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Evaluation of *Andrographis paniculata* Burm.f. (Family:Acanthaceae) extracts against *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.) (Diptera:Culicidae)

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ABSTRACT

Objective: To investigate the larvicidal and ovicidal efficacy of different extracts of *Andrographis paniculata (A. paniculata)* against *Culex quinquefasciatus (Cx. quinquefasciatus)* Say and *Aedes aegypti (Ae. aegypti)* L. (Diptera: Culicidae). **Methods:** Larvicidal efficacy of the crude leaf extracts of *A. paniculata* with five different solvents like benzene, hexane, ethyl acetate, methanol and chloroform was tested against the early third instar larvae of *Cx. quinquefasciatus* and *Ae. aegypti*. The ovicidal activity was determined against two mosquito species to various concentrations ranging from 50-300 ppm under the laboratory conditions. **Results:** The benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *A. paniculata* was found to be more effective against *Cx. quinquefasciatus* than *Ae. aegypti*. The LC_{50} values were 112.19, 137.48, 118.67, 102.05, 91.20 ppm and 119.58, 146.34, 124.24, 110.12, 99.54 ppm respectively. Among five tested solvent, methanol and ethyl acetate crude extract was found to be most effective for ovicidal activity against two mosquito species. The extract of methanol and ethyl acetate exerted 100% mortality at 200 ppm against *Cx. quinquefasciatus* and at 250 ppm against *Ae. aegypti*. **Conclusions:** From the results it can be concluded the crude extract of *A. paniculata* was a potential for controlling *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes.

1. Introduction

Mosquitoes are the major vector for the spreading of malaria, dengue fever, yellow fever, filariasis, and several other diseases [1]. Mosquitoes also cause allergic responses on humans including local skin and systemic reactions such as angioedema [2]. *Aedes aegypti* (*Ae. 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 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 [3]. An outbreak of chikungunya virus infection emerged in the southwest Indian Ocean islands in 2005, spread out to India, and resulted in an ongoing outbreak that has involved >1.5 million patients, including travelers who have visited these areas[4]. *Culex quinquefasciatus (Cx. quinquefasciatus)*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people being infected worldwide and 44 million people having common chronic manifestation[5].

An effective method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. However, one major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides [6], and for more detailed studies of naturally-occurring insecticides[7]. These problems have highlighted the need for the development of new strategies for selective mosquito larval control. Extracts

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or essential oils from plants may be alternative sources of mosquito larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae [8-11].

Hexane extract obtained from leaves of *Eucalyptus citriodora* was tested against larvae of *Anopheles stephensi (An. stephensi)*, *Cx. quinquefasciatus* and *Ae. aegypti* [12]; the crude carbon tetrachloride, methanol and petroleum ether extracts of *Ajuga remota* leaves were observed against the larvae of *An. stephensi* and *Cx. quinquefasciatus* [13] and the whole plant petroleum ether extract of *Citrullus colocynthis* was assayed for its toxicity against the early fourth instar larvae of *Cx. quinquefasciatus* [14] ; 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* [15]. 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* [16]. Larvicidal activity of the acetone, chloroform, ethyl acetate, hexane and methanol leaf extracts of *Rhinacanthus nasutus* were studied against fourth instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* [17] ; the dried root powder methanol extract of *Rhinacanthus nasutus* was tested against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*[18]; 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*, *An. stephensi* and *Ae. aegypti* [19]; the petroleum ether extract showed larvicidal activity against the *Ae. aegypti, Cx. quinquefasciatus, An. dirus* and *Mansonia uniformis* [20] .

Andrographis paniculata (A. paniculata) (Burm.f.) Wall. ex Nees., also known commonly as "King of Bitters (English) or Nilavembu (Tamil)," is a member of the plant family *Acanthaceae*. It is an annual herbaceous plant which is widely cultivated in southern Asia, China and some parts of Europe. *A. paniculata* extract is traditionally used as a medicine to treat different diseases in India, China and Southeast Asia including Malaysia. The leaves and roots have been traditionally used over the centuries in Asia and Europe as a folklore medicine for a wide variety of ailments or as herbal supplements for health promotion. In traditional Chinese medicine, it is widely used to get rid of body heat, as in fevers and to dispel toxins from the body. Previous studies have explicitly revealed that *A. paniculata* has a wide range of pharmacological effects and some of them extremely beneficial such as anti-inflammatory [21] , anti-diabetes [22] , antidiarrhoeal[23], antiviral [24] , hepatoprotective [25], anticancer[26], antihuman immunodeficiency virus (HIV) [27]. Diterpenoids and flavonoids are the main chemical constituents of *A. paniculata* which are believed to be responsible for the most biological activities of this plant [28]. 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 different solvent crude extracts from the medicinal plant *A. paniculata* against the medically important mosquito vectors, *Cx. quinquefasciatus* and *Ae. aegypti*.

