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Evaluation of *Solanum trilobatum* L. (Solanaceae) aerial extracts for mosquito larvicidal activity against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract
Crude petroleum ether, chloroform, and acetone aerial extracts of *Solanum trilobatum* were tested for larvicidal activity against the filarial vector, *Culex quinquefasciatus*. Standard W.H.O. protocols with minor modifications were adopted for the larvicidal bioassay. Larvicidal activity was evaluated at concentrations of 62.5, 125, 250, 500 and 1000 mg/L. Larval mortality was observed for 24 and 48 h. Amongst the solvent extracts tested, petroleum ether exhibited highest larvicidal activity and LC_{50} values was 203.87 and 165.04 followed by acetone and chloroform extract which were 295.87 and 186.38; 535.74 and 346.08 mg/L after 24 and 48 h respectively. Further investigations are needed to explore the larvicidal activity of the petroleum ether aerial extract of this plant against a wide range of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

Keywords: *Solanum trilobatum, crude aerial extracts, Culex quinquefasciatus, larvicidal activity*

1. Introduction
Mosquitoes are a serious threat to public health transmitting several dangerous diseases in the tropics [1]. *Culex quinquefasciatus*, the principle vector for filariasis is one of the cosmopolitan mosquitoes which occur in human habitations in the tropics and subtropics of the world. There has been a large increase in the insecticide resistance of this vector and it has become a global problem. Insecticides residues in the environment, as a result of chemical insecticide usage, have turned the researcher's attention towards natural products [2]. In the past years, the plant kingdom has been of great interest as a potential source of insecticidal products. Many species in the plant kingdom synthesize a variety of secondary metabolites which play a vital role in defense of plants against insects/mosquitoes. Phytochemicals obtained from plants are usually less environmentally harmful than synthetic chemicals and it has renewed the interest in the research on these compounds, considering them as an ecologically safe alternative for synthetic insecticides [3].
Crude plant extracts and inorganic larvicides were largely used as natural insecticides before the organic laboratory-synthesized insecticides became available in the 1940s [4-6]. Applications of phytochemicals in mosquito control were in use since the 1920s [7], but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities [8].
Unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioural and physiological processes.

Thus, there is a very little chance of pests developing resistance to such substances. Identifying bioinsecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. The botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative products to fight against mosquito-borne diseases [9].

Crude plant materials and botanical derivatives had primary role for pest control in agriculture, veterinary and public health in ancient era. Chemically synthesized insecticides, due to their magical success replaced the popularity at intermediate time. Over and injudicious application of synthetic insecticides for long term causing various impairment of eco-system has renewed the importance of insecticides of green origin recently. Different plants contain different complex chemicals with unique biological activities. Literature provides a good volume of documents on efficacy of plant derived materials against different mosquitoes [8-19].

Solanum trilobatum grows as a climbing undershrub widely distributed in India and Sri Lanka [20, 21]. The plant is known as alarka in Sanskrit, alarkapatramu in Telugu, tuduvalai in Tamil and tutuvalam in Malayalam [21]. *Solanum trilobatum* is used in the field of folk medicine for treating all kinds of cough [21] diabetes, vomiting with blood and leprosy [22]. Besides these, it is also used to increase male fertility and also counter acts snake poison [23]. *Solanum trilobatum* possess pharmacological properties *viz*., antiasthmatic [24], anticancer [25], antioxidant [26], antiinflamatory, antidiabetes, antimicrobial, analgesic, hepatoprotective [27-29], antiulcer [30] and antidandruff [31]. The plant is also rich in phytochemical constituent like alkaloids, phenolics, flavonoids, sterols, saponins and glycosides [30]. Further, the plant is reported for its mosquitocidal property which includes ovicidal activity against *Culex quinquefasciatus* and *Culex tritaeniorhynchus* [32], larvicidal activity [33], oviposition deterrent and skin repellent activity against *Anopheles stephensi* [32]. Recently, Premalatha *et al.* [34] reported the acetone, chloroform and methanol leaf extracts of *Solanum trilobatum* for its larvicidal, pupicidal and adult emergence inhibition properties against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi*. Therefore, in the present investigation, the crude aerial extracts of *Solanum trilobatum* has been evaluated for its larvicidal activity against *Culex quinquefasciatus.*

