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# LARVICIDAL, OVICIDAL AND PUPICIDAL ACTIVITY OF *ERANTHEMUM ROSEUM* (VAHL) R. BR. AGAINST MALARIAL VECTOR MOSQUITO, *ANOPHELES STEPHENSI* (LISTON) (DIPTERA: CULICIDAE)

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**ABSTRACT**

In the present investigation acetone and methanol extract of *Eranthemum roseum* leaves were tested for its larvicidal, ovicidal and pupicidal activities against the important malarial vector, *Anopheles stephensi*. The present experiments were designed and carried out by adapting the standard protocols. Results pertaining to the present investigation clearly revealed that the methanol extract was more prominent than the acetone extract and the results are on par with the control groups. The bioefficacy of *E. rosum* was due to the existence of several phytochemical groups and this needs further exploration of mosquitocidal compounds by various spectral analyses. The information given in this is the first report on mosquito about its bio efficacies. Thus, it could pave the way for possible utilization of *E. roseum* as an alternative potential agent in Vector Control Programme.



## INTRODUCTION

Ecologically, mosquitoes are important components of aquatic and terrestrial food chains as they serve as food for a number of animals, such as fish and birds. With respect to the human well-being, mosquitoes are of great economic impact because their bites are annoying and may cause skin allergies, and they are vectors for a number of diseases, such as malaria, yellow fever, dengue, filariasis, and certain types of encephalitis such as West Nile Fever (Service 1993; Nasci and Miller 1996). *Anopheles stephensi* (Liston) is the primary vector of malaria in India and other West Asian countries, and improved methods of control are urgently needed (Burfield and Reekie 2005). Insect vectors, especially mosquitoes are responsible for spreading serious human diseases like malaria (Halstead, 2000). The distribution and abundance of these diseases is strongly influenced by the presence of humans and by the level of poverty of the population (Mendonca *et al*. 2005). Malaria is by far the most important insect transmitted disease (Gilles and Warrell 1993), remaining a major health problem in many parts of the world and is responsible for high childhood mortality and morbidity in Africa and Asia (Kleinschmidt *et al.* 2000; Pates and Curtis 2005). *Anopheles stephensi* have, therefore, become a challenging problem to public health worldwide, and it has a serious social and economical impact especially in tropical and subtropical countries (Borovsky 2003; Spielman 2003; Bossche and Coetzer 2008). An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. It has also provoked undesirable effects, including toxicity to non-target organisms, and fostered environmental and human health concerns (Lee *et al.* 2001). Thus, the effort towards mosquito control continues to be an important strategy in preventing the mosquitoborne diseases (Billingsley et al. 2008).

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Over the past 50 years, more than 2,000 plant species belonging to different families and genera have been reported to contain toxic principles, which are effective against insects (Mathivanan *et al.* 2011).In India, there are various plants known for their insecticidal property and are popular as pesticides. Plant derived compounds (phytopesticides) in general have been recognized as an important natural resource of insecticides (Gbolade et al. 2000). Several phytochemicals have been reported to exhibit detrimental effects on mosquitoes (Kuo et al. 2007; Ghosh *et al*. 2008; Rahuman *et al*. 2009). With these backgrounds the present investigation was aimed to assess the mosquitocidal activity of acetone and methanol extracts of *Eranthemum roseum* (Vahl) R. Br. (Acanthaceae) against an important malarial vector mosquito, *Anopheles stephensi* Liston.

## MATERIALS AND METHODS

### Collection and processing of plants

Plant sample (leaves) was collected during the growing season (March– April) of 2011 from different places of Dindugal District, Tamilnadu. Bulk samples were air-dried in the shade and after drying the sample was ground to a fine powder. At the time of collection, two pressed voucher herbarium specimens were prepared per species and identified with the help of plant taxonomist, Department of Botany, Govt. Arts College, Nandanam, Chennai, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

### Extraction method

The dried leaf (200g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with acetone and methanol (1000 ml, Ranchem), in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22–26 mmHg at 45°C by 'Rotavapour' and the residue obtained was stored at 4°C.