2. Materials and methods

2.1. Collection of plants

Fully developed leaves of the *A. paniculata* 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. Extraction

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[29]. 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 *A. paniculata* with five different solvents yielded 81.20, 94.85, 79.40, 97.60 and 121.30 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

The mosquitoes, *Cx. quinquefasciatus* and *Ae. aegypti* were 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[30]. 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 40 to 250 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 LC_{50} value was calculated after 24 h by probit analysis[31].

2.5. Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla[32] was performed. The egg raft/eggs of *Cx. quinquefasciatus* and *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 50 to 300 ppm. Eggs of these 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.

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

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 botanical extracts from the plant leaves in their crude form have been used as conventional insecticides for centuries. The activity of crude plant extracts is often attributed to the complex mixture of active compounds. In the present investigation, the toxicity of different solvent extract of *A. paniculata* was tested against *Cx. quinquefasciatus* and *Ae. aegypti* (Table 1& 2). The data were recorded and statistical data regarding LC_{50}, LC_{90}, LCL, UCL and *chi*-square values were calculated. The LC_{50} value

Table 1
Larvicidal activity of different solvent extracts of *A. paniculata* against *Cx. quinquefasciatus*.

Name of the extract	Concentration(ppm)	% of mortality	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2
Benzene	control	0.0			
	50	31.6			
	100	49.0	112.19 (79.71-142.26)	213.34 (176.39-287.82)	17.691*
	150	66.2			
	200	52.2			
	250	96.9			
Hexane	control	0.0			
	50	23.2			
	100	38.0	137.48 (110.62-165.60)	251.83 (213.62-323.33)	12.135*
	150	56.4			
	200	71.0			
	250	89.9			
Ethyl acetate	control	0.0			
	50	30.0			
	100	47.2	118.67 (86.30-149.51)	225.29 (186.58-303.84)	16.989*
	150	61.2			
	200	80.0			
	250	94.9			
Methanol	control	0.0			
	40	28.6			
	80	41.0	102.05 (76.67-127.50)	190.59 (158.17-257.03)	16.382*
	120	57.0			
	160	76.2			
	200	93.4			
Chloroform	control	0.0			
	40	31.2			
	80	49.0	91.92 (65.75-116.33)	176.36 (145.87-237.92)	17.129*
	120	63.9			
	160	81.6			
	200	95.0			

*Significant at $P<0.05$

Table 2
Larvicidal activity of different solvent extracts of *A. paniculata* against *Ae. aegypti*.

Name of the extract	Concentration(ppm)	% of mortality	LC_{50} (ppm) (LCL–UCL)	LC_{90} (ppm) (LCL–UCL)	χ^2
Benzene	control	0.0	119.58 (87.36–150.37)	226.95 (188.14–305.53)	16.755*
	50	29.0			
	100	47.9			
	150	61.2			
	200	79.0			
	250	94.6			
Hexane	control	0.0	146.34 (122.18–172.59)	262.89 (225.84–329.03)	9.940*
	50	19.6			
	100	35.6			
	150	52.2			
	200	68.0			
	250	87.2			
Ethyl acetate	control	0.0	124.24 (93.73–154.18)	234.02 (195.39–310.24)	15.224*
	50	28.4			
	100	44.0			
	150	59.2			
	200	78.2			
	250	93.0			
Methanol	control	0.0	110.12 (87.28–134.19)	204.37 (171.87–267.58)	13.010*
	40	25.0			
	80	38.2			
	120	54.6			
	160	72.0			
	200	88.6			
Chloroform	control	0.0	99.54 (73.26–125.27)	190.10 (157.16–258.22)	16.515*
	40	29.0			
	80	45.6			
	120	58.8			
	160	77.0			
	200	92.2			

*Significant at $P<0.05$

Table 3
Ovicidal activity of A. paniculata plant extracts against *Cx. quinquefasciatus* and *Ae. aegypti*.

