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# MOSQUITO LARVICIDAL ACTIVITY OF *OXYSTELMA ESCULENTUM* PLANT EXTRACTS AGAINST *ANOPHELES STEPHENSI* (DIPTERA: CULICIDAE)

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**ABSTRACT**

Malaria is one of the most common vector-borne diseases in widespread in tropical and subtropical regions, including part of the Asia and Africa. It is the world's most dreadful tropical disease. The aim of this study was to investigate the larvicidal activity of acetone, benzene, chloroform, hexane and methanol leaf extract of *Oxystelma esculentum* against *Anopheles stephensi.* Twenty five early third instar larvae of *An. Stephensi* was exposed to various concentrations (30-180ppm) and was assayed in the laboratory by using the protocol of WHO 2005; the 24h $LC_{50}$ values of the *O. esculentum* leaf extract was determined by probit analysis. The $LC_{50}$ and $LC_{90}$ values of acetone, benzene, chloroform, hexane and methanol leaf extracts of *O. esculentum* against *An. Stephensi* larvae in 24 h were 75.46, 68.55, 98.47, 88.24, 63.84 and 140.66, 130.65, 184.10, 169.36 and 122.48 ppm, respectively. From the results it can be concluded the crude extract of *O. esculentum* as an excellent potential agent for controlling *An. Stephensi* mosquito



## INTRODUCTION

Mosquitoes are the vectors for the dreadful diseases of mankind. Of all the insects that transmit diseases, mosquitoes represent the greatest menace. WHO has declared the mosquito "public enemy number one" because mosquitoes are responsible for the transmission of various dreadful diseases (WHO, 1996).Mosquito-borne diseases such as malaria, filariasis, dengue, and viral encephalitis contribute to a larger proportion of health problems of developing countries. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It also resultedin the development of resistance, undesirable effects on non-target organisms, and fostered environmental and human health concern (Thomas *et al.*,2004).One of the methods available for the control of mosquitoes is the use of insecticides. Chemical control using synthetic insecticides had been favorable so far, because of their speedy action and easy application. The relative toxicity of insecticides to various mosquito species has been studied by entomologists in detail (Rajavel*et al.*, 1987; Saxena and Kaushik 1988). Synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air. There is a need to find alternatives to these synthetic pesticides. Botanical pesticides are promising in that they are effective,environment–friendly, easily biodegradable and also inexpensive. Botanical pesticides have been used traditionally by human communities in many parts of the world against pest species of insects (Jacobson 1958).The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicides, insect growth regulators, repellents, and ovipositional attractants, having deterrent activities observed by different researchers (Mathivanan*et al.,* 2010; Niraimathi*et al.,* 2010; Samidurai*et al.,* 2009).In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the different solvent crude extracts from the medicinal plants *O. esculentum* against the medically important vector mosquito *Anopheles Stephensi.*

## MATERIALS AND METHODS

### Plant material

Plant sampling was carried out during the growing season (March - April) of 2011 from different places of Poompuhar Village, Nagapattinam Districts of the Tamilnadu. Bulk samples were air-dried in the shade and

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after drying each 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, Poompuhar College, whenever possible, flowering or fruiting specimens were collected to facilitate taxonomic identification.

**Extraction method**

The dried leaf (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with acetone, benzene, chloroform, hexane and methanol (500 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 *An. Stephensi* were collected from ICMR centre, Virudachalam. The eggs 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 16:8 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 16:8 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 *An. Stephensi* were used in all bioassays.

**Larvicidal activity**

The larvicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO2005. From the stock solution, five different test concentrations (*viz.*, 30,60,90,120,150 and 180ppm) 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 $LC_{50}$ value was calculated by using probit analysis Finney (1971).

**Statistical analysis**

The average adult mortality data were subjected to probit analysis for calculating $LC_{50}$, $LC_{90}$ and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit, and Chi-square values were calculated using the SPSS 12.0 version software. Results with $P<0.05$ were considered to be statistically significant.

## RESULTS

The crude acetone, benzene, chloroform, hexane and methanol leaf extract of *O. esculentum* was assessed against *An. Stephensi.* Among the various concentrations tested methanol extract of *O. esculentum*, results clearly revealed that there is an increasing mortality percentage with the increasing concentration of the extracts. The larvae were more susceptible to 180ppm concentration. The $LC_{50}$ and $LC_{90}$ values of acetone, benzene, chloroform, hexane and methanol leaf extracts of *O. esculentum* against *An. Stephensi* larvae in 24 h were 75.46, 68.55, 98.47, 88.24, 63.84 and 140.66, 130.65, 184.10, 169.36 and 122.48 ppm, respectively. The data is statistically significant at $P$ <0.05.From the results it can be concluded the crude extract of *O. esculentum* was an excellent potential for controlling *An. Stephensi* mosquito.

