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## Larvicidal Efficacy of *Eugenia jambolana* Linn. Extracts in Three Mosquito Species at Mysore

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### ABSTRACT

The development of resistance to chemical insecticides among mosquito species has been considered as a setback in vector control. So, researchers have diverted their interest towards insecticides of plant origin as an alternative source. Thus the present investigation was undertaken to analyse the larvicidal activity of *Eugenia jambolana* leaf extracts by employing against the fourth instar larvae of three medically important mosquito species namely *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* at Mysore following the guideline of WHO larval bioassay methodology. The extraction process was carried with a soxhlet apparatus employing petroleum ether, ethyl acetate, acetone and methanol as a solvents. The results shows that among the mosquito species *Aedes aegypti* was found to be the most susceptible with the LC<sub>50</sub> value of 40.97 ppm compared to that of *Culex quinquefasciatus* and *Anopheles stephensi* with LC<sub>50</sub> 53.84 and 96.00 ppm, respectively. The crude petroleum ether extract of this plant with good larvicidal efficacy will be considered as a potent candidate for further analysis.

**Key words:** *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*, *Eugenia jambolana*, larvicidal efficacy

### INTRODUCTION

Mosquitoes are well known for their public health importance, since they act as vectors of many tropical and subtropical diseases, such as malaria, dengue, chikungunya, lymphatic filariasis and Japanese encephalitis. *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae) are the major urban vectors of malaria, dengue and lymphatic filariasis respectively in India. The resurgence of these diseases is mainly due to the ever increasing urbanization and associated anthropogenic activities. One of the effective methods to control these diseases has been to target the vectors for interrupting the transmission. Though such measures could target all stages of the mosquito life cycle, main focus was almost on adult stage by using conventional insecticides based on indoor residual house spraying (Manzava *et al.*, 1993) and Insecticide Treated Nets (Ukpong *et al.*, 2007). Control of mosquito at the larval stage is also in practice in integrated mosquito management, as they are relatively immobile, remaining more concentrated than they are in the adult stage (Rutledge *et al.*, 2003). However, the indiscriminate application of synthetic insecticides has created multifarious problems such as environmental pollution, insecticide resistance and toxic hazards to humans. Globally there has been many efforts to overcome these problems and great emphasis has been placed recently on ecofriendly and

economically viable methodologies for vector control. Thus, in recent years various studies on natural plant products against mosquito vectors revealed it as possible alternatives to synthetic chemical insecticides (Maria *et al.*, 1997; Mittal and Subbaroa, 2003; Nazar *et al.*, 2009). Quite a few of these are selective and have little or no harmful effect on non-target organisms and the environment (Sivagnaname and Kalyanasundaram, 2004). Thus many medicinally important plants were tested for their efficacy to kill larvae of different species of mosquito (Madhumathy *et al.*, 2007; Bagavan *et al.*, 2009; Madhu and Vijayan, 2010).

It is in this regard *Eugenia jambolana* commonly known as Jambul belonging to the family Myrtaceae, has been selected for the present study. It is a widely distributed and cultivated plant in many parts of India. The seeds of this plant are used in ulcer healing and gastro-protective properties (Chaturvedi *et al.*, 2009) and also used as hypoglycemic and hepatoprotective properties (Jasmine and Daisy, 2007). Leaves of *Eugenia jambolana* has been employed for the inhibition of Buffalopox virus (Bhanuprakash *et al.*, 2007). Although some medicinal properties of this plant are known, there has been no report of its biological activity against mosquito species. The present study was designed to explore possibility of using this plant for testing its efficacy against three species of mosquitoes. The results may help to reduce the chemical burden on the environment and to promote sustainable utilization of locally available bioresource.

## MATERIALS AND METHODS

**Plant material and extraction:** Fresh leaves of *Eugenia jambolana* were collected from in and around Mysore, Karnataka, India from October 2009 to May 2010 and shade dried. The extract was prepared from the fine powder by employing a soxhlet apparatus using petroleum ether, ethyl acetate, acetone and methanol as solvents. The pooled extract was evaporated in a rotary vacuum evaporator at 40°C to dryness and stored at 4°C in an air tight bottle for further analysis. After preliminary experiments, petroleum ether extract was selected for further bioassay as it exhibited maximum efficacy. The entire experiment was conducted in the Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore, Karnataka, India.

