Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com

Research Article | SOJ Microbiology and Infectious Diseases | Open Access

Screening of a Few traditionally used Medicinal Plants for their Larvicidal Efficacy against *Aedes aegypti* Linn (Diptera: Culicidae), a Dengue Fever Vector

Anoopkumar AN[1], Sreedev Puthur[2], Sharrel Rebello[2] and Embalil Mathachan Aneesh[2*]

[1]*Department of Zoology, Christ College, Irinjalakuda, University of Calicut*

[2]*Communicable Disease Research Laboratory, Department of Zoology, St Joseph's college, Irinjalakuda*

Received: 21 July, 2017; Accepted: 30 August, 2017; Published: 06 September, 2017

***Corresponding author:** Embalil Mathachan Aneesh, Communicable Disease Research Laboratory, Department of Zoology, St Joseph's college, Irinjalakuda. Tel: +91 9400741861; Email: aneeshembalil@gmail.com*

Abstract

Mosquitoes are the significant group of insects as they cause morbidity and mortality to human beings by severe diseases, such as zika, malaria, filariasis, dengue fever, Japanese encephalitis, West Nile virus and chikungunya. According to World Health Organization draft on global vector control response, 2017 more than 80 percentage of global human populations were significantly infected by at least one vector-borne disease. This vector - borne diseases may induce social and economic burden over the globe. Herbal products with larvicidal properties have been used as natural insecticides for eco-friendly vector control programme. The larvicidal efficacy of nine medicinal plants collected from Wayanad district of Kerala, a part of Western Ghats, India was assessed against fourth instar larvae of *Aedes aegypti*. The larvicidal bio assay had been carried out according to WHO standard protocol with acetone, methanol, petroleum ether and water as extracts. Out of nine traditionally used medicinal plants screened, *Persicaria hydropiper* and *Plectranthus hadiensis* have significant larvicidal efficacy with LC_{50} 489.278 Mg/ L and LC_{50} 411.746 Mg/ L respectively against the fourth instar larvae of this dengue fever vector. Hence the aforesaid plants can be used as a potential natural insecticide against mosquitoes thus, to control mosquito borne diseases.

Keywords: *Aedes aegypti*; Vector mosquitoes; medicinal plant; larvicidal efficacy; plant extracts

Introduction

Mosquitoes (Diptera: Culicidae) have significant impact on human health, principally because they act as vectors for ruinous pathogens and parasites causing many severe infectious diseases such as dengue, chikungunya, malaria, West Nile virus and filariasis [1- 3]. Dengue fever recognized as the most severe mosquito-borne disease, responsible for medical and economic burden together with defeat in commercial [4-11]. The World Health Organization in 2017 reported that roughly 65 to 136 million people were infected by dengue fever in every year [12]. The genus *Aedes* are liable to transmit various arboviral diseases including dengue fever all over the world [13]. *Aedes aegypti* has been reported as the principal vector of chikungunya and dengue in the United States and other regions of tropical and sub tropical countries [14]. There are only a small number of vaccines are available to treat the pathogens transmitted by mosquitoes and the scientific community is yet to discover the vaccines for severe mosquito borne diseases including dengue [15]. However, fight against mosquito transmitting diseases is a challenging problem to public health [3,16].

Mosquito control – targeting its larvae remains the most effective approach to prevent various mosquito borne diseases [17]. Control of such ailments is ending up progressively troublesome be-reason for expanding resistance in mosquitoes to synthetic insecticides [18]. Plant derived products are safer than synthetic insecticides [19].

Several people use either synthetic or plant based repellents to protect them from mosquito transmitting diseases. Bed nets treated with insecticide and indoor residual spraying may also be used for preventing mosquito-borne diseases [3,17,20-23]. Active, frequent use of synthetic insecticides in farming and health programs may leads to various harmful results such as ecosystem destabilization, environmental pollution, hazardous effects to human beings and non target organisms [24,25].

Use of plant based insecticides against mosquitoes becoming a significant approach for the prevention of various mosquito transmitted diseases because of a number of advantages rather than artificial repellents [26,27].

Plant derived products with insecticidal activity have been used in the recent years to control different types of vectors [28].Various methods have been implicated to control mosquito population. One of the methods to prevent mosquito transmitting disease is by killing its larvae at larval stage. The modern mosquito control method is based on artificial insecticides [29].

