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Ovicidal and pupicidal activity of *Andrographis paniculata* (Acanthaceae) against vector mosquitoes (Diptera : Culicidae)

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Abstract

The present investigation was aimed to determine the ovicidal and pupicidal activity of *Andrographis paniculata* leaf extracts against *Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus* . The ovicidal and pupicidal activity was determined against three mosquito species at concentrations of 50, 100, 150, 200 and 250 ppm and mortality was assessed after 24 hours. Highest percentage of ocividal activity was recorded in petroleum ether extract on *A. stephensi.* Maximum pupicidal activity was found in petroleum ether extracts on *A. stephensi* (94.6%). These results suggested that the leaf extracts of *A. paniculata* showed potential to be used as an ideal ecofriendly approach for the control of the *Ae. aegypti*, *An. Stephensi and Cu. quinquefasciatus.*

Keywords: Ovicidal, Pupicidal, *Andrographis paniculata, Aedes aegypti, Anopheles Stephensi* and *Culex quinquefasciatus*

Introduction

Mosquitoes are still representing the world's number-one vector of human and domestic animals. Comprising approximately 3500 species, mosquitoes are found beyond the tropical and subtropical regions of the world with which they are classically associated. Mosquito-borne diseases are especially important vector-borne diseases with malaria, dengue and yellow fever alone affecting millions of people every year. Worldwide, the most important mosquito vector species are members of three genera, *Aedes*, *Culex* and *Anopheles*, each having its own set of climatic and environmental drivers and constraints. Control of mosquito – borne diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to pesticides (Ranson *et al.*, 2001). Not only can a species occur within its natural geographical range (past or present) and Petroleum Ether extract dispersal potential (indigenous species), but it can also occur outside this range through various introduction routes (exotic species). An exotic (or invasive) species may subsequently establish and spread causing economic or environmental impact or harm to human health (Kasari *et al*., 2008). However, they are known to accept small and inconspicuous containers like tree holes, urban areas, flower vases, discarded tyres, cans, bottles, and paper cups as breeding sites (Seng and Jute, 1994). The mosquito *Ae. aegypti* is more widely dispersed now than any time in the past, placing billions of humans at risk of infection. It enjoys greater geographical distribution and is established virtually in all tropical countries (Halstead, 2008). 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 (Sharma, 2001).

Aedes aegypti (L.) is generally known as a vector for an arbovirus responsible for dengue fever, 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 hemorrhagic fever, and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Pancharoen et al., 2002). The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health. *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. Lymphatic filariasis is a major vector borne disease making about 120 million peoples in 83 countries physically disabled (WHO, 2006) which is transmitted by *C. quinquefasciatus* mosquito having cosmopolitan distribution. WHO (1992) have suggested various controlling strategies to control vector transmission at different levels. Among the available vector control methods, chemical control is decisively superior over environmental and biological control strategies that have limited applicability in mitigating sporadic unpredictable outbreaks of vector borne disease. However, Cx.quniquefasciatus has also shown resistance to different insecticides used in mosquito control such as organochlorines, organophosphorous, pyrethroids and microbial insecticides throughout the world (Tikar et al., 2008).

In India around 20,000 medicinal plants have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases (Kalaivani *et al*., 2012). *Andrographis paniculata* (Acanthaceae) is a plant that has been effectively used in traditional Asian medicines for centuries. It's perceived "blood purifying" property results in its use in diseases where blood "abnormalities" are considered causes of disease, such as skin eruptions, boils, scabies, and chronic undetermined fevers. The aerial part of the plant, used medicinally, contains a large number of chemical constituents, mainly lactones, diterpenoids, diterpene glycosides, flavonoids, and flavonoid glycosides. Controlled clinical trials report its safe and effective use for reducing symptoms of uncomplicated upper respiratory tract infections. Since many of the disease conditions commonly treated with *A. paniculata* in traditional medical systems are considered self-limiting, its purported benefits need critical evaluation. This review summarizes current scientific findings and suggests further research to verify the therapeutic efficacy of *A. paniculata*. *Therefore the present study was carried out to determine the* larvicidal activity of *A. paniculata* leaf extracts against important vectors *Ae. aegypti*, *An. Stephensi and Cx. quinquefasciatus.* 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 ovicidal and pupicidal potential of the petroleum ether, chloroform and ethy acetate extract of *A. paniculata* against the medically important vector mosquitoes.

Materials and Methods

Plant material

The leaves of *A. paniculata* were collected from Musiri, Tiruchirapalli District, Tamil Nadu, India during the July 2014. Collecetd plant specimen was identified by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Sytematics, St' Joseph's College, Tiruchirapalli, Tamil Nadu, India and The Voucher specimen (IPH-52) was deposited in Entomology lab, Arignar Anna Government Arts College, Musiri, Tamil Nadu, India.

Extraction method

The dried leaves (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with hexane, chloroform , and ethyl acetate (500ml, Ranchem), in a soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure of 22-26mm hg at 45º C by 'Rotavapour' and the residue obtained was stored at 4ºC in an amber vial. Then the vials were named and covered with silver foil and transported to the laboratory. Until use those vials were kept in cool and dark place at 4ºC.

