Research Article

Preliminary Larvicidal Potential of *Andrographis Echioides* against Dengue Vector *Aedes aegypti* Larvae

Singaravel Sengottuvelu[1,*], Sheik Haja Sherief[1], John Staines Mark[1], Chinnadurai Kathirvelan[2], Banupriya Senthamaraikannan[3]

[1]Department of Pharmacology, Nandha College of Pharmacy, Erode, Tamil Nadu, INDIA.
[2]Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, Tamil Nadu, INDIA.
[3]Department of Livestock Products Technology – Diary Science, Veterinary College and Research Institute, Namakkal, Tamil Nadu, INDIA.

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ABSTRACT

Aim/Background: The present study was conducted to assess the larvicidal potential of *Andrographis echiodies* against the dengue vector *Aedes aegypti* larvae. Alcoholic leaf extract of *Andrographis echiodies* was studied at 500, 250, 125, 62.5, 31.25, and 15.625 ppm concentrations against third-instar larvae of *Aedes aegypti*. **Materials and Methods:** 100 larvae of *Aedes aegypti* mosquitoes were exposed to each concentration of both ethanolic and methanolic crude extract of *Andrographis echiodies* in a volume of 100ml. To minimize the error five replicates were employed with respective diluted alcoholic solutions as control. The percentage mortality of larvae at different concentrations of both alcoholic extracts and control were recorded after 24 hr and 48 hr of constant exposure followed by LC_{50} and LC_{90} determined. **Results:** The effective and highest coefficient of determination (R_2) was calculated using regression analysis. The effective and highest coefficient of determination (R_2) was 0.88 in 24 hr of exposure of larvae in methanolic leaf extract of *Andrographis echioides*. Similarly, the effective and highest coefficient of determination (R_2) was 0.94 in 24 hr of exposure larvae in ethanolic leaf extract of *Andrographis echioides*. **Conclusion:** From the result it was concluded that both methanolic and ethanolic leaf extract of *Andrographis echioides* exhibited larvicidal activity against the dengue vector *Aedes aegypti* larvae and it may be due to its secondary metabolite diterpenoid lactone. Further studies may be needed to expose its exact mechanism of action.

Keywords: *Andrographis echioides*, Larvicidal activity, Dengue vector, *Aedes aegypti,* and Diterpenoid lactone.

Correspondence:
Dr. S Sengottuvelu,
M. Pharm., PhD
Professor and Head,
Department of Pharmacology, Nandha College of Pharmacy,
Erode – 638052,
Tamil Nadu, INDIA.

Email id: sehejan@gmail.com

INTRODUCTION

Emerging infectious illnesses are defined as new, resurfacing, or drug-resistant infections in humans that have grown in the last two decades or promise to increase in the near future. Changes in human industrial practices, economic growth, changes in land use, increased international travel and trade, and adaptability of bacteria, including the development of resistance to antimicrobial agents, are all factors contributing to the comeback of infectious illnesses. Mosquitoes are a major annoyance to humans and a hazard to public health as vectors of illnesses such as dengue fever, West Nile fever, malaria, filariasis, Japanese encephalitis, and. Malaria, spread by Anopheles mosquitos, is believed to infect 300 million people each year, with over one million deaths. The global impact of lymphatic filariasis is Emerging infectious illnesses are defined as new, resurfacing, or drug-resistant infections in humans that have grown in the last two decades or promise to increase in the near future. Changes in human industrial practices, economic growth, changes in

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land use, increased international travel and trade, and adaptability of bacteria, including the development of resistance to antimicrobial agents, are all factors contributing to the comeback of infectious illnesses. Mosquitoes are a major annoyance to humans and a hazard to public health as vectors of illnesses such as dengue fever, malaria, filariasis, West Nile fever and Japanese encephalitis. Malaria, spread by Anopheles mosquitos, is believed to infect 300 million people each year, with over one million deaths. The global impact of lymphatic filariasis is 250 million individuals are estimated. Dengue viruses, spread by Aedes mosquitos, infect approximately 20 million people each year, resulting in 24,000 fatalities.[1] The majority of mosquito control programs, particularly in third-world nations, rely on chemical pesticides. However, many mosquito species have acquired resistance to DDT and other pyrethroid family pesticides. As a result, mosquito-borne infections remain common. Furthermore, the overall negative impact of chemical pesticides on the ecosystem is disastrous.[2] Insecticides produced from plants are becoming increasingly important as plant-derived complex new chemicals with the potential to be sensitive insecticides. Because many mosquito-borne illnesses do not have a particular synthetic medicine, vector control is the best method. As a result, ongoing mosquito control measures are critical to preventing epidemics of these illnesses, and it should be treated as a plant-derived novel chemical entity.