World Health Organization in 2008 reported that 80 percentage of population of some countries in Asia and Africa may use traditional medicines to cure various diseases due to monetary and environmental constrain. Traditional medicines

 * **Corresponding author email:** *aneeshembalil@gmail.com*

are used to maintain health by preventing various severe diseases based on knowledge, experience and practice [30].

Extracts of plants constitute various bio active phyto compounds; hence they can be used as alternative approach to mosquito larval control. Many scientific studies have proven that the plant extracts or plant derived products can be used as an alternate approach to control mosquito population [31-33].

Various vector borne diseases can be prevented by means of traditionally used medicinal plants. Hence, the demand for traditional medicines is enhancing as they are usually recognized to be bio degradable, natural, safer than synthetic drugs [34]. Thus, searching for natural insecticides is of greatest significance in vector control. This study focused on screening of few traditionally used medicinal plants for their larvicidal efficacy against dengue fever vector Aedes aegypti to develop an efficient, natural, biodegradable insecticide of plant origin.

Methods

Plant Material

Fresh leaves of nine traditionally used medicinal plants were collected from different regions of Thirunelly (11°53'N, 76°0'E), Wayanad a part of Western Ghats, Kerala, India. The collection was performed during their dynamic growing season, June to September (monsoon season).

Preparation of Extracts

Fresh leaves of nine medicinal plants were collected and shade dried (Table 1). The dried plant materials were ground to fine powder using a mechanical grinder and proceeded for soxhlet extraction using different solvents such as petroleum ether, methanol acetone and water. The extracts obtained were evaporated and used for further study.

Table 1: List of plants collected for bioassay from Thirunelly,Western Ghats, Kerala, India

S No.	Plant Name	Family	Local Name	Parts used	GPS	
					Latitude	Longitude
1	***Sphaernthus indicus***	Asteraceae	Adakkamaniyan	Seed	11°53’29.20”N	76° 0’59.00”E
2	***Hydrocotyle javanica***	Apiaceae	Eranga	Leaf	11°53’29.50”N	76° 0’34.20”E
3	***Deris trifoliate Lour***	Fabaceae	Thuduthuduppankayi	Leaf	11°53’29.40”N	76° 0’34.20”E
4	***Persicaria hydropiper***	Polygonaceae	Kovvanenji	Leaf	1°53’25.00”N	76° 0’50.40”E
5	***Acanthospermum hispidum***	Asteraceae	Nherinjil	Leaf	11°53’27.80”N	76° 0’47.80”E
6	***Drymaria cordata***	Caryophyllaceae	Odivally	Leaf	11°53’21.14”N	76° 0’59.64”E
7	***Toddelia asiatica***	Rutaceae	Narinarakam	Leaf	11°53’7.30”N	76° 0’38.90”E
8	***Plectranthus hadiensis***	Lamiacea	Bhaya	Leaf	11°53’23.00”N	76° 1’1.90”E
9	***Triumfetta rhomboidea***	Malvaceae	Kodithoova	Leaf	11°53’27.64”N	76° 0’47.59”E

Mosquito’s Culture

Aedes aegypti were reared in the Communicable Disease Research Laboratory, Department of Zoology, St Josephs College, Irinjalakuda. The larvae were maintained and fed with dog biscuit and yeast in the ratio 3:1. Adult mosquitoes were provided with 10% sucrose solution and young chick was kept within the cage to offer blood meal. Mosquitoes were held at 27 ± 2°C and 75–85% relative humidity, with 12:12 Light and dark photoperiod cycle.

Larvicidal Bioassay

Larvicidal bioassay of plant extracts were tested against fourth instar larvae of *Aedes aegypti*. The tests were conducted in glass beakers with WHO standard protocol. Larvicidal efficacy was tested against fourth instar larvae of *Aedes aegypti* using petroleum ether, methanol, acetone and water extracts of the plant material (1000 mg/L). A set of control groups were included for each test. 1 ml of petroleum ether, acetone, methanol and water was mixed separately with 249ml of distilled water for control groups. Twenty five healthy larvae were released in each glass beaker and mortality was observed after 24 hours of exposure. The dead larvae in replicates were pooled and percentage of larval moratlity was calculated. The larvicidal bioassay was performed at 27 ± 2°C and 75–85% relative humidity, with 12:12 Light and dark photoperiod cycle.