Vector rearing

The mosquito larvae of *Ae. aegypti*, *An. Stephensi and Cx. quinquefasciatus* were collected from National centre for disease control, Government of India ministry of health and family welfare, Southern India branch, field station, Mettupalayam, Coimbatore district, Tamilnadu, India. The larvae were kept in the plastic buckets half filled with tap water and fed with dog biscuit once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

Ovicidal activity

The method of Su and Mulla (1998) was slightly modified 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*, *Ae. aegypti* and *C. quinquefasciatus* was counted individually with the help of hand lens. Freshly hatched eggs of these mosquito species (100) was 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 will be replicated five times. The hatchability will be assessed 48 h post treatment by the following formula.

$$\% \text{ Ovicidal Activity} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs treated}} \text{ X } 100$$

Pupicidal activity

The pupicidal activity of plant crude extract was assessed by using the standard method as prescribed by WHO (2005). Similar test concentrations as stated in the previous experiments was prepared and tested against the pupae of *An. stephensi*, *Ae. aegypti* and *C. quinquefasciatus*. DMSO (emulsifier) in water treated as control. The pupae of these mosquito species (25 pupae) was 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 pupal 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).

Determination of lethal concentrations

Lethal concentration (LC_{50}) represents the concentration of the test material that caused 50% mortality of all test organisms within the specified period of exposure 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} and LC_{90} was calculated along with their fiducial limits at 95% confidence level by probit analysis using SPSS software package 12.0 (Statistical Package of Social Sciences) software. Results with $p<0.05$ was considered to be statistically significant.

Results

The efficacy of leaf crude extracts such as petroleum ether, chloroform and ethyl acetate extracts of *A. paniculata* evaluvated for ovicidal (eggs) and pupicidal (pupae) activities against *Ae. aegypti* , *An. Stephensi* and *Cx. quinquefasciatus* and results are presented in tables.1-4.

Generally, as the concentration increases the rate of eggs and pupal mortality are also increases. It has been noticed that the higher concentrations of *A. paniculata* extracts of petroleum ether possesses strong ovicidal activity at 250ppm concentration against An. stephensi (100%) no egg hatchability was recorded and followed by *C.quinquefasciatus* and *Ae. aegypti*. In pupicidal activity, among the three solvent extracts tested against selected mosquitoes at 250ppm higher concentrations, the petroleum ether extract was found to be most effective for pupicidal activity provided 94.6 % (Lc50 27.63), 85.23 % (LC50 41.23) and 85.20% (LC50 42.12) against *An. stephensi, C. quinquefasciatus and Ae. aegypti* respectively (table 2-4). Results of this study show that the *A. paniculata* selected extracts may be a potent source of natural ovicidal and pupicidal activities against selected important vector mosquitoes.

Table 1. Ovicidal activity of different solvent extracts of *A. paniculata* against eggs (0-6 h old) of three mosquito species

Concentrations (ppm)	Crude extracts tested		
	Petroleum Ether extract	**Chloroform extract**	**Ethyl acetate extract**
Ae. aegypti			
Control	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
50	79.4 ±2.8	81.8 ±1.3	85.4 ±3.5
100	64.4 ±2.9	62.2 ±2.5	64.6 ±3.0
150	53.8 ±3.1	46.2 ±3.3	53.6 ±2.9
200	35.2 ±3.5	44.2 ±3.4	32.4 ±1.9
250	13.2 ±3.8	16.8 ±1.9	19.2 ±2.5
An. stephensi			
Control	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
50	85.8 ±2.5	88.8±2.3	87.6±2.0
100	76.6±2.3	74.2±1.9	69.2±1.9
150	56.8±2.5	65.6±3.2	48.6±3.2
200	22.8±2.3	28.8±2.7	28.4±2.1
250	0.00±0.00	6.6±2.7	8.4±2.5
Cx. quinquefasciatus			
Control	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
50	81.2 ±2.3	85.8 ±3.4	89.8 ±2.9
100	69.2 ±3.4	77.2 ±2.5	74.8 ±3.6
150	49.2 ±2.9	65.2 ±3.7	56.6 ±2.0
200	33.8 ±3.3	49.4 ±2.5	37.2 ±2.1
250	5.6 ±3.3	21.4 ±1.1	26.8 ±3.5

Value represents mean ± S.D. of five replications.

