Aqueous Plant Extracts As Potential Mosquito Larvicides Against Filariasis Fever Mosquito, Culex Quinquefasciatus (Say.) (Diptera: Culicidae)

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Abstract- *This study investigates the larvicidal potential of indigenous plant extracts from commonly used medicinal herbs as an environmentally safe measure to control the filarial vector, Culex quinquefasciatus Say (Diptera: Culicidae).The early fourth-instar larvae of C. quinquefasciatus, reared in the laboratory, were used for larvicidal assay with cold water, hot water, Anisomeles malabarica (Lamiaceae), Side acuta (Malvaceae), Piper betle (Piperaceae) and Vitex negundo (Lamiaceae). All plant extracts showed moderate larvicidal effects after 24 h of exposure at 1,000 ppm; however, the highest larval mortality was observed after 48 h cold water extracts A. malabarica (LC_{50}=165.86, 182.18 and 141.62 ppm), (LC_{90}=664.10, 801.11and 504.65 ppm) and hot water (LC_{50}=141.72, 172.11and130.12ppm) (LC_{90}=615.18, 731.81and 483.12ppm), S. acuta (LC_{50}=79.17, 88.66 and 68.95ppm), (LC_{90}=356.71, 426.44 and 285.12ppm V.negundo (LC_{50}=106.85,125.16 and 92.16ppm) ($LC_{90=}$531.10, 636.11and 420.18 ppm) Hot water (LC_{50}=85.28, 900.11 and 69.55 ppm) ($LC_{90=}$711.69, 911.11and 519.32 ppm) against the larvae of C. quinquefasciatus, respectively. This is an ideal ecofriendly approach for the control of lymphatic filariasis vector, C. quinquefasciatus.*

Keywords- Culex quinquefasciatus plant extracts cold water hot water larval mortality

I. INTRODUCTION

Lymphatic filariasis is a mosquito-borne disease caused by mosquito-transmitted filarial nematodes, including *Wuchereria bancrofti and Brugia malayi*. The infected people carry the nocturnally periodic *W. bancrofti*, which has *C. quinquefasciatus* as the main mosquito vector. *C. quinquefasciatus* is a vector of lymphatic filariasis, which is a widely distributed tropical disease with around 120 million people infected worldwide, and 44 million people have common chronic manifestation (Bernhard et al. 2003). The disease is a major health problem in many parts of the tropical world. Although the disease itself is rarely fatal, the disability caused by the swollen extremities, the acute attacks of adenolymphangitis, and the consequent sufferings of those afflicted are considerable. The functional impairment caused by lymphatic filariasis was assessed in rural areas of Tamil Nadu, South India, and about 66% of the patients said that the disease hampered their occupational activities. Lymphatic filariasis affects 119 million people living in 73 countries, with India accounting for 40% of of the global prevalence of infection (Ramaiah et al. 1997).

Lymphatic filariasis is second only to malaria as the most important vector-borne disease in India. It is a major public health and socio-economic problem; approximately 420 million people reside in endemic areas, and 48.11 million are infected. Bancroftian filariasis, caused by W. bancrofti and transmitted by the tropical house mosquito *C. quinquefasciatus*, accounts for 95% of the total lymphatic filariasis cases in India. There were 20.32 million chronic cases in India in 1996, 79% occurring in men, and 40.65 million adenolymphangitis episodes are suffered per year in India, 60% of which are suffered by men (Michael et al. 1996; Ramaiah et al. 2000).

The estimates of the proportion of adenolymphangitis episodes, chronic patients incurring treatment costs, the average expenditure for treatment, and total treatment costs were estimated as being US $23.01 million for men and US $8.07 million for women. More than 60% of these total costs are attributable to the chronic condition. In men and women, a total of US $811 million was estimated to be lost per year due to lymphatic filariasis. Overall, it is estimated that US $842 million are lost to patients and households every year in India from treatment costs and reduced working time (Ramaiah et al. 2000). Control of the mosquito larvae is frequently dependent on continued applications of organophosphates and insect growth regulators (Yang et al. 2002). An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many

synthetic agents have been developed and employed in the field with considerable success. It has also provoked undesirable effects, including toxicity to nontarget organisms, and fostered environmental and human health concerns (Lee et al. 2001). The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides (Macedo et al. 1997) and for more detailed studies of naturally occurring insecticides (Ansari et al. 2000). These problems have highlighted the need for the development of new strategies for selective mosquito larval control. Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae.