Statistical Analysis

Larval mortality was calculated in percentage and if the control mortality was ranged from 5-20%, it was corrected using Abbott’s formula [35].

$$corrected\ mortality = \frac{\%\ test\ \ mortality - \%\ control\ mortality}{100 - \%\ control\ mortality} \times 100$$

LC_{50} and LC_{90} values for different solvent extract such as petroleum ether, methanol, acetone and water was calculated by using IBM SPPSS Version.24.

Results

Results for screening of the nine traditionally used medicinal plants against *Aedes aegypti* fourth instar larvae were shown in Table 2. The highest mortality was observed in leaf extract of two plant species such as *Persicaria hydropiper* and *Plectranthus hadiensis*. The petroleum ether extract of *Plectranthus hadiensis* showed promising larvicidal efficacy with LC_{50} - 411.746 mg/L against fourth instar larvae of *Aedes aegypti* (Table 3). However, methanol, acetone and water extracts of the same may also showed considerable larval mortality. Methanol extract of *Persicaria hydropiper* also showed significant larvicidal property with LC_{50}-489.278 mg/L against *Aedes aegypti* (Table 3). Besides this, petroleum ether, acetone and water extracts of *Persicaria hydropiper* exhibit substantial larvicidal property against fourth instar larvae of *Aedes aegypti.* The medicinal plant *Sphaernthus indicus* showed less mortality in Petroleum ether, methanol, Acetone and Water extract against *Aedes aegypti*. Similarly the other medicinal plant species such as *Sphaernthus indicus*, *Hydrocotyle javanica, Deris trifoliate Lour, Acanthospermum hispidum Drymaria cordata, Toddelia Asiatic* and *Triumfetta rhomboide* showed relativiely less mortality against *Aedes aegypti* fourth instar larvae. Among the nine plants investigated, maximum larval mortality was obtained from the petroleum ether and methanol extract of *Plectranthus hadiensis and Persicaria hydropiper* respectively.

Table 2: Screening of plant extracts for larvicidal activity against *Aedes aegypti* at 1000 mg/L concentration

S No.	Plants tested	Petroleum ether	Acetone	Methanol	Water
1	***Sphaernthus indicus***	-	-	-	-
2	***Hydrocotyle javanica***	-	-	-	-
3	***Deris trifoliate Lour.***	-	-	-	-
4	***Persicaria hydropiper***	+	+	+	+
5	***Acanthospermum hispidum***	-	-	-	-
6	***Drymaria cordata***	-	-	-	-
7	***Toddelia asiatica***	-	-	-	-
8	***Plectranthus hadiensis***	+	+	+	+
9	***Triumfetta rhomboidea***	-	-	-	-

+ above 50 percent mortality at 24 hr, - No larval mortality

Table 3: Larvicidal activity of plant extracts against fourth instar larvae of *Aedes aegypti* after 24 h exposure (mg/L)

Plants	Acetone		Methanol		Petroleum ether		Water	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Persicaria hydropiper	658.005 (422.174-1625.366)	5572.689 (2027.987-143992.195)	489.278 (316.218-962.289)	4121.964 (1651.772-68018.777)	750.326 (494.333-1763.542)	5023.5 (2013.139-72991.339)	773.69 (469.252-2767.500)	8120.726 (2434.857-759740.251)
Plectranthus hadiensis	432.238 (262.951-874.953)	4555.766 (1662.711-148706.043)	500.655 (336.181-903.638)	3452.934 (1544.054-31689.036)	411.746 (285.842-633.040)	2250.134 (1194.474-10160.482)	485.791 (295.213-1112.471)	5512.748 (1857.242-303616.247)

Discussion

Natural insecticides are safer than synthetic ones as they posses little chance of developing insecticide resistance [1]. Vector control at its larval stage is the significant option as they are slow mobile and their habitats can easily be recognized [36]. However, frequent use of synthetic insecticides for mosquito control may leads to various harmful effects to human beings and non target organisms [24,25]. Hence, the demand for plant based products is enhancing as they are usually recognized to be bio degradable, natural, and safer than synthetic drugs [34].

Extracts of plants contain various active phyto compounds; hence they can be used as alternative approach to mosquito larval control. Many scientific studies have proven that plant derived products can be used as an alternate approach to control mosquito population [31,32].

