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Screening of *Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) crude leaf extracts for larvicidal efficacy against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract
Mosquitoes represent a significant threat because of their ability to vector pathogens that cause deadly diseases that afflict millions of people worldwide. Several species belonging to genera *Aedes*, *Anopheles* and *Culex* are vectors for the pathogens of various diseases like dengue, dengue hemorrhagic fever, chikungunya, malaria, Japanese encephalitis and filariasis. Mosquito control relies heavily on synthetic insecticide application. However, excess and injudicious application of synthetic insecticides has resulted in development of resistance to these insecticides by the mosquitoes and unwarranted toxic or lethal effects on non-target organisms, as well as environmental health problems. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs. In the present study, the crude hexane, ethyl acetate, methanol, benzene and aqueous leaf extracts of *Eichhornia crassipes* were tested for the larvicidal efficacy against the early fourth instar larvae of *Culex quinquefasciatus* at concentrations of 62.5, 125, 250 and 500 mg/L. Mortality was recorded after 48 hours. Amongst the crude leaf extracts of *Eichhornia crassipes* tested, the hexane and methanol extract was found to be the most effective at 62.5 and 500 mg/L and LC_{50} values were 80.54 and 137.50 mg/L respectively. Further investigations are needed to elucidate the larvicidal activity of *Eichhornia crassipes* crude hexane and methanolic leaf extract against all stages of mosquito species and also the active ingredient(s) of the extract responsible for larvicidal activity should be identified.

Keywords: *Eichhornia crassipes*, crude leaf extracts, larvicidal efficacy, *Culex quinquefasciatus*

1. Introduction

The world today is still plagued by a myriad of diseases and a number of these are caused by organisms which are vector-borne. The mosquito, having a cosmopolitan distribution, except the Antarctic regions [1] is one of the most despised creatures in the animal kingdom with a horrific reputation of being a vector of several diseases. Vector-borne diseases are illnesses caused by pathogens and parasites in human populations. Mosquitoes represent a significant threat because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide [2, 3]. Every year, more than one billion people are infected and more than one million people die from vector-borne diseases [4]. About 3000 species of mosquitoes have been recorded worldwide, out of which more than 100 species are reported to be capable of transmitting diseases to humans [5]. Several species belonging to genera *Aedes*, *Anopheles* and *Culex* are vectors for the pathogens of various diseases like dengue, dengue hemorrhagic fever, chikungunya, malaria, Japanese encephalitis and filariasis [6-8].

Mosquitoes are the oldest human enemy and controlling them is of prime importance in recent years because of the numerous diseases caused by them. Around the world, the medical and economic burden caused by vector-borne disease continues to grow as current control measures fail to cope. There is an urgent need to identify new control strategies that will remain effective, even in the face of growing insecticide and drug resistance [9]. Mosquito control relies heavily on synthetic insecticide application. However, excess and injudicious application of synthetic insecticides has resulted in development of resistance to these insecticides by the mosquitoes and unwarranted toxic or lethal effects on non-target organisms, as well as environmental health problems. This phenomenon has triggered and urged the development of alternative techniques using natural products. Natural products of plant origin are generally preferred because of their less harmful nature to non-target organisms and their innate biodegradability [10]. Botanicals can be used as alternative synthetic insecticides or along with other insecticides under integrated vector control programs and phytochemicals could be valuable weapons in the fight against mosquito-borne diseases [11]. Despite centuries of control efforts, mosquito-borne diseases are flourishing worldwide. Resistance to drugs as well as insecticides [12, 13] is the main hurdle to overcome so as to fulfill the dream of vector-borne disease free nation. To bridle these problems, safe and effective herbal stratagem is in focus against the vectors and vector-borne diseases [14]. A recent emphasis has been placed on plant material and various reports on the use of natural plant products against mosquito vectors have been reported [4, 15-29].