### Mosquito rearing

Eggs of *Anopheles stephensi* were collected from water bodies in the garden. The egg rafts were then brought to the laboratory. The eggs were placed in enamel trays (30×24×5 cm) each containing 2 *l* of tap water and kept at room temperature (28 ± 2°C) with a photoperiod of 12:12 h (L:D) for larval hatching. The larvae of each mosquito species were maintained in separate trays under the same laboratory conditions and fed with a powdered feed containing a mixture of dog biscuit and baker's yeast (3:1 ratio).The trays with pupae of each mosquito species were maintained in separate mosquito cages at 26±2°C and relative humidity of 85±3% under a photoperiod of 12:12 h (L:D) for adult emergence. Cotton soaked in 10% aqueous sucrose solution in a Petri dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized young chick was placed for 3 h inside the cage in order to provide blood meal especially for female mosquitoes. A plastic tray (11× 10×4 cm) filled with tap water with a lining of partially immersed filter paper was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched larvae / pupae of *Anopheles stephensi* were used in all bioassays.

### Bioassay

### Larvicidal activity

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005). From the stock solution, five different test concentrations (*viz.*, 50, 100, 150, 200 and 250 ppm were prepared and they were tested against the freshly moulted (0 – 6 hrs) third instar larvae of *An. stephensi*. The larvae of test species (25) were introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1ml of emulsifier) and the required amount of plant extract was added. The larval mortality was observed and recorded after 24 h of post treatment. For each experiment, five replicates were maintained at a time. The percentage of mortality was calculated by using Abbott's formula (Abbott 1925). The $LC_{50}$, $LC_{90}$, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), chi-square values and the degrees of freedom were calculated by using Probit analysis with Statistical Package for Social Sciences (SPSS) 17.0 Version in MS-Excel, 2007.

### Ovicidal activity

The method of Su and Mulla (1998) was slightly modified and used to test the ovicidal activity. The various concentrations as stated in the previous experiments were prepared from the stock solution. Before treatment, the eggs of *An. stephensi* were counted individually with the help of hand lens. Freshly hatched eggs (100) were exposed to each concentration of leaf extract until they hatched or died. Eggs exposed to DMSO in water served as control. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under a microscope. Each test was replicated five times. The hatchability was assessed 48 h post treatment by the following formula.

### Pupicidal assay

Batches of ten pupae were introduced into 500 ml of the test medium containing particular concentration of the crude extract in a plastic cups in five replications. In control, the same number of pupae was maintained in 500 ml of dechlorinated water containing appropriate volume of DMSO. All containers were maintained at room temperature (28±2°C) with naturally prevailing photoperiod (12: 12h / L: D) in the laboratory. Any pupa was considered to be dead if did not move when prodded

repeatedly with a soft brush. Mortality of each pupa was recorded after 24 of exposure to the extract.

**Determination of lethal concentrations**

Lethal concentration ($LC_{50}$) represents the concentration of the test material that caused 50% mortality of the test (target and non target) organisms within the specified period of exposure, and it was determined by exposing various developmental stages of the mosquitoes to different concentrations of the extract. Based on the mortality of the test organisms recorded in these bioassays, $LC_{50}$ was calculated along with their fiducial limits at 95% confidence level by probit analysis using SPSS software package.