2. Materials and Methods

2.1. Plant collection and extraction

Mature aerial parts of *Solanum trilobatum* collected from places in and around Chennai, Tamil Nadu, India were brought to the laboratory, shade dried at room temperature and powdered. Dried and powdered aerial parts (1 Kg) was macerated sequentially with 3 L of petroleum ether, chloroform and acetone for a period of 96 h and filtered. The filtrate was then concentrated at reduced temperature on a rotary vacuum evaporator. The crude petroleum ether, chloroform and acetone aerial extracts thus obtained were lyophilized and a stock solution of 1,00,000 mg/L prepared by adding adequate volume of Tween 80 was refrigerated at 4 ^{0}C until testing for bioassay.

2.2. Larvicidal bioassay

Bioassay was carried out against laboratory reared *Culex quinquefasciatus* mosquitoes free of exposure to insecticides. Standard W.H.O. [35] protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. Mosquito immature particularly third instar larvae were obtained from laboratory colonized mosquitoes of F_1 generation. From the stock solutions, concentrations of 62.5, 125, 250, 500 and 1000 mg/L was prepared. Ten healthy larvae were released into each 250 mL glass beaker containing 200 mL of water and test concentration. Larval mortality was observed for 24 and 48h after treatment. Larvae were considered dead when they showed no signs of movement when provoked on their respiratory siphon by a needle. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Treated control was prepared by the addition of Tween 80 to distilled water. Distilled water served as control. The larval per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's formula [36]. SPSS 11.5 version package was used for determination of LC_{50} and LC_{90} values [37]. One way ANOVA followed by Duncan multiple range test (DMRT) was performed to determine the difference in larval mortality between concentrations.

3. Results

The results of *Culex quinquefasciatus* larval mortality tested against *Solanum trilobatum* crude petroleum ether, chloroform and acetone aerial extracts are presented in Table 1 and 2. No larval mortality was observed in treated and untreated control after 24 and 48 h. The results of the present study revealed that among the solvent extracts tested, petroleum ether exhibited highest larvicidal activity and LC_{50} values was 203.87 and 165.04 followed by acetone and chloroform extract which were 295.87 and 186.38; 535.74 and 346.08 mg/L after 24 and 48 h respectively (Table 3). Further, one hundred per cent larval mortality was observed at 1000 mg/L in all the extracts after 24 h exposure and at 500 mg/L in petroleum ether extract only after 48 h.

Table 1: Larval mortality of *Culex quinquefasciatus* against crude aerial extracts of *Solanum trilobatum* at 24 h

Solvents	Concentration (mg/L)						
	Untreated control	Treated control	62.5	125	250	500	1000
Petroleum ether	0.00 ±0.00^a (0.0)	0.00 ±0.00^a (0.0)	1.33 ±0.57^b (13.3)	4.66 ±0.57^c (46.6)	6.33 ±0.57^d (63.3)	9.66 ±0.57^e (96.6)	10.00 ±0.00^e (100.0)
Chloroform	0.00 ±0.00^a (0.0)	0.00 ±0.00^a (0.0)	0.00 ±0.00^a (0.0)	1.33 ±0.57^b (13.3)	3.66 ±0.57^c (36.6)	4.33 ±0.57^d (43.3)	9.00 ±1.00^e (90.0)
Acetone	0.00 ±0.00^a (0.0)	0.00 ±0.00^a (0.0)	1.66 ±0.57^b (16.6)	3.66 ±0.57^c (36.6)	5.33 ±0.57^d (53.3)	7.33 ±1.52^e (73.3)	10.00 ±0.00^f (100.0)

Values are mean of the three replicates of three trials ±standard deviation. Figures in parenthesis denote per cent larval mortality. Different superscript alphabets within same row indicate statistical significant difference at P <0.05 level by one way ANOVA followed by DMRT.

