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Evaluation of Larvicidal Activity of Methanolic Extract of Citrullus Lanatus

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ABSTRACT

The present study was performed with the objectives of elaborating the larvicidal efficacy of Methanolic extract of *Citrullus lanatus* (MECL) leaves against *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti*. It has been reported used widely in traditional herbal medicine. The leaves of *Citrullus lanatus* is analgesic, anti-inflammatory, mosquitocidal, gonorrhea and anti-microbial property. The larvicidal activity of plant extract was carried out on late 3rd and early 4th instar larvae of *Anopheles stephensi*, a primary vector of urban malaria, *Culex quinquefaciatus*, a common vector of filariasis, *Aedes aegypti*, common vector of dengue and yellow fever. LC_{50} and LC_{90} of MECL were determined against *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti* by using Abbott's formula. LC50 and LC 90 of MECL against Anopheles stephensi were found to be 84.23ppm & 989.396ppm. LC50 and LC_{90} of MECL against *Culex quinquefasciatus* were found to be 51.31ppm &405.88ppm. The LC_{50} was found to be 2645ppm or 2.645%v/v for *Aedes agypti*. The LC_{90} value was found to be 138326ppm or 138.326%v/v for *Aedes agypti* The results of the present study suggest that methanolic extract of *Citrullus lanatus* showed promising larvicidal activity against important vectors of malaria, filarial, dengue, dengue haemorrhagic fever, yellow fever, chikungunya.

Keywords: Larvicidal activity, *Citrullus lanatus, Anopheles stephensi, Culex quinquefasciatus Aedes aegypti,*

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INTRODUCTION

Mosquito borne diseases are one of the most public health issues in the developing countries. Malaria and other vector-borne diseases contribute to the major disease burden in India. One of the methods to control these diseases is to control the vectors for the interruption of disease transmission. In the past, synthetic organic chemical insecticides based intervention measures for the control of insect pests and disease vectors have resulted in development of insecticide resistance in some medically important vectors of malaria, filariasis, Japanese encephalitis, dengue, hemorrhagic fever, chikungunya and yellow fever transmitted by mosquitoes which cause millions of death every year[1] Hence controlling the mosquitoes in larval stage is important and effective for preventing the above infections [2-3]. During the last decade, various studies on natural plant products against mosquito vectors indicate them as possible alternatives to synthetic chemical insecticides. Mosquito control programmes largely target the larval stage at their breeding sites with larvicides[4]. Larviciding is a successful method of reducing mosquito population in their breeding place before they emerge as adults. The screening of local medicinal plants for mosquito larvicidal activity may eventually lead to their use in natural product -based mosquito abatement practices[5]. Plants are well known to contain a complex of chemicals with bioactive potential like deterrents or attractants. Plant extracts are reported to be eco-friendly mosquito control agents [6-7]. *Citrullus lanatus* is well known as Watermelon plant (Family - Cucurbitaceae). Water melon is popular in indigenous system of folk medicine. It is a trailing annual plant with several herbaceous, firm and stout stems [8-9]. The leaves of *Citrullus lanatus* is used as anti-inflammatory, analgesic, gonorrhoea, mosquitocidal and anti- microbial property 10 . Leaves are available throughout the year could be easily collected without any additional cost. Therefore, leaves extracts could be used as a larvicidal agent in an integrated vector control program. The plant *Citrullus lanatus* has been selected (specially the leaves) for the present investigation on the basis of the ethnomedical information and the review of literature as the plant is widely cultivated throughout India. Larvicidal effect of *Citrullus colocynthis* leaves was previously reported.[11] This present work has attempted to study mosquito larvicidal property of *C. lanatus* against three mosquito species - *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti.*

MATERIALS AND METHOD

Collection and preparation of extract:

The leaves of *Citrullus lanatus* were collected in Chembarambakkam during the month of July 2023. The plant specimen was identified and authenticated as *'Citrullus lanatus'* (Curcubitaceae) by Taxonomist. The authenticated herbarium of plant has been kept in the Department of

Pharmacognosy, Sree Sastha Pharmacy College, Chennai-123.. The leaves were washed thoroughly and dried in shade. The shade dried leaves were powdered and used for further studies. Extraction of leaves of *Citrullus lanatus* was carried out by washing the plants and drying at room temperature in 14 days. After that, they were filtered with sieve analyzer to get homogeneous particles and defatted with 2.5L of petroleum ether (60-80˚C) by cold maceration method for 72hr.The solvent was then removed by filtration and the marc was dried. The dried marc was re-soaked with 2.5L of Methanol.The steps were performed three times and the combined filtrates were evaporated to a cohesive mass using rota vapour.

