Available online on www.ijcpr.com
International Journal of Current Pharmaceutical Review and Research; 9(5); 67-70

ISSN: 0976 822X

Research Article

Larvicidal Activity of Various Extracts of Selected Plants Against the Dengue Vector Larvae

Shibu George

Department of Zoology, St. Aloysius College, Edathua- Alappuzha District, Kerala-India

Available Online: 25th September, 2018

ABSTRACT

Vector born diseases, especially mosquito born diseases are increasing day by day due to environmental changes. This may adversely affect the socio-economic realms of the developing countries. One of the best remedies to overcome mosquito born diseases is to reduce the mosquito populations by using insecticides and larvicides. However, the use of chemical insecticides has some disadvantages. They may increase the chance of insecticide resistance and environmental pollutions. As an alternative, plant based drugs are preferred because they are safer than synthetic insecticides and larvicides. In this study, twelve different plants collected from the Kuttand areas of Alappuzha District of Kerala State were used for studying their larvicidal activity against 3rd and 4th instar larvae of dengue vector, the *Aedes* sp. The larvicidal activity was assessed by standard procedure, as described by the WHO, but with a slight modification. The results of the study showed that among the various extracts tested, only the methanolic extract of flowers of *Ipomoea cairica* was powerful to inhibit the growth of all the tested larvae (100%) at a concentration of 0.4 mg/ml. Its methanolic extracts were also showed 95% and 83% of larvicidal activities at a concentration of 0.2 mg/ml and 0.1 mg/ml respectively. Previous studies showed that the leaves of *Ipomoea cairica* possess larvicidal activity. But, this study suggests that the methanol extracts of flowers of *Ipomoea cairica* possess promising larvicidal activity against the dengue vector larvae of *Aedes* species.

Keywords: Dengue vector, *Ipomoea cairica*, larvicidal activity, *Aedes* larva, mosquito, methanolic extract, flower extract.

INTRODUCTION

In vector control research, *Aedes* mosquitoes are given prime importance as they are the main agent for spreading dengue fever; one of the life challenging vector born diseases of tropical countries, especially in India[1,2]. To control the dengue and other mosquito born diseases, the most suitable strategy is the controlling of mosquitoes at their breeding sites. This has been achieved by using synthetic chemicals; especially the organophosphates. But, such chemicals are not safe as they may increase the chance of insecticide resistance, environmental issues like water pollution, death of aquatic organisms like fishes and crustaceans etc. Moreover, they have the ability to stay on for a very long time in the nature without losing their biological activity[3]. As an alternative to synthetic chemicals, plant based drugs are in focus for developing as larvicides[4]. This is because of the fact that plants contain a variety of chemicals called as secondary metabolites that are useful as bio-control agents against bacteria, mosquito larvae, nematodes etc.[5] Many studies are going on in India in search of good, effective larvicidal drugs against mosquito larvae. More than two hundred plants are identified as effective sources for killing the larvae of many mosquitoes species[6]. Mosquito control programmes are primarily focused on their larvae rather than adults as the target organisms due to their low mobility in their habitats[7].

This study was primarily focused to evaluate the larvicidal activity of various extracts of selected plants from Kuttanad of Alappuzha District of Kerala State against the dengue vector larvae of *Aedes* sp. Kuttanad is an ecologically fragile land, spreads in three districts of Kerala; Alappuzha, Pathanamthitta and Kottayam. It is one of the World wonders as the land is lying below the sea level. In India, this is the one and only place with lowest altitude. This peculiar geography of the land mass of Kuttanad allows the stagnation of water and is favorable for the proliferation of vector species. Hence, vector borne diseases especially mosquito borne diseases like dengue are prevalent in Kuttanad. In order to overcome the problem of vector proliferation especially mosquito proliferation, new and effective natural sources are to be identified.

MATERIALS AND METHODS

Preparation of the plant extracts

The following Table No.1 shows the list of plants selected, their family and parts used for the larvicidal studies against *Aedes* mosquito larvae.

The selected plants were collected from different geographical areas of Kuttanad, Alappuzha district of

**Author for Correspondence: shibugeorgepala@gmail.com*

Table 1: Plants selected for the larvicidal studies against the dengue vector; *Aedes* mosquito larvae.

