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MOSQUITO LARVICIDAL ACTIVITY OF *ARISTOLOCHIAINDICA* LINN. (ARISTOLOCHIACEAE) EXTRACTS AGAINST *AEDESAEGYPTI* (LINN.), ANOPHELES STEPHENSI (LISTON) AND *CULEXQUINQUEFASCIATUS* (SAY) (DIPTERA: CULICIDAE)

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ABSTRACT

Mosquitoes are transmitting several diseases such as malaria, dengue, yellow fever, West Nile virus fever, Japanese encephalitis, filariasis and, more recently, Zika virus. Mosquitoes are the major vector for the transmission of several communicable diseases and causing millions of deaths every year. The eco-friendly control of mosquito vectors is a crucial challenge of public health importance. Therefore, the present study was aimed to investigate the mosquito larvicidal activity of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract of *Aristolochiaindica*Linn. (Aristolochiaceae) against vector mosquito *Ae.aegypti, An. stephensi* and *C. quinquefasciatus*. The larvicidal bioassay test was assessed under laboratory condition and experiments was designed according to the standards protocol. The larvicidal activity of leaf extracts of *Aristolochiaindica* was tested with 100 -500ppm concentrations against fourth instar larvae selected mosquitoes. The $LC_{50}$and LC_{90}value of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract of *Aristolochiaindica* were 260.80, 257.64, 255.88, 260.77, 261.06, 259.33, 263.98, 265.08, 173.45, 251.01, 256.31, 246.79, 226.59, 226.42, 230.05 and 462.57, 471.23, 464.96, 468.65, 470.83, 465.24, 465.25, 467.16, 379.42, 445.49, 461.26, 446.10, 426.56, 421.43 and 421.39*Ae. aegypti, An. stephensi* and *C. quinquefasciatus* respectively. Among the different extracts tested methonal extracts showed maximum activity against selected mosquitoes.

INTRODUCTION

Arthropods are important vectors of a great number of pathogens and parasites, which may hit as epidemics or pandemics in the increasing world populations of humans and animals. Mosquitoes (Diptera: Culicidae) represent a key threat for millions of organisms worldwide, since they act as vectors of the agents of malaria, dengue, yellow fever, West Nile virus fever, Japanese encephalitis, filariasis and, more recently, Zika virus. Mosquitoes are the major vector for the transmission of several communicable diseases and causing millions of deaths every and also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (WHO, 1999; WHO, 2009; 2010).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 (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, 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

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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; Baluselvakumar *et al*., 2012). 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 activity of differentl extracts of *Aristolochiaindica* against vector mosquitoes.

MATERIALS AND METHODS

Plant collection and processing

Several hundred medicinal plant species from the Indian sub-continent have been identified and their usage documented in the ethnobotanical literature. These literature reports guided the selection of plant for the present study. Plant sampling (*Aristolochiaindica*) was collected from in and around Yercaud hills, Salem district, Tamil Nadu, India. At the time of collection, two pressed voucher herbarium specimens were prepared per species. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder using an electric blender.

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 different solvents 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 100 and500ppm concentrations.

Test organisms

The larvae of mosquitoes, *Ae.aegypti, An. stephensi* and *C. quinquefasciatus* 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.

Larvicidalactivity

The larvicidal activity of the plants crude extracts was evaluated as per the method recommended by World Health Organization (2005). Batches of 25 fourth 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).

RESULTS

The larvicidal activity of different crude extracts of *Aristolochiaindica* was tested with 100 - 500ppm concentrations against fourth instar larvae of selected mosquitoes. The larvicidal bioassay test was assessed under laboratory condition and experiments was designed according to the standards protocol. The larvicidal activity of leaf extracts of *Aristolochiaindica* was tested with 100 -500ppm concentrations against fourth instar larvae selected mosquitoes. The LC_{50} and LC_{90} value of hexane, diethyl ether,

Table 1 Larvicidal activity of *A. indica*extracts against fourth instar larvae of selected mosquitoes

Extract tested	Concentration (ppm)	LC50 (ppm)	95%Confidence Limits (ppm)		LC90 (ppm)	95%Confidence Limits (ppm)		Slope	χ2value
			LCL	UCL		LCL	UCL		
Hexane	*Ae. aegypti*	260.80	239.87	280.72	462.57	231.27	503.41	3.5614033	0.542
	An. stephensi	257.64	235.59	278.47	471.23	437.89	515.25	3.3779586	1.249
	Cx. quinquefasciatus	255.88	234.18	276.38	464.96	432.47	507.66	3.4533497	1.737
Diethyl ether	*Ae. aegypti*	260.77	239.38	281.16	468.65	436.18	511.25	3.4156662	2.263
	An. stephensi	261.06	221.83	297.07	470.83	417.92	556.90	3.4076276	3.440
	Cx. quinquefasciatus	259.33	223.34	292.47	465.24	416.27	541.98	3.5418388	3.014
Dichloromethane	*Ae. aegypti*	263.98	229.14	296.53	465.25	417.34	539.57	3.5619372	3.002
	An. stephensi	265.08	244.32	285.01	467.16	435.51	508.48	3.5516477	2.179
	Cx. quinquefasciatus	173.45	112.59	215.85	379.42	328.45	467.27	3.6268557	4.217
Ethyl acetate	*Ae. aegypti*	251.01	197.26	298.08	445.49	383.19	565.00	3.9534708	6.367
	An. stephensi	256.31	220.37	289.19	461.26	412.94	536.65	3.5514839	2.991
	Cx. quinquefasciatus	246.79	200.26	287.57	446.10	389.94	544.32	3.716923	4.720
Methanol	*Ae. aegypti*	226.59	155.28	281.27	426.56	357.52	577.36	3.9249339	8.197
	An. stephensi	226.42	135.12	292.78	421.43	343.40	625.87	4.1397943	11.927
	Cx. quinquefasciatus	230.05	163.22	283.20	421.39	354.78	563.49	4.4299443	8.215

Value represents mean±S.D. of five replications. *mortality of the larvae observed after 24hrs of exposure period WHO (2005). 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 are significantly different at $P < 0.05$.

dichloromethane, ethyl acetate and methanol extract of *Aristolochiaindica* were 260.80, 257.64, 255.88, 260.77, 261.06, 259.33, 263.98, 265.08, 173.45, 251.01, 256.31, 246.79, 226.59, 226.42, 230.05 and 462.57, 471.23, 464.96, 468.65, 470.83, 465.24, 465.25, 467.16, 379.42, 445.49, 461.26, 446.10, 426.56, 421.43 and 421.39*Ae. aegypti, An. stephensi* and *C. quinquefasciatus* respectively. Among the different extracts tested methonal extracts showed maximum activity against selected mosquitoes.

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). 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 *Andrographispaniculata*was assessed by Kuppusamy *et al*.(2008) against *An. stephensi*. Larvicidal and ovipositionactivity 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 *Meliaazedarach* leaves and seeds were tested to explore the in vitro ovicidal and larvicidal activity against *Haemonchuscontortus* (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 *Artimisiaannua* 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 *Momordicacharantia* 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, *Aristolochiaindica* extract showed promising effect on selected mosquitoes 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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