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## Comparative Study of Larvicidal Activity of Leaf And Root Extracts of *Erythrina Indica* Lam against *Aedes Aegypti* and *Culex Quinquefasciatus*

Rathi Sre PR<sup>\*1</sup>, Reka M<sup>1</sup>, Elumalai D<sup>2</sup>, Kaleena PK<sup>2</sup>, Murugesan K<sup>1</sup>.

1 CAS in Botany, University of Madras, Guindy campus, Chennai-600 025, Tamilnadu, India.

2 Department of Zoology, Presidency College, Chennai-600 005, Tamilnadu, India.

### ABSTRACT

In view of an increasing interest in developing plant derived insecticides as an alternative to chemical insecticides, this study was undertaken to assess the larvicidal potential of *Erythrina indica* root and leaf extracts against two medically important species of mosquito vectors, *Aedes aegypti* and *Culex quinquefasciatus*. Larvicidal activity of methanol, aqueous, ethyl acetate, chloroform and petroleum ether extracts of leaf and root of *Erythrina indica* were tested against the early fourth instar larvae of *A.aegypti* and *C. quinquefasciatus*. Fourth instar larvae of *A. aegypti* and *C. quinquefasciatus* were used for assessing the larvicidal activity. A number of 125 larvae were exposed in five replicates of 25 larvae each. Experiments were maintained at 28±1° C, 65±2% relative humidity. The mortality data were subjected to probit analysis to determine the lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) to kill 50 and 90 per cent of the treated larvae of the respective species. Among the leaf and root extracts studied for mosquito larvicidal activity, both root and leaf extracts showed the moderate activity. The chloroform extract of the root was moderately effective against the larvae of *C. quinquefasciatus* and *A. aegypti*; the respective values for LC<sub>50</sub> and LC<sub>90</sub> were 125.55, 118.90 ppm and 375.27, 354.03 ppm. The methanol extract of the leaf was also moderately effective against the larvae of *C. quinquefasciatus* and *A. aegypti* with respective LC<sub>50</sub> and LC<sub>90</sub> values 126.60, 126.76 ppm. The finding of the present investigation revealed that the leaf and root extract possesses remarkable larvicidal activity against two medically important vector mosquitoes *C.quinquefasciatus* and *A. aegypti*. So this present ecofriendly paradigm proves the efficacy of *E.indica* as an botanical larvicide.

**Keywords:** *Aedes Aegypti*, *C. quinquefasciatus*, *Erythrina indica*, Larvicide, Mosquito, Phytochemical

\*Corresponding Author Email: [rathissk@gmail.com](mailto:rathissk@gmail.com)

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## INTRODUCTION

Lymphatic filariasis caused by *Wuchereria bancrofti* is transmitted by mosquito *Culex quinquefasciatus*, with around 120 million people infected Worldwide, and 44 million people have common chronic manifestation.<sup>1</sup>

It is reported that *Aedes aegypti*, a vector of dengue fever and its incidence has increased fourfold since 1970, and nearly half the world's population is now at risk<sup>2</sup>. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50%<sup>3</sup>.

Synthetic insecticides such as organochlorine, organophosphorus, carbamates, pyrethrins and pyrethroids are commonly used for controlling the increasing population of vectors. These insecticides although very slightly toxic, may also cause headaches, loss of memory and irritability, and risk of toxicity to humans<sup>4,5</sup>. Continuous application of insecticides poses serious threats to the environment in killing non target species such as larval predators, bioaccumulation, hampering biodiversity, pest resurgence, pest resistance to insecticides and environmental pollution<sup>6,7</sup>.

It has been found that herbal extracts (phytochemical insecticides) are one safer alternative method of control since they have<sup>8, 9,10</sup> phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, and oviposition attractants and can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level.<sup>11,12,13, 14,15,16,17,18</sup>

So the development of alternative, ecofriendly, and sustainable approaches for mosquito control is with the use of natural phytoproducts owning greater target specificity and lower bioaccumulation properties<sup>19</sup>. So, researchers are now looking for natural insecticides which do not have any ill effects on non target population and are easily degradable. Thus, the search has been directed extensively to the plant kingdom as many plant chemicals have larvicidal, pupicidal, and adulticidal activities, most being repellants, ovipositional deterrents, and antifeedants against both agricultural pests and medically important insect species. The search for herbal preparations that do not produce any adverse effects in the nontarget organisms and are easily biodegradable remains a top research issue for scientists associated with alternative vector control<sup>20</sup>.