Andrographis echiodies is a herbaceous plant of the Acanthaceae family that grows in the arid areas of South Asian countries. Andrographis echioides has traditionally been used to treat inflammation., Goitre, liver diseases,[3] fertility problems, bacterial/fungal infections,[4] and malarial disorders.[5] Leaf juice boiled with coconut oil controlled the falling and greying of hair[6] and the anti-inflammatory activity of this plant was thoroughly studied by Basu SK. *et al.*[7] *Andrographis echioides* (L.) is also used in febrifuge, cooling, and alternative for cuts and wounds. The entire plant extract is used to treat fever.[4] Andrographis echioides is well-known for its diuretic, anthelmintic, hepatoprotective, analgesic, anti-ulcer, antibacterial, and antivenom properties.[8] The current study will analyse Andrographis echioides' preliminary larvicidal ability against Aedes aegypti mosquito larvae.

MATERIALS AND METHODS

Plant Material

The plant Andrographis echioides was collected in the outskirts of Erode, Tamil Nadu. Scientist D, Botanical Survey of India, Southern Regional Center, Agricultural University, Coimbatore, India, validated and identified the plant as *Andrographis echioides*. The voucher specimen (BSI/SRC/12/42/2019-20/Sci/196) has been deposited in Herbarium for further reference.

Preparation of Extract

The leaves were washed with tap water to remove debris before being shade dried for seven days. Andrographis echioides leaves were cursed into coarse powder after being shade dried. Andrographis echioides powdered samples were extracted using the Soxhlet hot extraction procedure with 250 ml of 90% methanol and 90% ethanol. Separate filters were used to separate the resultant solution, and the solvent was evaporated at decreased pressure. The extracted materials were concentrated using a rotary evaporator, dried, and kept in desiccators for further studies.

Collection, identification, and maintenance of mosquito third instar larvae

The third instar larvae of Mosquito were collected from drains that are open in Erode and brought in sterile plastic containers to the Department of Zoology at Bharathiyar University in Coimbatore to be identified using a stereo microscope. Third instar larvae of Aedes aegypti mosquitos were placed in waterfilled plastic plates at 25°C-29°C and 80 percent -90 percent relative to larval feeding (biscuit and yeast in the ratio of 3:1).[9]

Larvicidal Assay

To test larvicidal efficacy, a 1000 ppm stock solution of Andrographis echioides was produced by dissolving 100 mg of crude extracts in 1 ml of methanol and ethanol. Twenty mosquito larvae were placed in 250 ml beakers typically contains 100 ml of 500, 250, 125, 62.5, 31.25, and 15.625 ppm concentration crude extracts with five repetitions (n = 100 larvae) for each treatment and 0.2 percent control at 20°C temperature for 24 and 48 hr. Triplicates were kept to avoid errors. As a control, tap water was used. The larval mortality at various concentrations and under control was recorded, as well as the number of surviving rate and mortality rate of larvae.[10]

$$\text{Mortality \%} = \frac{\text{Observed mortality (\%)} - \text{Control mortality (\%)}}{100 - \text{Control mortality \%}} \times 100$$

Statistical Analysis

The LC50 and LC90 values were calculated using GraphPad Prism (version 5.01) software and then subjected to a dose-response bioassay on Aedes aegypti mosquito larvae after 24 and 48 hr of activity. GraphPad

Prism (version 5.01) software was used to calculate the regression equation, R_2, and *p* value.

RESULTS

The larvicidal activity of ethanolic and methanolic extract of *Andrographis echioides* was studied against the third instar larvae of *Aedes aegypti* mosquitoes and the results were shown in Table 1. Mortality of *Aedes aegypti* mosquitoes' larvae was observed at 24hr and 48hr exposure to various concentrations (500, 250, 125, 62.5, 31.25, 15.625) PPM of ethanolic and methanolic crude extracts of Andrographis *echioides*. The result showed that LC_{50} of methanolic extract of *Andrographis echioides* at 24hr and 48 hr was 290.02 and 224.22 ppm respectively. Similarly, LC_{90} of methanolic extract of *Andrographis echioides* at 24hr and 48 hr was 522.04 and 402.86 ppm respectively. The effective and highest coefficient of determination (R_2) was 0.88 in 24 hr of exposure. In an ethanolic extract of *Andrographis echioides,* the LC_{50} at 24hr and 48 hr were 270.27 and 230.41 ppm respectively. Similarly, LC_{90} of ethanolic extract of *Andrographis echioides* at 24hr and 48 hr was 485.96 and 414.75 ppm respectively. The effective and highest coefficient of determination (R_2) was 0.94 in 24 hr of exposure.