Collection of mosquito:

The mosquito larvae (*Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti*) were obtained from ICMR, Chennai.

Preparation of stock solution of Methanolic extract of *C.lanatus*

A stock solution of 100mg/ml of the MECL was prepared by dissolving the required quantity of the extract in distilled water.

Larvicidal activity of MECL.[12-16]

The larvicidal activity of plant extract was carried out on late 3rd and early 4th instar larvae of Anopheles stephensi, a primary vector of urban malaria, *Culex quinquefaciatus*, a common vector of filariasis, *Aedes aegypti*, common vector of dengue and yellow fever. Twenty larvae were released in 500ml beaker containing 200ml distilled water with varying concentrations of plant extract. The larvae were provided with dog biscuit and yeast powder in a ratio of 3:2 as nutrients. The experiments were carried out a room temperature (26°C ± 2°C). Three replicates of each concentration were run under the same microclimate conditions along with untreated control. The mortality was monitored for 24hr.

Data analysis [17-19]

LC50 (lethal concentration to cause 50% mortality in the population) and LC90 (lethal concentration to cause 90% mortality in the population) were determined by plotting the regression line. The percentage mortality was calculated using Abbot's formula and the data so obtained was analyzed by probit analysis by using the software Minitab-15 for dose and time mortality regression lines. The purpose of the probit transformation is to straighten the line so we can estimate LC50 more easily.

$$\text{Percentage Mortality} = \frac{\%\text{ Mortality in treated} - \%\text{Mortality in control}}{100 - \%\text{ Mortality in control}} \times 100$$

RESULTS AND DISCUSSION

Larvicidal activity of MECL against *Anopheles stephensi*

The results obtained for the larvicidal effect of MECL against *Anopheles stephensi* & the graphical representation were presented (Table 1 & Figure 1). From the Table 1 it can be observed that a mortality of 100.00 ± 0.00 was observed for *Anopheles stephensi*. The LC50 and LC90 values were calculated using probit analysis. The percentage mortality calculated using Abbott's formula versus log concentration was plotted and Y=50 is substituted in the resulting linear equation to obtain the X value. The linear regression equation was found to be y = 37.387x - 21.988 for activity against *Anopheles stephensi*. The antilog of X was then the LC50 (conc. of 50% mortality) or LC90 (conc. of 90% mortality) value.

The LC50 & LC90 were found to be 84.23ppm or 0.084v/v & 989.3956ppm or 0.989%v/v for *Anopheles stephensi*. One hundred percent mortality was observed for the concentrations tested against the organisms. The extract was effective against *Anopheles stephensi*.

Table 1: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Anopheles stephensi*

S. No	Conc. (ppm)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Probit	Probit	Regression value
0	0	0	0	0	0	0		0
1	25	1.40	20	3.00	15.00	3.77	3.00	4.358
2	50	1.70	20	5.00	25.00	4.19	4.00	4.778
3	100	2.00	20	6.00	30.00	4.36	4.00	4.948
4	150	2.18	20	9.00	45.00	4.8	5.00	5.388
5	200	2.30	20	10.00	50.00	4.92	5.00	5.508
6	250	2.40	20	13.00	65.00	5.33	6.00	5.918
7	300	2.48	20	16.00	80.00	5.81	6.00	6.398
8	350	2.54	20	17.00	85.00	5.99	6.00	6.578
9	400	2.60	20	19.00	95.00	6.65	7.00	7.238
10	450	2.65	20	20.00	100.00			
LC_{50}				84.23ppm or 0.084v/v				
LC_{90}				989.396ppm or 0.989%v/v				