According to present scenario, it must be necessary to apply target specific, cost effective, and affordable biodegradable phytoproducts which are sustainable and ecofriendly<sup>21,19</sup>.

In the present paper, an attempt has been made to a step forward to observe rationalise impact of

leaf and root extracts of *Erythrina indica* against *A. aegypti* and *C. quinquefasciatus* to assess their larvicidal activity for the first time.

## MATERIALS AND METHODS

### Plant collection

The fresh leaves and roots of plant were collected from Mangadu, Chennai, Tamil Nadu, India. It was authenticated by Prof. K.Murugesan, CAS in Botany, University of Madras, Chennai. A voucher specimen was also deposited in the centre.



*Erythrina indica* tree.

### Preparation of the extract

Approximately one kilogram of the roots and leaves were collected and washed in running tap water. The roots and leaves were then chopped in to small pieces and shade dried for about a month and was ground to coarse powder in a blender. The powder was then sequentially extracted with petroleum ether, chloroform, ethyl acetate, methanol and aqueous using Soxhlet apparatus and then concentrated in a rotary evaporator under reduced pressure and dried. The dried powder was diluted with acetone and used for the study.

### Phytochemical screening of the extracts

The phytochemical screening was carried out as described by Harbourne, et al<sup>22</sup>. The samples were screened for carbohydrates, alkaloids, flavonoids, quinones, phytosterols, terpenoids, triterpenoids, coumarins, acids sterols, phytosterols, cardiac glycosides, cyanin, phenols, tannins, saponins and proteins.

### Evaluation of crude extracts for mosquito larvicidal activity

#### Larvicidal bioassay

During preliminary screening with the laboratory trial, the larvae of *A.aegypti* and *C. quinquefasciatus* were collected from the insect rearing cage and identified in Zonal Entomological Research Centre, Chennai-28. From the stock solution, 1000 mg/l extract was prepared with dechlorinated tap water.

One gram of crude extract was first dissolved in 100 ml of respective solvent (stock solution). From the stock solution, 1,000 ppm was prepared using dechlorinated tap water. The control was set up with acetone and polysorbate 80. A leaf dipping method<sup>30</sup> was used to evaluate the activity of the test samples. Fourth instar larvae of *A. aegypti* and *C. quinquefasciatus* were transferred individually on treated and control leaf disks placed in petri plates. A number of 125 larvae were exposed in five replicates of 25 larvae each. Experiments were maintained at  $28\pm 1^\circ$  C,  $65\pm 2\%$  relative humidity. Mortality was determined 24 h after larvae were placed on disks. All moribund mosquito larvae were considered as dead.

### Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC50, LC90, and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using the SPSS software. Results with  $p < 0.05$  were considered to be statistically significant.

## RESULT AND DISCUSSION

All extracts of leaf and root showed moderate larvicidal activity, with higher effect seen in methanolic and chloroform extracts followed by ethyl acetate and petroleum ether extracts. The phytochemical analysis of the promising chloroform extract of root and methanolic extract of leaf were positive for carbohydrates, phenols, quinones, saponins, terpenoids, tannins, coumarins, anthraquinones, alkaloids, Cardiac glycosides and sterols (Table I and II).

