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Phytochemical constituents and larvicidal activity of *Tragia involucrata* Linn. (Euphorbiaceae) leaf extracts against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae)

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ABSTRACT

Objective: To determine the phytochemical and larvicidal activity of *Tragia involucrata* (*T. involucrata*) leaf extracts against fourth instar larvae of *Ae. aegypti*.

Methods: Phytochemical analysis of hexane leaf extract was performed and larvicidal activity was determined against *Ae. aegypti* at concentrations of 50, 100, 150, 200 and 250 mg/L. Larval mortality was assessed after 24 h.

Results: The hexane extracts of *T. involucrata* was found to be higher mortality against the larvae of *Ae. aegypti* with a LC₅₀ value of 153.51 mg/L.

Conclusions: These results suggested that the leaf extracts of *T. involucrata* showed potential to be used as an ideal ecofriendly approach for the control of the *Ae. aegypti*.

1. Introduction

Mosquitoes are important pests because their biting activity often interferes with outdoor activities and can transmit disease organisms to people and domestic animals. Mosquitoes are the vectors for the dreadful diseases of mankind. Of all the insects that transmit diseases, mosquitoes represent the greatest menace. Mosquito control, in view of their medical importance, assumes global importance. Under the context of ever increasing trend to use more powerful synthetic insecticides to achieve immediate results in the control of mosquitoes, an alarming increase of physiological resistance in the vectors, its increased toxicity to non-target organism and high costs are noteworthy. Chikungunya is considered as a serious menace in southern India because it offers ambient environment for the proliferation of its vector *Aedes aegypti* (*Ae. aegypti*) (Diptera: Culicidae)[1,2]. Since *Ae. aegypti* breeds in fresh water it is cumbersome to control it during rainy season. It has been estimated that about 2500 million people are now at risk from Dengue fever[3].

Present scenario on the avoidance of breeding sites and synthetic insecticidal applications to combat with various forms such as larval and adult mosquito, have resulted in development of resistance without eliminating the risk of dengue outbreak[4,5]. Thus, this has necessitated the exploration of natural products for the control of vector insects in general and mosquitoes in particular[6-9].

Genus, *Tragia*, comprises of nearly hundred species belongs to the family Euphorbiaceae distributed throughout the tropical and subtropical part of the world. Plant extractives of *Tragia involucrata* (*T. involucrata*) have been used as ethnomedicine since prehistoric times and in India their use in "Sidhha" medicine by Tamil people is wide spread. *T. involucrata* root extractives are proven to act as potent antimicrobial compounds and a useful fungicide as well[10]. As per our literature survey was concerned, no information was available on the larvicidal activity against *Ae. aegypti*. Hence the investigation was to aimed to evaluate the larvicidal potential of selected plant extracts against fourth instar larvae of *Ae. aegypti*.

2. Materials and methods

2.1. Vector rearing

The mosquito larvae and pupae of selected species were collected from nearby water bodies in and around Musiri taluk,

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Tiruchirappalli District, Tamil Nadu, India and maintained in cages of dimension, $40\text{ cm}^3 \times 60\text{ cm}^3 \times 40\text{ cm}^3$ at ambient conditions [$(27 \pm 1)^\circ\text{C}$, $(75 \pm 2)\%$ relative humidity and 12 h light and 12 h dark photoperiod] in the laboratory. Yeast suspension (10% w/v) was served as food source for larval stages. Adult females were fed with chick blood and males with sucrose solution (10% w/v) soaked in cotton pads. The eggs were washed with 0.01% formaldehyde solution for 30–40 min as recommended by AI-Mashhadani[11]. This is imminent for the normal development of the further stages of mosquito[12].

2.2. Collection of plant material

The selected plant leaves were collected during growing season month of September 2013 from Thirunarayanapuram village, Thottiyam Taluk, Trichirappalli District, Tamil Nadu, India. Plant was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Sytematics, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India and The Voucher specimen (IPH 11) was deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

2.3. Extraction method

The dried leaves (100 g) were powdered mechanically by using commercial electrical stainless steel blender and extracted sequentially with hexane, chloroform, and ethyl acetate (500 mL, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22–26 mmHg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C in an amber vial. Then the vials were named and covered with silver foil and transported to the laboratory. Those vials were kept in cool and dark place at 4°C until use.

2.4. Larvicidal bioassay

The larvicidal activity of selected plant extracts was done as prescribed by WHO[13]. Based on the wide range and narrow range tests, all extracts tested ranging 50–250 mg/L were prepared and they were tested against the freshly moulted (0–6 h) third instar larvae of selected mosquito species. The plants extracts were dissolved in 2 drop of Tween 20 and then diluted in 100 mL of dechlorinated tap water to obtain each of the desired concentrations. The control was prepared using 2 drop of Tween 20 in 100 mL of dechlorinated water. Ten larvae of test species were introduced in 250 mL plastic cups containing 100 mL of aqueous medium (100 mL of dechlorinated and 2 drop of Tween 20) and the required amount of chemical compositions was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at one time. The LC_{50} value was calculated by using probit analysis[14]. The mean larval mortality values were depicted in probit analysis for calculating LC_{50} , LC_{90} and other statistics *Chi*-square values were calculated by using SPSS version 18.0 for windows, significance level was set at $P \leq 0.05$.

2.5. Phytochemical analysis

Since the hexane crude extract induced significant larval it was subjected to screen for the phytochemical analyses according to Harborne[15].