Eichhornia crassipes (Mart.) Solms commonly called water hyacinth is an aquatic perennial herb that belongs to the family Pontederiaceae (Fig. 1). It is indigenous to the New World tropics, and has its center of origin in Amazonia, Brazil [30], with anthropogenic spread to other areas *viz*., Venezuela, parts of Central South America, the larger Caribbean islands [31, 32], Egypt, India, Australia and Java [33]. The fresh juice of this plant is used to treat fresh wounds and along with vinegar, it is being used in treatment of septic wounds [34]. The plant possesses antimicrobial [35], antioxidant [36], antitumour [37], antiinflammatory [38] and wound healing activity [39]. The phytochemical constituents include anthraquinones, phenolics, alkaloids, flavonoids, sterols, anthocyanins, proteins, quinones, flavonoids, anthraquinones, carbohydrates [40, 41], stigmasterol, campesterol, β-sitosterol [42] and many phenalene compounds [43].

Fig 1: *Eichhornia crassipes*

Eichhornia crassipes is also reported to exhibit insecticidal activity. In Bangladesh, the dried whole plant of water hyacinth has been used to ward off insects in animal sheds [44]. The plant is reported to possess adult emergence inhibition activity on the rice moth *Corcyra cephalonica* [45], insect attractant property towards the maize weevil *Sitophilus zeamais* [46] and insecticidal activity against the larvae and pupae of the cotton leaf worm *Spodoptera littoralis* [47]. In addition, the petroleum ether extract of this plant has been shown to exhibit anti-juvenile hormone-like activity against the late fourth instar larvae and adult female mosquitoes of *Culex quinquefasciatus* [48]. The aqueous extract of this plant has revealed drastic effect on larval midgut and muscles of the second instar larvae of *Culex pipiens* [49]. Further, the ethanol fractionate of *Eichhornia crassipes* leaves was also reported to possess larvicidal and pupicidal activity against *Culex quinquefasciatus* [50]. Considering the above mentioned insecticidal activity particularly mosquitocidal, the present study was aimed to evaluate the larvicidal activity of *Eichhornia crassipes* crude leaf extracts against the filarial vector *Culex quinquefasciatus*.

2. Materials and Methods

2.1 Plant collection and extraction

Mature fresh plants of *Eichhornia crassipes* collected from Chembarambakkam lake, Kanchipuram district, Tamil Nadu, India (13.01158°N 80.06063°E) were brought to the laboratory. Taxonomical identity of the plant was confirmed at the Department of Plant Biology and Plant Biotechnology, Women's Christian College, Chennai, Tamil Nadu, India. The fresh and healthy leaves were isolated from the plant, washed with dechlorinated tap water and shade dried at room temperature. Dried leaves of *Eichhornia crassipes* were powdered with the aid of an electric blender. The powdered leaves (1 Kg) was sequentially extracted with 3 L of both non-polar and polar solvents *viz*., hexane, ethyl acetate, methanol, benzene and distilled water using a Soxhlet apparatus [51], and then evaporated to dryness in a rotary vacuum evaporator. The crude leaf extracts of *Eichhornia crassipes* (hexane, ethyl acetate, methanol, benzene and aqueous) thus obtained was refrigerated at 4 °C until testing for bioassay.

2.2 Test mosquitoes

Culex immatures collected from various places in Chennai, Tamil Nadu, India were transported to the laboratory in plastic containers. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. After emergence, the adult mosquitoes were identified upto species level and confirmed before rearing. Cyclic generations of *Culex quinquefasciatus* were maintained separately in two feet mosquito cages in an insectary with a mean room temperature of 27±2 °C and a relative humidity of 70-80%. The adult mosquitoes were fed on 10% glucose solution. The eggs laid were then transferred to enamel larval trays maintained in the larval rearing chamber. The larvae were fed with larval food (dog biscuits and yeast in the ratio 3:1). The larvae on becoming pupae were collected, transferred to plastic bowls and kept inside mosquito cage for adult emergence.

2.3 Larvicidal bioassay

Standard WHO [52] protocol with minor modifications was adopted for the study. The tests were conducted in glass beakers. *Culex quinquefasciatus* immature particularly early fourth instar larvae were obtained from laboratory colonized mosquitoes of F_1 generation. Larvicidal activity at test concentrations of 62.5, 125, 250 and 500 mg/L of the crude extract was assessed. The required test concentrations and quantity of test solution was prepared by serially diluting one per cent stock solution of the crude extract. Twenty healthy larvae were released into each 250 ml glass beaker containing 200 mL of water and test concentration. Mortality was observed for 48 hours after treatment. 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 acetone to distilled water. Distilled water served as untreated control. The larval per cent mortality was calculated and when control mortality ranged from 5-20% it was corrected using Abbott's formula [53].

