology, Thiruvalluvar University, Vellore 632 115, Tamil Nadu, India

A. Daniel Reegan
Entomology Research Institute, Loyola College, Chennai 600 034, Tamil Nadu, India

For Correspondence:
Dr. R. Raveen
Assistant Professor, Department of Zoology, Madras Christian College, Chennai 600 059, Tamil Nadu, India

Ovicidal and repellent activities of *Cereus hildmannianus* (K. Schum.) (Cactaceae) extracts against the dengue vector *Aedes aegypti* L. (Diptera: Culicidae)

K.T. Kamakshi, R. Raveen, Samuel Tennyson, S. Arivoli and A. Daniel Reegan

ABSTRACT
A preliminary screening was performed with hexane, petroleum ether, ethyl acetate, carbon tetrachloride and aqueous extracts of *Cereus hildmannianus* cladodes for ovicidal and repellent activities against the dengue vector, *Aedes aegypti*. Ovicidal bioassay was performed at concentrations of 62.5, 125, 250, 500 and 1000 mg/L and mortality observed after 96 h period of exposure. A moderate ovicidal activity was noted only in *Cereus hildmannianus* petroleum ether extract with 52.8% at 1000 mg/L. In the case of repellent studies, the petroleum ether extract showed repellent activity with a mean protection time of 137 min at 5.0 mg/cm^2 dosage against the adult female mosquitoes of *Aedes aegypti* where bioassays were carried at concentrations of 1.0, 2.5 and 5.0 mg/cm^2. Further studies are needed to elucidate the repellent activities of *Cereus hildmannianus* crude petroleum ether extract against a wide range of all stages of mosquito species and also the active ingredient(s) of the extract responsible for repellent activity should be identified.

Keywords: *Cereus hildmannianus*, crude extracts, ovicidal, repellent, *Aedes aegypti*.

1. Introduction
Mosquitoes are the principal vector of many vector-borne diseases affecting human beings and animals, in addition to nuisance. Vector-borne diseases in India, *viz*., dengue, chikungunya, malaria, filariasis, and Japanese encephalitis cause thousands of deaths per year [1-3]. Mosquitoes belonging to the genera *Aedes* are the vectors of dengue/dengue haemorrhagic fever and chikungunya [4]. *Aedes aegypti* is responsible for spreading dengue and chikungunya. 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 of central nervous system [5].

Dengue is prevalent throughout the tropics and subtropics. The World Health Organization estimates that around 2.5 billion people are at risk of dengue. Infections have dramatically increased in recent decades due to increased urbanization, trade, and travel. No effective drug or vaccine is available so far. The only solution is to prevent the disease carrying mosquito from breeding and biting humans. Dengue is the most important mosquito spread viral disease and a major international public health concern. It is a self-limiting disease found in tropical and subtropical regions around the world, predominantly in urban and semi urban areas. Dengue fever or dengue hemorrhagic fever is caused by dengue virus, which belongs to genus Flavivirus and family Flaviviridae, and include serotypes 1, 2, 3, and 4 (Den-1, Den-2, Den-3 and Den-4) [6]. Mosquito control methods mainly rely on the chemical insecticides, but has led to environmental pollution. Therefore, alternative biological mosquitocides are urgently needed.

Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal and adulticidal properties [7]. Plants could be an alternative source for mosquito repellents because they constitute a potential source of bioactive chemicals and typically are free from harmful effects [8].

The chemicals derived from plants have been projected as weapons in future mosquito control program as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents and oviposition-deterrent [9]. Utilizing endogenous knowledge concerning plants with traditional medicinal value has proven fruitful in identifying potential sources of phytoextracts with insecticidal activity [10]. There have been many attempts to assay the activity of plant extracts against vectors of human disease, in particular through the utilization of plants for which such knowledge exists [11]. In a previous study, the larvicidal activity of *Cereus hildmannianus* cladodes extracts against *Aedes aegypti* was reported [12]. Hence, the present study was aimed to screen the crude extracts of *Cereus hildmannianus* for their ovicidal and repellent activity against the eggs and adults of dengue vector, *Aedes aegypti*.

