



Larvicidal efficacy of indigenous plant *Ficus pumila* leaves extract against dengue vector *Aedes aegypti*

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Abstract

Aedes aegypti, dengue fever mosquito, is primarily associated with the transmission of dengue and chikungunya in tropical and subtropical regions of the world. The present investigations were carried out to assess the larvicidal efficiency of indigenous plant against *A. aegypti*. The different ppm (20 – 100) of ethanol extract prepared from the leaf of *Ficus pumila* was screened for their larvicidal activity against early fourth instars of dengue vector. The extracts which could cause 80–100% mortality were further investigated for their efficacy. The preliminary screening established the efficacy of ethanol extract. Further investigations revealed the highest concentration has significant activity was observed in 48hrs. The lethal concentration 50 (LC₅₀) is the concentration of a chemical that will kill 50 percent of the larvae. The lethal concentration (50%) was 97.37ppm for 12hr, 52.07ppm for 24hrs and 31.13ppm for 48hrs. The present investigations suggest the possible use of *A. aspera* as an ideal ecofriendly, larvicidal agent for the control of dengue vector, *Ae. aegypti*. Future studies are, however, required to explore and identify the bioactive component involved and its mode of action.

Keywords: *Ficus pumila*. phytochemicals, larvicidal activity, *aedes aegypti*, lethal concentration

Introduction

Mosquitoes have attracted considerable attention worldwide being the most prevalent vectors of several lethal diseases, malaria, filariasis, chikungunya, yellow fever, dengue, and encephalitis, accounting for enormous mortality and morbidity. Since last few years, dengue fever has become the major public-health concern in tropical and subtropical regions of the world. It is considered the most rapidly spreading mosquito-borne disease with 30-fold rise in global occurrence since the past 5 decades. The incidence of dengue infections estimated by World Health Organization is about 390 million annually of which 96 million are supposed to be manifested clinically (WHO, 2015) [11]. As per WHO reports, approximately 3900 million individuals, inhabiting over 128 endemic countries, are likely to be at the risk of dengue. In India, official records of the Union Health Ministry reveal a massive increase in dengue infections every year (NVBDCP, 2015) [5].

Till date, specific medications and vaccinations are not available commercially for treating dengue fever. The only approach followed to reduce the incidence of dengue is by the control of its vector, *Aedes aegypti* L., which is also the primary carrier of chikungunya virus and yellow fever virus. In the past, the control measures for mosquito vectors were based on the frequent and indiscriminate use of synthetic chemical-based insecticides, such as organochlorines, carbamates, organophosphates, and pyrethroids (Liu *et al.*, 2006) [4].

Botanicals are considered safe alternative to synthetic pesticides since they are biodegradable and safe for environment causing low toxicity to humans and non-target organisms (Rajmohan *et al.*, 2012) [8]. More than 2,000

plants species have already been known to possess chemical factors and metabolites of significance in pest control programs whilst products of approximate 344 species have been reported to encompass diverse activities against mosquitoes (Sukumar *et al.*, 1991; Ghosh *et al.*, 2012) [10, 11]. Keeping in view above properties and possible mosquito control potential of these weeds, the leaves of *Ficus pumila* (Tamil Name: Suvarooti) were assessed for their larvicidal potential against an Indian strain of dengue vector, *Aedes aegypti*. The investigations were carried out with an objective that the result of the study could be useful in promoting research aiming at the development of new ecofriendly alternative for mosquito control based on the biologically active plant sources.

Materials and Method

Collection and preparation of *Ficus pumila* leaves extract

The *Ficus pumila* (Family: Moraceae) leaves were collected from Tamil University campus, Thanjavur, Tamil Nadu. The selected healthy parts were shade-dried at room temperature (°C) for approximate three weeks till they were dried completely. Plant extracts were prepared by maceration method in which the dried plant parts were ground with electric blender and then sieved to get fine powder. The 10 g of dried powdered part was weighed and soaked in 50 mL of ethanol in a stoppered conical flask (100 mL) for 24 hrs with frequent agitation until soluble matter was dissolved. The extract, thus obtained, were concentrated using water bath keeping the temperature not more than their respective boiling point, that is, 55°C and 45°C. The concentrated extract was stored in a refrigerator at 4°C for phytochemical and larvicidal activities.

Mosquito culture

Aedes aegypti L. larvae were collected from rice field and stagnant water areas of Karukadipatti, Thanjavur and identified by Zoologist Dr. M. Sukumaran, Assistant Professor, Department of Zoology, Rajahs Serfoji Government College, Thanjavur, 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 $27 \pm 2^\circ\text{C}$ and 75–85 per cent relative humidity under 14:10 h 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 as per the method of Kamaraj *et al.*, (2009) [2].

