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LARVICIDAL ACTIVITY OF LEAF METHANOL EXTRACT OF *ACHYRANTHUS BIDENTATA* AGAINST DENGUE AND MALARIA VECTORS

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ABSTRACT

The present study was targeted to investigate the phytochemical compounds from crude methanolic leaf extract of *Achyranthus bidentata* and larvicidal activity against 4th instar larvae of *Aedes aegypti* and *Anopheles stephensi*. Larvicidal activity of the methanol crude extracts was determined by topical application to early 4th instar larvae and lethality was estimated by applying various concentrations (50, 100, 150, 200 and 250 ppm) of the crude extract. The methanol extract of *A. bidentata* was subjected to preliminary screening of phytochemicals using standard procedure. Screening of phytochemicals revealed the presence of flavonoid, phenol, tannins, triterpenoids, saponins, alkaloids, and carbohydrates listed in Table 1. The early 4th instar larvae of *A. bidentata* more susceptible to the methanol extracts with LC_{50} value of 110.68 ppm and 330.09 respectively. The LC_{90} value of methanol extract was 123.37 ppm and 369.42 ppm against 4th instar larvae of *A. stephenesi*. Methanol extracts showed a significant activity against 4th instar larvae of *A. aegypti*.

Keywords: *Achyranthes bidentata*, Phytochemicals, GC-MS analysis, Larvicidal activity.

INTRODUCTION

Mosquitoes constitute a major public health problem as vectors of serious human diseases like dengue fever, malaria, chikungunya and yellow fever. Mosquitoes alone transmit disease to more than 700 million people annually. Mosquito-borne diseases are endemic in more than 100 countries, its mortality rate was nearly two million every year. One million children were died as well as each year, 2100 million people at risk around the world. [1,2].

Aedes aegypti and *Anopheles stephensi* are the major urban vectors of dengue and malaria and about 90% of deaths occur in Africa and South Sahara because of all malarial attack. *Aedes aegypti* is the principal vector of dengue and hemorrhagic dengue fever [3]. The annual morbidity of malaria is about 4 - 5 million in developing countries like India as well as in endemic areas [4].

Some of the conventional pesticides such as malathian, DDT and pyrethroides were generally used to control mosquito are well known to cause the environmental pollution, residual effects and resistance of mosquito species. Development of resistance in *A. stephensi* and *A. aegypti* has been noted by [5] and by other studies [6, 7]. These problems forced to search for new, alternative and safer control measures especially from plant source. Because, plant derived molecules are eco-friendly, biodegradable and target specific [8, 9].

Chemical control of mosquitoes were linked with numerous drawbacks, like resistance development, environmentally effective sound alternatives is much needed [10]. Plant products act as insecticides or repellent, which can play an important role to interrupt the transmission of mosquito-borne diseases at the individual level. Natural products are best option due to its less harmful to environment and non-targeted organisms. Several extracts and compound from different plant families have been evaluated for new and promising larvicides [11].

These developments require efforts to prepare alternative insecticidal agents with high mosquito control activity that cause little or no harmful effect to human health and environment. The plant based herbal insecticides are found to be more efficient, safe and best substitute for chemical insecticides [12-15]. Natural products of plant origin are safe to use than the synthetic insecticides [16].

Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositor attractant and important role in the interruption

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towards transmission of mosquito-borne diseases at the individual as well as at the community level and ovipositor attractant which have different activities observed by many researchers [17, 18]. However, insecticides of plant origin have been extensively used on agricultural pests to a limited extent against insect vectors of public health importance.

Achyranthus bidentata is an herb belongs to Amaranthaceae family, has become one of the most important traditional plant widely distributed and grown in China, Korea, Japan and India and it has found that wide application in traditional and folk medicine [19]. The phytochemical studies of *Achyranthus bidentata* revealed rutin, saponins, achyranthine, caffeic acid, oleanolic acid, inokosterone, ecdysterone, rubrosterone and physcion [20, 21]. It also reduce cholesterol levels and have anti-cancer, anti-inflammatory and analgesic properties [22, 23, 24].

