Int. J. Adv. Res. Biol. Sci. 2(10): (2015): 156–160

International Journal of Advanced Research in Biological Sciences

ISSN: 2348-8069

www.ijarbs.com

Coden: IJARQG(USA)

Research Article

Larvicidal activity of *Andrographis paniculata* (Acanthaceae) against important human vector mosquitoes (Diptera: Culicidae)

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Abstract

To determine the larvicidal activity of *Andrographis paniculata* leaf extracts against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. The larvicidal activity was determined against three vector mosquito species at concentrations of 50, 100, 150, 200 and 250 ppm and mortality was assessed after 24 hours. The leaf extracts of *A. paniculata* was found to be more susceptible against the larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus.* However, the maximum larval mortality was detected in plant of Petroleum ether extract against *An. stephensi* of 94.2 % (LC_{50} 20.85 and LC_{95} 444.41). These results suggested that the leaf extracts of *A. paniculata* showed potential to be used as an ideal eco-friendly approach for the control of the *Ae. aegypti*, *An. stephensi* and *Cu. quinquefasciatus.*

Keywords: Larvicidal, *Andrographis paniculata*, Leaf extract, *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus.*

Introduction

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).

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.*

Materials and Methods

Plant material

The leaves of *A. paniculata* were collected from Musir, Tiruchirapalli District, Tamil Nadu, India during the July 2014. Collected 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.

Larvicidal bioassay

The larvicidal activity of selected plants extracts were evaluated as per the protocol previously described WHO,(2005) Based on the wide range and narrow range tests, all extracts tested ranging 30-200ppm were prepared and they were tested against the freshly moulted (0-6 hrs) third instar larvae of selected mosquito species. The plants oils were dissolved in 2 drop twin20 and then diluted in 100ml of dechlorinated tap water to obtain each of the desired concentrations. The control was prepared using 2 drop tween 20 in 100ml of dechlorinated water. The larvae of test species (10) were introduced in 250-ml plastic cups containing 100ml of aqueous medium (100ml of dechlorinated + 2 drop tween 20) and the required amount of chemical compositions 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). The average mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and other statistics chi-square values were calculated by using the software using statistical package of social science (SPSS) version 18.0 for windows, significance level was set at $p \leq 0.05$.

Results

The effect of petroleum ether, chloroform and ethyl acetate extracts of *A. paniculata* on fourth instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* is presented in table.1. Mosquito larvicidal effect of the plant extract was clearly dependent on the concentrations of the extract. All the larvae maintained in the control medium survived for 24 hr, thus no mortality was observed among the control group larvae. The experimental larvae were more susceptible to the maximum concentration of the treated hence, the maximum laral mortality was observed in 250ppm concentration of petroleum ether on *An. Stephensi* and followed by *Ae. aegypti* and *Cx. quinquefasciatus.* The highest larval mortality was detected in petroleum ether extracts of *A. paniculata* on *An. stephensi,* and, 94.2% (LC_{50} 20.85 and LC_{95} 444.41), *Ae. aegypt* 93.6%

Table 1. Larvicidal activities of crude extracts of *A. paniculata* at different concentrations against fourth instar larvae of Mosquito vectors.

Solvent Extracts	Concentration (ppm) % of larval mortality					LC50 (ppm)	95% Confidence Limit (ppm)		LC95 (ppm)	95% Confidence Limit (ppm)		$x2$ (df = 4)
	50 (ppm)	100 (ppm)	150 (ppm)	200 (ppm)	250 (ppm)		LCL	UCL		LCL	UCL	
An. aegypti												
Petroleum Ether extract	65.5± 3.9	71.2± 3.0	79.6 ±1.8	87.8±1.6	93.6±2.5	31.54	18.92	52.59	457.28	266.46	784.77	4.313
Chloroform extract	58.2±2.4	66.2±2.2	70.2±2.2	77.2±2.3	82.4±3.3	33.60	16.69	67.66	1753.87	465.39	6609.58	1.097
Ethyl acetate extract	44.4±2.3	51.8±3.0	56.8 ±2.3	62.8±3.9	70.4±2.9	81.04	56.25	116.76	5238.57	812.28	33784.37	1.065
An. stephensi												
Petroleum Ether extract	70.8± 2.5	77.6± 1.1	82.2 ±2.6	88.6±2.7	94.2±2.4	20.85	9.77	44.47	444.41	236.10	836.52	3.148
Chloroform extract	55.8±3.1	61.8±1.6	68.8±1.6	76.6±1.8	82.4±3.0	42.76	25.11	72.80	1516.73	492.01	4675.64	1.941
Ethyl acetate extract	48.8±1.9	53.8±3.3	65.2 ±3.2	74.8±2.3	80.2±1.7	63.62	45.89	88.19	1302.83	533.90	3179.20	2.978
Cx. quinquefasciatus.												
Petroleum Ether extract	57.2± 2.3	63.8± 1.7	74.4 ±3.0	80.8±2.1	88.6±2.6	43.0	28.11	65.78	732.13	368.38	1455.08	3.227
Chloroform extract	53.2±1.4	60.6±1.1	66.6±1.6	78.4±1.8	85.4±2.4	51.25	34.80	75.47	1008.52	449.24	2264.05	4.295
Ethyl acetate extract	38.4±1.1	45.4±2.8	53.2 ±2.1	63.2±2.5	71.8±3.1	104.43	82.97	131.43	2439.65	780.21	7628.59	2.572

