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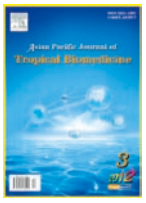

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Larvicidal activity of plant extracts on *Aedes Aegypti* L.

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ABSTRACT

Objective: To evaluate the larvicidal activity of plant extracts on *Aedes aegypti*. **Methods:** Petroleum ether, Chloroform and aqueous extracts obtained from *Acalypha indica, Aerva lanata, Boerhaavia diffusa, Commelina benghalensis, Gompherna sps, Datura stramonium, Euphorpia hirta, Cynodon dactylon, Lantana camara* and *Tridax procumbens* were used for larvicidal activity at concentration of 1000 μg/ml and the mortality rate was calculated after 24 and 48hrs . The LC_{50} for the extracts were also estimated after 24 hrs. **Results:** The petroleum ether extract of *Lantana camara, Tridax procumbens* and Datura stramonium showed 100% mortality after 48hrs of incubation. *Tridax procumbens* petroleum ether extract had the least LC_{50} of 219 μg/ml followed by *Lantana* and *Datura* with 251and 288 μg/ml respectively. A combination of petroleum ether extracts of *Aerva lanata* and *Cynodon dactylon, Boerhaavia diffusa* and *Commelina benghalensis* exhibited 100% mortality of larvae. Formulation-1 inhibited the metamorphosis of the larvae by retaining 60% in its larval stage. Petroleum ether extracts of *Lantana, Tridax, Datura* and a combination of extracts were effective larvicide. The formulations proved to be effective in inhibiting the metamorphosis. Alkaloids and flavonoids were present in datura petroleum ether extract . **Conclusions:** Either the crude extracts of *Datura stramonium, Lantana camara* and *Tridax procumbens* or its phytochemicals can be used as effective vector control agents individually or in combination.

1. Introduction

Dengue fever is considered as a serious public health problem in the world, mainly in tropical countries where the favorable environmental conditions are responsible for the proliferation of vectors *Aedes aegypti*. Among the arbovirus in India, distribution of all the dengue virus type is continuously expanding. Remarkably the reemergence of Chikungunya virus (CHIK) since 2005 is posing an additional concurrent diseases burden in the country. Both these virus are born by the mosquito *A. aegypti* (L) (Diptera: Culicidae)[1,2] .Since *Aedes aegypti* is a fresh water breeding mosquito it is very difficult to control it during rainy season .Approximately 2500 million people, two fifths of the world's population, are now at risk from Dengue fever. The WHO currently estimates there may be 50 million cases of Dengue fever infection worldwide every year [3]. Vaccine development is still at an early stage and therefore the only method available for reducing incidence of the disease is the control of its mosquito vector. One of the approaches for control of these mosquito borne diseases is the interruption of disease transmission, either killing, preventing mosquitoes to biting human beings or by causing larval mortality in a large scale at breeding centers of the vectors [4].

Current strategies based on the elimination of breeding sites and applications of chemical insecticides for larval and adult mosquito control have resulted in development of resistance without eliminating the constant risk of dengue epidemics [5]. Thus new approaches are urgently needed. Interest on possible use of environment friendly natural products such as extracts of plants or plant parts increased for vector control. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticide properties [6,7,8,9].Hence the objective of the present study was to evaluate the larvicidal activity of plant extracts and to evaluate the formulation prepared using basic raw materials.

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2. Materials and methods

2.1 Collection of plant material

The leaves of *Acalypha indica*, *Aerva lanata*, *Boerhaavia diffusa*, *Commelina benghalensis*, *Gompherna sps*, *Datura stramonium*, *Euphorbia hirta*, *Cynodon dactylon*, *Lantana camara and Tridax procumbens* were collected washed thoroughly , blotted and shade dried.

2.2 Extraction

About 10 gm of dry sample of each plant was macerated with sterile water, chloroform and petroleum ether and left to stand at room temperature for 48hrs. Then mixture was filtered through a Whatman no.1 filter paper by suction. Filtrate was evaporated under vacuum for 40°C until completely dried.

