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Larvicidal Activity and Molecular Effect of Stem/Leaf Extracts of *Opuntia dillenii* Against Dengue Vector *Aedes aegypti*

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ABSTRACT

The aim of the study was to investigate the effect of Opuntia delleni leaves on Dengue Vector Aedes aegypti. Larvae on molecular DNA extraction. The plant Opuntia delleni Linn (Verbenaceae) is described in Ayurveda and Siddha as a potent drug for a variety of ailments. The plant was collected, authenticated and extracted using hexane, chloroform, ethylacetate and ethanol solvent. The phytochemical, physico chemical and TLC studies were done and further larvicidal activity was performed. The results of the chloroform extract of Opuntia delleni showed the presence of terpenoids, flavanoids and glycoside. TLC studies showed the results of 7 spots with Rf value range from 0.06-0.96 in the mobile phase Chloroform: Ethanol (7:3). Opuntia dellen is extracts showed potent larvicidal activity. The LC50 value for 24 hr observation was very high indicating potent activity. Among the extracts, methanol showed comparatively better activity. The LC50 value of chloroform was 6 lesser than other extract against A. Aegypti. The toxic effects resulting in the mortality manifested differently on exposure to the extracts at different times. However, more than 80% mortality was observed only at higher concentrations. The larvae was subjected for DNA isolation by standard procedure and run in Agarose Gel Electrophoresis and observed in UV light. The DNA fragment pattern was observed for chloroform extract of Opuntia Delleni which showed potent effect on Larvae Aedes aegypti. Thus the molecular studies on Dengue vector Larvae Aedes aegypti was potent with chloroform extract of Opuntia delleni leaves.

Keywords: *Opuntia dillenii*, Dengue vector *Aedes aegypti*, Larvicidal, Gel Electrophoresis

INTRODUCTION

Mosquitoes are one of the most medicinally significant vectors and they transmit parasites pathogens which continue to have devastating impact on human beings. Several numbers of species belong to genera *Anopheles, Culex, Aedes* and vectors for the pathogens of various diseases like malaria, filarial, Japanese encephalitis, dengue and yellow fever. Thus, one of the approaches for control of these is interruption of disease transmission by killing mosquitoes or preventing mosquito bites [1].

In recent years, the top priority in finding a new insecticide is that, they must be of plant origin and should not have any ill effects on the ecosystem. Researchers have proved the effectiveness of plant derived secondary compounds, such as saponin, steroids, Isoflavonoids, essential oil, alkaloids and tannins, as mosquito larvicides. Plant compounds and their essential oils provide alternative source of mosquito repellents agents [2].

However, the use of synthetic larvicides imposes threats not only to human health but also to the ecosystem because when they are applied into the environment; they may stay on for a very long time or even remain there without end. Synthetic larvicides also disrupt natural Biological control systems that sometimes results into a widespread development of resistance. This phenomenon has triggered and urged the development of alternative techniques using Natural products. Current research trends use plant extracts as alternative larvicides because they contain various phytochemicals that are specific in killing mosquito larvae without harming other International Research Journal of Biological Sciences. Instead of using synthetic larvicides, the use of these plant-derived products in controlling mosquito larvae is inexpensive and Environment-friendly [3]. *Opuntia dillenii* (Ker Gawl) an important medicinal plant with several Medicinal uses in traditional medication system belongs to the family Cactaceae. The present study was design to determine the larvicidal activity of the stem/leaf extracts *of Opuntia dillenii* (Ker Gawl) and Measure the DNA by gel electrophoresis.

MATERIALS AND METHODS

Preparation of extract

The plant *Opuntia dillenii* was collected from the rural part in Kancheepuram District, Tamilnadu. The collected plant parts (Leafs) were washed in running water, cut into small pieces, shade dried, coarsely powdered and used for extraction. The solvents used are Hexane, Chloroform (100%), Ethylacetate (100%), ethanol (100%) and extracted through soxhlet apparatus. The different extracts obtained were stored and tested for preliminary phytochemical studies and physicochemical constant as per Indian Pharmacopoeia. The Extracts were studied for thin layer chromatography.