Values are mean ± SD for five replications. Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT).

(LC_{50} 31.54 and LC_{95} 457.28), and *Cx. quinquefasciatus* 88.6% (LC_{50} 43.0 and LC_{95} 732.13), respectively, followed by Chloroform and ethyl acetate extract 82.4% (LC_{50} 33.6 and LC_{95} 1753.87), 82.4% (LC_{50} 42.76 and LC_{95} 1516.73),85.4% (LC_{50} 51.25 and LC_{95} 1008.52) , 70.4% (LC_{50} 81.04 and LC_{95} 5238.57),80.2% (LC_{50} 63.62 and LC_{95} 1302.83) and 71.8% (LC_{50} 104.43 and LC_{95} 2439.65), respectively.

Phytochemical Analysis

The results of preliminary phytochemical analysis of the petroleum ether extract of *A. paniculata* are shown in Table 2. A qualitative test indicated the presence of saponins, steroids and tannins, amino acids, phenolics, flavonoids as major phytochemicals.

Table 2. Preliminary phytochemical analysis of Petroleum ether extract of *A. paniculata*

Constituents	Petroleum Ether extract
Alkaloids	-
Anthraquinones	-
Catechin	-
Coumarin	-
Flavonoids	+
Phenols	+
Quinines	-
Saponins	+
Steroids	+
Tannins	+
Terpenoids	-

+ Presence of compound - Absence of compound

Discussion

Plant derived chemicals may serve as suitable alternative to control the vectors than the chemical insecticides because they are relatively safe, inexpensive and available everywhere in the world. Nowadays, mosquito control is mostly directed against larvae and only against adults when necessary. This is for the reason that the fight against adult is temporary, unsatisfactory and polluting for the environment, while larval treatment is more localized in time and space resulting in less-dangerous outcomes. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are man-made and can be easily identified (Howard *et al*., 2007)

This work demonstrates the efficacy of leaf extract of *A. paniculata* as an effective larvicide against larvae of *Ae. aegypti* , *An. stephensi* and *Cx. quinquefasciatus*. The high rates of larval mortality observed at 250 ppm within 48 hrs with LC_{50} value 9.77ppm indicate the high toxicity of the petroleum ether leaf extract. The results of present study are comparable with similar reports of earlier workers. Ramar and Jeyasankar (2014) reported that, hexane extract of *Trgia involucrata* leaves exhibited larvicidal activity with LC_{50} value of 153.51ppm after 24 h of exposure. The toxicity to the third instar larvae of *Ae.aegypti*, *Cx.quinquefaciatus* and *An.stephensi* by the ethyl acetate leafe extract of *Breyenia vitis –idaea* showed the LC_{50} value of 98.2, 107.79 and 115.8ppm respectively (Jeyasankar and Ramar, 2014). Jeyasankar *et al* (2012) have reported that the ethyl acetate extract of *Phyllanthus emblica* exhibited more than 90% larval mortality at 250ppm on *Cx. quinquefaciatus*. The methanol extract of *Solanum trilobatum* against larvae of mosquito was observed to be most toxic with LC_{50} value of 125.43, 127.77 and 116.64ppm at 48 h after application, respectively vector mosquito's *Ae.aegypti*, *Cx.quinquefaciatus* and *An.stephensi* (Premalatha *et al*., 2013). Several researchers reported, phytochemical based experiments for exploring the larvicidal activity on mosquito vectors (Elumalai *et al* ,2012a; Elumalai *et al*, 2012b; Krishnappa *et al.*, 2012; Krishnappa *et al.*, 2013)

The present investigation revealed that the presence of several bioactive compounds in *A. paniculata*, which is responsible for larval toxicity. 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.

Acknowledgments

This research was financially supported by grants from TNSCST/S&T Project/VR/MS/2013-14 (Ref. No. 2480).

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