2.3 Raring of Aedes aegypti larvae

The eggs of *A. aegypti* were procured from the Central Research Medical Entomology Institute at Madurai, Tamilnadu, India. The egg rafts of *A. aegypti* were kept in the tray containing tap water (culture medium) at laboratory condition (29±1°C). after 24 hrs of incubation, the eggs were observed to hatch out into first instar larvae. Appropriate amount of nutrient (sterilized yeast powder and dog biscuit in 1 :1 ratio) were added to enhance the growth of larvae. The 4th instar larvae was used in the study.

2.4 Larvicidal bioassay

The plant extracts were dissolved in 10μl of DMSO for its solubility in water. Larvicidal activity was determined according to WHO protocol [10]. The larvae were treated with the plant extracts of 1000μg/ml concentration . A corresponding control was maintained. The larval mortality of fourth instar of *A. aegypti* was observed. The number of larvae surviving at the end of 24 and 48 hours were recorded and the percent mortality was calculated.

The percentage of mortality was calculated by

(No. of larva dead /No. of larvae)*100

corrected mortality was accounted for by [11]. The extract which showed 80%-60% mortality were combined to check the larvicidal activity by similar procedure as mentioned above.

2.5 Lethal Concentration

The LC_{50} of the plant extract that showed 100% mortality was determined by a similar procedure as mentioned above. 100, 250, 500, and 750 μg/ml concentration were tested and the observation was recorded after 24 hrs of incubation. The LC_{50} was determined by a Probit analysis [12].

2.6 Phytochemical analysis

The plant extracts that showed 100 % mortality of *A.aegypti* larvae were screened for the phytochemicals present according to [13].

2.7 Formulation

Two formulations were prepared by taking individual and combination of plant extracts which shows 100% mortality was designated as a formulation1 (Petroleum ether extracts of *Tridax*, *Datura* and *Lantana*) & formulation 2 (*Aerva lanata* and *Cynodon dactylon*, *Boerhaavia diffusa* and *Commelina benghalensis*) respectively. The sterilized raw materials were saw dusk, Hay powder, starch and filter sterilized plant extract (1mg/ml). The saw dusk and hay powder were mixed in 1:1 ratio and starch was added as binder and mixed thoroughly along with the extract. The Pellets obtained were air dried. About 20ml of water was taken in a beaker and added 3 pellets of formulation 1 and formulation 2 in to it. Twenty 4th instar larvae of *A.aegypti* were introduced into the beakers with formulation . A corresponding control was also maintained. Observations were recorded after 72 hrs of incubation.

3. Results

3.1 Larvicidal Activity

Petroleum ether, chloroform and aqueous extract of *Acalypha indica*, *Aerva lanata*, *Boerhaavia diffusa*, *Commelina benghalensis*, *Gompherna sps*, *Datura stramonium*, *Euphorbia hitra*, *Cynodon dactylon*, *Lantana camara*, *Tridax procumbens* were screened for larvicidal activity. It was found that petroleum ether extract was highly toxic to the 4th instar larvae *Aedes aegypti*. Among the petroleum ether extract *Lantana camara*, *Tridax procumbens* and *Datura stramonium* shows 100% mortality after 48hrs of incubation.While in 24hrs incubation mortality % was 80,60,100 respectively. *Euphorbia hitra*, *Cynodon dactylon*, *Aerva lanata*, *Gompherna sps*, has no effect on larvae (Table-1). The mosquito larvae exposed to plant extracts showed significant behavioral changes. The most obvious sign of behavioral changes observed in *Aedes aegypti* was restlessness, loss of equilibrium which finally led to death. Chloroform extract of *Euphorbia hirta* exhibited 78.94% mortality. While *Datura stramonium* and other extracts had 15.78% mortality after 48hrs of incubation. In aqueous extract *Lantana camara* showed 100% mortality after 24hrs of incubation. All other plant extracts had no effect on the larvae.