2. Materials and Methods

2.1. Plant collection and extraction

Mature fresh cladodes of *Cereus hildmannianus* collected from Madras Christian College campus, Chennai, Tamil Nadu, India were brought to the laboratory, shade dried at room temperature and powdered. Dried and powdered cladodes (1 Kg) was macerated sequentially with 3 L of hexane, petroleum ether, ethyl acetate, carbon tetrachloride and distilled water for a period of 96 h each and filtered using Soxhlet apparatus. The filtrate was then concentrated at reduced temperature on a rotary evaporator. The crude extracts thus obtained were lyophilized and a stock solution of 1,00,000 mg/L prepared by adding adequate volume of acetone was refrigerated at 4 ^{0}C until testing for bioassay.

2.2. Test mosquitoes

The eggs of *Aedes aegypti* were obtained from Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India. In the laboratory, the immature mosquitoes were reared in enamel larval trays until adult emergence. Cyclic generations of *Aedes aegypti* were maintained separately in two feet mosquito cages in an insectary. Mean room temperature of 27±2 ^{0}C and a relative humidity of 70-80% were maintained in the insectary. The adult mosquitoes were fed on ten per cent glucose solution. For continuous maintenance of mosquito colony, the adult female mosquitoes were blood fed with laboratory reared albino mice. Ovitraps were placed inside the cages for egg laying. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside mosquito cage for adult emergence.

2.3. Ovicidal activity

Egg hatchability was studied following the method of Elango *et al*. [13]. Twenty five freshly laid eggs of *Aedes aegypti* were exposed to concentrations, *viz*., 62.5, 125, 250, 500 and 1000 mg/L. A total of three trials with five replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of acetone to distilled water. Distilled water served as untreated control. The hatchability of eggs were observed with the aid of a microscope and the results was assessed in percentage at 96 h post treatment. The ovicidal activity was assessed in terms of Egg mortality rate (EMR) using the formula given below.

$$\frac{\text{Number of unhatched eggs}}{\text{Total number of eggs introduced}} \times 100$$

One way ANOVA followed by Duncan’s multiple range test (DMRT) was performed to determine the difference in egg mortality rate between concentrations.

2.4. Repellent activity

Repellent activity was conducted as per the guidelines of W.H.O. [14] with slight modifications. One hundred blood-starved adult female mosquitoes (three to six days old) were introduced into separate laboratory cages (45×45×50 cm). Before each test, the forearms of human volunteers were washed with unscented neutral soap, thoroughly rinsed, and allowed to dry before the application of the extract. Three concentrations *viz*., 1, 2.5 and 5 mg/cm^2 for each extract and five replicates (five volunteers) were maintained for each concentration. The extracts were applied on the right upper forearm and the remaining regions covered with gloves. *N*,*N*-Diethyl-*meta*-toluamide (DEET 12%, w/w) was used as standard reference control on the sixth volunteer forearm (treated control). The left arm of all six volunteers served as untreated control. Mosquito repellency was observed for every three full minutes of fifteen minutes by inserting the right hand inside the cage. Likewise, the same methodology was followed for the left hand. The protection time of each concentration of each extract was calculated. The percentage protection was calculated using the following formula [15].

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

3. Results

The ovicidal activity of *Cereus hildmannianus* extracts on *Aedes aegypti* eggs are presented in Table 1. Moderate ovicidal activity was noted only in the petroleum ether extract on the eggs of *Aedes aegypti* with 52.8% EMR at 1000 mg/L at 96 h post treatment period. The lowest concentration (62.5 mg/L) of petroleum ether extract caused 28.8% egg mortality against the eggs of *Aedes aegypti*. The carbon tetrachloride extract showed 38.4% and hexane, ethyl acetate and aqueous extracts recorded egg mortality of 21.6%, 24.8% and 20.0% respectively at 1000 mg/L concentration against *Aedes aegypti*. No mortality was observed in untreated control but very few egg mortality in treated control was observed (Table 1).