The finding of the present investigation indicated that *Persicaria hydropiper* can be used as an alternative to synthetic insecticides to control mosquito transmitting diseases. The

petroleum ether, methanol, acetone and water extract of *Persicaria hydropiper* showed highest mortality against fourth instar larvae of *Aedes aegypti*. Similarly *Plectranthus hadiensis* showed potential mortality againt fourth instar larvae of *Aedes aegypti.* The remaining plant species such as *Sphaernthus indicus, Hydrocotyle javanica, Deris trifoliate Lour, Acanthospermum hispidum Drymaria cordata, Toddelia asiatica, Triumfetta rhomboidea* comparitvely showed less mortality than the other two plant species.

The present investigation obviously proved that the leaf extracts of *Plectranthus hadiensis* and *Persicaria hydropiper* has remarkable larvicidal property against *Aedes aegypti* vector mosquitoes. The flora vegetation of India has prosperous aromatic plant diversity; hence they can be used for the development of natural insecticides for controlling mosquito population and to prevent mosquito transmitting diseases. The results from the present investigation might encourage the search for novel, natural insecticides offering an alternative to synthetic insecticeds from traditionally used medicinal plants. The leaf extracts of *Plectranthus hadiensis* and *Persicaria hydropiper* have the potential to be used as an ideal approach for the mosquito control programmes.

Acknowledgements

We thank Principal, St. Joseph's College, Irinjalakuda for the laboratory facilities provided. We are greatly thankful to University Grants Commission (UGC), New Delhi for providing funds under UGC Major Research Project (F.NO.42-609/2013 (SR). We also thank Kerala State Council for Science, Technology and Environment (KSCSTE) for funds under Major Research Project (No.27/SRSLS/2013/CSTE).

References

1. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J Med Res. 2012;135(5):581-598.
2. Mehlhorn H. Encyclopedia of parasitology, 3rd edn. Springer, Heidelberg; 2008.
3. Mehlhorn H, Al Rasheid KAS, Al-Quraishy S, Abdel-Ghaffar F. Research and increase of expertise in arachno-entomology are urgently needed. Parasitol Res. 2012;110(1):259-265. doi: 10.1007/s00436-011-2480-7
4. Anders KL, Nguyet NM, Chau NV, Hung NT, Thuy TT, Lien le B, et al. Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. Am J Trop Med Hyg. 2011;84:127-134. doi:10.4269/ajtmh.2011.10-0476
5. Coudeville L, Shepard DS, Zambrano B, Dayan G. Dengue economic burden in the Americas: estimates from dengue illness. Am J Trop Med Hyg. 2009;81:127-127.
6. Beaute J, Vong S. Cost and disease burden of dengue in Cambodia. BMC Public Health. 2010;10:521. doi: 10.1186/1471-2458-10-521
7. Shepard DS, Coudeville L, Halasa YA, Zambrano B, Dayan GH. Economic impact of dengue illness in the Americans. Am J Trop Med Hyg. 2011;84(2):200-207. doi: 10.4269/ajtmh.2011.10-0503
8. Halasa YA, Shepard DS, ZengW. Economic cost of dengue in Puerto Rico. Am J Trop Med Hyg. 2012;86(5):745-752. doi: 10.4269/ajtmh.2012.11-0784
9. Mogasale V, Maskery B, Lim J, Thiem VD, Tho LH, Anh DD, et al. Economic evi- dence for policy makers in Vietnam: dengue fever economic burden of disease and household willingness to pay for dengue vaccines. Int J Infect Dis.2012;16:e310. doi:10.1016/j.ijid.2012.05.1005
10. Wettstein ZS, Fleming M, Chang AY, Copenhaver DJ, Wateska AR, Bartsch SM, et al. Total economic cost and burden of dengue in Nicaragua: 1996-2010. Am J Trop Med Hyg. 2012;87(4):616-622. doi: 10.4269/ajtmh.2012.12-0146
11. Ali KM, Asha A V, Aneesh EM. Bioecology and Vectorial Capacity of Aedes mosquitoes (Diptera : Culicidae) in Irinjalakuda Municipality , Kerala , India in Relation to Disease Transmission. Int J Curr Res Acad Rev. 2014;2(4):43-49.
12. WHO. Global Vector Control Response - Background document to inform deliberations during the 70th session of the World Health Assembly. 2017.
13. Division of Vector-Borne Disease. Dengue and Aedes aegypti mosquito. San Juan. 2012.
14. Surveillance and Control of Aedes aegypti and Aedes albopictus in the United States. Division of Vector-Borne Diseases: Centers for Disease Control. 2016.
15. Farag SA, Osama H, Mohamed R, Mohamed H. Development of longer-lasting repellence cellulosic based curtain fabrics. Material Sciences and Applications. 2011;2(3):200-208. doi:10.4236/msa.2011.23025
16. Jensen M, Mehlhorn H. Seventy-five years of Resochin in the fight against malaria. Parasitol Res. 2009;105(3):609-627. doi: 10.1007/s00436-009-1524-8
17. Amer A, Mehlhorn H. Larvicidal effects of various essential oils against Aedes, Anopheles,and Culex larvae (Diptera, Culicidae). Parasitol Res. 2006;99(4):466-472.
18. Ranson H, Rossiter L, Ortelli F, Jensen B, Wang X, Roth CW, et al. Identification of a novel class oil of insect glutathione S- transferase involved in resistance to DDT in the malaria vector Anopheles gambiae. Biochm J. 2001;359:295-304.
19. Bowers WS. Biorational approaches for insect control. Korean J Appl Entomol. 1992;31(3):289-303.
20. Mehlhorn H, Schmahl G, Schmidt J. Extract of the seeds of the plant Vitex agnus castus proven to be highly efficacious as a repellent against ticks, fleas,mosquitoes and biting flies. ParasitolRes. 2005;95(5):363-365.
21. Amer A, Mehlhorn H. The sensilla of Aedes and Anopheles mosquitoes and their importance in repellency. Parasitol Res. 2006;99(4):491-499.
22. Lees RS, Knols B, Bellini R, Benedict MQ, Bheecarry A, Bossin HC et al. Review: improving our knowledge of male mosquito biology in relation to genetic control programmes. Acta Trop. 2014 Apr;132 Suppl:S2-11. doi: 10.1016/j.actatropica.2013.11.005
23. Benelli G .Research inmosquito control: current challenges for a brighter future. Parasitol Res. 2015;114(8):2801-2805. doi: 10.1007/s00436-015-4586-9