Thin layer chromatography

Procedure

The solvent system is taken in the T.L.C chamber and kept for saturation. The extracts of crude drug were spotted in the pre-coated TLC plate and placed inside the chamber. After development of the spots the plate is taken out, then dried in hot-air oven and observed in UV chamber to detect the number of spots developed. The Stationary phase used was pre-coated TLC plate, Mobile phase: Chloroform: Ethanol, Detecting Reagent: UV visible light and iodine vapour. The Rf value was taken with colour obtained Bluish green, Yellow, fluorescence, Orange colour spots [5].

Larvicidal activity

Test organisms: The larvae of the mosquito species *Aedes aegypti* were collected from the aquatic environments of Chennai District. Biscuits were served as larval food. The larvae were kept at 25 ± 2ºC and proper photoperiod was given for their growth. Late third and early fourth instars larvae were (4-5 mm in length) used for larval bioassay purpose. The

morphological and anatomical characteristics of the collected larvae were observed and identified through microscopic analysis and by comparing to the standard keys [6-8].

Bioassay: Larvicidal bioassay was performed based on WHO protocol. *A. aegypti* mosquito larvae were exposed to a wide range of concentration of crude extracts i.e. 10, 25, 50, 100 and 200 µg/ml and control to find out activity. Batches of 25 healthy fourth instar larvae were transferred to the 250 ml water containing chambers and different concentrations of three non-polar and polar extracts of crude extracts were added to assess the desired target dosage. Three replicates were performed for each concentrations and equal number of controls were also setup with tap water. Larval mortality was observed after 24 h. Mortality percentage was calculated using Abbott's formula. LC_{50} and LC_{90} values were calculated using Probit analysis

$$\text{Mortality}(\%)=\frac{\text{X-Y}}{\text{X}}\times 100$$

Where, X= Survival in the untreated control. Y= Survival in treated sample.

Dose-response bioassay

The crude extracts were subjected to dose-response bioassay for larvicidal activity against the larvae of *A. aegypti*. The number of dead larvae was counted after 24 h and the selected samples turned out to be equal in their toxic potential.

Statistical analysis

The larvicidal activity of plant extracts were expressed in terms of lethal concentrations (LC_{50} and LC_{90}) of the average of larval mortality data that were subjected to Probit analysis (Finney, 1971) 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 using the software type, SPSS (2007). Results with $p<0.05$ were considered to be statistically significant [9,10].

Isolation of Genomic DNA

The 24 h dead larvae 1.5 ml was added in to an eppendorf tube and centrifuged at 10000 rpm for 10 minutes. Then pellet was collected and resuspended in to 450 µl of TE buffer (vortex mixer).To that pellet 5 µl of lysozyme and 50 µl of 10% SDS (sodium dodecyl sulphate) was added and incubated at 37ºC for 1 hours with regular interval. After incubation, equal volume of phenol; chloroform (1:1) was added and mixed well by inverting the tubes and centrifuged at 10000 rpm for 10 minutes. The aqueous phase was transferred to fresh tube and phenol: chloroform extraction step was repeated. The supernatant was transferred to a fresh tube without disturbing the bottom layer. To the aqueous solution 50 µl of 3M NacH3 was added and 300 µl of isopropanol was added to precipitate the DNA. Then centrifuged at 1000 rpm for 10 minutes and the supernatant were discarded. Pellets were washed with 70% ethanol and centrifuged at 8000rpm for 1-2 minutes.Ethanol was discarded and evaporated the excess of ethanol without losing DNA.Then dissolved it with 50 µl of TE buffer.Dissolved genomic DNA samples were stored at -20°C [11,12].