In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Sharma et al. 2006; Rasheed et al. 2005; Amer and Mehlhorn 2006 ; Rahuman et al. 2008). Elango et al. (2010) reported that the hexane extracts of *Aegle marmelos* and *A. paniculata* served as a potential repellent, ovicidal, and oviposition deterrent against *Culex tritaeniorhynchus*. *C. hirsutus* is a widely growing plant found in the plains of India in dry localities and the methanol extract showed effective oviposition repellency against *A. subpictus* (Elango et al. 2009a). *Eclipt. prostrata*, a member of the Asteraceae family and commonly known as False Daisy and the ethyl acetate extract of *E. prostrata* and leaf hexane extract of *A. paniculata* have the potential to be used against the fourth-instar larvae of *Anophles subpictus* and *C. tritaeniorhynchus* (Elango et al. 2009b).The aqueous crude extract from the roots of *Hibiscus abelmoschus* (Dua et al. 2006); the long pepper, *Piper retrofractum* (Chansang et al. 2005); dry powder of *Cuscuta hyalina* on the water surface (Mehra and Hiradhar 2002); extracts of *Cussonia barteri*, *Glinus oppositifolius*, and *Lannea velutina* (Diallo et al. 2001); the toxic compounds involved in the dietary toxicity of decomposed arborescent leaf litter against larval mosquito, where a toxic fraction was extracted from crude leaf litter by using hot water (Tilquin et al. 2002), have been tested against the larvae of *C. quinquefasciatus*. The acetone extracts of *Ageratum conyzoides*, *Cleome icosandra*, *Tagetes erectes*, and *Tridax procumbens* showed growth inhibitory and juvenile hormone mimicking activity to the treated larvae of *C. quinquefasciatus* (Saxena et al. 1992). the latex and stem bark of *Euphorbia tirucalli* (Yadav et al. 2002) have been tested against the larvae of *C. quinquefasciatus.* Aqueous and polar/non-polar solvent extract of fresh, mature, green berries of *Solanum villosum* was tested against *Stegomyia aegypti* (Chowdhury et al. 2008); crude carbon-tetra-chloride, methanol, and petroleum ether extracts of *S. xanthocarpum* fruits were examined against *A.stephensi* and *C. quinquefasciatus* (Mohan et al. 2005); the ethyl acetate leaf extract of *Solanum suratense and* petroleum ether fraction of *Solanum trilobatum* were tested against the larvae of *C. quinquefasciatus* (Muthukrishnan et al. 1997).

In the light of earlier literature, it is known that larvicides play a vital role in controlling mosquitoes in their breeding sites, but still, vectors' resistance to them remains unanswered. Though various biocontrol measures are in vogue, their effective control of larval mosquitoes has not been hitherto highlighted, whereas possibilities of plant extract and isolation of active components have been fragmentally documented. The results of the present study would be useful in promoting research aiming at the development of new agent for mosquito control based on bioactive chemical compounds from indigenous plant source. In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the extracts from the medicinal plant against the medically important mosquito vector, *C. quinquefasciatus*

II. AIM OF THE PRESENT STUDY

The present study aims to evaluate the Larvicidal activity of experimental plant aqueous cold and Hot water extracts against *C. quinquefasciatus.*

III. MATERIALS AND METHODS

3.1Plant collection

The fully developed leaves of *Anisomeles malabarica* (Lamiaceae), *Piper betle* (Piperaceae) *Side acuta* **(**Malvaceae) and *Vitex negundo* (Lamiaceae) (Fig 1), were selected on the basis of aromatic smell, bitter taste, resistance to damage by insect pests, ethnopharmacological, traditionally used medicinal value and ethnobotanical literature survey. The plant materials were collected from the Tamil Nadu Medical Plant Farms and Herbal Medicine Corporation Limited, medicinal plant farm, Arumbakkam (13º13'4 N, 79º59'7E; Altitude 118 feet), Chennai, Tamil Nadu, and the taxonomic identification was made by Dr. A.Thalavai pandiyan, Department of Botany, Government Thirumagal Mills college Gudiyatham, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

