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## Larvicidal activity of *Andrographis paniculata* (Burm.f) Nees against *Culex quinquefasciatus* Say (Insecta: Diptera–Culicidae), a filarial vector

Sheeja BD<sup>1</sup>, Sindhu D<sup>2</sup>, Ebanasar J<sup>3</sup>, Jeeva S<sup>4\*</sup><sup>1</sup>Department of Botany, Government Arts College, Udhamandalam, The Nilgiris, Tamilnadu, India<sup>2</sup>Department of Botany, Nesamony Memorial Christian College, Marthandam, Tamilnadu, India<sup>3</sup>Department of Zoology and Wildlife Biology, Government Arts College, Udhamandalam, The Nilgiris, Tamilnadu, India<sup>4</sup>Department of Botany Scott Christian College Nagercoil, Kanyakumari, Tamilnadu, India

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### ABSTRACT

**Objective:** The purpose of the present study was to assess the acetone and ethanol extracts of *Andrographis paniculata* against the filarial vector *Culex quinquefasciatus*. **Methods:** The ethanol and acetone extracts of plant leaves was used at 25, 50, 100, 200 and 300 ppm dilution in bioassays against second instar larvae of *Culex quinquefasciatus*. Ten larvae were exposed to the leaf extract at different concentration in a final volume of 100 mL formulation taken in 500 mL of glass bowl. For avoiding error triplicates were maintained. Tap water was taken as control. The larval mortality at different concentrations and in control was recorded 24 hour continuous exposure. **Results:** The toxicity of ethanol extract of *Andrographis paniculata* on survival of *Culex quinquefasciatus* reveals that the mortality is dependent on the dose of the plant extract and also on time of exposure. Highest mortality rate of 20% and 73.3% was recorded in 300 ppm at 3 and 24 h respectively. The similar trend was observed in case of the toxicity of acetone extract. **Conclusions:** The results of the present study suggest that the leaf extracts of *Andrographis paniculata* may have the potential to be used as an ideal eco-friendly approach for the control of the filarial vector *Culex quinquefasciatus*.

## 1. Introduction

Emergence and resurgence of mosquito-borne diseases such as malaria, dengue, Japanese encephalitis and filariasis in south Asian countries are well known because of their tropical or subtropical climate, the frequently poor drainage system which provides abundant mosquito breeding places[1,2]. More than two billion people, mostly in tropical countries, are at risk from mosquito-borne diseases, such as malaria, dengue, haemorrhagic fever and filariasis[3]. Control of such mosquito-borne diseases is becoming more and more difficult because of increasing resistance to pesticides, lack of effective vaccines and drugs against disease-causing mosquitoes. Biological control at the larval stage of development of mosquitoes is one of the techniques which affords a cheap, easy to use, and

environment friendly method of malaria control. Natural insecticides are less phytotoxic and do not accumulate chemical residues in flora, fauna and soil. Phytochemicals with mosquito larvicidal activity occur in the oils, leaves and roots of plants[4–6]. Hence, an alternative approach for mosquito control is the use of extracts of plant origin[7–9].

Plant products have been used by traditionally human communities in many parts of the earth against the vectors and species of insects[10–14]. The phyto-chemicals derived from plant sources can act as larvicides, insect growth regulators, repellents, ovipositional attractants and have deterrent actions as observed by many researchers[15–19]. Kamaraj and Rahuman[20] studied the larvicidal and adulticidal activities of hexane, ethyl acetate and methanol extracts of *Momordica charantia*, *Moringa oleifera*, *Ocimum gratissimum*, *Ocimum tenuiflorum*, *Punica granatum* and *Tribulus terrestris* against *Culex gelidus* and *Culex quinquefasciatus*. Zahir *et al.*[21] studied the adult emergence inhibition and adulticidal activities of hexane, chloroform, ethyl acetate, and acetone leaves extracts of *Anisomeles malabarica*, *Euphorbia hirta*, *Ocimum basilicum*,