The complete protection times for all the five extracts of *Cereus hildmannianus* against *Aedes aegypti* female mosquitoes were recorded and the results are given in Table 2. The repellence was directly proportional to the dose and protection time (min) for each extract and showed variations against *Aedes aegypti* mosquitoes. The petroleum ether extract gave maximum protection time against *Aedes aegypti* when compared to other extracts. Petroleum ether extract gave protection time up to 137 min against *Aedes aegypti* at a dose of 5 mg/cm^2 followed by ethyl acetate which provided 77 min protection (Table 2). The lowest concentration (1 mg/cm^2) of petroleum ether extract provided protection up to 46 minutes. These results when compared with reference control (*N*,*N*-Diethyl-meta-toluamide 12%, w/w), showed maximum of 213 min protection time at 5 mg/cm^{2}dosage against *Aedes aegypti* mosquitoes. The hexane, carbon tetrachloride and aqueous extracts provided a protection time upto 47, 18 and 48 min at 5 mg/cm^{2}dosage against *Aedes aegypti* mosquitoes.

Table 1: Ovicidal activity of *Cereus hildmannianus* extracts against *Aedes aegypti*

Solvents	Egg mortality rate (EMR) (%)						
	Concentration (mg/L)						
	Untreated control	**Treated control**	**62.5**	**125**	**250**	**500**	**1000**
Hexane	0.0 ±0.00[a]	0.6 ±0.89[a]	8.8 ±0.83[b]	10.4 ±1.51[b]	12.0 ±1.58[b]	20.0 ±0.70[c]	21.6 ±1.14[c]
Ethyl acetate	0.0 ±0.00[a]	0.8 ±1.09[a]	16.8 ±0.83[b]	20.0 ±0.70[bc]	23.2 ±0.83[cd]	23.2 ±0.44[cd]	24.8 ±1.30[d]
Carbon tetrachloride	0.0 ±0.00[a]	0.0 ±0.00[a]	20.8 ±1.48[b]	26.4 ±1.14[c]	32.0 ±0.70[cd]	29.6 ±1.5 [d]	38.4 ±0.54[e]
Petroleum ether	0.0 ±0.00[a]	0.0 ±0.00[a]	28.8 ±1.92[b]	31.2 ±0.83[bc]	37.6 ±0.89[c]	44.8 ±1.30[d]	52.8 ±2.38[e]
Aqueous	0.0 ±0.00[a]	0.2 ±0.44[a]	4.0 ±0.70[b]	8.0 ±0.71[c]	16.0 ±0.70[d]	16.0 ±0.70[d]	20.0 ±1.00[e]

Different superscript alphabets within the column indicate statistical significant difference in EMR between concentrations at $P<0.05$ level by one way ANOVA followed by DMRT

Table 2: Repellent activity of *Cereus hildmannianus* extracts against *Aedes aegypti*

Solvents	Concentration (mg/cm^2)	Repellency	
		Complete protection time (min)	
		Control	**Treated**
Hexane	1.0	1.20 ±0.44	16.00 ±1.09
	2.5	1.20 ±0.44	31.00 ±1.26
	5.0	1.40 ±0.54	47.00 ±1.67
Petroleum ether	1.0	1.60 ±0.54	46.00 ±1.26
	2.5	1.20 ±0.44	91.00 ±0.89
	5.0	1.40 ±0.54	137.00 ±1.41
Carbon tetrachloride	1.0	1.80 ±1.09	16.00 ±1.26
	2.5	1.80 ±0.83	16.00 ±1.26
	5.0	1.60 ±0.54	18.00 ±1.09
Ethyl acetate	1.0	1.60 ±0.89	16.00 ±0.63
	2.5	1.80 ±0.44	46.00 ±0.89
	5.0	1.60 ±0.54	77.00 ±1.26
Aqueous	1.0	2.40 ±0.89	16.00 ±0.89
	2.5	1.80 ±0.83	36.00 ±3.09
	5.0	2.00 ±1.41	48.00 ±1.26
N,N-Diethyl-*meta*-toluamide 12%	1.0	2.20 ±0.83	56.00 ±1.41
	2.5	1.80 ±0.83	105.00 ±3.00
	5.0	2.40 ±1.14	213.00 ±1.00