S. No.	Selected Plant	Family	Part Used
1	*Vernonia cinerea* L.	Compositae	Whole plant
2	*Ixora finlaysoniana* Wall.ex G.Don.	Rubiaceae	Flower
3	*Tiliacora acuminate* (Lam.)	Menispermaceae	Leaf & Stem
4	*Gliricidia sepium* Jacq.	Fabaceae	Leaf
5	*Ludwigia parviflora* L.	Onagraceae	Whole plant
6	*Triumfetta rhomboidea* Jacq.	Malvaceae	Leaf & Stem
7	*Sphagneticola trilobata*	Asteraceae	Leaf & Stem
8	*Waltheria indica* L.	Malvaceae	Whole plant
9	*Eryngium foetidum* L.	Apiaceae	Whole plant
10	*Senna alata*	Fabaceae	Flower and leaf
11	*Cerbera odollam* Gaertn.	Apocynaceae	Leaf
12	*Ipomoea cairica* (Linn.) Sweet.	Convolvulaceae	Flower

Table 2: Effect of acetone & methanol extracts of selected plants against the 3rd and 4th instar larvae of *Aedes* sp.

Sl. No.	Plants Selected	No. of larvae died* in Acetone Extract			No. of larvae died* in Methanol Extract		
		0.4 mg/ml	0.2 mg/ml	0.1 mg/ml	0.4 mg/ml	0.2 mg/ml	0.1 mg/ml
1	*Vernonia cinerea*	1.3	-	-	5.3	1	-
2	*Ixora finlaysoniana*	2	-	-	9.6	2	-
3	*Tiliacora acuminate*	-	-	-	-	-	-
4	*Gliricidia sepium*	-	-	-	-	-	-
5	*Ludwigia parviflora*	2.6	-	-	8	2	-
6	*Triumfetta rhomboidea*	-	-	-	-	-	-
7	*Sphagneticola trilobata*	8.6	5	3	18.6	15.6	9
8	*Waltheria indica*	-	-	-	9	5	1
9	*Eryngium foetidum*	-	-	-	4.3	1.3	-
10	*Senna alata*	-	-	-	-	-	-
11	*Cerbera odollam*	-	-	-	-	-	-
12	*Ipomoea cairica*	16	14.3	11.6	20	19	16.6

*Mean value of the triplicate experiments.

Kerala State. The taxonomic positions of the selected plants were identified by Sri. Bijesh P.P., Botanist, Sreedhareeyam Ayurvedic Research Centre, Koothattukulam, Ernakulam-Kerala. The collected plants were washed thoroughly under tap water, dried under sunlight and powdered using a mixer grinder. Serial extraction of the powder was made by a Soxhlet extractor using solvents such as petroleum ether, acetone, methanol and distilled water. The soxhlet extracts were filtered using Whatman No.1 filter paper and then concentrated. The concentrate was considered as stock and kept in refrigerator. From the stock, a concentration of 100mg/ml, 50 mg/ml and 25 mg/ml was prepared in de-chlorinated tap water and was used for the preliminary larvicidal screening.

Mosquito larvae culture

3rd and 4th instar larvae of *Aedes* sp. were collected from the ditch water/ stagnant water of Kuttanad and they were identified in the Department of Zoology, St. Aloysius College, Edathua, Alappuzha District, Kerala State. Larvae were kept in plastic trays containing tap water at 27 ± 2°C and with a humidity range of 75–85%. A mixture of Brewer's yeast, dog biscuits and algae (collected from ditch water/pond water) in a ratio of 3:1:1 was given to the larvae as food.

Larvicidal Bioassay

The 3rd and 4th instar larvae of *Aedes* sp. were used for the preliminary screening. Sample concentrations of 100mg/ml, 50 mg/ml and 25 mg/ml were used for preliminary larvicidal screening. The larvicidal activity was assessed according to the procedure of WHO, but with a slight modification as described by Kamaraj *et al*. [8-10] For the screening test, 20 larvae were taken in 249 ml of water and 1.0 ml of the plant extract with desired concentration (100mg/ml, 50 mg/ml and 25 mg/ml respectively) was added. Therefore, the final concentration of plant extract in the testing sample water was 0.4mg/ml, 0.2mg/ml and 0.1mg/ml respectively. In control experiment, 1 ml of distilled water was added without the plant extract. The numbers of dead larvae were counted after 24 h of exposure to the plant extract; the larvae were considered as dead, if they showed no sign of motility when touched with a glass rod. The percentage of mortality was recorded from the average of three replicates using the following formula,

$$\frac{n}{N} \times 100$$

where 'n' is the number of dead larvae and 'N' is the total number of larvae taken for the study.

RESULT AND DISCUSSION

Table 3: Percentage of *Aedes* larvae died in acetone & methanol extracts of selected plants.