DISCUSSION

Mosquitoes transmit various deadly illnesses, including malaria, filaria, dengue, chikungunya, and encephalitis. Every year, millions of people die as a consequence of mosquito bites throughout the world. Dengue fever has been increasingly common in recent decades all across the world. According to WHO reports, Over the last two decades, the frequency of dengue illnesses has increased more than eightfold., from 505,430 cases in 2000 to more than 2.4 million in 2010, and 5.2 million in 2019. Globally, reported fatalities climbed from 960 to 4032 between 2000 and 2015.[11]

Mosquito control is the only way to get out of these sticky circumstances. The use of synthetic chemical sources may result in resistance development and be hazardous to the environment. Drugs derived from nature, particularly plants and their secondary metabolites, are thought to be environmentally benign, conveniently accessible, cost-effective, and physiologically safe. Andrographolide is a diterpenoid lactone found in abundance in Andrographis species that is commonly utilized in Indian medicine as a bitter tonic, snake bite therapy, parasite problems, and hepatitis treatment. It is also known as the "king of bitters" due to its intense bitterness.[12] *Andrographis echioides* was tested for

Table 1: Larvicidal activity of Methanolic and Ethanolic extract of *Andrographis echioides* against *Aedes aegypti*.

Concentration (PPM) 24 hr		Percentage Mortality		Regression Equation		LC_{50}		LC_{90}		R^2		P	
		48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	
Control (Methanol)		0	0	y = 0.1724x	y = 0.2234x	290.02	224.22	522.04	402.86	0.88	0.85	0.0015	0.0004
Methanolic Extract of *Andrographis echioides*	500	75.62±2.34	99.1±0.22										
	250	56.55±1.54	69.7±0.42										
	125	32.45±0.95	40.12±1.32										
	62.5	17.23±0.65	27.3±0.11										
	31.25	9.3±0.03	18.74±0.96										
	15.625	5.25±0.01	10.2±0.64										
Control (Ethanol)		0	0	y = 0.1852x	y = 0.2169x	270.27	230.41	485.96	414.75	0.94	0.81	< 0.0001	< 0.0001
Ethanolic Extract of *Andrographis echioides*	500	87.22±2.45	99.54±3.36										
	250	52.2±2.24	60.14±2.26										
	125	26.14±1.47	37.87±2.89										
	62.5	18.21±0.98	28.32±1.66										
	31.25	14.22±0.74	22.34±1.75										
	15.625	12.33±0.66	18.2±0.95										

preliminary larvicidal effectiveness against third-instar Aedes aegypti mosquito larvae. Secondary metabolites carbohydrates, alkaloids, glycosides, flavonoids, steroids, proteins and amino acids, and terpenes were identified to be the primary components of Andrographis echioides.[13] *Andrographis paniculata* shown larvicidal action against filarial vector *Culex quinquefasciatus*[14] and dengue vector Aedes aegypti.[15] The presence of the diterpenoid lactone, andrographolide, in the ethanolic and methanolic extracts of *Andrographis echioides* showed larvicidal action against *Aedes aegypti* mosquito larvae in the current study.

CONCLUSION AND SUMMARY

In order to validate the traditional use of *Andrographis echioides* as an antimalarial agent, the study was conducted to substantiate the anti-parasitic potential of *Andrographis echioides* against third-instar larvae of *Aedes aegypti*. From the findings, it was concluded that, *Andrographis echioides* exhibited larvicidal activity by enhancing the mortality of larvae while its exposure after 24hr and 48hr and it may be due to its secondary metabolite diterpenoid lactone. Additionally, the isolation of bioactive principles from *Andrographis echioides* liable for its promising role in larvicidal potential and its structural elucidation may be necessitated to explore its mode of action.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DDT: Dichlorodiphenyltrichloroethane; **WHO:** World Health organization; **BSI/SRC:** Botanical Survey of India/Southern regional centre; **PPM:** Parts per million; **R^2:** R-square (Regression Coefficient); **LC_{50}/ LC_{90}:** Lethal concentration.

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