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Mosquito larvicidal and ovicidal properties of *Eclipta alba* (L.) Hassk (Asteraceae) against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae)

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ABSTRACT

Objective: The present study deals with the investigation of larvicidal and ovicidal activities of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Eclipta alba (E. alba)* against dengue vector, *Aedes aegypti (Ae. Aegypti)*. **Methods:** Twenty five early III instar larvae of *Ae. aegypti* was exposed to various concentrations (50-300 ppm) and was assayed in the laboratory by using the protocol of WHO 2005; the 24 h LC_{50} values of the *E. alba* leaf extract was determined by Probit analysis. For ovicidal activity, slightly modified method of Su and Mulla was performed. The ovicidal activity was determined against *Ae. aegypti* to various concentrations ranging from 100-350 ppm under the laboratory conditions. The egg hatch rates were assessed 48 h post treatment. **Results:** The LC_{50} values of benzene, hexane, ethyl acetate, methanol and chloroform extract of *E. alba* against early third instar larvae of *Ae. aegypti* were 151.38, 165.10, 154.88, 127.64 and 146.28 ppm, respectively. Maximum larvicidal activity was observed in the methanol extract followed by chloroform, benzene, ethyl acetate and hexane extract. No mortality was observed in control. Among five solvent tested the methanol extract was found to be most effective for ovicidal activity against *Ae. aegypti*. The methanol extracts exerted 100% mortality (zero hatchability) at 300 ppm. **Conclusions:** From the results it can be concluded the crude extract of *E. alba* was an excellent potential for controlling *Ae. aegypti* mosquito.

1. Introduction

Mosquitoes constitute a major public health problem as vectors of serious human diseases like malaria, flariasis, Japanese encephalitis, dengue fever, chikungunya and yellow fever[1] cause substantial mortality and morbidity among people living in tropical and sub tropical zones. *Aedes aegypti (Ae. aegypti)*(L.) is generally known as a vector for an arbovirus responsible for dengue fever and chikungunya, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. This mosquito is also the vector of yellow fever in Central and South America and West Africa. Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue haemorrhagic fever and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement[2]. Chemical control is an effective strategy used extensively in daily life. There are many kinds of compounds toxic to mosquitoes, including organochlorine, organophosphorus, carbamates, phyrethroids and so on. They are both effective and residual. However, the environmental threat these chemicals pose and the resistance of mosquitoes to insecticides has increased during the last five decades. There is an urgent need to develop new material for controlling mosquitoes with environment safety, rapid biodegradation and low cost[3]. Plants are one potentially important source of candidates.

Plants have been used in traditional medicine for several thousand years. Medicinal plants as a group comprise approximately 8 000 species and account for about 50% of all the higher flowering plant species in India. The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In a large number of countries, human population depends on medicinal plants for treating various illnesses as well as a source for livelihood. The World Health Organization (WHO) estimated that 80% of

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populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs[4]. Recently, some of the researchers have reported the bioactivity of extracts/essential oils from various plants against medically important vectors. The methanol extracts of *Abutilon indicum, Aegle marmelos, Euphorbia thymifolia, Jatropha gossypifolia* and *Solanum torvum* were assayed for their toxicity against the early fourth-instar larvae of *Culex quinquefasciatus (Cx. quinquefasciatus)*[5]. The acetone extracts of *Citrullus colocynthis, Coccinia indica, Cucumis sativus, Momordica charantia, Trichosanthes anguina* were tested against the early fourth instar larvae of *Ae. Aegypti* and *Cx. quinquefasciatus*[6]. *Rhinacanthus nasutus* dried root powder methanol extract showed acute toxicity against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*[7]. Palsson and Jaenson[8] conducted research in several villages in Guinea Bissau (West Africa) on *Ocimum canum* being used traditionally as mosquito repellents by native people. Essential oils exhibited larvicidal activity against larvae of field collected *Ae. aegypti*[9].