**Mosquito larvae:** Larvae of the three mosquito species *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* were reared in enamel or plastic trays (30×24×5 cm) containing dechlorinated water. *Culex quinquefasciatus* and *Anopheles stephensi* larvae were fed with finely powdered mixture having 2:1 parts of dog biscuits and dry yeast, whereas *Aedes aegypti* larvae were fed with powdered dry yeast. The rearing water was changed daily until pupation.

**Larval bioassay:** Bioassays on mosquito larvae were performed on late third or early fourth instars, according to the standard guidelines of WHO (2005). The required quantities of *Eugenia jambolana* leaf extract of different concentrations were prepared in acetone as solvent. One milliliter of each of the concentration was mixed thoroughly with 249 mL of dechlorinated water in 500 mL glass beakers. Larvae were exposed to an ascending series of five concentrations according to log dose. Parallel control tests were also maintained by adding one mL of the solvent to 249 mL of dechlorinated water. Finally, 25 early fourth instar larvae were transferred to each of the beakers. A minimum of three replicates were kept for each concentration along with the control. Observation for the dead or moribund larvae was carried out after 24 h duration at 25°C and 14 h light and 10 h dark regime.

**Data analysis:** Larval mortality counts were adjusted for the mortality in control, if any employing Abbott's formula (Abbott, 1925) to give an estimate of the plant extract attributable mortality. The corrected mortality data were subjected to regression analysis of probit mortality on log dosage (Finney, 1971). The significant difference in LC<sub>50</sub> is based on the non-overlapping of 95% fiducial limits (Yang *et al.*, 2002).

**RESULTS AND DISCUSSION**

The results showing the toxicity of the *Eugenia jambolana* leaf extracts obtained with different solvents tested against three mosquito species are presented in Table 1 along with the log dose-probit mortality responses of all extracts in Fig. 1. Out of the four organic solvent extracts petroleum ether extract was found to be highly effective against all the three mosquito species

Table 1: Efficacy of different solvents of *Eugenia jambolana* leaf extracts against larvae of three mosquito species

Species	Extraction solvent	LC <sub>50</sub> (ppm)	95% FL	LC <sub>90</sub> (ppm)	95% FL	Slope±SE	Heterogeneity (df)	Regression equation
<i>Aedes aegypti</i>	Petroleum ether*	40.97	38.12-43.80	83.29	74.19-97.6	84.15±0.76	6.84 (3)	Y = 4.15X±1.707
	Ethyl acetate	58.25	51.59-65.41	198.66	162.2-262.0	2.40±0.217	3.52 (3)	Y = 2.405X 0.75
	Acetone	117.33	102.50-132.1	412.40	335.8-552.1	2.34±0.23	2.36 (3)	Y = 2.34X±0.14
	Methanol	402.82	360.6-449.32	1283.20	1046.7-1704.7	2.54±0.23	1.52 (3)	Y = 2.546X±1.63
<i>Culex quinquefasciatus</i>	Petroleum ether*	53.84	48.97-58.58	127.49	111.82-152.15	3.423±0.92	1.58 (3)	Y = 3.423X± 0.92
	Ethyl acetate	90.97	84.28-98.08	195.33	172.67-229.56	3.861±0.61	2.59 (3)	Y = 3.86X±.56
	Acetone	188.26	172.18-204.98	449.55	392.41-537.39	3.39±0.54	5.87 (3)	Y = 3.39X±2.7
	Methanol	433.93	390.68-480.05	1261.20	1056.5-1605.4	2.765±0.48	7.41 (3)	Y = 2.76X±2.29
<i>Anopheles stephensi</i>	Petroleum ether*	96.00	91.06-100.68	156.42	145.19-172.84	6.144±2.01	1.46 (3)	Y = 6.04X±6.98
	Ethyl acetate	102.27	95.82-108.93	195.92	176.54-224.84	4.53±0.74	3.18 (3)	Y = 4.53X±4.12
	Acetone	229.27	204.99-255.64	718.94	594.57-929.75	2.58±0.44	2.98 (3)	Y = 2.58X±2.58
	Methanol	428.37	386.21-473.38	1211.70	1024.1-1506.8	2.83±0.47	3.54 (3)	Y = 2.83X±2.46

LC<sub>50</sub> median lethal concentration; FL Fiducial limits; LC<sub>90</sub> 90% lethal concentration; df degree of freedom. \*The difference in LC<sub>50</sub> is significant based on the non-overlapping of 95% fiducial limits (p<0.05)