Agarose Gel Electrophoresis

0.25 g of 0.8% agarose was dissolved 1x Tris Acetate Ethylene Diamine tetra acetic acid (TAE). Agarose mixture was heated in the microwave oven for 90 sec with constant shaking. The mixture was swirled and was made sure that agarose has melted without any formation of lumps or particles. Agarose was allowed to cool for several minutes and 1 drop of ethidium bromide (4 mg/ml) was added in to it. Agarose solution was poured into a sealed gel tray without any air bubbles. After the gel had completely hardened, the comb was carefully removed and the gel was immersed using 1x TAE

running buffer. Sample DNA was mixed with loading dye (Bromophenol blue- xylene mixture) and loaded in the wells. The marker DNA was added for the reference. Electrical leads were connected to the electrophoresis chamber and the gel was allowed to run with a current of 50-100 V for 30 min. The gel was carefully removed and viewed with gel documentation [13-24].

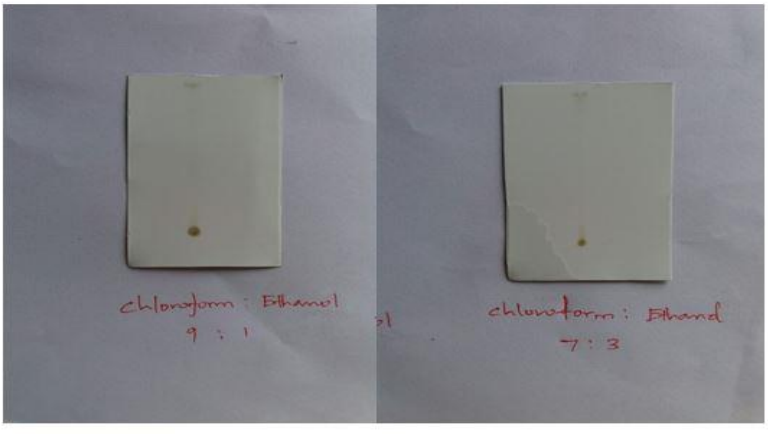
RESULTS

Hexane extract showed the presence of Steroids. Chloroform extract showed the presence of Glycoside and Phenol. Ethyl acetate extract showed the presence of protein. Ethanol extract showed the presence of Sugar (Table 1).

Table 1: Preliminary phytochemical studies of *Opuntia dillinii*.

S. No.	Test	Hexane extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
1	Alkaloid	-	-	-	-
2	Glycoside	-	+	+	+
3	Anthraquinone	-	-	-	-
4	Terpinoids	-	-	-	-
5	Steroids	+	+	+	+
6	Flavanoids	-	-	-	-
7	Phenol	-		+	+
8	Tannins	-	-	-	-
9	Sugar	-	-	-	+
10	Quinones	-	-	-	-
11	Saponins	-	-	-	-
12	Proteins	-	-	+	+
13	Resin	-	-	-	-
‘+’ indicates presence ‘-‘ indicates absence					

TLC studies showed the results of 7 spots (Figure 1) with Rf value range from 0.06-0.96 in the mobile phase chloroform: Ethanol (7:3) (Table 2). This reveals many phytoconstituents was present in *Opuntia delleni*. The physico chemical studies revealed the drug was standard (Table 3).



9:1 (Chloroform: Ethanol) 7:3(Chloroform: Ethanol)

Figure 1: Thin layer chromatography photos.

Table 2: Thin layer chromatography.

S.no.	Extract	Solvent system	No. of spots	Rf Values
1	Chloroform	7:3(Chloroform:Ethanol)	7	1.02, 1.11 1.18, 1.27, 1.34 5.22, 9.40
		9:1(Chloroform: Ethanol)	--	--

Table 3: Physico chemical constants.