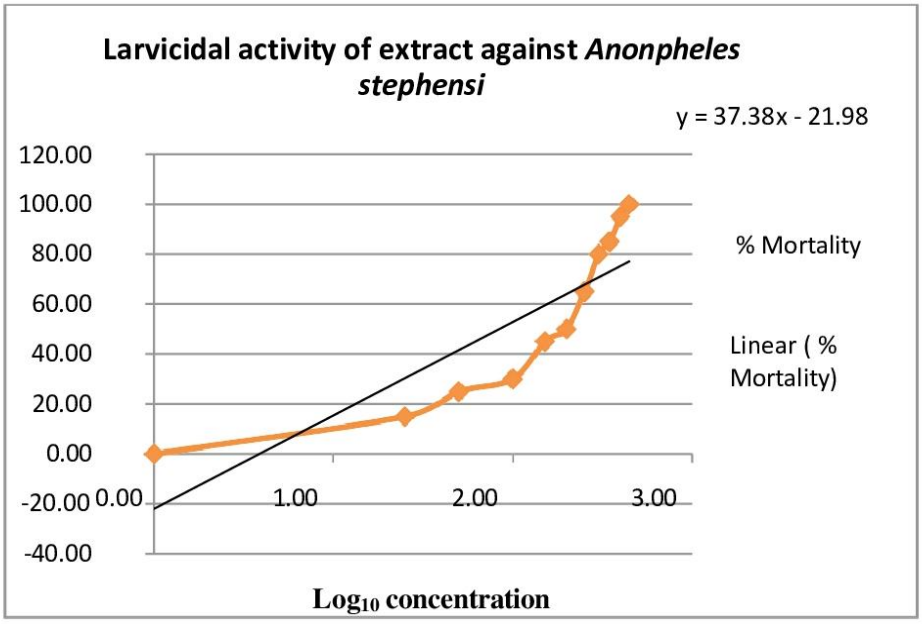

Figure 1: **Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Anopheles stephensi***

Larvicidal activity of MECL against *Culex quinquefasciatus*

The results obtained for the larvicidal effect of MECL against *Culex quinquefasciatus* & the graphical representation were presented (Table 2 & Figure 2)

From table 2, it can be observed that a mortality of 100.00 ± 0.00 was observed for *Culex quinquefasciatus*. The LC50 and LC90 values were calculated using Probit analysis. The percentage mortality calculated using Abbott's formula versus log concentration was plotted and Y=50 is substituted in the resulting linear equation to obtain the X value. The linear regression equation was found to be y = 44.533x - 26.161 for activity against *Culex quinquefasciatus*. The antilog of X was then the LC50 (conc. of 50%mortality) or LC90 (conc. of 90% mortality) value. The LC50 & LC90 were found to be 51.31ppm or 0.051%v/v & 405.88ppm or 0.405%v/v for *Culex quinquefasciatus*. One hundred percent mortality was observed for the concentrations tested against the organisms. The extract was effective against *Culex quinquefasciatus*

Table 2: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Culex quinquefasciatus*

S. No	Conc. (ppm)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Probit	Probit	Regression value
0	0	0	0	0	0	0	0	0.00
1	5	0.70	20	1.00	5.00	0	0.00	0.65
2	10	1.00	20	1.00	5.00	0	0.00	0.65
3	25	1.40	20	3.00	15.00	3.77	4.00	4.42
4	50	1.70	20	6.00	30.00	4.36	4.00	5.01
5	100	2.00	20	10.00	50.00	4.92	5.00	5.57

6	150	2.18	20	13.00	65.00	5.33	5.00	5.98
7	200	2.30	20	16.00	80.00	5.81	6.00	6.46
8	250	2.40	20	17.00	85.00	5.99	6.00	6.64
9	300	2.48	20	19.00	95.00	6.75	7.00	7.40
10	350	2.54	20	20.00	100.00			
LC50				51.31ppm or 0.051%v/v				
LC90				405.88ppm or 0.405%v/v				

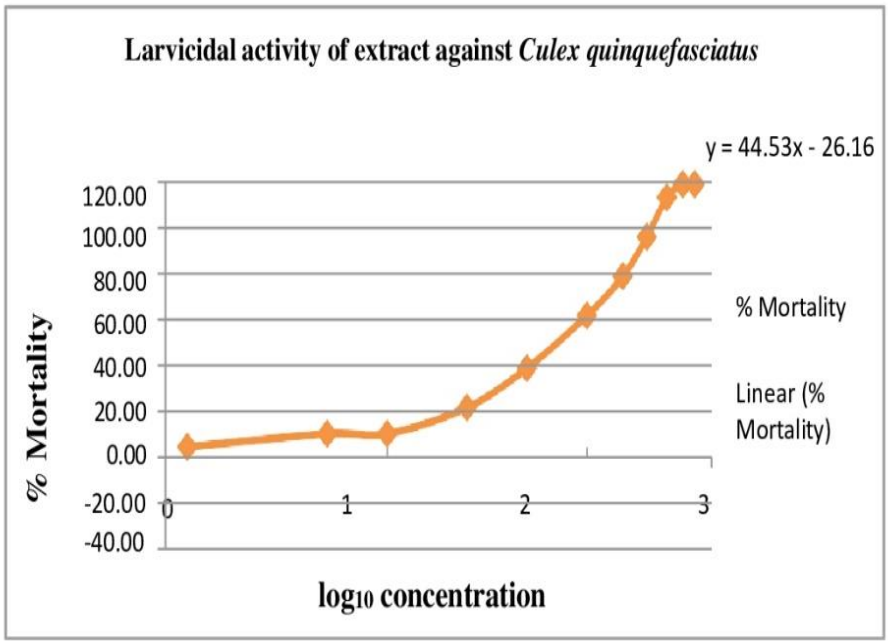

Figure 2: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Culex quinquefasciatus*