\*Corresponding author: Dr. S. Jeeva Assistant Professor Department of Botany Scott Christian College Nagercoil, Kanyakumari – 629 003 Tamilnadu, India  
Tel: +91–9952202112  
E-mail: [solomonjeeva@gmail.com](mailto:solomonjeeva@gmail.com)

*Ricinus communis*, *Solanum trilobatum*, *Tridax procumbens* and seeds of *Gloriosa superba* against *Anopheles stephensi*. The study revealed that there are probabilities that the active principles contained in these plant extracts, especially the chloroform, ethyl acetate and acetone extracted fractions are further more mosquito adult emergence inhibition and adulticidal as compared with their crude forms. The larvicidal and adult emergence inhibition activities of castor (*Ricinus communis*) seed extract against three potential mosquito vectors *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes albopictus* in India was studied by Mandal<sup>[19]</sup> and the findings of the study suggest that the seed extract provided an excellent potential for controlling mosquito vectors.

Larvicidal activity of *Abrus precatorius*, *Croton bonplandianum*, *Cynodon dactylon*, *Musa paradisiaca* and *Syzygium aromaticum* were tested against fourth instar larvae of *Anopheles vagus*, *Armigeres subalbatus* and *Culex vishnui* was reported by Bagavan and Rahuman<sup>[22]</sup>. Govindarajan and Karuppannan<sup>[23]</sup> studied the mosquito larvicidal and ovicidal properties of *Eclipta alba* (L.) Hassk (Asteraceae) against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae) and concluded that the crude extract of *Eclipta alba* was an excellent potential for controlling *Aedes aegypti* mosquito. Past studies reveal the need for screening potential of herbal larvicides against mosquito larvae. Hence the present study was planned to evaluate the phytochemical rich plant on control of *Culex quinquefasciatus* larvae.

## 2. Materials and methods

### 2.1. Plant collection and extraction of crude bioactive

The leaves of *Andrographis paniculata* (Burm.f) Nees (Acanthaceae) was collected in and around Puthalam village, Kanyakumari, Tamilnadu, India. Prior to the extraction, the leaves of respective species were washed with sterile water to remove any associated debris, shade dried in order to prevent photolysis and thermal degradation, chopped into small pieces and ground coarsely to powder form in mortar and pestle. For extraction of crude bioactives, 100 g of powdered plant material were exhaustively extracted with 200 mL of ethanol and acetone using soxhlet apparatus. The extract was further concentrated by recovering excess solvents to thick oily natured crude in a rotary evaporator at reduced pressure. The extract was stored at 4 °C in air-tight plastic vials for further studies.

### 2.2. Collection and maintenance of test organism

The egg rafts of *Culex quinquefasciatus* were collected from the puddles in the vicinity of Puthalam village, and allowed to hatch. The young 2nd instar larvae were chosen as test organism.

### 2.3. Larvicidal bioassay

Standard methods for testing the susceptibility of mosquito larvae to insecticides were followed in all the experiments with slight modifications. The ethanol and acetone extracts of plant leaves was used at 25, 50, 100, 200 and 300 ppm dilution in bioassays against second instar larvae of *Culex quinquefasciatus*. Ten larvae were exposed to the leaf extract at different concentration in a final volume of 100 ml formulation taken in 500 mL of glass bowl. For avoiding error triplicates were maintained. Tap water was taken as control. The larval mortality at different concentrations and in control was recorded 24 h continuous exposure.

### 2.4. Statistical analysis

The percent mortality values for second instar larvae *Culex quinquefasciatus* treated with various concentrations (ranging from 25 to 300) of the leaf extract of *Andrographis paniculata* was recorded and the percentage mortality was calculated and the data was analyzed using curve expert software for finding the LC<sub>50</sub>, LC<sub>95</sub> and LC<sub>100</sub> values. The third degree polynomial fit was used as a suitable mathematic model in the curve expert software.