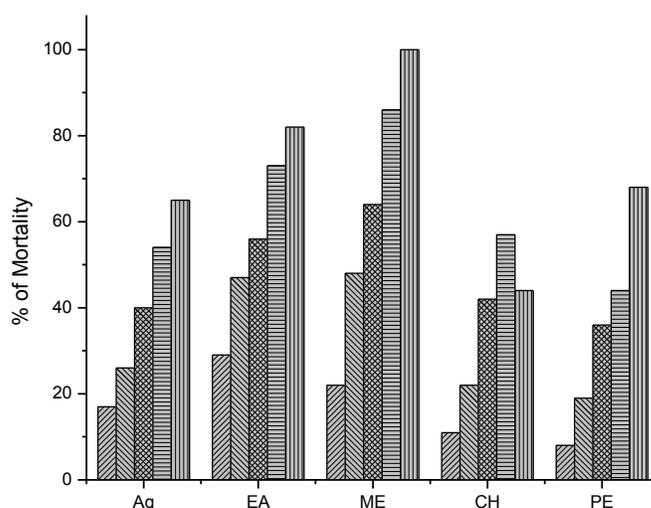
The LC50 and LC90 values and their 95% upper and lower confidence limits and slope values and  $r^2$  values of leaves and roots of *E.indica* extracts are given in Table III, IV, V and VI. Results of the study shows that the mortality of mosquito larvae of *A. aegypti* and *C. quinquefasciatus* treated with leaf and root extract of *E.indica* increased with increase in concentration of extract (Fig I,II,III and IV). The ethyl acetate, chloroform, aqueous and petroleum ether extracts of leaf of *E.indica* showed larvicidal activity against larvae of *C. quinquefasciatus* and *A. aegypti* with the respective LC50 and LC90 values as shown in Table III and IV. The chloroform extract of root was followed by petroleum ether, ethyl acetate, methanol and aqueous extracts that showed larvicidal activity against *C. quinquefasciatus* and *A. aegypti* with the respective LC50 and LC90

values as shown in Table V and VI. The increased mortality with time may be due to the individual or synergistic effect of several factors like time provides better chance for accumulation of active moiety of compound in the larval body or with the time the active compound turns in to more toxic substance for the larvae of mosquitos<sup>23</sup>. Several authors had reported that methanol and chloroform extracts of plants exhibit potent larvicidal activity.

Current study reveals that the methanolic extract of leaf and chloroformic extract of root showed excellent larvicidal properties with mortality rate of 98-100% at 200ppm concentration for 24 h (Figure I,II,III and IV). The LC50 and LC90 for *E.indica* methanolic leaves were 126.60,126.76 ppm and 378.94, 376.89 ppm for *C. quinquefasciatus* and *A. aegypti* respectively. And for chloroform extract of root it is 125.55, 118.90 ppm and 375.27, 354.03 ppm respectively.

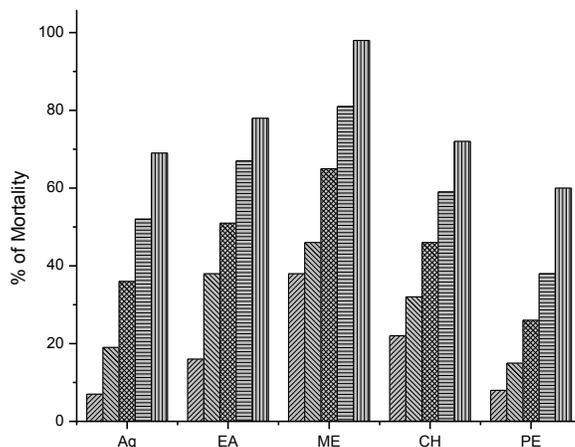
Though the different parts of this plant extracts have been studied for various medicinal properties such as cardiovascular effect<sup>24</sup>, antihelminthic activity<sup>25</sup>, Antioxidant activity<sup>26</sup>, antiulcer activity<sup>27</sup>, diuretic activity<sup>28</sup>, so far no study on the mosquito larvicidal properties of this plant leaf and root extract has been reported to our knowledge. This is the first report on the mosquito larvicidal properties of the leaf and root extracts of *E.indica* against the larvae of *C. quinquefasciatus* and *A. aegypti*.

The finding of the present investigation revealed that the leaf and root extract possesses remarkable larvicidal activity against two medically important vector mosquitoes *C.quinquefasciatus* and *A. aegypti*. Further detailed study on the isolation of the active principles responsible for the mosquito larvicidal activity may pave the way for the development of an environmentally safe botanical insecticide for the control of mosquito larvae.