Larvicidal activity of MECL against *Aedes agypti*

The results obtained for the larvicidal effect of MECL against *Aedes agypti* & the graphical representation were presented (Table 3 & Figure 3). From the Table 3, it was observed that a mortality of 100.00 ± 0.00 was observed for *Aedes agypti.* The LC50 and LC90 values were calculated using Probit analysis. The percentage mortality calculated using Abbott's formula versus log concentration was plotted and Y=50 is substituted in the resulting linear equation to obtain the X value. The linear regression equation was found to be y = 23.278x - 29.67 for activity against *Aedes agypti*. The antilog of X was then the LC50 (conc. of 50% mortality) or LC90 (conc. of 90% mortality) value. The LC50 & LC90 were found to be 2645ppm or 2.645%v/v & 138326ppm or 138.326%v/v for *Aedes agypti*. One hundred percent mortality was observed for the concentrations tested against the organisms. The extract was effective against *Aedes agypti*. One hundred percent mortality was observed for various concentrations ranging for above organisms tested were observed within 24hr of the start of the experiment.

Table 3: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Aedes agypti*

S. No	Conc. (ppm)	Log_{10} Conc.	Total No.	No. Dead	% Mortality	Probit	Probit	Regression value
0	0	0	0	0	0	0	0	0
1	100	2.00	20	1.00	5.00	3.35	3.00	4.624
2	150	2.18	20	1.00	5.00	3.35	3.00	4.624
3	300	2.48	20	3.00	15.00	3.96	4.00	5.234
4	500	2.70	20	4.00	20.00	4.16	4.00	5.434
5	1000	3.00	20	6.00	30.00	4.48	4.00	5.754
6	1500	3.18	20	7.00	35.00	4.62	5.00	5.894
7	3000	3.48	20	9.00	45.00	4.87	5.00	6.144
8	6000	3.78	20	10.00	50.00	5	5.00	6.274
9	12000	4.08	20	18.00	90.00	6.28	6.00	7.554
10	13000	4.11	20	20.00	100.00			
LC50				51.31ppm or 0.051%v/v				
LC90				405.88ppm or 0.405%v/v				

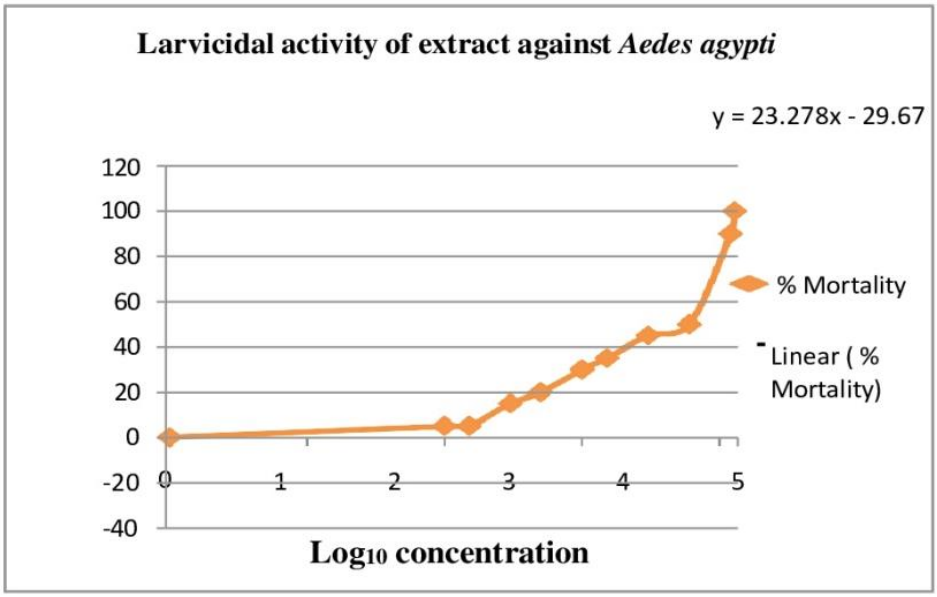

Figure 3: Larvicidal activity of methanolic extract of *Citrullus lanatus* against *Aedes agypti*

CONCLUSION

In the present study, Methanolic extract of *Citrullus lanatus* (MECL) showed promising larvicidal activity against important vectors of Malaria, Filarial, Dengue and Yellow fever. MECL may be used as biodegradable, ecofriendly and cost effective alternative mosquito control tool instead of using synthetic chemicals.

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Competing interests

Author has declared that no competing interests exist

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