Table 1

Larvicidal activity of plant extracts at different time intervals.

All values in triplicate .*Corrected mortality calculated by the formula of Abbott (1925).

Plants	Petroleum Ether Extract		Chloroform Extract		Aqueous Extract	
	% Mortality *at 1000 μ g/ml concentration					
	24 Hrs	48 Hrs	24 Hrs	48 Hrs	24 Hrs	48 Hrs
Acalypha indica	60%	80%	15.78%	40%	Nil	Nil
Aerva lanata	20%	60%	15.78%	15.78%	20%	20%
Boerhaavia diffusa	60%	60%	Nil	Nil	Nil	Nil
Commelina benghalensis	80%	80%	11.11%	11.11%	Nil	Nil
Gompherna sps	20%	60%	Nil	Nil	Nil	Nil
Datura stramonium	100%	–	15.78%	15.78%	100%	–
Euphorpia hirta	Nil	Nil	15.78%	78.94%	20%	20%
Cynodon dactylon	20%	40%	20%	36.84%	Nil	Nil
Lantana camara	100%	–	36.89%	40%	100%	–
Tridax procumbens	60%	100%	15.78%	15.78%	Nil	Nil

3.2 Larvicidal Activity Of Plant Extract In Combination

The petroleum ether extracts of plants which had around 60 and 80% mortality were selected in random combination. *Aerva lanata* and *Cynodon dactylon*, *Boerhaavia diffusa* and *Commelina benghalensis exhibited* 100% *mortality*.

3.3 Lethal concentration

The 50% lethal concentration of *Lantana camara* petroleum ether extract was between 251 μ g/ml and *Datura stramonium* had 288 μ g/ml. LC_{50} for *Tridax procumbens* was less than 220 μ g/ml. (Table–2).

Table 2

Lethal concentration of plants extracts after 24 hrs

* According To Probit Analysis (Finney, 1971). % Mortality = mean ± SD

Plant Extracts	Concentration (mg/ml)	% mortality	LC_{50} (mg/ml)*
Commelina benghalensis	0.750	73.3±2.88	0.302
& *Boerhaavia diffusa*	0.500	70 ±5	
(1:1)	0.250	56.6±2.88	
	0.100	36.6±2.88	
Cynodon dactylon&	0.750	70±5	0.363
Aerva lanata (1:1)	0.500	68.3±7.63	
	0.250	56.6±2.88	
	0.100	43.3±2.88	
Datura stramonium	0.750	81.6±2.88	0.288
	0.500	70±5	
	0.250	60±5	
	0.100	43.3±2.88	
Lantana camara	0.750	83.3±2.88	0.251
	0.500	73.3±2.88	
	0.250	50±5	
	0.100	35±5	
Tridax procumbens	0.750	78.3±2.88	0.219
	0.500	71.6±2.88	
	0.250	60±5	
	0.100	48.3±7.63	

3.4 Phytochemical Test

Phytochemical analysis indicated the presence or absences metabolites present in extract. Petroleum ether extract was effective on larvae due to the presence of specific plant secretion. Phytochemical test of plants extract which showed 100% mortality revealed that Lantana petroleum ether extract contained phenol, flavonoid, resin, phlobatannin and saponins. While Tridax contained Terpeniods , Anthraquinone and saponins and Datura had Alkaloids and Flavonoids. (Table– 3)

Table 3

Phytochemical analysis of plant extracts

Phytochemicals	*Datura stramonium*	*Tridax procumbens*	Lantana camara
Alkaloid	+	–	+
Phenol	–	–	+
Flavonoid	+	–	+
Tannin	–	–	–
Terpenoid	–	+	–
Gum	–	–	–
Anthraquinone	–	+	–
Phlobatannin	–	–	+
Resin	–	–	+
Saponin	–	+	+

+/– indicates presence or absence.