Recently, it has been reported that ABB extract prevents glutamate-induced cell damage in cultured hippocampal neurons and induces nerve growth and neural differentiation [25, 26, 27]. The aim of this study was to evaluate phytochemicals and larvicidal activity of crude methanolic extract.

MATERIALS AND METHODS

Collection and Identification of plant materials

The medicinal plant *Achyranthes bidenta* was collected from Government Siddha Medical College campus, Arumbakkam, Chennai, Tamilnadu, India in a sterile polythene bag. Morphological characters of the selected plant were recorded and it was authenticated by the Chief Botanist, Tamil Nadu Aromatic and Medicinal Plants Corporation Limited (TAMPCOL) at Government Siddha Medical College, Arumbakkam, Chennai, India.

Preparation of extracts

A. bidentata was collected from the field and washed thoroughly with running tap water and rinsed in sterile distilled water by following method of [28]. The washed plant materials were shade dried at room temperature for 10 days. The shade dried plant parts were made into a coarse powder using a mechanical grinder. The powder was extracted with methanol in a Soxhlet apparatus for 8 to 16 h. The extract was concentrated using rotary evaporator (Heidolph laborata, Germany) at various temperature under reduced pressure.

Multiple screening of secondary metabolites

The methanol extract of *A. bidentata* was subjected to preliminary screening of phytochemicals using standard procedure [29].

Detection of Alkaloids

100 mg of powdered sample was dissolved in 5 mL of methanol and then filtered. Then 2 mL of filtrate was mixed with 5 mL of 1% aqueous HCl. 1 mL of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange-red precipitate was taken as positive. To the second tube Mayer's reagent was added and appearance of buff-colored precipitate was taken as positive test for the presence of alkaloids.

Shinoda's test for flavonoids

Five hundred milligram of sample was dissolved in 5 mL of ethanol, slightly warmed and then filtered. Few pieces of magnesium chips were added to the filtrate followed by addition of few drops of concentrated hydrochloric acid. A pink, orange, or red to purple coloration was taken as a confirmation for the presence of flavonoids [30].

Molisch's test – carbohydrates

Five hundred milligram of powdered sample was taken and dissolved in 5 mL of distilled water and then filtered. Filtrate was added with few drops of Molisch's reagent, followed by addition of 1 mL of conc. H_2SO_4 by the side of the test tube. After two minutes, 5 mL of distilled water was added. Red or dull violet color formation at the interphase of the two layers was taken as positive test.

Legal's test - glycosides

The extract was hydrolyzed with HCL for few hours on a water bath and the hydrolysate was subjected to Legal's or Borntrager's test to detect the presence of glycosides. To the hydrolysate added 1 mL of pyridine and a few drops of sodium nitroprusside solution was added and then it was made alkaline with solution hydroxide solution. Appearance of pink to red color showed the presence of glycosides.

Ferric chloride test - tannins

1 to 2 mL of the extract and few drops of 5% aqueous ferric chloride solution were added. A violet color formation indicates the presence of tannins.

KOH test - phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residue was tested for the presence of phytosterol.

Ninhydrin test - protein and amino acids

1 mL of the extract was treated with few drops of ninhydrin reagent. Appearance of purple color shows the presence of amino acids.

Detection of Triterpenoids

10 mg extract was dissolved in 1 mL of chloroform and 1 mL of acetic anhydride was added following of 2 mL of concentrated sulphuric acid. Formation of reddish violet color indicates the presence of triterpenoid.

Detection of Anthraquinones

5 mL of the extract solution was hydrolyzed with diluted concentrated sulphuric acid extracted with benzene. One mL of dilute ammonia was added to it. The result was

indicating the rose pink coloration suggests the positive response for anthraquinones.

Liebermann–Burchard test for steroids

200 mg of powder sample was dissolved in 2 mL of acetic acid separately; solutions were cooled followed by the addition of few drops conc. H2SO4. Color development from violet to blue or bluish-green was taken as positive test steroidal ring.

Test for saponins

One gram of powdered sample was boiled in 10 mL of distilled water and then filtered. 3 mL of distilled water was added to filtrate and shaken vigorously for about 5 min. Formation of foam after shaking was taken as a confirmation for the presence of saponins.