## DISCUSSIONS

The results of present study are comparable with earlier reports. The toxicity to the late third instar larvae of *Cx. quinquefasciatus* by methanolic leaf extract of *M.charantia*, *T.anguina, L.acutangula, B.cerifera* and *C. vulgaris* showed the $LC_{50}$ values of 465.85, 567.81, 839.81, 1189.30 and 1636.04 ppm respectively (Prabakarand Jebanesan 2004).Ansari *et al.* (2000) suggested that the peppermint oil (*M.piperita*) showed strong repellent activity against adult mosquitoes when applied on the human skin. The protection obtained against *An. annularis, An. culicifacies, and Cx. quinquefasciatus* was 100.0%, 92.3%, and 84.5%, respectively. The root extract of *V.jatamansi* which exhibited adulticidal activity of 90% lethal concentration against adult *An. stephensi, An. culicifacies, Ae. aegypti, An. albopictus, and Cx. quinquefasciatus* were 0.14, 0.16,

**Table 1** Larvicidal activity of crude extracts of *O. esculentum* against *An. stephensi*

| Name of the Solvent | $LC_{50}$ (ppm) | 95% Confidence Limits(ppm) | | $LC_{90}$ (ppm) | $\chi^2$ | *d f* |
|---|---|---|---|---|---|---|
| | | LCL | UCL | | | |
| Acetone | 75.46 | 64.92 | 85.37 | 140.66 | 12.636* | 5 |
| Benzene | 68.55 | 55.94 | 78.45 | 130.65 | 13.578* | 5 |
| Chloroform | 98.47 | 87.69 | 103.68 | 184.10 | 11.297* | 5 |
| Hexane | 88.24 | 75.28 | 104.59 | 169.36 | 16.594* | 5 |
| Methanol | 63.84 | 48.33 | 76.48 | 122.48 | 20.487* | 5 |

*Statistically significant at $P$ <0.05. $LC_{50}$, $LC_{90}$, LCL-Lower confidence limit, UCL-Upper confidence limit $\chi 2$ chi-suuare and degree of freedom.

0.09, 0.08, and 0.17 and 0.24, 0.34, 0.25, 0.21, and 0.28 mg/cm$^2$, respectively(Dua*et al.,* 2008).Mullai and Jebanesan (2006) reported the larvicidal efficacy of the leaf extract of *C.pubescens* with four different solvents against late third instar larvae of *An.stephensi, Cx. quinquefasciatus* and *Ae.aegypti*. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available in many areas of the world. Different parts of plants contain a complex of chemicals with unique biological activity which is thought to be due to toxins and secondary metabolites, which act as mosquitocidal agent (WHO 1981). Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors (Banerjee *et al.,* 2011).

The bioactive compound Azadirachtin (*A.indica*) showed complete ovicidal activity in the eggs of *Cx. Tarsalis* and *Cx.quinquefasciatus* exposed to 10 ppm concentration (Su and Mulla 1998). Nathan *et al.*(2005) considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activity against *An. Stephensi* and the larval mortality was dose-dependent with the highest dose of 1 ppm azadirachtin, evoking almost 100% mortality, affecting pupicidal and adulticidal activity and significantly decreased fecundity and longevity of *An. stephensi*. The ovicidal activity of *M.polystachyum* leaf extract against the egg rafts of *Cx. quinquefasciatus* showed 100% mortality at 0-3 h and 3-6 h with concentrations of 125, 150, 175 and 200 mg/l (Rajkumarand Jebanesan 2004). The volatile oil of *M. polystachyum* and *S.xanthocarpum* possess effective skin repellent activity against *Cx. quinquefasciatus* (Rajkumar and Jebanesan 2005). The mean protection time and total percentage protection in relation to dose of *F.elephantum* leaf extract showed the percentage protection in relation to dose and time (h) (Venkatachalam and Jebanesan 2001).Simple crude extracts from plants have been used as insecticides in many countries for centuries. Crude plant extracts often consist of complex mixtures of active compounds. Advances of using complete mixture may act synergistically; they may show greater overall bioactivity compared to the individual constituents (Chen *et al.,* 1995).The mosquito larvicidal properties of the leaf extract of a herbaceous plant *O.canum* against *Ae.aegypti*. The $LC_{50}$ values for 2nd, 3rd and 4th larvae were 177.82, 229.08 and 331.13ppm respectively(Singh *et al.,* 2003).These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

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