3.5 Formulation

The two formulation were used in the study and larvicidal efficiency was observed. Growth retardation and delay in the development of larvae to the pupal stage was observed at 72 hrs of incubation. This may due to chemical compounds present in the extract that prevented normal pupation and adult emergence from occurring. When treated with formulation 1, only 15 % of adults, 25 % of pupa and 60% of them remained as larvae even after 72 hrs of treatment. It is very evident that inhibition in the metamorphosis from the larval to pupal stage took place. Moreover the feeding

ability of the larvae was reduced greatly and exhibited slow movements. In formulation- 2, 10 % of the larvae had developed into adults, while 50 % in its pupal stage and 40 % were in its larval stage. Theses studies have shown clearly that formulation 1 inhibited the developmental stages of *A.aegypti* very effectively than formulation 2 (Fig-1). It is probably due to the synergistic effect of phytochemical constituent of the extracts.

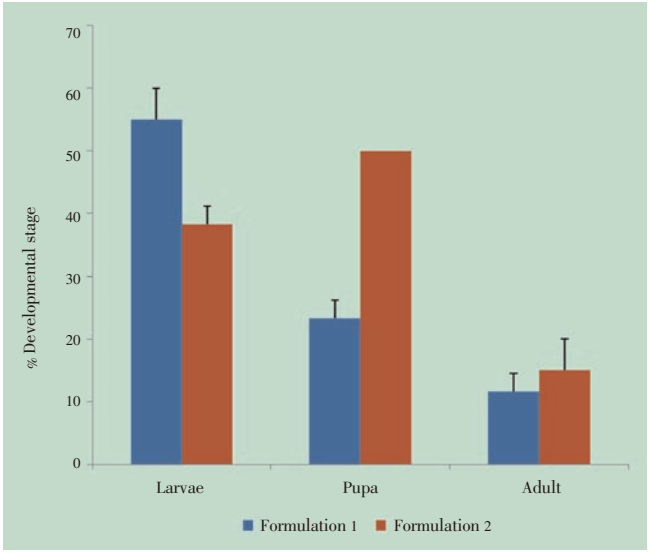

Fig 1. Effect of formulations on the metamorphosis of A.aegypti larvae Formulation 1 (Petroleum ether extracts of Tridax, Datura and Lantana 1:1 ratio), formulation-2 (Petroleum ether extracts of *Aerva lanata* and *Cynodon dactylon*, Boerhaavia diffusa and Commelina benghalensis in 1:1 ratio). All values mean of trplicates ± Standard deviation.

4. Discussion

Secondary metabolites produced in plants for its protection against microorganisms and predator insects are natural candidates for the discovery of new products to combat *A. aegypti*. Several studies have focused on natural products for controlling Aedes mosquitoes as insecticides and larvicides, but with varied results [14,15,16,17,18,19].

In the present study the petroleum ether extracts of *Lantana camara*, *Tridax procumbens* and *Datura stramonium* shows 100% mortality after 48hrs of incubation. Moreover behavioural changes were observed in the movement of the larvae. These effects may be due the presence of neurotoxin compounds in plant extracts. No behavioral changes were obtained in control group. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, repellent and ovipositor attractant and have different activities observed by many researchers [20]. However, insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent, against insect vectors of public health importance.

LC_{50} of Ageratina adenophora on *Aedes aegypti* was found to 50 μg/ml [4]. Similar results was obtained for *Datura stramonium* petroleum ether extract. It is concluded that Lantana camara, *Tridax procumbens* and *Datura stramonium* showed toxic effect on *Aedes aegypti* and also combination of extracts were also effective and can be used for control of mosquito larvae.

Crude extracts or isolated bioactive phytochemicals from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. However, further studies on the identification of the active principals involved and their mode of action and field trials are usually needed to recommend any of these plant materials as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

Plant could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further analysis is required to isolate the active principles and its mode of action in inhibiting the developmental stages in *Aedes aegypti*. The phytochemicals of Lantana, Datura and Tridax extracts can be well utilized for preparing biocides or insecticidal formulation.

Conflict of interest statement

We declare that we have no conflict of interest

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