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LARVICIDAL AND OVICIDAL ACTIVITIES OF *EXACUM PEDUNCULATUM* (LINN.) (GENTINACEAE) AGAINST A COMMON MALARIAL VECTOR, *ANOPHELES STEPHENSI* LISTON (DIPTERA : CULICIDAE)

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ABSTRACT

The present study was aimed to investigate the mosquitocidal activity hexane, chloroform, petroleum ether and ethanol extract of *Exacum pedunculatum* Linn. against an important malarial vector mosquito, *Anopheles stephensi* Liston under laboratory condition. This experiment was designed according to the standards protocols. The larvicidal activity of different crude extracts of *E. pedunculatum* was tested with 50, 100,150,200 and 250ppm concentrations against fourth instar larvae *of An. stephens*i. The experimental larvae exposed to 250ppm concentration of *E. pedunculatum* showed more susceptibility to hexane extract, followed by petroleum ether, ethanol and chloroform. The LC_{50} value of hexane extract was recorded to be 127.45ppm; for chloroform 127.39ppm; petroleum ether with 151.96ppm and the least LC_{50} value of 121.24ppm was recorded with ethanol extract. Likewise, the LC_{90} values and their LCL-UCL concentrations were determined as 231.20 (215.07-252.44ppm), 249.13 (203.11-373.03ppm), 255.49 (234.22-271.74ppm) and 240.57 (194.81- 368.30pp) were noticed against hexane, chloroform, petroleum ether and ethanol extracts respectively towards the fourth instar larvae of *An. stephensi*. The data obtained in this experiment was pertinent to note that 200-300ppm concentration of the petroleum and ethanol extracts showed strong ovicidal activity with no hatchability in the experimental group eggs, contrarily 92.8 -100% egg hatchability was recorded with control. The percentage of egg hatchability was decreased with increasing concentration of the plant extracts. The information's given are first report on *E. pedunculatum* against *An. Stephensi*

INTRODUCTION

Mosquitoes are the major vector for the transmission of several communicable diseases like malaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis (JE), etc., causing millions of deaths every and also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (WHO, 2009; 2010). *Anopheles stephensi* Liston is the common vector of malaria in India and other West Asian countries. Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes upto2.7 million deaths (WHO, 1999). The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health.

Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides may enter into the food chain, and thereby, the liver, kidney, etc., may be irreversibly damaged. They even result in mutation of genes and these changes become prominent only after a few generations (Gosh, 1991). Mosquito control is very costly. In larval mosquito control, application of insecticides in ponds, wells, and other water bodies may cause health hazards to human and larvivorus fishes. Nowadays, mosquito coils containing synthetic pyrethroids and other organophosphorus compounds cause so many side effects,

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such as breathing problem, eye irritation, headache, asthma, itching, and sneezing to the users. With the use mosquito repellent, people complained of ill health effect and sometimes required medical treatment. In addition, pests were becoming resistant to chemical treatments. Indoor residual spraying of insecticides stains the walls and leaves a long lasting unpleasant odor.

These problems have highlighted the need for the development of new strategies for selective mosquito control. Phytochemicals are advantageous due to their eco-safety, target-specificity, non development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Phytochemicals obtained from the whole plant or specific part of the plant by the extraction with different types of solvent such as aqueous, methanol, chloroform, benzene, acetone, etc., depending on the polarity of the phytochemical. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and growth inhibitor or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant (Markouk *et al.,* 2001).

Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable. They are potentially suitable for use in integrated pest management programs (Alkofahi, 1989; Dharmshaktu *et al.,* 1987; Green *et al.,* 1991). 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 and ovicidal potential of the hexane, chloroform, petroleum ether and ethanol crude extract of *Exacum pedunculatum* against the medically important mosquito vector, *Anopheles stephensi.*

MATERIALS AND METHODS

Collection of plants

Matured leaves of *Exacum pedunculatum* were collected during the flowering season (From January 2011-April 2011) in and around Dindugal District of Tamil Nadu, India. The collected plants were authenticated by a plant taxonomist in the Department of Botany, Poompuhar College, Tamilnadu, India.

Extraction

The leaves were washed with tap water, shade-dried, and finely ground with the help of electrical blender. The finely ground plant leaf powder (1.0 kg) was loaded in Soxhlet apparatus and was extracted with ethanol by adapting a standard protocol (Vogel, 1978). The solvents from the extracts were removed using a rotary vacuum evaporator (Rota vapour, Systronics India Ltd., Chennai, India) to collect the crude extract. Standard stock solutions were prepared to 50, 100, 150, 200 and 250ppm (for larvicidal activity; and 50-300 ppm for ovicidal activity) by dissolving the residues in their respective solvent.