The petroleum ether extract showed larvicidal activity against the *Ae. aegypti, Cx. quinquefasciatus, Anopheles dirus (An. dirus)* and *Mansonia uniformis*[10]. The methanol extracts from *Calophyllum inophyllum* and *Rhinacanthus nasutus* seeds and leaves showed significant larvicidal and growth-regulatory activities even at very low concentrations on the juveniles of *Cx. quinquefasciatus*, *Anopheles stephensi (An. Stephensi)* and *Ae. aegypti*[11]. The methanolic leaf extract of *Cassia fistula* was tested for larvicidal and ovicidal activity against *Cx. quinquefasciatus* and *An. stephensi*[12]; and the larvicidal activity of methanol extracts of *Cassia obtusifolia, Cassia tora* and *Vicia tetrasperma* were tested against early fourth stage larvae of *Ae. aegypti* and *Culex pipiens*[1]. The acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Leucas aspera* were studied against the early fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*[13]. The leaf benzene, chloroform, ethyl acetate and methanol extract of *Acalypha indica* were tested for larvicidal activity, ovicidal activity and oviposition attractancy against *Ae. Stephensi*[14]; Hexane extract obtained from leaves of *Eucalyptus citriodora* was tested against larvae of *An. stephensi, Cx. quinquefasciatus* and *Ae. aegypti*[15].

Eclipta alba (E. alba) (L.) Hassk (Asteraceae) is a widely distributed herb in tropical countries. The herb is rich source of ascorbic acid. It is a good source of thiophene derivatives which are effective against nematodes. From this; ecliptal, wedelolactone[16], desmethyl wedelolactone[17] and its 7-O-glucoside, β - amyrin[18] were isolated. Polypeptides isolated from whole plant gave five amino acids on hydrolysis viz.; cystine, glutamic acid, phenylalanine, tyrosine and metionine (leaves/aerial parts)[19]. A new dithienyl acetylene ester isolated from roots and characterized as 5′-isovaleryloxymethylene-2-(4-isovaleryloxybut-3-ynl) dithiophene[20] , 5′-tigloyloxymethylene-2-(4-isovaleryloxybut-3-ynyl) ditiophene isolated from aerial parts and roots. It gives glycosides along with eclalbasaponins[21]. Wedelolactone and desmethyl wedelolactone possesses potent antihepatotoxic property[22]. Alcoholic extract of the herb has antiviral activity against Ranikhet disease. The leaves alone or in combination with ajowan seeds are used in diseases of gall bladder. Plant juice cures skin infections. *E. alba* is reported to be effective in the treatment of peptic ulcers. Immunoactive property has also been observed against surface antigen of hepatitis B- virus[23]. The fresh plant is used as a tonic and deobstruent in enlargement of the liver and spleen[24]. It is widely used to improve colour and luster of the hair[25,26]. Furthermore, a mosquitocidal property of *E. alba* has not yet reported. Therefore, the present study was carried out to determine the larvicidal and ovicidal efficacy of *E. alba* leaves extract against *Ae. aegypti*.

2.0 Materials and methods

2.1. Plant collection

Fully developed leaves of the *E. alba* were collected from different regions of Cuddalore District, Tamilnadu, India. It was authenticated by a plant taxonomist from the Department of Botany, Annamalai University. A voucher specimen is deposited at the herbarium of plant phytochemistry division, Department of Zoology, Annamalai University.

2.2. Preparation of the extract

The leaves were washed with tap water, shade dried and finely ground. The finely ground plant material (3.0 kg/solvent) was loaded in soxhlet apparatus and was extracted with five different solvents namely benzene, hexane, ethyl acetate, methanol and chloroform individually[27]. The solvent from the extract was removed using a rotary vacuum evaporator to collect the crude extract. The crude residue of this plant varies with the solvents used. The *E. alba* with five different solvents yielded 83.40, 102.32, 97.36, 134.27 and 112.95 g of crude residue respectively. Standard stock solutions were prepared at 1% by dissolving the residues in acetone. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal and ovicidal bioassays.