Table 2. Pupicidal activity of different crude extracts of *Andrographis paniculata* against *Ae. aegypti.*

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC50 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC95 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Petroleum Ether extract									
Control	91.2 ± 2.1	0± 0.0	41.23	24.82	68.47	1123.94	437.94	2884.51	2.738
50	34.8± 1.7	57.2± 2.2							
100	32.4 ± 3.9	63.2± 2.1							
150	28.6 ± 1.6	70.4± 2.0							
200	20.8± 3.1	78.8± 1.3							
250	12.8± 1.6	85.2 ± 3.6							
Chloroform extract									
Control	90.2± 0.4	0± 0.0	117.64	92.13	150.21	4112.65	926.56	18254.40	0.906
50	62.6± 1.3	36.8± 2.5							
100	51.4 ± 2.3	44.4± 3.2							
150	45.2 ± 2.7	52.4 ± 2.0							
200	37.2± 2.4	59.6 ± 1.6							
250	24.4 ± 1.5	66.2 ± 1.4							
Ethyl acetate extract									
Control	89.6± 1,1	0± 0.0	132.48	99.26	176.81	9593.14	1041.75	88339.87	0.165
50	60.8± 2.4	36.4 ±1.1							
100	53.8± 3.8	44.6 ±2.3							
150	47.8± 2.9	50.8± 3.5							
200	36.2 ± 2.1	56.8 ±1.9							
250	31.2 ± 1.3	60.6± 3.4							

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{95} = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 3. Pupicidal activity of different crude extracts of *Andrographis paniculata* against *An. stephensi*

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC50 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC95 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Petroleum Ether extract									
Control	92.6 ± 2.3	0± 0.0	27.63	15.86	48.14	392.99	236.84	652.07	3.934
50	31.4± 2.0	67.8± 1.3							
100	24.2 ± 2.5	75.4± 3.2							
150	16.4 ± 2.1	81.4± 1.8							
200	9.4± 1.1	89.6± 1.1							
250	4.6± 1.3	94.6 ± 1.8							
Chloroform extract									
Control	93.2± 2.8	0± 0.0	76.36	54.71	106.58	2829.08	725.02	11039.26	3.471
50	50.8± 2.1	46.4± 1.5							
100	44.6 ± 1.9	50.6± 2.7							
150	40.4 ± 2.0	57.4 ± 2.4							
200	29.8± 1.6	66.8 ± 2.1							
250	22.8 ± 1.3	75.4 ± 3.1							
Ethyl acetate extract									
Control	94.2± 2.7	0± 0.0	93.51	73.71	118.64	1950.07	706.26	5384.34	1.60
50	59.8± 1.0	39.2 ±1.6							
100	50.8± 3.4	48.8 ±2.7							
150	39.4± 4.0	56.4± 3.5							
200	32.8 ±3.2	66.2 ±2.2							
250	24.4 ± 2.7	73.4± 3.6							

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{95} = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table. 4. Pupicidal activity of different crude extracts of *Andrographis paniculata* against *Cx. Quinquefasciatus*

Concentration (ppm)	Adult Emergence (%)	Pupal Mortality (%)	LC50 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		LC95 (ppm) Pupal Mortality	95% Confidence Limit (ppm)		*x*2 (*df* = 4)
				LCL	UCL		LCL	UCL	
Petroleum Ether extract									
Control	95.4 ± 2.3	0± 0.0	66.87	51.78	86.35	776.42	430.15	1401.42	2.465
50	52.8± 2.1	45.4± 3.0							
100	41.8 ± 3.8	57.6± 1.8							
150	30.2 ± 2.5	67.2± 3.7							
200	22.6± 2.4	76.4± 2.6							
250	11.2± 1.9	85.2± 2.7							
Chloroform extract									
Control	97.2± 2.0	0± 0.0	90.16	69.06	117.71	2458.98	743.65	8130.92	1.264
50	58.4± 3.6	40.8± 1.9							
100	48.8± 1.7	49.4± 1.5							
150	41.4 ± 1.8	57.2 ± 1.7							
200	33.2± 3.5	65.4 ± 3.0							
250	25.2±2.1	72.2 ± 2.3							
Ethyl acetate extract									
Control	90.8± 1.3	0± 0.0	115.27	85.87	154.73	8236.38	978.99	69293.72	0.951
50	58.2± 2.3	39.4 ±1.1							
100	50.8± 0.8	45.6 ±3.2							
150	45.4± 3.0	51.4± 2.3							
200	39.8 ±1.3	58.4 ±2.0							
250	23.2 ± 1.6	64.4± 2.5							

Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 48h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{95} = Lethal Concentration brings out 95% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Discussion

Indiscriminate use of synthetic chemicals insecticides to control the mosquitoes in the natural habitats, they have developed strong resistance to almost all the chemicals that are available today. Moreover, chemical insecticides 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 derived insecticides in nature. Today, the environmental safety of an insecticide is considered to be of more important milestone in the field of 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 in particular petroleum ether extract. Similar kind of observation was also noted earlier by several workers (Rajkumar et al., 2011; Aarthi and Murugan, 2011; Balu et al., 2015). 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 *A. paniculata* could be used for mosquito control. We observed a functional response of the ovicidal activity exhibited by the petroleum ether 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* 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* sp. with an LC50 of 16.85 ppm and 11.45 ppm after 24 and 48 h of exposure, respectively.

The present investigation revealed that the presence of several bioactive compounds in *A. paniculata*, which is responsible for ovicidal and pupicidal activity. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in –depth laboratory and field bioassays are needed. In conclusion, an attempt has been made to evaluate the role of *A. paniculata* against an alternative approach to combat with the important human vector mosquitoes.

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