Test organisms

The larvae of mosquito, *Anopheles stephensi* were collected from the agricultural gardens and field, reared in the laboratory. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week-old chick for blood meal. Mosquitoes were held at 28 ± 2°C, 70–85% RH, with a photo period of 12L: 12D.

Larvicidal bioassay

The larvicidal activity of the plants crude extracts was evaluated as per the method recommended by World Health Organization (2005). Batches of 25 third instar larvae were transferred to a small disposable test cups, each containing 200 ml of water. The appropriate volume of dilution was added to 200 ml water in the cups to obtain the desired target dosage, starting with the lowest concentration. Five replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. To this, 1 ml of appropriate solvent was added. The larval mortality was calculated by using the formula of Abbott (1925) and LC_{50} value was calculated after 24 h by probit analysis (Finney, 1971).

Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla (1998) was performed. The eggs of *Anopheles stephensi* were collected from vector control laboratory, Annamalai University. The different leaf extracts diluted in the appropriate solvent to achieve various concentrations ranging from 50 to 450 ppm. Eggs of these mosquito species (100) were exposed to each concentration of leaf extracts. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated six times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

$$\% \text{ of egg mortality} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} X100$$

RESULTS

The larvicidal activity of different crude extracts of *E. pedunculatum* was tested with 50, 100,150,200 and 250ppm concentrations against fourth instar larvae of An. stephensi. Perusal of the data clearly revealed that at 50ppm concentration ethanol extracts showed strong larvicidal activity among the other tested extracts. At 100ppm concentration chloroform extract of *E. pedunculatum* showed potential larvicidal effect followed by ethanol extract. Whereas, at 150ppm concentration hexane extract showed high larvicidal activity against the

fourth instar larvae of *An. stephensi*. Similar trend was also observed among the 200ppm concentration. The experimental larvae exposed to 250ppm concentration of *E. pedunculatum* showed more susceptibility to hexane extract, followed by petroleum ether, ethanol and chloroform (Fig.1). The determination of LC_{50} and LC_{90} for the above experiments was derived by probit analysis and the data pertaining to it was depicted in table 1. It is very clear that the LC_{50} value of hexane extract was recorded to be 127.45ppm; for chloroform 127.39ppm; petroleum ether with 151.96ppm and the least LC_{50} value of 121.24ppm was recorded with ethanol extract. Likewise, the LC_{90} values and their LCL-UCL concentrations were determined as 231.20 (215.07-252.44ppm), 249.13 (203.11-373.03ppm), 255.49 (234.22-271.74ppm) and 240.57 (194.81- 368.30pp) were noticed against hexane, chloroform, petroleum ether and ethanol extracts respectively towards the fourth instar larvae of *An. stephensi*.

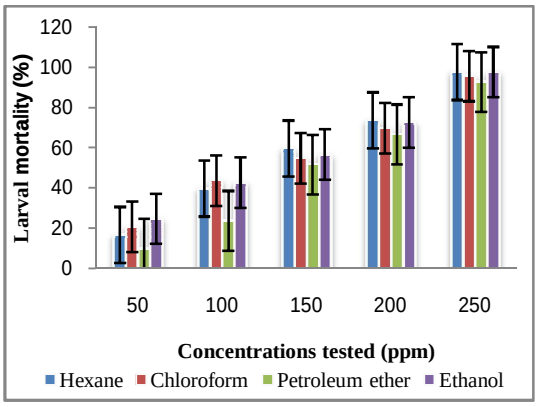

Fig. 1 Larvicidal activity of different crude extracts of *Exacum pedunculatum* (leaves) tested with various concentrations against freshly moulted fourth instar larvae of *Anopheles stephensi.*

terms of % egg hatchability. The data obtained in this experiment was pertinent to note that 200-300ppm concentration of the petroleum and ethanol extracts showed strong ovicidal activity with no hatchability in the experimental group eggs, contrarily 92.8 -100% egg hatchability was recorded with control groups (control group eggs were treated with respective solvent + DMSO; Table 2). The percentage of egg hatchability was decreased with increasing concentration of the plant extracts.

DISCUSSIONS

Due to indiscriminate use of synthetic chemicals to control the mosquitoes in the natural habitats, they have developed strong resistance to almost all the chemicals that are available today. Moreover, chemical pesticides gradually altered the behaviour of non-target organisms. Thus, in this context, the world scientific community intensively searching for the alternative mosquitocidal agent preferably from plants available in nature. Today, the environmental safety of an insecticide is considered to be of important milestone in the field of pest control in general and vector control programme in particular. An insecticide must not cause high mortality in target organisms in order to be acceptable (Kabaru and Gichia, 2001). The extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion. It is evident from the present study that exposure of *An. stephensi* eggs to the leaf extracts of various solvents not only elicited egg mortality but also delayed hatchability to larval stages. Similar kind of observation was also noted earlier by several workers (Rajkumar *et al.*, 2011; Aarthi and Murugan, 2011).