Table 2: Larval mortality of *Culex quinquefasciatus* against crude aerial extracts of *Solanum trilobatum* at 48 h

Solvents	Concentration (mg/L)						
	Untreated control	Treated control	62.5	125	250	500	1000
Petroleum ether	0.00 ±0.00[a] (0.0)	0.00 ±0.00[a] (0.0)	2.00 ±0.00[b] (20.0)	5.33 ±0.57[c] (53.3)	7.00 ±0.00[d] (70.0)	10.00 ±0.00[e] (100.0)	10.00 ±0.00[e] (100.0)
Chloroform	0.00 ±0.00[a] (0.0)	0.00 ±0.00[a] (0.0)	0.66 ±0.57[a] (6.6)	1.66 ±0.57[b] (16.6)	4.66 ±0.57[c] (46.6)	7.00 ±1.00[d] (70.0)	10.00 ±0.00[e] (100.0)
Acetone	0.00 ±0.00[a] (0.0)	0.00 ±0.00[a] (0.0)	2.33 ±0.57[b] (23.3)	5.33 ±0.57[c] (53.3)	7.33 ±0.57[d] (73.3)	9.33 ±1.52[e] (93.3)	10.00 ±0.00[e] (100.0)

Values are mean of the three replicates of three trials ±standard deviation. Figures in parenthesis denote per cent larval mortality. Different superscript alphabets within same row indicate statistical significant difference at $P < 0.05$ level by one way ANOVA followed by DMRT.

Table 3: Probit analysis of larvicidal efficacy of crude aerial extracts of *Solanum trilobatum* against *Culex quinquefasciatus*

Solvents	LC_{50} (mg/L)		LC_{90} (mg/L)	
	24 h	48 h	24 h	48 h
Petroleum ether	203.87	165.04	360.96	293.48
Chloroform	535.74	346.08	931.56	595.57
Acetone	295.87	186.38	571.23	366.49

4. Discussion

Vector-borne diseases caused by mosquitoes have become a global health problem. A review of botanical phytochemicals with mosquitocidal potential has been published [8], demonstrating identification of novel effective mosquitocidal from botanicals containing active phytochemicals. Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic potential insecticides or along with other insecticides under the integrated vector control. Botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative product to fight against mosquito-borne diseases [9].

One of the earliest reports on the use of plant extracts against mosquito larvae is credited to Campbell *et al.* [38] who found the plant alkaloids like nicotine, anabasine, methyl anabasine and lupinie extract from *Anabasis aphylla* killed larvae of *Culex pipiens, Culex territans* and *Culex quinquefasciatus*. Screening of botanical material if attempted, the solvents for phytochemical extractions should be carefully selected because different solvent types can significantly affect the potency of extracted plant compounds [39]. A converse relationship is said to exist between extract effectiveness and solvent polarity where efficacy increases with decreasing polarity [40]. In the present study, the larvicidal activity of *Solanum trilobatum* crude aerial extracts was studied against *Culex quinquefasciatus*. The petroleum ether aerial extract was found to be the effective and LC_{50} value was 203.87 and 165.04 followed by acetone and chloroform which were 295.87 and 186.38; 535.74 and 346.08 mg/L after 24 and 48h respectively. The results of the present study were comparable with the reports of earlier studies.

The acetone, chloroform and methanol leaf extracts of *Solanum trilobatum* exhibited larvicidal activity against *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* with LC_{50} values of 125.67, 125.87 and 125.43; 123.50, 121.06 and 122.77; 121.99, 119.52 and 116.64 ppm respectively after 24 h exposure [34]. The petroleum ether stem extract of *Euphorbia hirta* was effective against the larvae of *Culex quinquefasciatus* and LC_{50} was 424.94 ppm [41]. Rajmohan and Ramaswamy [42] reported the acetone extract of *Ageratina adenophora* twigs to be effective against the larvae of *Culex quinquefasciatus* (LC_{50} 227.20 ppm). Arivoli and Samuel [43] reported that the ethyl acetate leaf extract of *Strychnos nux-vomica* was effective against the larvae of *Culex quinquefasciatus* and LC_{50} values were 222.28 and 146.99 ppm after 24 and 48 h. The dichloromethane extract of *Citrullus colocynthis* whole plant provided hundred per cent mortality and LC_{50} value was 240.36 ppm [44] and diethyl ether extract of *Abutilon indicum* leaf exhibited a LC_{50} value of 395.90 ppm [45] and the hexane extract of *Hyptis suaveolens* aerial parts a LC_{50} value of 203.37 ppm [46] after 24 h of exposure against the third instar larvae of *Culex quinquefasciatus*. The acetonic leaf extract of *Ocimum sanctum* exhibited LC_{50} 592.60 ppm against *Culex quinquefasciatus* [47]. The toxicity to the third instar larvae of *Culex quinquefasciatus* by methanolic leaf extract of *Momordica charantia, Trichosanthes anguina* and *Luffa acutangula* showed LC_{50} values of 465.85, 567.81 and 839.81 ppm, respectively [48].