4. Discussion

Vector control is facing a serious threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting counter measures such as developmental of novel insecticides [16]. Vector control has experienced a paradigm shift over time as public health officials have come to better appreciate the potential applications of natural products in the mission of disease control [17]. Indian flora comprises a rich storehouse of phytochemicals/botanical insecticides which serve as suitable alternatives to synthetic insecticides [18] as they are relatively safe, degradable, and are readily available in many areas of the world. Secondary metabolites present in plant act as key candidate with insecticidal properties and can be explored to develop the natural compounds to control mosquito population [19]. In the present study, *Cereus hildmannianus* crude extracts were tested for their ovicidal and repellent activities against *Aedes aegypti*. Among the solvent extracts of *Cereus hildmannianus* tested, the petroleum ether extract showed more moderate ovicidal and pronounced repellency activity against *Aedes aegypti*.

Plant extracts have been screened and studied for their ovicidal activity against mosquitoes [20]. Ovicidal compounds are able to interrupt embryo development, impair the survival of larva inside the egg or block egg hatching. Fresh eggs from control showed embryogenesis in progress while impairment of embryo development was detected in treated eggs, reflecting ovicidal activity [21, 22].

Thavara *et al*. [23] reported that the phytochemicals provided protection for 7 h against *Aedes aegypti*, and at least 8 h against *Culex quinquefasciatus* and *Anopheles dirus* under laboratory conditions. Bream *et al*. [24] reported that the repellent action of the plant extracts tested varied depending on the plant parts, solvent used in extraction and the dose of the extract and further reported the petroleum ether extracts of the leaf, stem and root of *Echinochloa stagninum* at 5, 5 and 4.3 mg/cm^2 to exhibit 100% repellency against mosquitoes. Venkatachalam and Jebanesan [15] reported the repellent activity of methanol extract of *Feronia elephantum* leaves against *Aedes aegypti* at 1.0 and 2.5 mg/cm^2 concentrations which 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. Yang *et al*. [25] tested the methanol extracts from 23 aromatic medicinal plant species for their repellent activity against female blood starved *Aedes aegypti*. Skin repellency test at 1, 2.5 and 5 mg/cm^2 concentrations of *Cymbopogon citratus* gave 100% protection up to 3, 4 and 5 h, respectively, while the total protection percentage of the essential oil was recorded as 49.64% at 1 mg/cm^2, 62.19% at 2.5 mg/cm^2 and 74.03% at 5 mg/cm^2 against *Culex quinquefasciatus* for 12 h [26]. Mullai *et al*. [27] also reported skin repellent test at 1.0, 2.5 and 5.0 mg/cm^2 concentration gave a mean complete protection time ranging from 119.17 to 387.83 min against *Anopheles* *stephensi* with the benzene, petroleum ether, ethyl acetate and methanol extracts of *Citrullus vulgaris* tested. Govindarajan [28] reported the repellent activity of methanol extract of *Feronia elephantum* leaves up to 150 min against *Aedes aegypti* female adults at 5 mg/cm^2 concentration and also the crude extracts of *Sida acuta* had strong repellent action against three species of vector mosquitoes as it provided 100% protection against *Anopheles stephensi* for 180 min followed by *Aedes aegypti* (150 min) and *Culex quinquefasciatus* (120 min). The methanol extracts of *Ervatamia coronaria* and *Caesalpinia pulcherrima* at a higher concentration of 5.0 mg/cm^2 provided 100% protection up to 150, 180 and 210 min against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*, respectively [22]. Govindarajan and Sivakumar [29] tested the repellent activities of crude hexane, ethyl acetate, benzene, chloroform, and methanolic leaf extracts of *Eclipta alba* and *Andrographis paniculata* at three different concentrations of 1.0, 2.5, and 5.0 mg/cm^2 against *Aedes aegypti* and suggested that the leaf solvent plant extracts have the potential to be used as an ideal eco-friendly approach for the control of mosquitoes. The seed extracts of *Tribulus terrestris* exhibited 100% repellent protection at 1.0 mg/cm^2 against *Anopheles arabiensis* [30]. Complete protection was provided by leaves of *Adansonia digitata* benzene up to 150 min at 4 and 6 mg/cm^2 and in chloroform up to 180 at all concentrations; hexane extract with repellency up to 120, and methanol extract up to 210 min against *Anopheles stephensi* [31].