2.3. Test organisms

Ae. aegypti was reared in the vector control laboratory, Department of zoology, Annamalai University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and one week old chick for blood meal. Mosquitoes were held at 28 ± 2 ℃, 70% - 85 % relative humidity (RH), with a photo period of 14 h light, 10 h dark.

2.4. Larvicidal bioassay

The larvicidal activity of the plant crude extracts was evaluated as per the method recommended by WHO[27]. 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 (concentration ranging from 50 to 300 ppm), starting with the lowest concentration. Six replicate were set up for each concentration and an equal number of control were set up simultaneously using tap water. To this 1 mL of appropriate solvent was added. The LC50 value was calculated after 24 h by probit analysis[29].

2.5. Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla[30] was performed. The eggs of *Ae. aegypti* were collected from vector control laboratory, Annamalai University. The different leaf extract diluted in the appropriate solvent to achieve various concentrations ranging from 100 to 350 ppm. Eggs of this mosquito species (100 nos.) were exposed to each concentrations of leaf extract until they hatched or died. 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.

$$\% \text{ of egg mortality} = \frac{\text{Number of hatched larvae}}{\text{Total no. of eggs}} \times 100$$

2.6. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit and *chi*-square values were calculated using the SPSS12.0 (Statistical Package of Social Sciences) software. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The toxicity of different solvent crude extract of *E. alba* was tested against larvae of *Ae. aegypti* (Table 1). The data were recorded and statistical data regarding LC_{50}, LC_{90}, LCL, UCL and *chi*-square values were calculated. The LC_{50} and LC_{90} values of benzene, hexane, ethyl acetate, methanol and chloroform extract of *E. alba* against early third instar larvae of *Ae. aegypti* were 151.38, 165.10, 154.88, 127.64 and 146.28 ppm and 274.34, 297.70, 288.61, 245.73 and 274.42 ppm respectively. Maximum larvicidal activity was observed in the methanol extract followed by chloroform, benzene, ethyl acetate and hexane extract. No mortality was observed in control. *Chi*-square values were significant at $P<0.05$ level. The percentage of egg hatchability of *Ae. aegypti* with the leaf extract of *E. alba* is presented in Table 2. The methanol extracts exerted 100% mortality (zero hatchability) at 300 ppm. In control experiments 100 % hatchability.

Table 1
Larvicidal activity of different solvent extracts of *E. alba* against *Ae. aegypti*.

Name of the extract	Concentration (ppm)	% of mortality±SD	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2
Benzene	control	0.0±0.0	151.38 (110.65-192.21)	274.34 (225.42-380.96)	20.208*
	60	26.6±1.5			
	120	41.0±1.3			
	180	57.6±1.2			
	240	75.0±1.8			
	300	97.9±1.4			
Hexane	control	0.0±0.0	165.10 (128.31-204.20)	297.70 (247.99-400.37)	16.139*
	60	23.0±0.8			
	120	39.3±1.5			
	180	51.0±1.4			
	240	70.3±1.9			
	300	93.6±2.2			
Ethyl acetate	control	0.0±0.0	154.88 (122.38-187.33)	288.61 (245.02-368.64)	12.324*
	60	25.0±1.6			
	120	40.4±1.8			
	180	62.0±1.2			
	240	77.9±0.8			
	300	89.2±1.2			
Methanol	control	0.0±0.0	127.64 (94.72-160.26)	245.73 (203.20-333.85)	15.802*
	50	27.2±1.6			
	100	46.0±1.4			
	150	58.6±1.2			
	200	77.3±0.8			
	250	88.2±1.6			
Chloroform	control	0.0±0.0	146.28 (111.00-180.56)	274.42 (230.25-359.07)	14.685*
	60	29.4±1.6			
	120	42.0±1.4			
	180	61.6±1.8			
	240	81.0±1.6			
	300	93.2±1.2			

*Significant at $P<0.05$, LCL – Lower confidence limits ; UCL – Upper confidence limits.

Table 2
Ovicidal activity of *E. alba* plant extracts against *Ae. aegypti*.