3. Results

The preliminary screening is a good means to the evaluation of the potential larvicidal activity of plant popularly used for this purpose. The effect of *T. involucrata* leaf extracts of hexane, chloroform, and ethyl acetate were tested at 50, 100, 150, 200, 250 mg/L and showed activity against the fourth instar larvae of *Ae. aegypti* (Table 1). The plant extracts showed promising larvicidal effects after 24 h; however, the highest larval mortality was found in hexane extracts of *T. involucrata* against the fourth instar larvae of *Ae. aegypti* ($\text{LC}_{50} = 153.51\text{ mg/L}$, $\text{LCL} = 135.42\text{ mg/L}$, $\text{UCL} = 174.03\text{ mg/L}$, $\text{LC}_{95} = 884.35\text{ mg/L}$, $\text{LCL} = 564.85\text{ mg/L}$, $\text{UCL} = 1384.57\text{ mg/L}$) followed by chloroform and ethyl acetate ($\text{LC}_{50} = 233.26\text{ mg/L}$, $\text{LCL} = 176.10\text{ mg/L}$, $\text{UCL} = 308.99\text{ mg/L}$, $\text{LC}_{95} = 4273.58\text{ mg/L}$, $\text{LCL} = 1168.52\text{ mg/L}$, $\text{UCL} = 1562.52\text{ mg/L}$ and $\text{LC}_{50} = 190.17\text{ mg/L}$, $\text{LCL} = 159.87\text{ mg/L}$, $\text{UCL} = 226.22\text{ mg/L}$, $\text{LC}_{95} = 1626.31\text{ mg/L}$, $\text{LCL} = 801.61\text{ mg/L}$, $\text{UCL} = 3299.44\text{ mg/L}$, respectively).

Table 1
Larvicidal activity of *T. involucrata* against 4th instar larvae of *Ae. aegypti*.

Concentration (mg/L)	Mortality (%)	LC_{50} (mg/L)	95% Confidence limit (mg/L)		LC_{95} (mg/L)	95% Confidence limit (mg/L)		df	χ^2 value	
			LCL	UCL		LCL	UCL			
Hexane extract	Control	0.0 ± 0.0 ^a								
	50	17.6 ± 1.5 ^b								
	100	32.6 ± 2.7 ^c								
	150	41.6 ± 2.4 ^d	153.51	135.42	174.03	884.35	564.85	1384.57	4	4.171
	200	60.4 ± 1.1 ^e								
Chloroform extract	Control	0.0 ± 0.0 ^a								
	50	21.4 ± 1.6 ^b								
	100	30.4 ± 1.1 ^c								
	150	36.2 ± 1.3 ^d	233.26	176.10	308.99	4273.58	1168.52	1562.52	4	3.113
	200	43.2 ± 2.1 ^e								
Ethyl acetate extract	Control	0.0 ± 0.0 ^a								
	50	18.6 ± 2.4 ^b								
	100	26.2 ± 2.2 ^c								
	150	40.0 ± 2.9 ^d	190.17	159.87	226.22	1626.31	801.61	3299.44	4	2.722
	200	53.2 ± 2.8 ^e								
250	60.8 ± 1.6 ^f									

Value represents mean ± SD of five replications. Values in the column with a different superscript alphabet are significantly different at $P < 0.05$ level by DMRT test.

Phytochemical analyses revealed the presence or absence of certain plant secondary metabolites, which are shown in Table 2.

Table 2
Phytochemicals screenings of *T. involucrata* hexane leaf extract.

S. No.	Phytochemicals	Hexane extract
1	Steroids	+
2	Triterpenoides	+
3	Glycosides	+
4	Carbohydrates	+
5	Alkaloids	+
6	Phenolic compound	-
7	Catachins	-
8	Flavonoids	+
9	Saponins	-
10	Tannins	-

+: Presence; -: Absence.

4. Discussion

Reducing mosquito borne diseases remains a big challenge even at the most advancement of modern sciences. Personal protection from mosquito bites through several synthetic and herbal products are

often practiced. But synthetics are mostly non biodegradable with having harmful residual hazards as well as costly[16].

The results obtained in the present study are in accordance with the earlier works of Bhattacharya *et al.* and Rahuman *et al.*[17,18]. Jeyasankar *et al.* who have been reported that ethyl acetate extracts of *Phyllanthus emblica* showed highest larval mortality against *Ae. Aegypti* and *Culex quinquefasciatus* with LC₅₀ = 80.04 mg/L, LC₉₀ = 323.53 mg/L and LC₅₀ = 78.89 mg/L, LC₉₀ = 502.10 mg/L, respectively[19]. Hexane and diethyl ether extracts was found in leaf extracts against *Ae. Aegypti* and *Culex quinquefasciatus* with LC₅₀ = 111.34 mg/L, LC₉₀ = 617.50 mg/L, LC₅₀ = 136.78 mg/L, C₉₀ = 939.01 mg/L, LC₅₀ = 114.77 mg/L, LC₉₀ = 333.50 mg/L, LC₅₀ = 82.65 mg/L and LC₉₀ = 206.65 mg/L, respectively. Venketachalam *et al.* have also reported that the repellent activity of methanol extract of *Ferronia elephantum* leaves against *Ae. Aegypti* and *Zingiber officinale* against *Culex tritaeniorhynchus* and *Anopheles subpictus*[20,21].

Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, and repellent and ovipositor attractant and have different activities observed by many researchers[22]. The findings of the present investigation revealed that the leaf hexane extracts of *T. involucrata* posses larvicidal activity against vector mosquitoes. It may conclude that natural products as extracts from parts of plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their larvicidal toxicity. Further studies on the screening, isolation and purification of bioactive phytochemical constituents or compounds followed by in-depth laboratory and field bioassays are needed as the present study which shows that there is scope to use *T. involucrata* leaf extracts to control the immature stages of vector mosquitoes. In conclusion, an attempt has been made to evaluate the role of *T. involucrata* against an alternative approach to combat with the important human vector mosquitoes.

Conflict of interest statement

We declare that we have no conflict of interest.

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