## 3. Results

The toxicity of ethanol extract of *Andrographis paniculata* on survival of *Culex quinquefasciatus* reveals that the mortality is dependent on the dose of the plant extract and also on time of exposure. Highest mortality rate of 20% and 73.3% was recorded in 300 ppm at 3 and 24 h respectively. No mortality was recorded up to 50 ppm in 3 h. The polynomial fit was found to be the best fit model with the highest coefficient of determination ( $r^2$ ) of 0.8248 in 3 h of exposure. The relationship can be expressed as,

$$Y = -0.030 - 0.004x + 0.00035x^2 - 4.111x3e-007$$

where, x = concentration and y = mortality. From this LC<sub>50</sub> value was found to be 54 ppm and LC<sub>100</sub> was found to be 206 ppm. In case of 24 hour exposure, the highest coefficient of determination ( $r^2$ ) of 0.9500. The relationship can be expressed as,

$$Y = -3.994 - 0.639x + 0.0026x^2 - 4.164x3e-006.$$

From this LC<sub>50</sub> value was found to be 117 ppm and LC<sub>100</sub> was found to be 381 ppm.

The similar trend was observed in case of the toxicity of acetone extract, i.e. the mortality is dependent on the dose of the plant extract on survival of *Culex quinquefasciatus*. Highest mortality rate of 50% and 83.3% was noted in 300ppm in 3 and 24 h correspondingly. The polynomial fit with the

highest coefficient of determination ( $r^2$ ) of 0.9381 and the relationship can be expressed as,

$$Y = -0.209 - 0.096x + 0.0024x^2 - 5.212 \times 10^{-6}.$$

From this  $LC_{50}$  value was found to be 15 ppm and  $LC_{100}$  was found to be 31 ppm. Polynomial fit also shows that the highest coefficient of determination ( $r^2$ ) of 0.9647 was noted in 24 h of exposure and can be expressed as,

$$Y = -1.772 - 0.293x + 0.00033x^2 - 1.590 \times 10^{-6}.$$

From this,  $LC_{50}$  value was found to be 17 ppm and  $LC_{100}$  was found to be 32 ppm.

**Table 1.**  
Effect of ethanol extract of *A. paniculata* on survival of *C. quinquefasciatus* during different period of exposure

Dose (ppm)	Percentage mortality		
	3 h	6 h	24 h
25	0	10	23.3
50	0	13.3	33.3
100	3.3	20	40
200	10	40	63.3
300	20	56.6	73.3

**Table 2.**  
Effect of ethanol extract of *A. paniculata* on survival of *C. quinquefasciatus* during different period of exposure

Dose (ppm)	Percentage mortality		
	3 h	6 h	24 h
25	0	10	26.6
50	0	23.3	36.6
100	10	26.6	46.6
200	36.6	63.3	73.3
300	50	76.6	83.3

#### 4. Discussion

Medicinal plants have been recognized as a repository of bioactive compounds[24–28]. Biologically active plants show great promise for their potential efficiency as larvicides. Present study showed clearly that alcoholic and acetone extracts of *Andrographis paniculata* had a pronounced larvicidal effect on mosquito *Culex quinquefasciatus*. It may be due the presence of the phytochemicals such as alkaloids, carboxylic acids, flavanoids, phenols, proteins, quinines, resins, steroids, and saponins[29]. This larvicidal effect may be due to the highest amount of andrographolide (2.39%), the most medicinally active phytochemical in the plant, *Andrographis paniculata*. Besides this, other active components include 14–deoxy–11,12–didehydroandrographolide (andrographolide D), homoandrographolide, andrographan, andrographosterin, and stigmasterol are also present in the leaves of *Andrographis paniculata*[30,31]. The acetone extract of *Andrographis paniculata*, methanol extract of *Eclipta*

*prostrata* and *Tagetes erecta* showed good oviposition–deterrent, ovicidal and repellent activities against malaria vector, *Anopheles subpictus* Grassi (Diptera: Culicidae)[32]. Elango *et al.*[33] reported that the hexane and chloroform extracts of *Andrographis paniculata* exerted 100% mortality (no hatchability) at 1000 ppm and at 250 ppm a very low hatchability.