Name of the solvent	Percentage of egg hatch ability						
	Concentration (ppm)						
	Control	100	150	200	250	300	350
Benzene	100.0±1.7	67.6±1.5	56.9±1.6	43.4±1.9	31.3±1.4	19.2±1.9	NH
Hexane	100.0± 0.0	83.8±1.2	71.2±1.5	57.6±1.2	42.8±2.1	33.2±0.8	21.4±1.6
Ethyl acetate	99.2±1.3	73.8±0.8	62.6±1.9	49.7±0.8	35.8±1.5	23.4±1.2	NH
Methanol	100.0±0.0	55.8±1.8	44.2±1.8	29.6±1.2	18.9±1.8	NH	NH
Chloroform	98.6±1.9	63.9±1.4	51.2±1.4	39.6±1.7	27.2±1.6	16.8±1.4	NH

NH– No hatch ability

4. Discussion

Recently, bio-pesticides with plant origins are given for use against several insect species, especially disease transmitting vectors, based on the fact that compounds of plant origin are safer to use, without phototoxic properties and leave no scum in the environment[31]. Ruskin[32] has reported that in mosquitoes, the compounds extracted from *Azadirachta indica* showed mortality for fourth instar larvae of *An. stephensi* with LC_{50} values of 60 and 43 ppm. The LC_{50} and LC_{90} values for Neemarin were 0.35 and 1.81 mg/L for *An. stephensi* and 0.69 and 3.18 mg/L for *Cx. quinquefasciatus*[33]. *Solanum elaeagnifolium* and *Luffa cylindrica* showed more toxic effects when the first instar of *Ae. aegypti* was treated for 24 h. The LC_{50} values observed were 0.0586 and 0.812 mg/mL respectively[34]. Broadbent and Pree[35] reported that when eggs were directly exposed to high concentrations of the compounds, more chemicals entered the egg shell, which affected the embryogenesis; similarly, longer exposure periods also facilitated the increased penetration of the compounds into the shells, thus increasing their effectiveness. The bioactive compound azadirachtin (*A. indica*) showed complete ovicidal activity in the eggs of *Cx. tarsalis* and *Cx. quinquefasciatus* exposed to 10-ppm concentration[30].

In our previous study, we have reported the methanol extract of *Cassia fistula* exhibited LC_{50} values of 17.97 and 20.57 mg/L against *An. stephensi* and *Cx. quinquefasciatus*, respectively[12]. The crude leaf extract of *Azadirachta indica* with different solvents, viz. benzene, chloroform, ethyl acetate and methanol were tested for larvicidal activity against An. stephensi. The LC_{50} values were 19.25, 27.76, 23.26 and 15.03 ppm, respectively[14]. The LC_{50} of leaf extract of *C. fistula* with different solvents, viz. methanol, benzene and acetone against *Ae. aegypti* were 10.69, 18.27 and 23.95 mg/L respectively[36]. Larvicidal efficacy of the crude leaf extracts of *Ficus benghalensis* with three different solvents like methanol, benzene and acetone was tested against the early second, third, fourth instar larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*. Among the three solvents the maximum efficacy was observed in methanol. The lethal concentration (LC_{50}) values of *Ficus benghalensis* against early second, third and fourth larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were 41.43, 58.21 and 74.32 ppm, 56.54, 70.29 and 80.85 ppm and 60.44, 76.41 and 89.55 ppm respectively[37]. The LC_{50} and LC_{90} values of crude methanol extract of leaves of *Eria coronaria* on *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* larvae in 24 h were 72.41, 65.67, 62.08 and 136.55, 127.24 and 120.86 mg/L, respectively[38]. Larvicidal activity of crude extract of Sida acuta against *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* with LC_{50} values ranging between 38 to 48 mg/L[39]. The current investigation revealed that the crude extract of *E. alba* possesses remarkable larvicidal and ovicidal activities against *Ae. aegypti*. This is the first report on the mosquito larvicidal and ovicidal activity of leaf extract of *E. alba* plant. Further purification and characterization of the bioactive fraction of *E. alba* are underway in our laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

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