Larvicidal test

Method for testing larvicidal action of the *Ficus pumila* leaves sample was slightly modified from those of World Health Organization (WHO, 1996) [12]. A stock solution was prepared dissolving a known amount of sample in water and stored in a refrigerator at 15°C . Twenty healthy late 4th instar larvae were introduced into each testing cup (sterilized beaker of 100 ml capacity) containing 100 ml of dechlorinated tap water with stock solution. A measured volume of stock solution was added to obtain the desired concentrations. Experiments were carried out with a series of five concentrations viz. 20, 40, 60, 80 and 100ppm in

triplicates. Each batch of replicates contained one control of 100 ml of water alone and another of 100 ml of water containing a volume of solvent corresponding to the maximum volume of *Ficus pumila* leaves sample tested. As very few larvae succumbed within 12 hours of exposure to the test solutions, mortality was recorded after 6 hours of exposure, during which no feed was given to the larvae. The mortalities of mosquito larvae were recorded if moribund larvae were incapable of rising to the surface or moving when they were disturbed.

Data Analysis

The obtained data was analysed by regression analysis using computerized statistical program SPSS (Version 22.0). The LC₅₀ values with 95% Confidence limits were calculated in each bioassay for the assessment of significance and measurement of difference between the test samples.

Results and Discussion

Phytochemical analysis

Wide variety of natural compounds like alkaloids, glycosides, saponins, phytosterols, phenolics, steroids, terpenoids, flavonoids, coumarins and tannins which exert physiological activity as synthesized in *Ficus pumila* leaves extract. Results of phytochemical analysis on ethanolic extract are summarized in Table 1.

Table 1: Qualitative analysis of phytochemicals in *Ficus pumila* leaves extract

S. No.	Name of the phytochemicals	Results
1	Alkaloids	+
2	Glycosides	+
3	Saponins	++
4	Phytosterols	+
5	Phenolics	++
6	Steroids	++
7	Terpenoids	++
8	Flavonoids	++
9	Coumarins	++
10	Tannins	++

+ Presence and ++ high concentration

Larvicidal activity of *Ficus pumila* leaves against mosquito larvae

The extensive use of synthetic chemical insecticides results in environmental degradation, hazards, and resistance in major vector species and this has necessitated leading the way towards the development of a more potent and environmentally friendly insecticide. Nowadays, the control of mosquitoes at larval stage is focused on the use of plant extracts. Plants produce various chemicals, many of which have medicinal, insecticidal, repellent, and growth regulatory properties (Ghosh *et al.*, 2012) [1].

The present investigations clearly revealed that when 100 ppm ethanol leaf extract was screened for their larvicidal efficacy against early IV instars of *Ae. aegypti*, the extract exhibited significant larvicidal efficacy causing 100% larval mortality. Similar results were reported by Kumar *et al.* (2012) [3] who performed an initial screening of fifteen local plant species to explore their potential as a mosquito larvicidal agent against early fourth instars of dengue vector *Ae. aegypti* and reported the effectual larvicidal potential of extracts of selected plant species resulting in 100% mortality at 100 ppm.

Figure 1 shows the experimental set up of larvicidal activity

of *Ficus pumila* leaves against mosquito larvae. Larvicidal activity of *Ficus pumila* leaves was directly proportional to the concentration and time duration of the *Ficus pumila* leaves exposure. The highest concentration has significant activity was observed in 48hrs. The lethal concentration 50 (LC₅₀) is the concentration of a chemical that will kill 50 percent of the larvae. The lethal concentration (50%) was 97.37ppm for 12hr, 52.07ppm for 24hrs and 31.13ppm for 48hrs. Table 2b shows LC₅₀ value of *Ficus pumila* leaves against mosquito larvae. Figure 1 shows the graphical represent the larvicidal activity of *Ficus pumila* leaves against mosquito larvae.

Likewise, evaluation of the larvicidal activity of extract prepared from fourteen medicinal plants against *Ae. Aegypti* revealed that eight out of fourteen plant species resulted in 100% larval mortality with their LC₅₀ values ranging between 4.1 $\mu\text{g/mL}$ and 89.4 $\mu\text{g/mL}$ (Promsiri *et al.*, 2006) [7]. Similar studies performed by Sakthivadivel and Daniel (2008) [9] evidently proved the toxicity of six plant extracts, *Acacia nilotica* (leaf), *A. mexicana* (leaves and seeds), *Citrullus colocynthis* (leaf), *Jatropha curcas* (leaf), and *Withania somnifera* (leaf), resulting in an LC₅₀ value of less than 100 ppm against 3rd instars of *Cx. quinquefasciatus*,

An. stephensi, and *Ae. aegypti*. Comparable screening assays with a total of 94 extract prepared from ten plant species

belonging to eight families widely found in the Northeast of Brazil were performed by Oliveira *et al.* (2010) [6].