Sl.No.	Plants Selected	Percentage of larvae died in Acetone Extract			Percentage of larvae died in Methanol Extract		
		0.4 mg/ml	0.2 mg/ml	0.1 mg/ml	0.4 mg/ml	0.2 mg/ml	0.1 mg/ml
1	*Vernonia cinerea*	6.5	0	0	26.5	5	0
2	*Ixora finlaysoniana*	10	0	0	48	10	0
3	*Tiliacora acuminate*	-	-	-	-	-	-
4	*Gliricidia sepium*	-	-	-	-	-	-
5	*Ludwigia parviflora*	13	0	0	40	10	0
6	*Triumfetta rhomboidea*	-	-	-	-	-	-
7	*Sphagneticola trilobata*	43	25	15	93	78	45
8	*Waltheria indica*	-	-	-	45	25	5
9	*Eryngium foetidum*	-	-	-	21.5	6.5	0
10	*Senna alata*	-	-	-	-	-	-
11	*Cerbera odollam*	-	-	-	-	-	-
12	*Ipomoea cairica*	80	71.5	58	100	95	83

The result of the preliminary screening of selected plants for determining their larvicidal activity was given in the Table No.2 and the percentage of mortality of larvae against respective plant extract was given in Table No.3. Triplicate screening studies showed that the petroleum ether extracts and aqueous extracts of the selected plants did not show any activity against the tested larvae. In control experiments, more than 90% of the tested larvae were alive. However, the acetone and methanol extracts of plants showed larvicidal activity. Hence, the effects of acetone and methanol extracts of selected plants were only noted in the Table No.2.

Among the active extracts of acetone and methanol, only the methanol extracts showed significant larvicidal activities against the tested larvae. This may be due to the fact that the methanol is an effective polar solvent than acetone, so that more active phytochemicals may dissolve in the methanol than acetone. Analysis of the results on the effect of methanol extracts against 3rd and 4th instar larvae of *Aedes* sp. showed that only the methanolic extract of *Ipomoea cairica* was powerful to inhibit the growth of all the tested larvae (100%) at a concentration of 0.4 mg/ml. However, the methanolic extract of *Sphagneticola trilobata* at a concentration of 0.4 mg/ml was also active against the tested larvae but, the effect cannot be considered as a significant one in the larvicidal studies. About 95% and 83% of larvae died in 0.2 and 0.1 mg/ml methanolic extracts of *Ipomoea cairica* respectively. This shows that the methanolic extract of *Ipomoea cairica* retains its activity even at lower concentrations. Therefore, it can be concluded that, among the tested plant extracts, the methanol extracts of flower of *Ipomoea cairica* with a concentration of 0.2 mg/ml has 95% larvicidal activity and 0.1 mg/ml has83% larvicidal activity. These activities are promising in larvicidal studies.

Vector borne diseases especially mosquito born diseases are prevalent in low land areas like Kuttanad because this region acts as a home ground for the proliferation of vectors like mosquitoes. Kerala, especially Kuttanad has increased incidence of Dengue fever since the last few years. *Aedes* is the vector for dengue fever in Kerala. Even though, massive mosquito eradication programs have been organized by both Government and Non Governmental institutions, 100% success is not yet achieved. One of the hindrances to this aim is the lack of eco-friendly larvicides to kill the mosquito larvae at their natural habitat. If toxic chemicals are utilized for the eradications of larvae, the chances for biological magnification of such chemicals are also high. This problem can be overcome only through the discovery of plant based natural products.

Ipomoea cairica (Linn.) Sweet. is commonly known as railroad creeper. It is an evergreen, herbaceous, perennial climbing plant, belonging to the family Convolvulaceae. It is considered as a weed of waste area. Tropical Africa or Asia may be the place of its origin[11]. Even though it is a weed, evaluations of its medicinal properties revealed that it is an excellent herb in medicine. Its leaves possess antioxidant activity, antibacterial activity against *E.coli*, *Klebsiella pneumoniae*, *Bacillus subtilus*, *Salmonella typhi* etc., antifungal activity against *Aspergillus nigar*, *Candida albicans*, *Penicillium* sp. etc.[12] Its leaves have effective anti-inflammatory activity as they contain tremendous anti-inflammatory chemicals like Saponins[13]. Its main bioactive compound is tannin; a powerful anticancer agent. This indicates that this plant has a good future in anticancer therapy[14]. Biochemical analysis showed that the flavonoids present in the leaves of *Ipomoea cairica* are responsible for its medicinal properties like antimicrobial, antioxidant and anti-allergic etc.[15] Previous studies also showed that the various extracts, especially leaves of this plant possess effective larvicidal activity[16]. In the present investigation, its flowers were tested for detecting the larvicidal activities and the study showed that its flower methanol extract was very effective against the 3rd and 4th instar larvae of *Aedes* mosquito. The extract was active at smaller concentrations; 0.2 mg/ml (95% larvicidal activity) and 0.1 mg/ml (83% larvicidal activity) against the larvae. Hence, this study suggests that the flower of this plant can be considered for studying its active larvicidal principles for selecting this plant in larvicidal treatment.