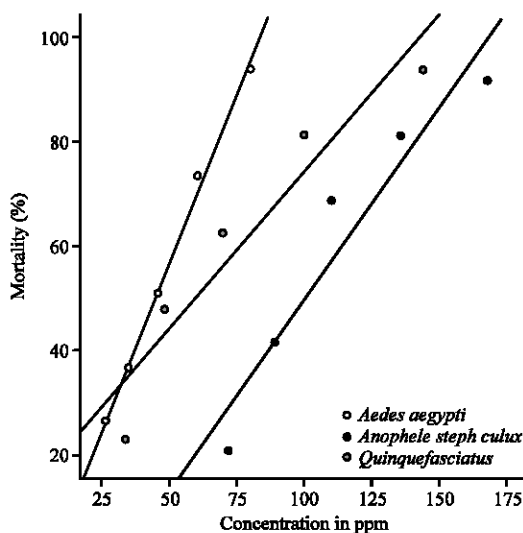


Fig. 1: Effect of Petroleum ether leaf extract of *Eugenia jambolana* against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* larvae

tested followed by ethyl acetate, acetone and methanol. The results show an  $LC_{50}$  of 40.97, 53.84 and 96.00 ppm for *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* respectively by the petroleum ether extract. Similarly,  $LC_{90}$  were found to be 83.29, 127.49 and 156.42 ppm, respectively. The larval sensitivity towards the crude extract was found in the order of *Aedes aegypti*>*Culex quinquefasciatus*>*Anopheles stephensi*. Further the larvicidal efficacy was found to be significantly different among all the extracts ( $p < 0.05$ ). The fig too depicts the log dose-probit mortality responses and slopes of regression lines of tested crude extract of *Eugenia jambolana* leaf extracts with different solvents.

The results of the larvicidal bioassay employing crude *Eugenia jambolana* extracts by different solvents employed against three different mosquito species revealed that all the organic extracts of *Eugenia jambolana* were bioactive (Table 1). However, significant ( $p < 0.05$ ) larvicidal activity was observed with petroleum ether followed by ethyl acetate, acetone and methanol extracts ( $p < 0.05$ ). The biological activity of this plant extract may be due to various compounds, including phenolics, terpenoids, flavonoids and alkaloids (Gohil *et al.*, 2010). These compounds may jointly or independently contribute to produce toxic activity against the mosquito species. It was earlier reported that petroleum ether of the whole plant such *Citrullus colocynthis* is effective against mosquito larvae (Rahuman *et al.*, 2008). Likewise, Latha and Ammini (2000) have studied petroleum ether extract of *Curcuma raktakanda* leaf against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. The present result and the earlier report on other plants indicate that petroleum ether extract may be more effective among organic solvents. Data presented in Table 1 and Fig. 1 further show that a converse relationship exists between extract efficacy and solvent polarity, which is in line with the observation made by Mulla and Su (1999) in neem plant extracts.

Among the three species tested by the present authors, maximum effect was on *Aedes aegypti* compared to the other mosquito species. This finding is in agreement with that of Rahuman *et al.* (2000) who have tested *Feronia limonia* extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* larvae. The varying susceptibility of the three species of mosquito is probably due to difference in the physiological characteristics of the three species of mosquito. This agrees with the report of Raghavendra *et al.* (2009), who have reported the existence of such differences among four mosquitoes assayed with *Solanum nigrum* extracts. This also agrees with the report of Kumar and Maneemegalai (2008), who have tested *Lantana camara* extract against *Aedes aegypti* and *Culex quinquefasciatus*. Maheswaran *et al.* (2008) too have reported variation toxicological efficacy with *Aedes aegypti* and *Culex quinquefasciatus* to the leaves of *Leucas aspera*. Similar observations were made by Bagavan *et al.* (2009) with *Achyranthes aspera* against *Aedes aegypti* and *Culex quinquefasciatus*. Mathew *et al.* (2009) also have reported variations in larvicidal efficacy from *Saraca indica*, *Nyctanthes arbor-tristis* and *Clitotia ternatea* extracts against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* larvae. Thus the present findings on *Eugenia jambolana* showed some promise for further chemical isolation of the active ingredient in future and it could be considered as a potent resource for local people for controlling mosquito larvae. Such practice would not only reduce the chemical burden on the environment but also promote sustainable utilization of locally available bioresource by rural communities.

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