**RESULTS**

Larvicidal activity of *E. roseum* ethyl acetate and ethanol extracts were tested against fourth instar larvae of *Anopheles stephensi*. Perusal of the data clearly revealed that minimum larval mortality was observed in ethyl acetate extract of *G. sepium* with 33.2 ± 0.6 at 50 ppm concentration and the maximum mortality was observed from the same extract with 96.0 ± 2.4%. Furthermore, the $LC_{50}$ and $LC_{90}$ value for ethyl acetate extract was found to be 121.65 and 237.38ppm. Similarly larvae exposed to 50 ppm concentration of methanol extract showed less susceptibility whereas, experimental larvae exposed to 250ppm concentration showed more susceptibility to the same extract. Furthermore, the $LC_{50}$ was found to be 139.86 (LCL=105.12 and UCL=178.38) and the $LC_{90}$ value was recorded to be 255.51ppm (LCL=208.61 and UCL=363.85ppm). The recorded data were found statistically significant (Table1; DMRT, $p<0.05$). Ovicidal activity of ethyl acetate and ethanol extract was assessed by assessing the egg hatchability. It was noted that 100% hatchability was noted from the control groups, which means 0% ovicidal activity. Highest concentration of both solvent extracts exhibited 100% ovicidal activity as it was evident from the table 2. Further, as the concentration increased the mortality of the eggs were also increased with decreased hatching percentage. The data obtained in the experiments were statistically significant over the control (Table 2). Effect of ethyl acetate and ethanol crude extract of the *E. roseum* tested on the pupae of *An. stephensi*, data obtained from the experiment is presented in table 3. About 13.62 pupae were found dead with 54.60% adult emergence when it was treated with 25 ppm concentration of ethyl acetate extract of *E. roseum*. Similarly, 17.43 ± 1.38 (n=30; 58.10%); 20.33 ± 1.38 (67.76) and 25.48 ± 2.33 (84.93) pupal mortality was recorded from the experimental pupae treated with 50, 75 and 100ppm concentration of extracts. Pupae exposed to different concentrations of ethanol extract were found dead with 58.10% adult emergence when it was treated with 25 ppm concentration. Similarly, 19.58 ± 2.62 (n=30; 65.26%); 23.64 ± 1.65 (78.80) and 23.38 ± 2.83 (77.93) pupal mortality was recorded from the experimental pupae treated with 50, 75 and 100ppm concentration of extracts.

**DISCUSSIONS**

With increasing legislative restrictions being implemented concerning the use of pesticides, safe, but efficient alternatives and application techniques must be developed to allow the least-toxic but most efficient means of integrated vector control, especially during emergency situations (Chavasse and Yap 1997; Anonymous 2006). In the present study, methanol extract of *E. roseum* was found to have significant mosquitocidal activity than the acetone extract tested against *An. stephensi*. The methanolic extracts of few plants exhibited larvicidal activity against *C. quinquefasciatus* (Venkatachalam and Jebanesan 2001). Rajkumar and Jebanesan (2002) reported that increase in the concentration of leaf extract of *Solanum aerianthum* induced the oviposition attractant activity in *C. quinquefasciatus*. Exposure of *A. stephensi* larvae to sub-lethal doses of neem extracts in the laboratory prolonged larval development, reduced pupal weight, high oviposition deterrence and high mortality (Su and Mulla 1998). Recently Mathivanan *et al.* (2010) reported that the methanol extract of *Ervatamia coronaria* showed promising larvicidal and ovicidal activity agains *An. stephensi*. The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for management of *An. stephensi*. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future (Wandscheer *et al.* 2004). Although the botanical insecticides are the lesser of many hazards in terms of general pesticide toxicities, they are toxins nonetheless. All toxins used in pest control pose some hazards to the user and also to the aquatic environment (Kreutzweiser 1997).

The biological activity of the experimental plant extracts may be due to various compounds, including phenolics, terpenoides, flavonoids and alkaloids present in plants, of these compounds may jointly or independently contribute to produce adult emergence inhibition, and adulticidal effect against malarial vector, *An. stephensi*. Undoubtedly, plant derived toxicants are a valuable source of potential insecticides. These and other naturally occurring insecticides play a prominent role in mosquito control programs in the future (Mordue and Blackwell, 1993). The results of this study may contribute to a great reduction in the application of synthetic insecticides, which in turn increase the opportunity for natural control of various medicinally important vectors by botanical chemicals. Since these are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control programme (Alkofahi et al., 1989), they could lead to the development of new classes of possible safer insect control agents. Our results agree with some previous studies, such as the aqueous leaves extract of *Calotropis procera* reported that the 50% of adult emergence inhibition ($EI_{50}$) was shown at 277.90 and 183.65 ppm for *An. arabiensis* and *C. quinquefasciatus*, respectively, and the pupal stage was not affected till a concentration of 5000 ppm (Elimam et al., 2009b).