Table 1 lethal concentration (LC_{50} and LC_{90}) of different crude extracts of *Exacum pedunculatum* (leaves) against freshly moulted fourth instar larvae of *Anopheles stephensi.*

Extracts tested	LC_{50}	95% Confidence Limit		LC_{90}	95% Confidence Limit		χ^2 value
		LCL	**UCL**		**LCL**	**UCL**	
Hexane	127.45	116.64	137.64	231.20	215.07	252.44	7.557
Chloroform	127.39	82.74	162.26	249.13	203.11	373.03	9.347
Petroleum ether	151.96	142.14	161.84	255.49	234.22	271.74	3.643
Ethanol	121.24	72.26	156.82	240.57	194.81	368.30	10.179

LC_{50}=Lethal Concentration causes 50% larval mortality; LC_{90}= Lethal Concentration causes 90% larval mortality.
LCL=Lower Confidence Limit; UCL=Upper Confidence Limit.

Table 2 Ovicidal activity (% egg hatchability) of different crude extracts of *Exacum pedunculatum* (leaves) against freshly freshly laid eggs (0-6h) of *Anopheles stephensi.*

Extracts tested	**Concentrations of the extract tested (ppm)**						
	50	100	150	200	250	300	Control
Hexane	90.6 ± 3.2	70.2 ± 2.9	65.8 ± 2.1	40.6 ± 1.4	10.8 ± 1.0	NH	92.8 ± 1.0
Chloroform	92.2 ± 2.7	80.6 ± 2.1	66.8 ± 1.9	47.5 ± 1.2	20.5 ± 1.3	10.8 ±1.2	100.0 ± 0.0
Petroleum ether	80.8 ±1.8	60.2 ± 1.4	30.8 ± 1.4	NH	NH	NH	100.0 ± 0.0
Ethanol	70.8 ±1.6	52.6 ± 1.2	14.8 ± 1.0	NH	NH	NH	97.8 ± 1.2

Values represent mean ± S.D. of five replications. Eggs in control groups were sprayed with no phytochemicals. NH - No hatchability (100% mortality)

The ovicidal activity indicated an important finding that the larvae which hatched out of the treated eggs were succumbed to death within an hour or two. In the present study, our aim was to determine whether *E. pedunculatum* could be used for mosquito control. We observed a functional response of the ovicidal activity exhibited by the ethanol extract. In the case of ovicidal activity, exposure to the freshly laid eggs was more effective than that to the older eggs. Similarly, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* was assessed by Kuppusamy *et al. (*2008) against *An. stephensi*. Larvicidal and oviposition activity of *Cassia obtusifolia* leaf extract against *An. stephensi* Liston was also evaluated by Rajkumar and Jebanesan (2009). Similarly, the aqueous and hydro-alcoholic extracts of *Melia azedarach* leaves and seeds were tested to explore the in vitro ovicidal and larvicidal activity against *Haemonchus contortus* (Kamaraj *et al.,* 2010) and the results were comparable with our results. Additionally, through screening several plants for their larvicidal activity, Sharma et al (2006) found that *Artimisia annua* was the most toxic against anopheles with an LC_{50} of 16.85 ppm and 11.45 ppm after 24 and 48 h of exposure, respectively. In addition, the larvicidal effects of *Momordica charantia* fruit on *An. stephensi* (LC_{50} of 66.05 ppm) were also investigated by Singh et al, (2006).

The biological activity of the plant extract might be due to a variety of compounds in *E. pedunculatum* may jointly or independently contribute to cause larvicdal and ovicidal activity against *An. stephensi*. The main chemical compounds present in the *E. pedunculatum* might responsible for the activities recorded in the present experiments. It would have been suggested that the direct and indirect contributions of such compounds to treatment efficacy while on the use of botanical insecticides for the control of *An. stephensi*. These and other naturally occurring insecticides may play a crucial role in vector control programs in the near future (Wandscheer *et al.*, 2004). Since *An. stephensi* breeds in drinking water tank, many of the plant extracts are subject to risk factors in mosquito control (Ahmed *et al*., 2011). The plant extracts which are highly toxic against *An. stephensi* are also toxic to human beings. In the present study, *E. pedunculatum* extract showed promising effect on *An. stephensi* and it has no deleterious effects against human beings since it has been used in Indian auyurvedhic medicine for several ailments.

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