3.2 Mosquito culture

C.quinquefasciatus larvae were collected from stagnant water area of Gudiyatham (12°56'41.05"N, 78°52'15.25"E) and identified in Zonal Entomological Research Centre, Vellore (12º552 483N, 79º72483E), Tamil Nadu, to start the colony, and larvae were kept in plastic and enamel trays containing tap water. They were maintained, and all the experiments were carried out, at 27±2ºC and 75–85% relative humidity under 14:10 light and dark cycles. Larvae were fed a diet of brewer's yeast, dog biscuits, and algae collected from ponds in a ratio of 3:1:1, respectively.

3.3 Preparation of plant extracts

The leaves were dried for 7-14 days in the shade at the environmental temperature (27-37ºC days time). The dried leaves (800 g) were powdered mechanically using commercial electrical stainless steel blender and extracted. For aqueous extracts, fresh parts of leaves were initially rinsed with distilled water and dried on a paper towel. The crude extracts were prepared by grinding the plant material in a mortar and pestle and passing the ground material through Whatman No 1 filter paper. Required concentrations of aqueous extracts were prepared by mixing the crude extract with a suitable amount of sterilized distilled water (Chowdhury et al. 2008). For hot water extract, the plant material was completely immersed in water in a round-bottomed flask, the water was brought to boiling, the distillate was collected, and required concentrations were prepared for the experimental test (Ross and Brian 1977). Finally a series of filtrations to have an aqueous extract sterile. One gram of crude extract was first dissolved in 100 ml of acetone (stock solution). From the stock solution, 1,000 and 500 ppm were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05% in the final test solution. After this process the aqueous extract is ready to be used in bioassays of antilarvicidal activity.

The *C. quinquefasciatus* larvicidal activity was assessed by the procedure of WHO (1996) with some modification and as per the method of. For Bioassay test, larvae were taken in five batches of 20 in 249 ml of water and 1.0 ml of the desired plant extract concentration. The control was set up with respective solvent and polysorbate 80. The numbers of dead larvae were counted after 24 and 48 h of exposure, and the percentage mortality was reported from the average of five replicates. The experimental media, in which 100% mortality of larvae occurs alone, were selected for dose–response bioassay

3.4 Dose–response bioassay

From the stock solution, different concentrations ranging from 31.25 to 1,000 ppm were prepared. Based on the preliminary screening results, crude different aqueous of leaf, extracts prepared from *A. malabarica P. betle, S. acuta* and *V. negundo* were subjected to dose–response bioassay for larvicidal activity against *A. subpictus*. The numbers of dead larvae were counted after 24 and 48 h of exposure, and the percentage mortality was reported from the average of five replicates. However, at the end of 24 and 48 h, the selected test samples turned out to be equal in their toxic potential.

3.5Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using the software developed by Reddy et al. (1992).