**Table 1** Larvicidal activity of *Eranthemum roseum* at different concentration tested against freshly moulted (0-6h old) 4th instar larvae of *Anopheles stephensi*

| Concentration (ppm) | Mortality (%) | $LC_{50}$ | 95% Confidence Limits (ppm) | | $LC_{90}$ ppm | 95% Confidence Limits (ppm) | | Degree of freedom | $\chi^2$ value |
|---|---|---|---|---|---|---|---|---|---|
| | | | LCL | UCL | | LCL | UCL | | |
| Acetone | | | | | | | | | |
| Control | 1.8 ± 0.6[a] | | | | | | | | |
| 50 | 33.2 ± 0.6[b] | | | | | | | | |
| 100 | 44.8 ± 1.6[c] | | | | | | | | |
| 150 | 55.4 ± 1.8[d] | 121.65 | 82.38 | 159.87 | 237.38 | 190.87 | 347.55 | 5 | 21.264 |
| 200 | 75.2 ± 1.6[e] | | | | | | | | |
| 250 | 96.0 ± 2.4[f] | | | | | | | | |
| Methanol | | | | | | | | | |
| Control | 1.8 ± 0.8[a] | | | | | | | | |
| 50 | 24.2 ± 1.6[b] | | | | | | | | |
| 100 | 36.6 ± 3.6[c] | | | | | | | | |
| 150 | 47.4 ± 2.6[d] | 139.86 | 105.12 | 178.38 | 255.51 | 208.61 | 363.85 | 5 | 19.127 |
| 200 | 66.6 ± 3.6[e] | | | | | | | | |
| 250 | 97.2 ± 3.2[f] | | | | | | | | |

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 24h of exposure period. $LC_{50}$=Lethal Concentration brings out 50% mortality and $LC_{90}$ = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with a different superscript alphabet are significantly different at $P \leq 0.05$ level DMRT Test.

**Table 2** Ovicidal activity of *Eranthemum roseum* crude extract on eggs (0-6h old) of *Anopheles stephensi*

| Concentrations tested | Solvents used | |
|---|---|---|
| | Acetone | Methanol |
| Control | 100.00±0.00[e] (0.00) | |
| 25ppm | 82.64±5.8[d] | 87.34±3.62[d] |
| 50ppm | 67.63±3.6[c] | 45.25±2.83[c] |
| 75ppm | 45.42±2.4[b] | 35.66±3.44[b] |
| 100ppm | 0.00±0.00[a] | 0.00±0.00[a] |

Values represent mean ± S.D. of five replications. Different alphabets in the column are statistically significant at $p \leq 0.05$level DMRT Test. Eggs in control groups were sprayed with no phytochemicals.

**Table 3** Pupicidal activity of ethyl acetate and ethanol extract of *Eranthemum roseum* at different concentrations tested against the pupae of *Anopheles stephensi*.

| Concentration (pppm) | n* | Mortality ** | | Adult Emergence | |
|---|---|---|---|---|---|
| | | Pupal Mortality | % Mortality | Adult | % Emergence |
| **Acetone extract** | | | | | |
| 50 | 30 | 13.62 ± 1.56[b] | 45.40 | 16.38 ± 1.34[d] | 54.60 |
| 100 | 30 | 17.43 ± 1.38[c] | 58.10 | 12.57 ± 1.65[c] | 41.90 |
| 200 | 30 | 20.33 ± 1.69[d] | 67.76 | 9.67 ± 0.87[b] | 32.23 |
| 400 | 30 | 25.48 ± 2.33[e] | 84.93 | 4.52 ± 1.23[a] | 15.06 |
| Control | 30 | 3.45 ± 0.98[a] | 11.50 | 26.55 ± 2.33[e] | 88.50 |
| **Methanol Extract** | | | | | |
| 50 | 30 | 12.57 ± 1.25[b] | 41.90 | 17.43 ± 1.22[d] | 58.10 |
| 100 | 30 | 18.36 ± 1.38[c] | 61.20 | 11.64 ± 1.36[c] | 38.8 |
| 200 | 30 | 21.28 ± 1.29[d] | 70.93 | 8.72 ± 1.45[b] | 29.06 |
| 400 | 30 | 27.33 ± 1.36[e] | 91.10 | 2.67 ± 0.32[a] | 8.9 |
| Control | 30 | 1.83 ± 1.87[a] | 6.10 | 28.17 ± 1.37[e] | 93.9 |

Value represents mean ± S.D. of five replications.* Number of pupae subjected to the experiment. **Mortality of the pupae observed after 7 days of exposure period). Values in the column with a different superscript alphabet are significantly different at $P \leq 0.05$ level DMRT Test).