CONCLUSION

Twelve plants selected from Kuttanad region of Alappuzha District of Kerala State were used for studying their larvicidal effect against the 3rd and 4th instar larvae of *Aedes* mosquito. Petroleum ether, acetone, methanol and aqueous extracts were prepared and tested against the larvae by following the standard procedure of WHO, but with a slight modification. The results of the study indicated that only the methanolic extract of flowers of *Ipomoea cairica* was powerful to inhibit the growth of tested larvae. The extract was active to inhibit the growth of 100% larvae at a concentration of 0.4 mg/ml, 95% of larvae at a concentration of 0.2 mg/ml and 83% of larvae at a concentration of 0.1 mg/ml. All other plant extracts showed insignificant larvicidal activity. Therefore, this study can be concluded that the flower extracts of *Ipomoea cairica* possess effective larvicidal activity against the *Aedes* larvae, an important vector of dengue fever.

ACKNOWLEDGEMENT

The author is thankful to the University Grants Commission (SWRO) for the financial assistance for carrying out this project under the scheme Minor Research Project (12th Plan).

REFERENCES

1. Kandasamy K, Kadarkarai M, Chellasamy P, Jiang SH. Mosquito larvicidal activity of *Cadaba indica lam* leaf extracts against the dengue vector, *Aedes aegypti*. Asian Journal of Plant Science and Research 2012; 2 (5):633-637.
2. Dhanya P, Benny P.J. Larvicidal action of *Garcinia Gummi-Gutta*.Robs. Var. *Gummi-Gutta* on dengue vector *Aedes aegypti*. Indian Journal of Applied Research 2014; 4(1):21-22.
3. Xin CL, Qi YL, Ligang Z, Zhi L L. Larvicidal activity of essential oil derived from *Illicium henryi* Diels (Illiciaceae) leaf. Tropical Journal of Pharmaceutical Research January 2015; 14 (1): 111-116.
4. Pavela R. Larvicidal activities of some Euro-Asiatic plants against *Culex quinquefasciatus* Say (Diptera: Culicidae). Journal of Biopesticides 2008; 1: 81–85.
5. Sun R, Sacalis JN, Chin CK, Still CC. Bioactive aromatic compounds from leaves and stems of vanilla fragrans. Journal of Agricultural and Food Chemistry 2006; 49: 51-61.
6. Govindarajan M, Jebanesan A, Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. Parasitology Research 2008; 102(2): 289-292.
7. Howard AFB, Zhou G, Omlin FX. Malaria mosquito control using edible fish in Western Kenya: preliminary findings of a controlled study. BMC Public Health 2007; 7:199-204.
8. Kamaraj C, Bagavan A, Elango G, Abduz Zahir A, Rajakumar G, Marimuthu S, Santhoshkumar T, Abdul Rahuman A. Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus.* Indian J Med Res 2011; 134 (1): 101–106.
9. World Health Organization. Guideliness for laboratory and field testing of mosquito larvicdes. WHO, Geneva 2005.
10. WHO. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC/81.807 Geneva: World Health Organization. 1981: 7.
11. Austin DF, Huaman Z. A synopsis of *Ipomoea* (Convolvulaceae) in the Americas. Taxon 1996; 45:5-6.
12. Arora S, Kumar D, Shiba. Phytochemical, antimicrobial and antioxidant activities of methanol extracts of leaves and flowers of *Ipomoea cairica*. Int. J. Pharm Pharm Sci 2013; 5(1):198-202.
13. Deepa S, Shukla K. *Ipomoea cairica*: a medicinal weed with promising health benefits. International Journal of Information Research and Review 2015; 2 (5): 687-694.
14. Li D, Wang P. Antifungal activity of Paraguayan plant used in traditional medicine. J. Ethanopharmacol 2003; 76: 93-98.
15. Vanlalhruaii R. Evaluation of phytochemical contents of *Ipomoea cairica* (L.) Sweet – a qualitative approach. Science Vision 2014; 14 (3): 145-151.
16. AhbiRami R, Zuharah WF, Thiagaletchumi M, Subramaniam S, Sundarasekar J. Larvicidal efficacy of different plant parts of railway creeper, *Ipomoea cairica* extract against dengue vector mosquitoes.
17. *Aedes albopictus* (Diptera:Culcidae) and *Aedes aegypti* (Diptera:Culcidae). J Insect Sci 2014; 14: 180.