2.4 Statistical analysis
Data from all replicates were pooled for analysis. LC_{50} and LC_{90} values were calculated using SPSS software by probit analysis [54]. One way ANOVA followed by Tukey's test was performed to determine the difference in larval mortality between concentrations. Results with $P<0.05$ level were considered to be statistically significant.

3. Results
Results of the larvicidal effects of crude leaf extracts of *Eichhornia crassipes* against *Culex quinquefasciatus* are presented in Table 1 and 2. No larval mortality was observed in treated and untreated control. The extracts showed a dose-dependent toxicity to *Culex quinquefasciatus* larvae. Amongst the crude extracts tested, the hexane and methanol were found to be effective at 62.5 and 500 mg/L at 48 hours (Table 1) and LC_{50} values were 80.54 and 135.70 mg/L respectively (Table 2).

Table 1: Larvicidal activity of *Eichhornia crassipes* crude leaf extracts against *Culex quinquefasciatus*

Solvents	Concentration (mg/L)			
	62.5	125	250	500
Hexane	54.0 ±2.4^{a}	54.9 ±3.7^{a}	58.1 ±2.0^{a}	62.2 ±1.5^{a}
Benzene	6.0 ±1.3^{a}	7.0 ±1.1^{a}	7.0 ±0.5^{a}	13.1 ±2.1^{a}
Ethyl acetate	23.0 ±1.3^{a}	29.0 ±1.5^{ab}	35.0 ±4.5^{b}	37.0 ±1.8^{b}
Methanol	34.1 ±4.4^{ab}	61.2 ±4.4^{ab}	68.5 ±2.7^{ab}	72.2 ±2.1^{b}
Aqueous	17.0 ±2.1^{a}	21.0 ±2.4^{a}	26.0 ±2.2^{a}	26.0 ±2.2^{a}

Values are mean (%) of three replicates of three trials ±standard deviation. Different superscript alphabets indicate statistical significant difference in larval mortality between concentrations at $P<0.05$ level by one way ANOVA followed by Tukey's test.

Table 2: Probit analysis of *Eichhornia crassipes* crude leaf extracts against *Culex quinquefasciatus*

Solvents	LC_{50} (mg/L)	95% confidence limit		LC_{90} (mg/L)	95% confidence limit	
		LL (mg/L)	UL (mg/L)		LL (mg/L)	UL (mg/L)
Hexane	80.54	27.68	146.20	1433.89	1116.66	2091.43
Benzene	1371.78	1072.51	2010.43	2398.85	1826.95	3627.57
Ethyl acetate	645.33	544.87	820.25	1744.50	1403.10	2364.79
Methanol	135.70	49.46	195.35	777.21	621.86	1098.90
Aqueous	983.18	779.47	1413.27	2297.08	1746.30	3479.24

LC_{50}: Lethal concentration that kills 50% of the exposed larvae; LC_{90}: Lethal concentration that kills 90% of the exposed larvae; LL: Lower limit; UL: Upper limit.

4. Discussion
Vector control is facing a serious risk due to the development of resistance in vector mosquitoes to conventional synthetic insecticides. There is an urgent need to develop new materials to control mosquitoes in an environmentally safe way, using biodegradable and target-specific insecticides against them [55]. Plants contain thousands of compounds which are virtually an untapped reservoir of new molecules having pesticidal potential [56]. Considerable interest has been shown in biologically active plant materials in mosquito control programmes because of their biodegradable nature. These phytochemicals have a major role in mosquito control programmes and they can be derived from any part of the plant (root, bark, stem, leaf, flower, fruit and seed) [57]. Samuel *et al*. [25] have pointed out that a considerable number of plant derivatives exhibited mosquitocidal activity in a safe manner, and the screening of plants for mosquito larvicidal activity may eventually lead to their use in natural product based mosquito abatement practices. Kumuda *et al*. [58] also emphasized that the purpose of a general screening for bioactivity is to extract as many potentially active constituents as possible. This is achieved by using solvents ranging from water with a polarity index (P) (10.2) to hexane (0.1) including a number of intermediary solvents *viz*., methanol (6.1), acetone (5.1), ethyl acetate (4.4) and chloroform (4.1). Scrutiny of literature reveals that not much work has been done using hydrophytes as larvicides against mosquitoes. However, some Indian coastal plants were screened for the larvicidal activity against *Culex quinquefasciatus* [59]. Recently, Vijayakumar *et al*. [60] tested the ethanolic extracts of sea grass *viz*., *Halodule pinifolia* roots and *Cymodocea serrulata* and *Thalasia testudinum* leaves for larvicidal activity against *Aedes aegypti* and found *Halodule pinifolia* to show promising results.