Similar study was conducted by Elimam et al. (2009a) and they reported that aqueous extracts from leaves of *Ricinus communis* showed 50% of adult emergence inhibition ($EI_{50}$) was 374.97 and 1180.32 ppm against 3rd instar larvae of *An. arabiensis* and *C. quinquefasciatus* and the extract showed oviposition deterrent effect against both species. The larvicidal, growth inhibitor and repellent actions of *Dalbergia sissoo* oil was evaluated against *A. stephensi, A. aegypti and C. quinquefasciatus* under laboratory conditions and observed no adult emergence was observed at 4 ml/m2 (Ansari *et al.*, 2000).

The present study results are in agree with Howard et al. (2009) who had reported that 50% inhibition of adult emergence ($IE_{50}$) of all larval instars obtained with <0.4 g of neem bark chippings of *A. indica* in 1 L of distilled water. The hexane extract obtained from leaves of *Eucalyptus citriodora* tested at lowest concentration viz. 10 ppm, 73% larvae of *An. stephensi* failed to emerge as adult mosquito while in *C. quinquefasciatus* and *A. aegypti* only 10% and 6% larvae failed to emerge (Singh *et al.*, 2007). Similar result was obtained in the root extract of *Valeriana jatamansi* which exhibited 90% lethal concentration against adult *An. stephensi, An. culicifacies, A. aegypti, An. albopictus*, and *C. quinquefasciatus* and were 0.14, 0.16, 0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm2,

respectively (Dua *et al.,* 2008). The highest adulticidal effect was established from *Piper sarmentosum*, followed by Piper ribesoides and *Piper longum*, with $LD_{50}$ values of 0.14, 0.15 and 0.26 microg/female, respectively (Choochote et al., 2006). In testing for adulticidal activity, the crude seed extract of celery, *Apium graveolens*, exhibited a slightly adulticidal potency on *A. aegypti* with $LD_{50}$ and $LD_{95}$ values of 6.6 and 66.4 mg/cm2, respectively, (Choochote *et al.*, 2004). This result is also comparable to earlier reports of Dua *et al.* (2010) who observed that the adulticidal activity of the essential oil of *Lantana camara* essential oil were 20, 18, 15, 12 and 14 min and 35, 28, 25, 18 and 23 min against *A. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluvialitis and An. stephensi* with their percent mortality of 93.3%, 95.2%, 100%, 100% and 100%, respectively. Also the result agrees with the finding of Halim (2008) who have reported the insecticidal activity of *Zingiber officinale* against the adult emergency of *A. pharoensis*. Elango et al. (2010) reported that the hexane extracts of *A. marmelos* and *A. paniculata* served as a potential repellent, ovicidal, and oviposition deterrent against *C. tritaeniorhynchus. C. hirsutus* is a widely growing plant found in the plains of India in dry localities and the methanol extract showed effective oviposition repellency against *An. subpictus* (Elango *et al.,* 2009a). *E. prostrata*, a member of the Asteraceae family and commonly known as False Daisy and the ethyl acetate extract of E. prostrata and leaf hexane extract of *A. paniculata* have the potential to be used against the fourth-instar larvae of *A. subpictus* and *C. tritaeniorhynchus* (Elango et al., 2009b).

Since there is no previous record of literature available about the mosquitocidal activity of the selected plant *E. roseum* these present investigations serve as first hand information. The finding of the present investigation revealed that the leaf extract of *E. roseum* possessed remarkable larvicidal, ovicidal activity and pupicidal activity against the malarial vector *An. stephensi.*

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