IV. RESULTS AND DISCUSSION

The early fourth-instar larvae of *C. quinquefasciatus*, reared in the laboratory, were used for larvicidal assay with leaf extracts of cold water, hot water, *A. malabarica P. betle, S. acuta* and *V. negundo* . All plant extracts showed moderate larvicidal effects after 24 h of exposure at 1,000 ppm; (Table 1and Fig 1a), the lowest larval mortality was observed leaf cold water extracts of *S. acuta* 58±1.70 and hot water extracts of *P.betle* 57±1.48; However, the 100% larval mortality was observed after 48 h (Table 2 and Fig 2). The leaf cold water extracts of *A.malabarica* (LC_{50}=165.86, 182.18 and 141.62 ppm), (LC_{90}=664.10, 801.11and 504.65 ppm) and hot water extracts (LC_{50}=141.72, 172.11and130.12ppm) (LC_{90}=615.18, 731.81 and4 83.12ppm), *S.acuta* (LC_{50}=79.17, 88.66 and 68.95ppm), (LC_{90}=356.71, 426.44 and 285.12ppm) and *V.negundo* (LC_{50}=106.85,125.16 and 92.16ppm) ($LC_{90=}$531.10, 636.11and 420.18 ppm), Hot water extracts (LC_{50}=85.28, 900.11 and 69.55 ppm) ($LC_{90=}$711.69, 911.11and 519.32 ppm) against the larvae of *C. quinquefasciatus*, respectively. Chi-square value was Significant at $P<0.05$ level (Table 3 and Fig 3). This is an ideal ecofriendly approach for the control of lymphatic filariasis vector, *C. quinquefasciatus*. The mortality values were significantly greater than the values of control. Based on the preliminary screening results, 100% larval mortality obtained crude extracts alone were selected and subjected to dose–response bioassay for larvicidal activity against *C. quinquefasciatus*. The present observation showed that all extracts showed moderate significant toxicity, while cold and hot water, extracts of *A. malabarica, S.acuta* and *V.negundo* 100% mortality against the larvae of *C. quinquefasciatus* in 48 h at 1,000 ppm. Earlier authors reported that the effect of

water extract of citrus-seed extract showed LC_{50} values of 135,319.40 and 127,411.88 ppm against the larvae of *A. aegypti* and *C. quinquefasciatus* (Sumroiphon et al. 2006). Dua et al. (2006) have reported that the mean median lethal concentration values of the aqueous extract from the roots of *H. abelmoschus* against the larvae of *A. culicifacies*, and *C. quinquefasciatus* were 52.6, and 43.8 ppm, respectively. The aqueous extract of *R. nasutus* showed LC_{50} values 9,681 (mg/l) against *C. quinquefasciatus* respectively (Chansang et al. 2005). A preliminary screening of crude acetone extract of *C. hyalina* was conducted against the laboratory-reared preadult stages of common house mosquito *C. quinquefasciatus* (Say) (Diptera: Culicidae). Twenty-four-hour LC_{50} of third and fourth-instar larvae and pupae were 303, 306.44, and 97.66 ppm, respectively (Mehra and Hiradhar 2002). Sharma et al. (2005) reported that the acetone extract of *Nerium indicum* and *Thuja oriertelis* have been studied with LC_{50} values of 200.87, 127.53, 209.00, and 155.97 ppm against III instar larvae of *C. quinquefasciatus*, respectively. Earlier authors reported that the methanol leaf extracts of *V.negundo*, *Vitex trifolia*, *Vitex peduncularis*, and *Vitex altissima* were used for larvicidal assay with LC_{50} values of 212.57, 41.41, 76.28, and 128.04 ppm, respectively, against the early fourth-instar larvae of *C. quinquefasciatus* (Kannathasan et al. 2007). The same extracts of *E. tirucalli* latex and stem bark were evaluated for larvicidal activity against laboratory-reared larvae of *C. quinquefasciatus* with LC_{50} values of 177.14 and 513.387 mg/L, respectively

(Yadav et al. 2002). Mullai and Jebanesan (2007) have reported that the methanol leaf extracts of *C. colocynthis* and Cucurbita maxima showed the LC_{50} values were 117.73 and 171.64 ppm, respectively, against *C. quinquefasciatus* larvae. Larvicidal efficacies of methanol extracts of *M. charantia, T. anguina, Luffa acutangula, Benincasa cerifera, and Citrullus vulgaris* tested with LC_{50} values were 465.85, 567.81, 839.81, 1,189.30, and 1,636.04 ppm, respectively, against the late third larval age group of *C. quinquefasciatus* (Prabakar and Jebanesan 2004). The results reported here open the possibility of further investigations of efficacy on their larvicidal properties of natural product extracts.