The present study showed that crude hexane and methanolic leaf extracts of *Eichhornia crassipes* was found to be the most effective and LC_{50} values were 80.54 and 135.70 mg/L. Results of the present study were comparable with earlier reports of larvicidal activity against *Culex quinquefasciatus*. Baranidharan and Dhanasekaran [61, 62] reported larvicidal activity of ethyl acetate followed by hexane, chloroform and acetone extracts of *Commiphora caudata* and *Ageratina adenophora* and their LC_{50} values were 94.76, 102.95, 95.98 and 105.09 mg/L, and 136.75, 145.69, 139.49 and 143.64 mg/L respectively. Raveen *et al*. [14] reported the hexane flower extract of *Nerium oleander* to exhibit larvicidal activity with LC_{50} value of 102.54 and 61.11 ppm after 24 and 48 hours respectively. Sakthivadivel *et al*. [63] reported *Hyptis suaveolens* aerial extracts to exhibit larvicidal activity. Amongst the solvent extracts, acetone exhibited highest larvicidal activity and LC_{50} value was 485.61 followed by petroleum ether and chloroform extract whose LC_{50} values were 493.44 and 625.97 mg/L after 24 hours whereas for 48 hours, petroleum ether extract (LC_{50} 298.76 mg/L) was found to exhibit highest larvicidal activity followed by acetone (LC_{50} 344.03 mg/L) and chloroform extract (LC_{50} 429.50 mg/L). Raveen *et al*. [28] reported that the crude chloroform flower extract of *Jasminum grandiflorum* was found to be effective showing 100% mortality at 500 mg/L and LC_{50} value was 212.10 mg/L after 48 hours exposure. The methanol extract of *Ocimum sanctum* leaves exhibited larvicidal activity with LC_{50}

value of 101.32 mg/L [64]. Jayanthi *et al*. [50] reported that the ethanol fractionate of *Eichhornia crassipes* showed the highest larvicidal activity against *Culex quinquefasciatus* when compared to other solvent extracts, since it contained phytochemicals *viz*., alkaloids, flavonoids, anthroquinones, anthocyanins and quinines [41]. In line with the above results of Lalitha *et al*. [41], the present study with *Eichhornia crassipes* leaves revealed that the hexane and methanol extracts exhibited larvicidal activity against *Culex quinquefasciatus*. This might be due to the synergistic or additive effect of the phytochemicals present in the crude extracts since hexane, ethyl acetate, methanol and benzene extracts of water hyacinth contains carbohydrates, tannins, flavonoids, quinones, phenols and steroids compounds [41]. Further, anthraquinone class of compounds has been reported to exhibit larvicidal activity [65] and sterols, in particular β-sitosterol, have been reported to possess larvicidal activity [66].

Mosquito control is vital for many countries and is still in a state of evolution. During the last decades, it depended upon synthetic organic insecticides, many of which have been removed from the arsenal of weapons [67] and botanicals are the new weapons of mosquito control under exploration. Natural pesticides derived from plants are a promising tool especially for targeting mosquitoes in their larval stage [68]. Vector control is one of the most powerful weapons in the process of managing vector populations to reduce/ interrupt the transmission of disease. As a result, vector control remains considered to be a cornerstone in the vector-borne disease control programme due to a lack of reliable vaccines [69]. The use of natural products may be considered an important alternative insecticide for the control of vector-borne diseases since they constitute a rich source of bioactive compounds that are biodegradable, nontoxic and potentially suitable for use in integrated mosquito management programmes. In conclusion, the present study has shown that the hexane and methanolic leaf extracts of *Eichhornia crassipes* has a promising effect to control *Culex quinquefasciatus* larvae. The results could be useful in search for newer, safer and more effective natural compounds as larvicides.

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