The blood-feeding contact or response is prevented with the application of the phytochemical extract on the skin, and the mosquito could not bite because the active ingredients does not allow it to smell the attractant (lactic acids) and could not therefore identify the human as its source of meal. This suggests that the active ingredients confused the olfactory receptors and the mosquito simply could not smell the host. It is suspected that the active ingredients in the *Cereus hildmannianus* phytochemical extracts when worn on the bare skin evaporate and are released with carbon dioxide from the host, thereby changing the human carbon dioxide signature to that of plants. By this, the visiting mosquito now perceives plants' carbon dioxide and not that of human that it is looking for [32, 33].

In conclusion, the results of the present study showed the petroleum ether extract of *Cereus hildmannianus* to exhibit repellent property against *Aedes aegypti* when compared to the other solvents tested which might be due to the polarity index and nature of the solvent. Further studies are needed to elucidate the activity of *Cereus hildmannianus* petroleum ether extract against a wide range of all stages of mosquito species and also to identify the active ingredient(s) of the extract responsible for repellent activity.

5. Acknowledgments

The authors are thankful to Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India for providing *Aedes aegypti* egg strips.

6. References

1. W.H.O. Combating waterborne diseases at the household level. Part1. WHO, Geneva, 2007.
2. Gopalan SS, Das A. Household economic impact of an emerging disease in terms of catastrophic out-of-pocket health care expenditure and loss of productivity: investigation of an outbreak of chikungunya in Orissa, India. Journal of Vector Borne Diseases 2009; 46: 57–64.
3. Dhiman CR, Pahwa S, Dhillon GPS, Dash AP. Climate change and threat of vector-borne diseases in India: are we prepared? Parasitology Research 2010; 106:763–773.
4. Borah R, Kalita MC, Kar A, Talukdar AK. Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vector *Aedes aegypti* and *Culex quinquefasciatus*. African Journal of Biotechnology 2010; 9:2527–2530.
5. Pancharoen C, Kulwichit W, Tantawichien T, Thisyakorn U, Thisyakorn C. Dengue infection: a global concern. Journal of the Medical Association of Thailand 2002; 85:25–33.
6. W.H.O. Dengue transmission research in WHO bulletin 2010. http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.13.pdf.
7. Babu R, Murugan K. Interactive effect of neem seed kernel and neem gum extracts on the control of *Culex quinquefasciatus* Say. Neem Newsletters 1998; 15(2):9-11.
8. Isman MB. Leads and prospects for the development of new botanical insecticides. Reviews in Pesticide Toxicology 1995; 3:1-20.
9. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: A review. Journal of the American Mosquito Control Association 1991; 7(2):210-237.
10. Rahuman A, Bagavan A, Kamaraj C, Vadivelu M, Zahir A, Elango G. Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitology Research 2009; 104:637-643.
11. Mathew N, Anitha M, Bala T, Sivakumar S, Narmadha R, Kalyanasundaram M. Larvicidal activity of *Saraca indica*, *Nyctanthes arbor-tristis*, and *Clitoria ternatea* extracts against three mosquito vector species. Parasitology Research 2009; 104: 1017-1025.
12. Kamakshi KT, Raveen R, Reegan AD, Samuel T. Larvicidal activity of *Cereus hildmannianus* (K. Schum.) extracts against dengue vector *Aedes aegypti* (Diptera: Culicidae). International Journal of Applied Biology 2013; 1:66–68.
13. Elango G, Bagavan A, Kamaraj C, Zahir AA, Rahuman AA. Oviposition deterrent, ovicidal, and repellent activities of indigenous plant extracts against *Anopheles subpictus* Grassi (Diptera: Culicidae). Parasitology Research 2009; 105:1567–1576.
14. W.H.O. Guidelines for efficacy testing of household insecticide products, 2009.
15. Venkatachalam MR, Jebanesan A. Repellent activity of *Feronia elephantum* Corr. (Rutaceae) leaf extract against *Aedes aegypti*. Bioresource Technology 2001; 76(3):287–288.
16. Chandre F, Darriet F, Darder M, Cuany A, Doannio JMC, Pasteur N. Pyrethroid resistance in *Culex quinquefasciatus* from West Africa. Medical and Veterinary Entomology 1998; 12: 359–366.
17. Hardin JA, Jackson FLC. Applications of natural products in the control of mosquito-transmitted diseases. African Journal of Biotechnology 2009; 8(25):7373-7378.
18. Vatandoost H, Dehkordi SA, Sadeghi SMT, Davari B, Karimian F, Abai MR. Identification of chemical constituents and larvicidal activity of *Kelussia odoratissima* Mozzaffarian essential oil against two mosquito vectors *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae). Experimental Parasitology 2012; 132(4):470-474.