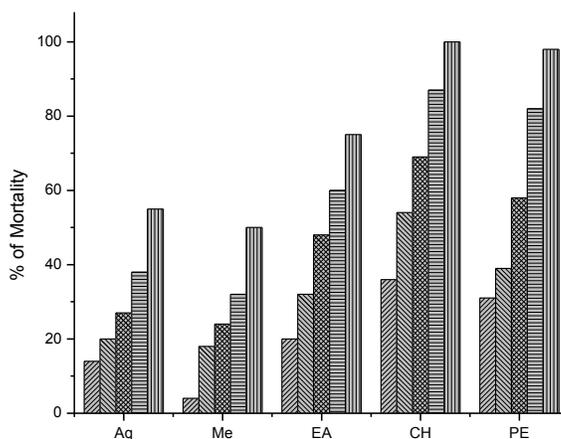
**Figure I: Graph Showing the Mortality of Different Extracts of Leaf Against Fourth Instar Larvae Of Culex Quinquefasciatus**

Graph showing the larvicidal activity of leaf of *E.indica* against *Culex quinquefasciatus*. The mortality rate was higher in methanol extract followed by ethylacetate, petroleum ether, aqueous and chloroform extract. Values are mean  $\pm$  SD of 5 replicates.



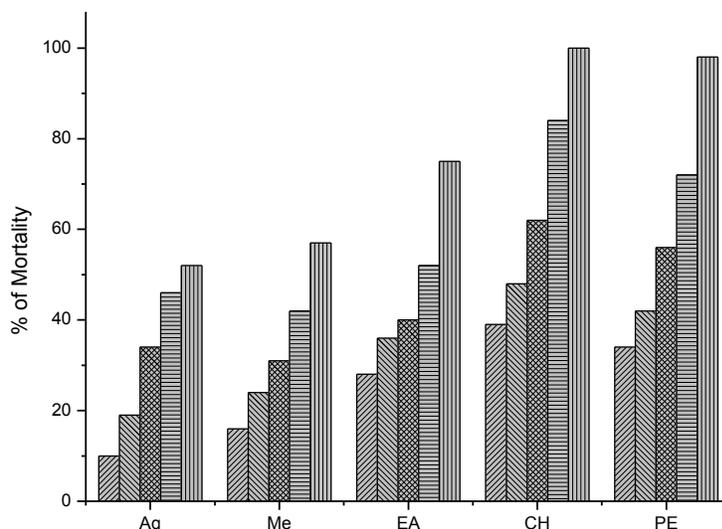
**Figure II: Graph Showing the Mortality of Different Extracts of Leaf Against Fourth Instar Larvae of Aedes Aegypti**

Graph showing the larvicidal activity of leaf of *E.indica* against *Aedes aegypti*. The mortality rate was higher in methanol extract followed by ethylacetate, chloroform, aqueous and petroleum ether extract. Values are mean  $\pm$  SD of 5 replicates.



**Figure III: Graph Showing the Mortality of Different Extracts of Root Against Fourth Instar Larvae of Culex Quinquefasciatus**

Graph showing the larvicidal activity of root of *E.indica* against *Culex quinquefasciatus*. The mortality rate was higher in chloroform extract followed by petroleum ether, ethylacetate, aqueous and methanol extract. Values are mean  $\pm$  SD of 5 replicates.



**Figure IV: Graph Showing the Mortality of Different Extracts of Root Against Fourth Instar Larvae of *Aedes Aegypti***

**Table I: Phytochemical screening of plant Leaf extracts of *Erythrina.indica***

S.No	phytochemicals	Aqueous	Ethyl acetate	Methanol	Chloroform	P.Ether
1	Carbohydrates	+	+++	+++	+++	-
2	Tannins	+	+++	+++	+++	-
3	Saponin	-	++	+++	-	+
4	Flavonoid	+	+++	++	+	-
5	Alkaloids	+++	+	-	++	-
6	Quinones	-	+++	+++	++	-
7	Glycoside	-	-	+	-	+
8	Terpenoids	-	+	-	-	-
9	Triterpenoids	-	-	-	-	-
10	Phenols	+	+++	+++	-	-
11	Coumarins	-	++	++	+	-
12	Acids	-	-	-	-	-
13	Proteins	-	-	+	-	-
14	Cyanin	+	+	++	-	-
15	Cardiac glycosides	-	++	++	+	++

**Table II: Phytochemical screening of plant roots extracts of *Erythrina.indica***

S.No	phytochemicals	Aqueous	Chloroform	Ethyl acetate	Methanol	P.Ether
1	Carbohydrates	+	+++	+++	+++	+
2	Tannins	+	++	++	+++	-
3	Saponin	-	++	-	-	++
4	Flavonoids	+	++	++	++	++
5	Alkaloids	-	+++	+	+	+
6	Quinones	-	+++	+	+++	-

