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Evaluation of Larvicidal Effect of *Euodia ridleyi* Hochr. Leaf Extract Against Three Mosquito Species at Mysore

K.P. Prathibha, B.S. Raghavendra and V.A. Vijayan
Vector Biology Research Lab, Department of Studies in Zoology, University of Mysore, Manasagangothri, 570006 Mysore, Karnataka, India

Abstract: With the development of resistance to conventionally used synthetic insecticides, vector management has become very difficult. Hence, scientists have shown interest on botanicals. It is in this regard, present study was aimed to evaluate the efficacy of extracts of *Euodia ridleyi* leaves against the larvae of vectors of filariasis, malaria and dengue fever vectors namely *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*, respectively employing the standard WHO bioassay method. Non-polar organic solvents such as petroleum ether, ethyl acetate, acetone and methanol were employed for soxhlet extraction. The results project that efficacy of ethyl acetate extract is the most effective among the four solvents. The efficacy was found to be maximum on the larvae of *Culex quinquefasciatus* with LC_{50} of 64.59 ppm than the other two species. Similarly the LC_{50} against *Anopheles stephensi* and *Aedes aegypti* were 120.07 and 139.88 ppm, respectively. The data highlights the importance of *Euodia ridleyi* as a promising local plants with larvicidal potency for further studies to isolate the active molecule in it.

Key words: *Culex quinquefasciatus*, *Anopheles stephensi*, *Aedes aegypti*, *Euodia ridleyi*, larvicidal, India

INTRODUCTION

Mosquito borne diseases such as malaria, lymphatic filariasis, dengue, yellow fever and Japanese encephalitis contribute significantly to human disease burden and death, in addition to poverty and social delibility in tropical countries. Lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by *Culex quinquefasciatus* is found to be more endemic in the Indian subcontinent. It is reported that *Culex quinquefasciatus* infects >100 million individuals worldwide annually (Rajasekaraiah *et al*., 1991). *Anopheles stephensi* is the primary vector of malaria in India and other West Asian countries. Every year, an estimated 300-500 million new infections and 1-3 million deaths result from malaria world wide (Muturi *et al*., 2008).

Aedes aegypti L., a vector of yellow fever, dengue and chickungunya is widely distributed in the tropical and subtropical zones. About two-thirds of the world's population lives in areas infested with dengue vectors, mainly *Aedes aegypti* (Hahn *et al*., 2001).

Many approaches have been developed to prevent mosquito menace and communicable diseases. One such strategy has been mainly based on synthetic insecticides to control mosquitoes at larval stages. Even though, these are effective, created many problems like insecticide resistance, pollution and toxic side effect on non-target organisms including human beings. Therefore, it is necessary to develop environmentally safe, biodegradable, economical and indigenous method for the control of vectors that can be used in minimum care by individuals and communities (Mittal and Subbarao, 2003). A review indicated that the assessment of the efficacy of different phytochemicals obtained from various plants is the best way to develop novel synthetic insecticides (Sukumar *et al*., 1991). In this regard, India has a rich flora that is widely distributed throughout the country. In the light of the said observations, the present investigation on the mosquito larvicidal activity of *Euodia ridleyi* (Rutaceae) leaf extracts were carried out. The jungle of South East Asia have produced this colourful citrus relative.

It is purely an ornamental plant. *Euodia*, In Greek means sweet smelling. *Euodia*, a native of tropical forests is very much at home in partially shaded garden areas where its yellow-green foliage forms a bright coloured accent. Some contain aromatic oils that act as coolants for fevers as lotions for the improvement of complexions and as tonics in the treatment of stomach complaints (Clay and Hubbard, 1977).

Corresponding Author: K.P. Prathibha, Vector Biology Research Lab, Department of Studies in Zoology, University of Mysore, Manasagangothri, 570006 Mysore, Karnataka, India

MATERIALS AND METHODS

Plant extracts: The leaves of *Euodia ridleyi* were collected from Wayanad district of Kerala state, India from November 2009 to August 2010. These were shade dried, powdered manually and subjected to solvent extraction in a soxhlet apparatus until exhaustion to obtain non polar bioactive constituents. Solvents from the plant extracts were removed using a rotatory evaporator under reduced pressure at 40°C to yield a dark, greenish, gummy extract and stored at 4°C in an air tight bottle. This was later dissolved in acetone and employed to prepare different concentrations.

Mosquito colony: *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* larvae were collected and maintained in an insectary at the Vector Biology Research Lab, Department of studies in Zoology, University of Mysore. The adults were reared in cages (30×30×30 cm) fitted with mosquito netting. *Culex quinquefasciatus* males were fed on 10% sucrose solution, those of *Anopheles stephensi* and *Aedes aegypti* were fed on dry grapes soaked in water.

Females were blood fed on a mouse obtained from animal house of the Zoology department after taking the approval of the institutional animal ethics committee. Small bowls with dechlorinated water were used to collect eggs of *Culex quinquefasciatus* and the one lined with filter paper bits was employed to collect the eggs in case of *Anopheles* and *Aedes* species.