S.NO	Binomial Name	Local Name	Photos
1	*Anisomeless malabarica*	Pei miratti & Perum thumbi	
2	*Piper betle*	Vetrilai	
3	*Sid acuta*	Arivalmanai poondu	
4	*Vitex negndo*	Notchi	

Fig 1 Selected Ethanomedicinal Plants

Table 1 Larvicidal activity of crude extracts against early fourth-instar larvae of *C. quinquefasciatus* 24 hr at 1,000 ppm

Botanical name/family	Parts used	% Mortality (ppm) [a] ± SD 1	2
Anisomeles malabarica /(Lamiaceae)	Leaf	85±1.72	86±1.00
(Piper betle / (Piperaceae)	Leaf	65±1.60	57±1.48
Side acuta /**(**Malvaceae)	Leaf	58±1.70	81±1.72
Vitex negundo (Lamiaceae)	Leaf	78±1.71	84±1.83

Control—nil mortality

1Cold water, 2 Hot water

[a] Mean value of five replicates

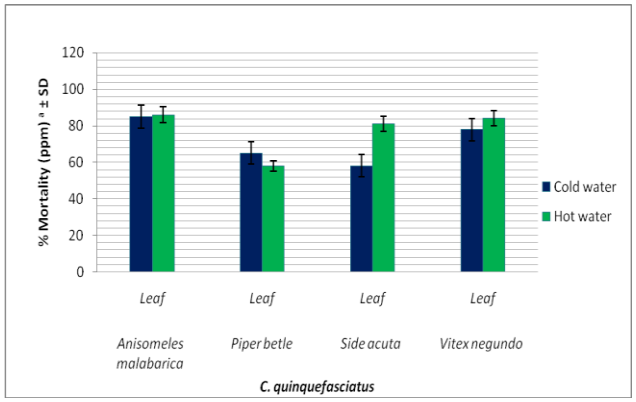

Fig 1a Larvicidal activity of crude extracts against early fourth-instar larvae of *C.quinquefasciatus* 24 hr at 1,000 ppm

Table 2 Larvicidal activity of crude extracts against early fourth-instar larvae of *C. quinquefasciatus* 48 hr at 1,000 ppm

Botanical name/family	Parts used	% Mortality (ppm) [a] ± SD 1	2
A. malabarica	Leaf	100±0.00	100±0.00
P.betle	Leaf	80±1.20	70±1.00
S. acuta	Leaf	82±1.01	100±0.00
V.negundo	Leaf	100±0.00	100±0.00

Control—nil mortality
1 Cold water, 2 Hot water
[a] Mean value of five replicates

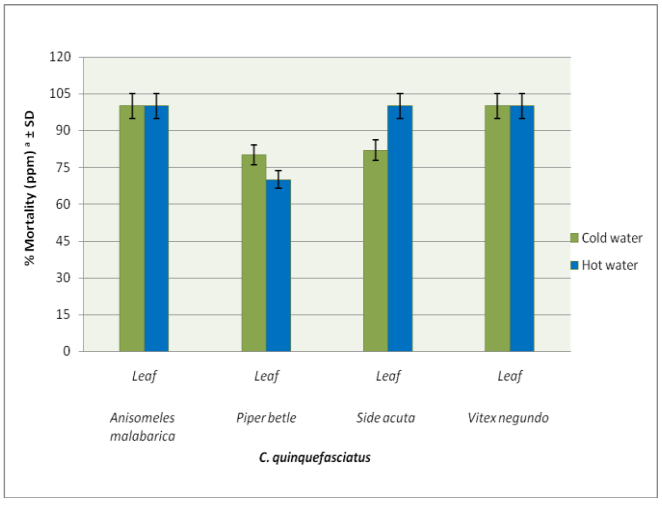

Fig 2 Larvicidal activity of crude extracts against early fourth-instar larvae of *C. quinquefasciatus* 48hr at 1,000 ppm

Table 3 Larvicidal activity of different solvent crude extracts against fourth-instar larvae of *C. quinquefasciatus*