Vector control is one of the most powerful weapons in the process of managing vector populations so as to reduce/interrupt the transmission of disease. As a result, vector control remains considered to be a cornerstone in the vector-borne disease control program due to lack of reliable vaccine, drug resistance parasites and insecticide resistance of insect vectors [49]. Plants and plant parts have provided a good source of inspiration for novel drug compounds, as plant derived drugs have made large contribution to human health. The use of plant extracts, as well as other alternative forms of medical treatment, are enjoying great popularity since the late 1990s [50]. Different parts of plants contain a complex of chemicals with unique biological activity [51]. Preliminary screening is a good approach to evaluate the potential larvicidal activity of plants [17, 52] and the activity of crude plant extracts subjected further to partial purification with respective solvent washed fraction is often distributed to the complex mixture of active compounds [53]. Natural product research using botanicals are progressively going on to identify novel bio-potency compounds to replace environmentally hazardous synthetic pesticides. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed as the present study shows that there is scope to use *Solanum trilobatum* petroleum ether aerial extracts to control the immature stages of vector mosquitoes.

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6. References

1. W.H.O. Malaria fact sheets No. 94. WHO Report, 2010.
2. Murty US, Jamil K. Effect of South Indian vetiver oil (*Vetiveria zizanioides* (L) Nash) against the immatures of *Culex quinquefasciatus* Say (Diptera: Culicidae). Journal of Entomology 1987; 2(1):8-9.
3. Isman M. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 2006; 55:45-66.
4. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. Science 1985; 228:1154-60.
5. Rawls RL. Experts probe issues: Chemistry of light-activated pesticides. Chemical and Engineering News 1986; 22:21-24.
6. Rozendaal JA. Vector control methods for use by individuals and communities. Geneva: World Health Organization 1997; 412.
7. Shahi M, Hanafi-Bojd AA, Iranshahi M, Vatandoost H, Hanafi-Bojd MY. Larvicidal efficacy of latex and extract of *Calotropis procera* (Gentianales: Asclepiadaceae) against *Culex quinquefasciatus* and *Anopheles stephensi* (Diptera: Culicidae). Journal of Vector Borne Diseases 2010; 47:185-8.
8. Shaalan EAS, Canyonb D, Younesc MWF, Abdel-Wahaba H, Mansoura AH. A review of botanical phytochemicals with mosquitocidal potential. Environment International 2005; 3:1149-66.
9. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian Journal of Medical Research 2012; 135(5):581-598.
10. Sukumar K, Perich MJ, Boober LR. Botanical derivatives in mosquito control: A review. Journal of the American Mosquito Control Association 1991; 30:122-124.
11. Ghosh A, Chowdhury N, Chandra G. Laboratory evaluation of a phytosteroid compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and nontarget organisms. Parasitology Research 2008; 103(2):271-277.
12. Singha S, Adhikari U, Chandra G. Smoke repellency and mosquito larvicidal potentiality of *Mesua ferrea* L. leaf extract against filarial vector *Culex quinquefasciatus* Say. Asian Pacific Journal of Tropical Biomedicine 2011; 1(Suppl 1):S119-S123.
13. Adhikari U, Chandra G. Laboratory evaluation of ethyl acetate and chloroform: methanol (1:1 v/v) extract of *Swietenia mahagoni* leaf against Japanese encephalitis vector *Culex vishuni* group. Asian Pacific Journal of Tropical Disease 2012; 2(6):451-455.
14. Arivoli S, Ravindran KJ, Samuel T. Larvicidal efficacy of plant extracts against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). World Journal of Medical Sciences 2012; 7(2):77-80.