Table 2a: Larvicidal activity of *Ficus pumila* leaves against mosquito larvae

Concentration (ppm)	Log ₁₀ base concentration (ppm)	Number of exposed larvae	12 Hours			24 Hours			48 Hours		
			Number of mortality	% of mortality	Probits	Number of mortality	% of mortality	Probits	Number of mortality	% of mortality	Probits
20	1.30	20	0	0	0	3	15	3.96	7	35	4.61
40	1.60	20	1	5	3.36	5	25	4.33	11	55	5.13
60	1.77	20	4	20	4.16	9	45	4.87	15	75	5.67
80	1.90	20	6	30	4.48	15	75	5.67	17	85	6.04
100	2.00	20	11	55	5.13	19	95	6.64	20	100	7.33
Control	Control	20	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table 2b: LC₅₀ value of *Ficus pumila* leaves against mosquito larvae

Hours	LC ₅₀ value (ppm)			Regression equation	r ² value
	LC ₅₀	LCL	UCL		
12	97.37	81.70	145.51	y = 7.0757x - 8.722	0.9362
24	52.07	42.40	63.06	y = 3.579x - 1.0507	0.8409
48	31.13	20.99	39.45	y = 3.4187x - 0.1135	0.8339

Probits analysed by SPSS ver. 22, Regression equation and r² value was calculated by log. Concentration vs probit value, Significant level alpha 0.05, LCL: 95% of Lower Confidence Limits, UCL: 95% of Upper Confidence Limits.

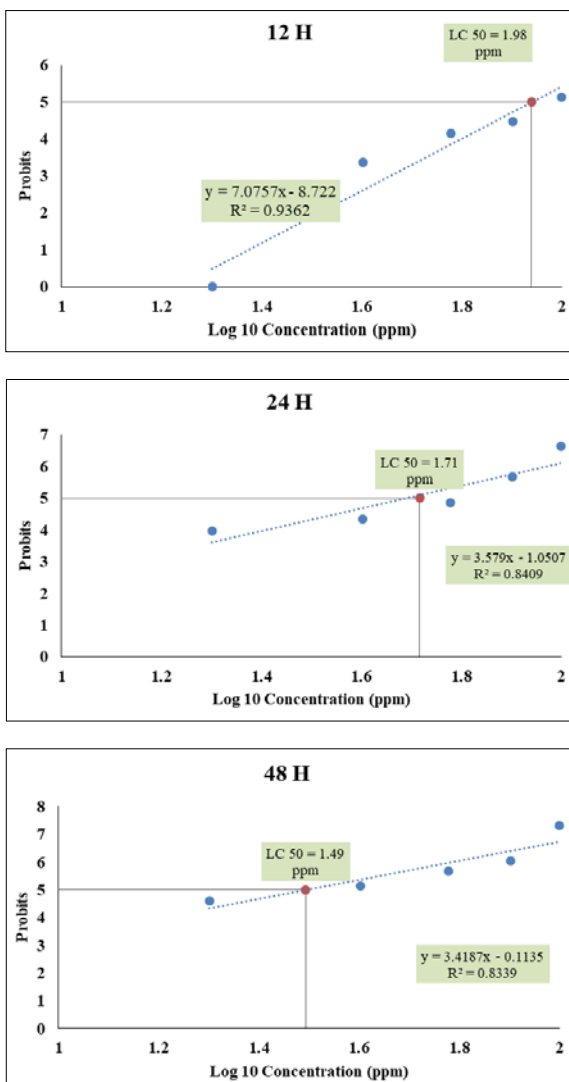


Fig 1: Show the graphs Larvicidal activity of *Ficus pumila* leaves against mosquito larvae

Conclusions

Our investigations have established the potential of ethanol leaf extract prepared from *Ficus pumila* leaves against early fourth instars of *Ae. aegypti* and their possible use in the development of larvicides for mosquito management. The variety of types and levels of active constituents in extract may be responsible for the variability in their potential against *Ae. aegypti*. However, the mechanism involved causing mortality of mosquito larvae is still unknown and needs to be studied further. Our investigations need further exploration to find out and identify the bioactive components involved and their mode of action.

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