19. Kumar G, Karthik L, Rao KVB, Kirthi AV, Rahuman AA. Larvicidal, repellent and ovicidal activity of *Calotropis gigantean* against *Culex gelidus* and *Culex tritaeniorhynchus* (Diptera: Culicidae). Journal of Agricultural Technology 2012; 8(3):869-880.
20. Samuel T, Ravindran KJ, Arivoli S. Screening of plant extracts for ovicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Applied Botany 2011; 40:5456-5460.
21. Madhiyazhagan P, Murugan K, Kumar AN, Nataraj T. Larvicidal, pupicidal and ovicidal activity of *Coccinia grandis*, against malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti*, filarial vector, *Culex quinquefasciatus*. Asian Pacific Journal of Tropical Biomedicine 2012; 2:1-7.
22. Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine 2011; 1:43-48.
23. Thavara U, Tawatsin A, Chompoosri J. Phytochemicals as repellents against mosquitoes in Thailand. Proceedings of the International Conference Biopesticide, 2002, 244-250.
24. Bream AS, El-Sheikh TMY, Fouda MA, Hassan MI. Larvicidal and repellent activity of extracts derived from aquatic plant *Echinochloa stagninum* against *Culex pipiens*. Tunisian Journal of Plant Protection 2010; 5:107-124.
25. Yang YC, Lee EH, Lee HS, Lee DK, Ahn YG. Repellency of aromatic medicinal plant extracts and a steam distillate to *Aedes aegypti*. Journal of the American Mosquito Control Association 2004; 20(2):146-149.
26. Pushpanathan T, Jebanesan A, Govindarajan M. Larvicidal, ovicidal and repellent activities of *Cymbopogon citratus* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Tropical Biomedicine 2006; 23:208-212.
27. Mullai K, Jebanesan A, Pushpanathan T. Mosquitocidal and repellent activity of the leaf extract of *Citrullus vulgaris* (cucurbitaceae) against the malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). European Review for Medical and Pharmacological Sciences 2008; 12(1):1–7.
28. Govindarajan M. Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes. Asian Pacific Journal of Tropical Medicine 2010; 1: 691-695.
29. Govnidarajan M, Sivakumar R. Adulticidal and repellent properties of indigenous plant extracts against *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). Parasitology Research 2011; 109:353–367.
30. El-Sheikh TMY, Hanan A, Bosly AM Shalaby NM. Insecticidal and repellent activities of methanolic extract of *Tribulus terrestris* L. (Zygophyllaceae) against the malarial vector *Anopheles arabiensis* (Diptera: Culicidae). Egyptian Academic Journal of Biological Sciences 2012; 5(2):13-22.
31. Krishnappa K, Elumalai K, Dhanasekaran S, Gokulakrishnan J. Larvicidal and repellent properties of *Adansonia digitata* against medically important human malarial vector mosquito *Anopheles stephensi* (Diptera: Culicidae). Journal of Vector Borne Diseases 2012; 49:86–90.
32. Foster S, Duke JA. “Naptalactose, a mild sedative compound in Catnip, also possesses herbicidal and insect-repellent properties”. In: Peterson Field Guides, Eastern/Central Medicinal plants. Houghton Mifflin Co. New York 1990.
33. Jacobson M. Glossary of plant derived insect deterrents. CRC Press, Inc., Boca Raton, Florida 1990.