Past studies suggested that the benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Andrographis paniculata* was found to be more effective against *Culex quinquefasciatus* than *Aedes aegypti*[34]. The treatment of different products of *A. paniculata* greatly affected the larval growth of *Anopheles stephensi* and caused malformation and mortality in a dose dependent manner[30]. Oviposition deterrent, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* caused moderate ovicidal activity against various age groups of *Aedes stephensi*, but it inflicted delayed effects such as high larval, pupal and adult mortality, thereby suppressing the vector population and adversely influencing transmission of the disease pathogen[35].

Topical studies on the adult emergence inhibition and adulticidal activity of the leaf hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *Aegle marmelos* (Linn.) Correa ex Roxb, *Andrographis lineata* Wallich ex Nees., *Andrographis paniculata* (Burm.f.) Wall. ex Nees., *Cocculus hirsutus* L. Diels, *Eclipta prostrata* L. and *Tagetes erecta* L. were tested against Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles (Diptera: Culicidae) showed that emergence inhibition of leaf methanol extract of *Aegle marmelos*, *Andrographis paniculata*, *Tagetes erecta* and chloroform extract of *Eclipta prostrata* ( $EL_{50}$ =141.94, 214.17, 166.43, and 184.58 ppm;  $EL_{90}$ =590.26, 882.34, 532.00 and 571.81 ppm), and the maximum adulticidal activity observed in acetone extract of *Aegle marmelos*, hexane extract of *Andrographis lineata*, ethyl acetate extract of *Andrographis paniculata*, methanol extract of *Cocculus hirsutus*, *Eclipta prostrata*, and *Tagetes erecta* ( $LD_{50}$  = 139.05, 251.24, 205.06, 222.10, 166.73, and 232.74 ppm;  $LD_{90}$  = 426.19, 837.09, 813.59, 794.42, 579.43 and 807.41 ppm), respectively against *Culex tritaeniorhynchus*[36].

Our results agreed with some previous studies, undoubtedly, plant derived toxicants are a valuable source of potential larvicidal activities. Similar study was conducted by Medhi *et al.*[37] in *Eucalyptus camaldulensis* against malaria vector, *Anopheles stephensi* and reported that adult emergence inhibition and adulticidal activity was found in ethyl acetate extracts of *Anisomeles malabarica*, chloroform extracts of *Ocimum basilicum*, *Solanum trilobatum*, acetone extract of *Ricinus communis*, *Tridax procumbens* and seed extracts of *Gloriosa superba* with  $EL_{50}$  values 143.12, 119.82, 157.87, 139.39, 111.19 and 134.85  $\mu$ g/mL, and the effective adulticidal activity was observed in chloroform, acetone extracts with  $LD_{50}$  values 120.17, 108.77, 127.22, 163.11, 118.27 and 93.02  $\mu$ g/mL, respectively.

Ovicidal and repellent activities of methanol leaf extract of *Ervatamia coronaria* and *Caesalpinia pulcherrima* against *Culex quinquefasciatus*, *Aedes aegypti* and *nopheles stephensi* showed that the crude extract of these botanicals were an excellent potential for controlling mosquitoes<sup>[38]</sup>. Larvicidal activity of *Moringa oleifera* exhibited in the first to fourth instar larvae of the *Anopheles stephensi*, and the LC 50 and LC90 values were 57.79 ppm and 125.93 ppm for first instar, 63.90 ppm and 133.07 ppm for second instar, 72.45 ppm and 139.82 ppm for third instar, 78.93 ppm and 143.20 ppm for fourth instar respectively<sup>[39]</sup>.

According to Kumar *et al.*<sup>[40]</sup> the essential oil extracted from *Mentha piperita* possessed excellent larvicidal efficiency against dengue vector. The bioassays showed an LC 50 and LC90 value of 111.9 and 295.18 ppm, respectively after 24h exposure. The toxicity of the oil increased 11.8% when the larvae were exposed to the oil for 48h. The remarkable repellent properties of *M. piperita* essential oil were established against adults *Aedes aegypti*.

The findings of the present study along with the previous studies suggested that the leaves of *Andrographis paniculata* may be exposed as potential eco-friendly mosquito larvicidal agent. Further studies on isolation of bioactive fraction/constituent may provide futuristic lead products for field application of mosquito control.

### Conflict of interest statement

We declare that we have no conflict of interest.

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