24. Jirakanjanakit N, Rongnoparut P, Saengtharatip S, Chareonviriyaphap T, Duchon S, Bellec C, et al. Insecticide susceptible/resistance status in Aedes (Stegomyia) aegypti and Aedes (Stegomyia) albopictus (Diptera: Culicidae) in Thailand during 2003–2005. J Econ Entomol. 2007;100(2):545-550.

25. Sarwar M, Ahmad N,Toufiq M. Host plant resistance relationships in chickpea (Cicer arietinum L) against gram pod borer (Helicoverpa armigera Hubner). Pak J Bot. 2009;41:3047-3052.

26. Moore SJ, Lenglet AD. An overview of plants used as insect repellents. In Traditional Medicinal Plants and Malaria. CRC Press. 2004;344-363.

27. Maia MF, Moore SJ. Plant-based insect repellents: a review of their efficacy, development and testing. Malar J. 2011;10 Suppl 1:S11. doi: 10.1186/1475-2875-10-S1-S11

28. Sreedev P, Aneesh EM. A Comparative Analysis Of Larvicidal Efficacy Of Piperlongum And Ruta Graveolens Against Aedes Aegypti A Dengue Virus Vector. Asia Pac j res. 2016;1(36):200-205.

29. Liu H, Xu Q, Zhang L, Liu N. Chlorpyrifos resistance in mosquito Culex quinquefasciatus J Med Entomol. 2005;42(5):815-820.

30. WHO World Health Organization. Traditional medicine. Fact Sheet. 2008.

31. Sharma P, Mohan L, Srivastava CN. Phytoextract-induced developmental deformities in malaria vector. Bioresour Technol. 2006;97(14):1599-1604.

32. Amer A, Mehlhorn H. Larvicidal effects of various essential oils against Aedes, Anopheles,and Culex larvae (Diptera, Culicidae). Parasitol Res. 2006;99(4):466-472.

33. Vinu Rajan PK, Sreedev P, Misvar Ali K, Aneesh EM. Larvicidal Efficacy of Piper Longum Fruit Extract against Aedes Aegypti, a Dengue Fever Vector. 2015;4(1):123-127.

34. Abd Kadir SL, Yaakob H, Mohamed Zulkifli R. Potential anti-dengue medicinal plants: A review. J Nat Med. 2013;67(4):677-689. doi: 10.1007/s11418-013-0767-y

35. Abbott WS. A method of computing of the effectiveness of an insecticide. J Econ Entomol. 1925;8(2):265-267.

36. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. Indian J Med Res. 2012;135(5):581-598.