The larvae were reared in large enamel or plastic trays (30×24×5 cm) containing dechlorinated water and fed using finely powered dog biscuits and dry yeast in the ratio of 2:1, the test population was maintained in the laboratory under environmentally controlled conditions (26±2°C and 70±5% RH) with a photoperiod of 14th light and 10th dark.

Larval bioassay: The larvicidal activity of a leaf extract of *Euodia ridleyi* was evaluated as per the method recommended by WHO (2005). Tests concentrations were conducted by adding 1 mL of known concentration of the phytoextract to 249 mL of dechlorinated tap water. For the control experiment, 1 mL of acetone was added to 249 mL of dechlorinated tap water. About 25 early 4th instar larvae were released into the test concentration and the control with the help of a strainer and mortality was recorded after 24 h. No food was provided either to the test or control during the experiment. Two replicates were maintained for both test and control. The moribund and dead larvae in the replicates were combined and expressed as percentage mortality at each concentration.

The larvae were considered as dead or moribund if they do not respond to a gentle prodding with a fine needle. All bioassays were carried out at room temperature of 25±2°C and 75±5% of relative humidity.

Data analysis: Larval counts were adjusted for the mortality in control if present, employing Abbott (1925)'s formula to give an estimate of plant extract attributable mortality. The corrected mortality data were subjected to regression analysis of probit mortality on log dosage Finney (1971). LC_{50} values were considered to be significantly different if the 95% FLs of two LC_{50} values did not overlap each other (Yang *et al*., 2002).

RESULTS AND DISCUSSION

Table 1 show the data on the efficacy of petroleum ether, ethyl acetate, acetone and methanol leaf extracts of *Euodia ridleyi* leaf against *Culex quinquefasciatus* with LC_{50} values of 71.30, 64.59, 74.66 and 427.9 ppm, respectively. Likewise, the LC_{50} values against *Anopheles stephensi* are 184.97, 120.87, 194.51 and 477.8 ppm, respectively and that against *Aedes aegypti* are 203.65, 139.88, 203.39 and 477.8 ppm, respectively. Out of the 4 solvents used for extraction, ethyl acetate extract was found to be more effective against *culex quinquefasciatus* with LC_{50} of 64.59, *Anopheles stephensi* with LC_{50} of 120.07 and *Aedes aegypti* with LC_{50} of 139.88 ppm. The log dose probit mortality responses of all the 3 species are shown in Fig. 1. The ethyl acetate extract against *Culex quinquefasciatus* larvae showed 18 and 98% mortality at 55 and 95 ppm, respectively and against *Anopheles stephensi* the extract showed 30 and 98% mortality at 100 and 200 ppm, respectively as against *Aedes aegypti,* it showed 36 and 98% mortality at 120 and 200 ppm, respectively. The larvicidal efficacy was found to be significantly different between the extracts ($p<0.05$).

Environmental safety of an insecticide is considered to be of paramount importance while employing it against pests and vectors. An insecticide need not cause high mortality on target organisms in order to be acceptable (Kabaru and Gichia, 2001). Resistance to insecticides dates back to the beginning of application of chemicals, since DDT was initially introduced for mosquito control in 1946 and just in 1 year (1947), the first cases of DDT resistance occurred in *Aedes tritaeniorhynchus* and *Aedes aolicitians* (Hemingway and Ranson, 2000). More than 500 species of arthropods are reported to be resistant to various insecticides (Shelton *et al*., 2007). In this regard, Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive and are readily available throughout the

Table 1: Efficacy of different solvents of *Euodia ridleyi* leaf extracts against larvae of three mosquito species

Species	Extraction solvent	LC_{50} (ppm)	(95% FL)	LC_{90} (ppm)	(95%FL)	Slope±SE	Heterogeneity X^2 (df)	Regression equation
Culex quinquefasciatus	Petroleum ether	71.30	69.71-72.83	89.36	86.59-92.96	13.07±0.95	0.39 (3)	Y = 13.07X±19.20
	Ethyl acetate	64.59	62.86-66.23	81.67	78.85-85.43	12.57±1.01	1.04 (2)	Y = 12.57X±1.770
	Acetone	74.66	72.65-76.60	98.68	94.91-103.6	19.57±0.77	1.64 (3)	Y = 10.56X±14.80
	Methanol	427.90	422.2-433.50	496.00	485.3-510.30	19.99±1.55	1.00 (4)	Y = 19.99X±47.60
Anopheles stephensi	Petroleum ether	184.90	180.2-189.70	240.40	230.4-254.40	11.25±0.97	1.38 (3)	Y = 11.2X± 20.50
	Ethyl acetate	120.00	115.0-124.60	178.20	168.9-191.20	7.46±0.63	0.54 (3)	Y = 7.46X ±10.50
	Acetone	194.50	189.9-199.00	246.20	237.1-258.60	12.56±1.03	2.57 (3)	Y = 12.5X± 23.60
	Methanol	477.80	471.0-484.60	552.20	539.2-569.80	20.38±1.69	3.44 (3)	Y = 20.38X±49.60
Aedes aegypti	Petroleum ether	203.60	200.5-206.60	236.70	231.1-244.20	19.61±1.61	2.23 (3)	Y = 19.6X ±40.20
	Ethyl acetate	139.80	134.0-144.90	203.50	191.6-222.00	7.86±0.82	7.24 (3)	Y = 7.86X±11.87
	Acetone	203.30	226.3-234.30	273.80	266.5-283.70	17.08±1.39	2.00 (3)	Y = 17.08X±35.30
	Methanol	477.80	471.0-484.60	552.20	539.2-569.80	20.38±1.64	3.44 (3)	Y =20.38X±49.60