Name of the plants	Solvents	LC_{50} ± SE (ppm)	(LCL-UCL)	LC_{90} ± SE (ppm)	(LCL–UCL)	χ^2 (df=4)
A.malabarica	Cold water	165.86±11.20	(182.18-141.62)	664.10±62.12	(801.11–504.65)	8.10
	Hot water	141.72±12.12	(172.11-130.12)	615.18±59.11	(731.81–483.12)	9.87
S. acuta	Hot water	79.17±6.23	(88.66-68.95)	356.71±31.11	(426.44–285.12)	12.8
	Cold water	106.85±7.11	(125.16-92.16)	531.10±58.11	(636.11–420.18)	8.08
V. negundo	Hot water	85.28±7.54	(900.11-69.55)	711.61±99.11	(911.11–519.32)	13.11

Control—nil mortality. Significant at P<0.05 level

LC_{50} lethal concentration that kills 50% of the exposed larvae, LC_{90} lethal concentration that kills 90% of the exposed larvae, *UCL* upper confidence limit, *LCL* lower confidence limit, $\chi 2$ chi-square; *df* degree of freedom

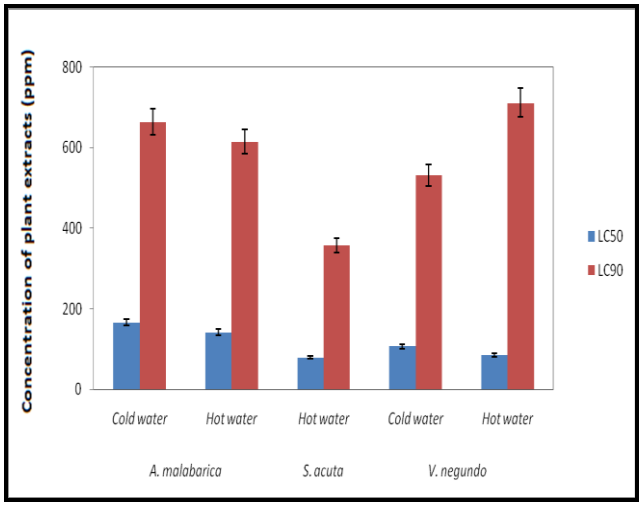

Figure 3 Larvicidal activity of different plant extracts against *C. quinquefasciatus* expressed as LC_{50} and LC_{90}

V. SUMMARY

- Filariasis is the most common diseases worldwide. It is also the most common type of Filariasis in males and remains the most common cause of malaria related mortality in both sexes.
- In the present study, the plants were selected on the basis of the Indian entnophrmocolgical information and traditional uses provided by different literature sources.
- Four medicinal plants were collected from Tamil Nadu Medical Plant Farms and Herbal Medicine Corporation Limited, medicinal plant farm, Arumbakkam (13º13'4 N, 79º59'7E; Altitude 118 feet), Chennai, Tamil Nadu, of South India.
- The experimental plants leaf was dried for 7-14 days in the shade at the environmental temperatures (27-37°Cday time). The dried leaf was powdered mechanically using commercial electrical stainless steel blender and extracted with cold and hot water.

- In the present study, larvicidal analysis and the *in vitro* activity against *C. quinquefasciatus* of leaf cold and hot water extracts of 4 medicinal plants.
- The outcome of this study strongly supports the development of new drug compound derived from plant products in the prevention against *C. quinquefasciatus*, which remains a devastating disease.
- The results of the study revealed that the *A. malabarica S. acuta* and *V. negundo* showed a significant larvicidal effect with IC_{50} values of (LC_{50}=165.86±11.20, 141.72±12.12, 141.72±12.12), 106.85±7.11, 85.28±7.54 µg/mL, respectively against *C. quinquefasciatus*.

VI. CONCLUSIONS

In conclusion, an attempt has been made to evaluate the role of plant extracts in mosquito larvicidal activity. The results reported here open the possibility of further investigations of efficacy on their larvicidal properties of natural product extracts. The present study revealed the larvicidal activity of some Indian medicinal plants. These plant extracts have potential for the development of new and safe control products for *A. subpictus*. As naturally occurring insecticides, these plant-derived materials could be useful as an alternative for synthetic insecticides controlling field populations of *A. subpictus*. The present study plants are easily available, accessible, and affordable therefore the usage of traditional plants should be promoted among the local residents in order to reduce the man–vector contact as well as vector-borne diseases.

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