7	Glycosides	-	-	-	+	-
8	Terpenoids	-	+++	-	+++	-
9	Triterpenoids	-	++	+	-	-
10	Phenols	+	+++	+++	+	-
11	Coumarins	-	++	-	+	-
12	Anthroquinones	-	+++	++	-	-
13	Sterols and phytosterols	-	+	+	++	++
14	Cyanin	-	+++	-	-	-
15	Cardiac glycosides	-	++	+	+	+

+++ - Strongly Positive ; ++ - Positive; + - Trace; - -Not detected.

**Table III: Larvicidal activity of leaf by *Culex quinquefasciatus*:**

Extract/Solvent	LC50	LCL-UCL	LC90	LCL-UCL	slope	r <sup>2</sup>
Aqueous	167.70	156.74-179.42	422.89	391.41-435.17	26	0.995
Ethyl Acetate	132.88	124.14-142.23	394.21	376.19-419.42	56	0.987
Methanol	126.60	117.99-135.85	378.94	363.29-392.76	64	0.996
Chloroform	163.19	154.51-172.36	488.16	463.42-513.49	22	0.993
Petroleum Ether	176.38	167.27-185.99	528.32	494.34-549.35	44	0.975

**Table IV: Larvicidal activity of leaf by *Aedes aegypti***

Extract/Solvent	LC <sub>50</sub>	LCL-UCL	LC90	LCL-UCL	Slope	r <sup>2</sup>
Aqueous	170.44	161.87-179.46	411.92	501.04-527.82	52	0.997
Ethyl Acetate	145.42	137.95-153.29	435.27	417.39-452.68	67	0.986
Methanol	126.76	114.91-139.81	376.89	362.43-393.64	65	0.987
Chloroform	155.87	146.87-165.88	466.13	449.38-483.19	46	0.998
Pet. Ether	188.22	180.47-196.31	565.94	542.27-583.89	38	0.956

**Table V: Larvicidal activity of Root by *Culex quinquefasciatus***

Extract/Solvent	LC50	LCL-UCL	LC90	LCL-UCL	Slope	r <sup>2</sup>
Aqueous	191.29	162.99-225.76	573.09	564.22-591.19	19	0.983
Methanol	187.92	176.55-200.01	561.24	544.09-582.82	31	0.975
Chloroform	125.55	112.48-140.14	375.27	352.19-394.36	62	0.980
Ethyl Acetate	158.60	147.74-170.25	474.88	462.03-432.91	52	0.905
Petroleum Ether	135.96	124.71-148.22	405.47	392.21-426.34	56	0.959

**Table VI: Larvicidal activity of Root by *Aedes aegypti***

Extract/Solvent	Conc. (ppm)	LC50	LCL-UCL	LC90	LCL-UCL	Slope	r <sup>2</sup>
Aqueous	200	192.30	181.18-204.10	576.34	562.41-584.09	20	0.952
Methanol	175	200.04	189.42-209.18	598.41	581.70-607.14	24	0.966
Chloroform	150	118.90	109.80-128.76	354.03	342.16-361.33	84	0.938
Ethyl Acetate	125	153.18	144.67-162.19	459.81	443.07-472.43	48	0.988
Petroleum Ether	100	136.10	126.91-145.96	408.71	392.42-414.42	58	0.993

Values are mean  $\pm$  SD of five replicates. 95% LCL = Lower Confidence Limit, 95% UCL = Upper Confidence Limit, Lc50 = Lethal Concentration Of 50% kills, Lc90 = Lethal Concentration Of 90% kills, r<sup>2</sup> = Regression Coefficient.

Graph showing the larvicidal activity of root of *E.indica* against *Aedes aegypti*. The mortality rate was higher in chloroform extract followed by petroleum ether, ethylacetate, methanol and

aqueous extract. Values are mean  $\pm$  SD of 5 replicates.

Aq- Aqueous extract, ME- Methanol extract, EA- Ethylacetate extract, CH- Chloroform extract, PE- Petroleum ether extract.

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