15. Kovendan K, Murugan K, Vincent S, Barnard DR. Studies on larvicidal and pupicidal activity of *Leucas aspera* Willd. (Lamiaceae) and bacterial insecticide, *Bacillus sphaericus*, against malarial vector, *Anopheles stephensi* Liston. (Diptera: Culicidae). Parasitology Research 2012; 110(1):195-203.
16. Samuel T, Ravindran KJ, Arivoli S. Bioefficacy of botanical insecticides against the dengue and chikungunya vector *Aedes aegypti* (L.) (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine 2012; 2:S1842-S1844.
17. Samuel T, Ravindran KJ, Arivoli S. Screening of twenty five plant extracts for larvicidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Biomedicine 2012; 2:S1130-S1134.
18. Sakthivadivel M, Gunasekaran P, Therasa JA, Samraj DA, Arivoli S, Samuel T. Larvicidal activity of *Wrightia tinctoria* R. BR. (Apocynaceae) fruit and leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Disease 2014; 4(1):S373-S377.
19. Sakthivadivel M, Gunasekaran P, Jothis M, Samraj A, Arivoli S, Samuel T. Evaluation of larvicidal efficacy of *Cleome viscosa* L. (Capparaceae) aerial extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). Asian Pacific Journal of Tropical Disease 2014; 4(Suppl 2): S795-S798.
20. Pandurangan A, Khosa RL, Hemalatha S. Evaluation of anti-inflammatory activity of the leaf extracts of *Solanum trilobatum.* Journal of Pharmacuetical Science and Research 2009; 1(1):16-21.
21. Mihira V, Ramana KV, Ramakrishna S, Kumar P. Evaluation of antimicrobial activity of *Solanum trilobatum* Linn. roots. Research Journal of Pharmaceutical Biology and chemical sciences 2011.
22. Doss A, Palaniswamy M, Angayarkanni J, Dhanabalan R. Antidiabetic activity of water extract of *Solanum trilobatum* (Linn) in alloxan-induced diabetes in rats. African Journal of Biotechnology 2011; 8(20):5562-5564.
23. Govindhan S, Viswanathan S, Viyasekaran V, Alagappan R. Clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. International Journal of Phytotherapy Research 2004; 18(10):805-809.
24. Govindan S, Viswanathan S, Vijayasekaran V, Alagappan R. Pilot study on the clinical efficacy of *Solanum xanthocarpum* and *Solanum trilobatum* in bronchial asthma. Journal of Ethnopharmacology 1999; 66:205-210.
25. Mohanan PV, Devi KS. Toxicological evaluation of *Solanum trilobatum*. Cancer Letter 1997; 1127:135-40.
26. Shahjahan M, Vani G, Shyamaladevi CS. Effect of *Solanum trilobatum* on the antioxidant status during diethyl nitrosamine induced and phenobarbital promoted hepatocarcinogenesis in rat. Chemico-Biological Interactions 2005; 156(2-3):113-123.
27. Shahjahan M, Sabitha KE, Devi MR, Shyamala CS. Effect of medicinal plants on tumurogenesis. Indian Journal of Medical Research 2004; 123:23-27.
28. Emmanuel S, Ignacimuthu S, Perumalsamy R, Amalraj T. Anti-inflammatory activity of *Solanum trilobatum.* Fitoterapia 2006; 77:611-612.
29. Pandurangan A, Khosa RL, Hemalatha S, Iranian J. Evaluation of anti-inflammatory and analgesic activity of root extract of *Solanum trilobatum* Linn. Indian Journal of Pharmaceutical Research 2008; 7:217-221.
30. Amir M, Kumar S. Possible industrial application of genus *Solanum* in twenty first century- A review. Journal of Scientific India Research 2004; 63:116-124.
31. Pant G, Nayak NR, Prasuna G. Enhancement of antidandruff activity of shampoo by biosynthesized silver nanoparticles from *Solanum trilobatum* plant leaf. Journal of Nanoscience and Nanotechnology 2013; 3:431-439.
32. Rajkumar S, Jebanesan A. Ovicidal activity of *Solanum trilobatum* Linn (Solanaceae) leaf extract against *Culex quinquefasciatus* Say and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). International Journal of Tropical