S. No.	Parameters	Crude drug
1	Total ash	12%W/W
2	Acid insoluble ash	10.2%W/W
3	Water soluble ash	8.4%W/W
4	Sulphated ash	24%W/W
5	Extractive value	
	• Water Soluble Extractive	8%W/W
	• Alcohol Soluble Extract	7.5%W/W
6	Loss on drying	14%W/W

Plant extracts that causes high level of mortality at reduced concentratioand those which can cause effective mortality within a short span of time can be considered to possess potential phytotoxicity. In the present study, *Opuntia delleni* extracts showed potent larvicidal activity (Table 4). The LC_{50} value for 24 hr observation was very high indicating potent activity (Table 5). Further it was confirmed by Gel Electrophoresis studies with DNA fragmentation pattern (Figure 2).

Table 4: Larvicidal activity of extracts against *A. aegypti.*

Test larvae	Extracts	Conc. (µg/ml)	LC_{50} 95%confidence (LCL-UCL)	LC_{90} 95%confidence (LCL-UCL)	χ^2 Value
A. aegypti	Hexane	Control	52.615 (42.107-66.072)	411.675 (266.755-793.776)	0.712
		10			
		25			
		50			
		100			
		200			
	Chloroform	Control	23.940 (18.257-29.882)	166.986 (119.671-337.622)	9.944
		10			
		25			
		50			
		100			
		200			
	Ethyl acetate	Control	37.378 (28.876-47.144)	337.622 (218.029-662.255)	2.243
		10			
		25			

		50			
		100			
		200			
	Ethanol	10	34.374 (27.028-42.675)	256.670 (175.818-450.424)	6.552
		25			
		50			
		100			
		200			
	Standard (Bleeching powder)	10	60.913 (47.676-79.569)	621.678 (362.126-1474.746)	0.653
		25			
		50			
		100			
		200			
Where, LC_{50} and LC_{90} - lethal concentration at 50 % and 90 % of samples, respectively; LCL- lower confidential limit; UCL- upper confidential limit; χ^2 value – Chi square value at $p > 0.05$ significant level.					

Table 5: Effect of chloroform extract against *A. aegypti*.

Chloroform	
Conc	Mean
10	8.666666667
25	12
50	15.333333333
100	19.666666667
200	24.666666667

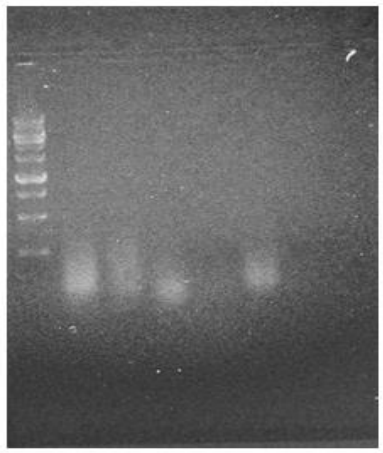


Figure 2: Agarose gel image

DISCUSSION

Among the extracts, chloroform showed comparatively better activity. The LC50 value of chloroform was 5.5 lesser than other extract against A. Aegypti. The toxic effects resulting in the mortality manifested differently on exposure to the extracts at different times. However, more than 80% mortality was observed only at higher concentrations. Comparatively the methanol extracts showed early toxicity resulting in mortality in a short duration of time on exposure to the extracts against *A. aegypti*. The larvicidal activity of *Opuntia delleni* may be due to the phytoconstituents present in it. The larvae was subjected for DNA isolation by standard procedure and run in Agarose Gel Electrophoresis and observed in UV light. The DNA fragment pattern was observed for chloroform extract of *Opuntia delleni* which showed potent effect on Larvae *Aedes aegypti*. Thus the molecular studies on Dengue vector Larvae *Aedes aegypti* was potent with chloroform extract of *Opuntia delleni* leaves. This is the first report on this plant on DNA fragmentation.

CONCLUSION

The chloroform extract showed potent larvicidal activity and good DNA fragmention pattern on gel electrophoresis studies. Further this study was explored for lead compound through chromatographic and spectroscopic studies. This will lead to get a new compound for Dengue larvae *Aedes aegypti*.

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