Mosquito	Name of the solvent	Percentage of egg hatch ability						
		Concentration (ppm)						
		Control	50	100	150	200	250	300
Cx. quinquefasciatus	Hexane	100.0±0.0	50.6±1.6	44.2±2.2	39.2±1.7	31.4±1.2	19.2±0.8	NH
	Ethyl acetate	100.0±0.0	44.2±1.4	37.4±2.4	28.6±0.9	NH	NH	NH
	Benzene	98.6±1.2	45.6±1.9	38.2±1.8	32.8±1.5	27.2±1.5	16.8±1.6	NH
	Chloroform	100.0±0.0	39.2±1.8	32.6±1.6	25.2±1.4	18.4±0.8	NH	NH
	Methanol	97.4±0.8	35.4±1.4	26.4±1.2	19.6±0.8	NH	NH	NH
Ae. aegypti	Hexane	99.6±1.2	72.2±0.6	57.5±1.5	48.2±1.3	39.9±1.2	26.6±1.2	NH
	Ethyl acetate	100.0±0.0	64.3±1.4	51.8±1.3	42.6±1.4	31.2±1.6	NH	NH
	Benzene	100.0±0.0	67.2±1.8	56.9±1.9	45.6±1.8	32.5±0.9	23.3±0.8	NH
	Chloroform	100.0±0.0	57.2±0.8	48.5±1.2	38.8±0.7	24.6±0.7	17.5±1.4	NH
	Methanol	99.6±0.8	49.3±0.6	37.9±1.6	28.6±1.2	21.2±1.3	NH	NH

NH– No hatch ability.

of benzene, hexane, ethyl acetate, methanol and chloroform extract of *A. paniculata* against early third instar larvae of *Cx. quinquefasciatus* were 112.19, 137.48, 118.67, 102.05, 91.92 ppm and against *Ae. aegypti* value were 119.58, 146.34, 124.24, 110.12, 99.54 ppm, respectively. No mortality was observed in control. *Chi*–square values were significant at $P<0.05$ level. Table 3 shows the mean percent hatchability of *Cx. quinquefasciatus* and *Ae. aegypti*. Among five tested solvent the methanol and ethyl acetate crude extract was found to be most effective for ovicidal activity against two mosquito species. The extract of methanol and ethyl acetate exerted 100% mortality at 200 ppm against *Cx. quinquefasciatus* and at 250 ppm against *Ae. aegypti*.

4. Discussion

The results showed that crude extract of *A. paniculata* have significant larvicidal and ovicidal activity against *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes. This result is also comparable to earlier reports of Rahuman and Venkatesan [33] who observed the petroleum ether extract of *Citrullus colocynthis*, methanol extracts of *Cannabis indica, Cannabis sativus, Momordica charantia* and acetone extract of *Trichosanthes anguina* against the larvae of *Ae. aegypti* (LC_{50}=74.57, 309.46, 492.73, 199.14, and 554.20 ppm) and against *Cx. quinquefasciatus* (LC_{50}=88.24, 377.69, 623.80, 207.61, and 842.34 ppm), respectively. Shaalan *et al*. [34] have reported that the LC_{50} value of acetone extracts of *Khaya saenegalensis* and *Daucus carota* were 20.12 and 236.00 mg/L, respectively, against 4th-instars of *Cx. annulirostris*. Larvicidal activity of acetone extracts of *Murraya koenigii, Coriandrum sativum, Ferula asafoetida,* and *Trigonella foenum* graceum reported maximum activity ranging 25 - 900 ppm against *Ae. aegypti* [35]. Mullai and Jebanesan [36] have reported that ethyl acetate, petroleum ether and methanol leaf extracts of *C. colocynthis* and *Cucurbita maxima* showed LC_{50} values of 47.58, 66.92, 118.74 ppm and 75.91, 117.73, 171.64 ppm, respectively, against *Cx. quinquefasciatus* larvae.

Jang *et al* [15] have reported that the methanol extracts of *Cassia obtusifolia, Cassia tora* and *Vicia tetrasperma* exhibited more than 90% larval mortality at 200 ppm on *Ae. aegypti* and *Cx. pipiens*; Komalamisra *et al*. [20] have reported that the petroleum ether and methanol (MeOH) extracts of *R. nasutus* and *Derris elliptica* exhibited larvicidal effects against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. dirus* and *Mansonia uniformis* with LC_{50} values between 3.9 and 11.5 mg/L, whilst the MeOH extract gave LC_{50} values of between 8.1 and 14.7 mg/L. *D. elliptica* petroleum ether extract showed LC_{50} values of between 11.2 and 18.84 mg/L and the MeOH extract exhibited LC_{50} values between 13.2 and 45.2 mg/L.

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[37]. The crude leaf extract of *Acalypha 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[38]. The LC_{50} of leaf extract of *Cassia fistula* with different solvents, viz. methanol, benzene and acetone against *Ae. aegypti* were 10.69, 18.27 and 23.95 mg/L respectively[39]. 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[40]. The LC_{50} and LC_{90} values of crude methanol extract of leaves of *Ervatamia 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[8]. 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 [41]. This study reveals that the leaf crude extract of *A. paniculata* has remarkable larvicidal as well as ovicidal properties. The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. These results could encourage the search for new active natural compounds offering an alternative to synthetic insecticides from other medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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