Science 2004; 24:340-342.
33. Rajkumar S, Jebanesan A. Oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract against the malarial vector *Anopheles stephensi*. Journal of Insect Science 2005; 5:1-3.
34. Premalatha S, Elumalai K, Jeyasankar A. Mosquitocidal properties of *Solanum trilobatum* L. (Solanaceae) leaf extracts against three important human vector mosquitoes (Diptera: Culicidae). Asian Pacific Journal of Tropical Medicine 2013; 3:854-858.
35. WHO. Guidelines for laboratory and field testing of mosquito larvicides. Geneva 2005, 10-12.
36. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925; 18:265-267.
37. SPSS. SPSS for windows, version 11.5. SPPS, Chicago, IL, 2007.
38. Campbell FL, Sullivan WW, Smith LN. The relative toxicity of nicotinic, anabasine, methyl anabasin and lupine for Culicine mosquito larvae. Journal of Economic Entomology 1933; 26:500-509.
39. Karmegam N, Sakthivadivel M, Anuradha V, Daniel T. Indigenous plant extracts as larvicidal agents against *Culex quinquefasciatus* Say. Bioresource Technology 1997; 59:137-140.
40. Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual Review of Entomology 1990; 35:271-97.
41. Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K. Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitology Research 2007; 102: 867-873.
42. Rajmohan D, Ramaswamy M. Evaluation of larvicidal activity of the leaf extract of a weed plant, *Ageratina adenophora* against two important species of mosquitoes, *Aedes aegypti* and *Culex quinquefasciatus*. African Journal of Biotechnology 2007; 6:631-638.
43. Arivoli S, Samuel T. Larvicidal efficacy of *Strychnos nuxvomica* Linn. (Loganiaceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). World Journal of Zoology 2012; 7(1):6-11.
44. Arivoli S, Samuel T. Bioefficacy of *Citrullus colocynthis* (L.) Schrad (Cucurbitaceae) whole plant extracts against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). International Journal of Current Research 2011; 3(4):296-304.
45. Arivoli S, Samuel T. Larvicidal and adult emergence inhibition activity of *Abutilon indicum* (Linn.) (Malvaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae). Journal of Biopesticides 2011; 4(1):27-35.
46. Arivoli S, Samuel T. Mosquitocidal activity of *Hyptis suaveolens* (L.) Poit (Lamiaceae) extracts against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). International Journal of Recent Scientific Research 2011; 2(5):143-149.
47. Anees AM. Larvicidal activity of *Ocimum sanctum* Linn (Labiatae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say. Parasitology Research 2008; 101:1451-1453.
48. Prabakar K, Jebanesan A. Larvicidal efficacy of some cucurbitaceous plant leaf extracts against *Culex quinquefasciatus*. Bioresource Technology 2004; 95:113-114.
49. Karunamoorthi K. Vector control: a cornerstone in the malaria elimination campaign. Clinical Microbiology and Infection 2011; 17:1608-1616.
50. Ali MS, Ravikumar S, Beula JM. Spatial and temporal distribution of mosquito larvicidal compounds in mangroves. Asian Pacific Journal of Tropical Disease 2012; 2:1-4.
51. Fransworth NR, Bingel AS. Natural products and plant drugs with pharmacological, biological or therapeutic activity. Springer, Berlin 1977.
52. Sakthivadivel M, Daniel T. Evaluation of certain insecticidal plants for the control of vector mosquitoes *viz.*, *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. Applied Entomology and Zoology 2008; 43(1):57-63.
53. Sakthivadivel M, Daniel T. Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana* L. seed. Bioresource Technology 2003; 89:213-216.