LC_{50} median lethal concentration; FL Fiducial Limit; LC_{90} 90% Lethal Concentration, df degree of freedom, *the difference in LC_{50} is significant based on the non-overlapping of 95% fiducial limit ($p<0.05$)

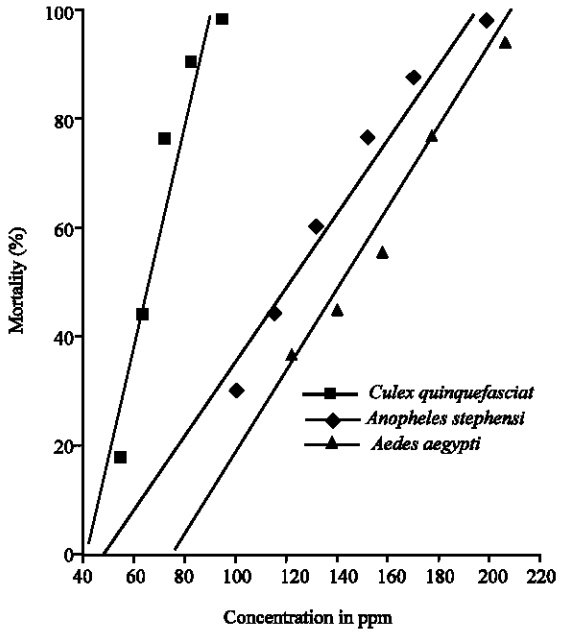

Fig. 1: Effect of *Euodia ridleyi* ethyl acetate extract on *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*

world. According to Bowers *et al.* (1995), the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. It is in this regard, the present study adds to the knowledge on the efficacy of the local plant, *Euodia ridleyi*. The present study highlight that out of 4 organic solvent extracts obtained from *Euodia ridleyi*, significant larvicidal activity against *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* was observed with ethyl acetate followed by petroleum ether, acetone and methanol (Table 1; Fig. 1). The type of solvents selected affects extract efficacy between different phytochemicals of varying volatility are present in the final extraction (Shaalan *et al.*, 2005). Different solvents are used in plant extracts screening, since polar solvents extract polar molecules and non polar solvents extract non polar molecules. The purpose of a general screening for bioactivity is to extract as many potentially active constituents as possible.

This is achieved by using solvents ranging from water, the most polar one with a Polarity index (P) of 10.2 to hexane (non polar, p = 0.1) including a number of intermediary solvents such as methanol (p = 6.1), acetone (p = 5.1), ethyl acetate (p = 4.4) (Shaalan *et al.*, 2005). Data on *Euodia ridleyi* leaf extracts showed that a converse relationship exists between extract efficacy and solvent polarity where efficacy increases with decreasing polarity. This is in line with the observation made by Aivazi and Vijayan (2009) in oak gall extracts.

However, this may not be consistent due to differences between the characteristics of active chemicals among plants. Similarly, acetone extract of *Millingtonia hortensis* leaves showed efficacy against the larval stages of three mosquito species *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti* with LC_{50} of 138, 223.9, 208.9 ppm, respectively (Kaushik and Saini, 2008).

Here, the larval sensitivity towards the crude extract was found in the order of *Culex quinquefasciatus>Aedes aegypti>Anopheles stephensi*. Whereas in the present study, the order is *Culex quinquefasciatus>Anopheles stephensi>*A*edes aegypti*. Similar observations were made by Sujatha *et al.* (1988) with petroleum ether extracts of 6 plants namely *Acorus calamus*, *Ageratum conyzoides*, *Annona squamosa*, *Bambusa arundanasia*, *Citrus medica* and *Madhuca longifolia* against 3 species of mosquitoes, *Anopheles gambiae*, *Aedes aegypti* and *culex quinquefasciatus*. In this regard, *Euodia ridleyi* is found to be remarkable, economical and environmentally

friendly with mosquito larvicidal properties. So, it could form a new source for managing mosquito larvae. Therefore, its bioactive components being explored for further investigations as this is the initial research carried out on the plant *Euodia ridleyi.*

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