Selection of Mosquito species

Aedes aegypti and *Anopheles stephensi*, vectors were selected for the present study. These are nocturnal and crepuscular in nature and also transmit the filarial worm causing filariasis (Dean, 2001).

Mosquito Culture

All tests were carried out against laboratory reared vector mosquitoes viz., *Aedes aegypti* and *Anopheles stephensi* free of exposure to insecticides and pathogens. Cyclic generation of vector mosquitoes was maintained at 25-29^{o} C in the insectariums. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio 3:1) and adult mosquitoes on 10 per cent glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

Larvicidal Bioassay

A total of three trials were carried out with five replicates per trial against vector mosquitoes for larval susceptibility test. Larvicidal assay of the crude extract were conducted separately using the fourth instar larvae of *Aedes aegypti* and *Anopheles stephensi*. Stock solution (1000 ppm) of the extract was prepared by dissolving 100 mg of crude extract in 1 mL acetone and volume raised to 100 mL with distilled water.

From this different dilution of 50, 100, 150, 200 and 250 ppm were prepared in 200 mL deionized water and 25 fourth instar larvae were released in it and mortality was scored after 24 h and 48 h. The beakers were kept in a temperature control room at 28^{o} C ± 2^{o} C and the larvae were exposed to 200 mL water containing 0.1 mL of acetone served as control. Each treatment was replicated five times.

Larval susceptibility tests

The larval susceptibility tests were carried according to standard procedures (WHO, 2005). Different concentrations of extract were prepared and larvae of *A. aegypti*, and *A. stephensi* were placed in each test solution to observe the larvicidal property as per the following procedure. Groups of 25 larvae were placed in glass beakers containing 200 mL of the plant extract solution. Control experiments without extract were run in parallel. The larvae in each solution were then left for 24 h the number of dead larvae was counted after 24 h of exposure and the percentage mortality was reported from the average of five replicates. The assay was extended to 24 h percentage mortality was recorded. Mortality was recorded when a control mortality ranged from 5-20 per cent, it was corrected by Abbott's (1925) formula.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC50, LC90 and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit and chi-square values were calculated using the SPSS 11.5 (Statistical Package of Social Sciences) software. Results with $P < 0.05$ were considered to be statistically significant.

RESULTS

The preliminary phytochemical analysis of methanol extract of *A. bidentata* showed a positive response for the appearance of the reddish pink color was strongly exhibited that flavonoids, a violet color formation indicates the presence of tannins, formation of reddish violet color indicates the presence of triterpenoids, formation of white precipitate indicates the presence of saponins, appearance of pink to red color shows the absence of glycosides, The yellow formation or brown precipitate confirmed the presence of alkaloids, and carbohydrates highlight of this study is screening of phytochemicals in methanol extract (Table 1).

Larvicidal activity

The results clearly indicate the plant extracts of *A. bidentata* exhibits potent lethality at concentrations ranging between 110. 68 ppm and 330.09 ppm against mosquito species tested. Methanol extract of *A. bidentata* was found to be more potent and showed 100% mortality at 250 ppm against *A. aegypti* but in 200 ppm methanol extract showed 100% mortality against *A. stephensi*.

Methanol extract were also found to be effective against fourth instar larvae of *Aedes aegypti* and *A. stephensi* with LC_{50} and LC_{90} values of 110.68 ppm and 330.09 ppm for *A. stephensi*, 123. 37 ppm and 369.42 ppm respectively. The methanol extracts were also effective against *A. aegypti* and *A. stephensi* mosquito larvae tested at a slightly higher concentration.

DISCUSSION

Traditional healers use successful prediction of botanical compounds from primarily water as the solvent but in our studies we found that plant extracts in organic solvent (methanol) provided more consistent larvicidal activity when compared to those extracted in water.

Today environmental safety of an insecticide is considered to be of paramount importance and should not cause mortality on non-target organism in order to be acceptable [32].

Table 1. Qualitative analysis of phytochemicals from *A. bidentata*

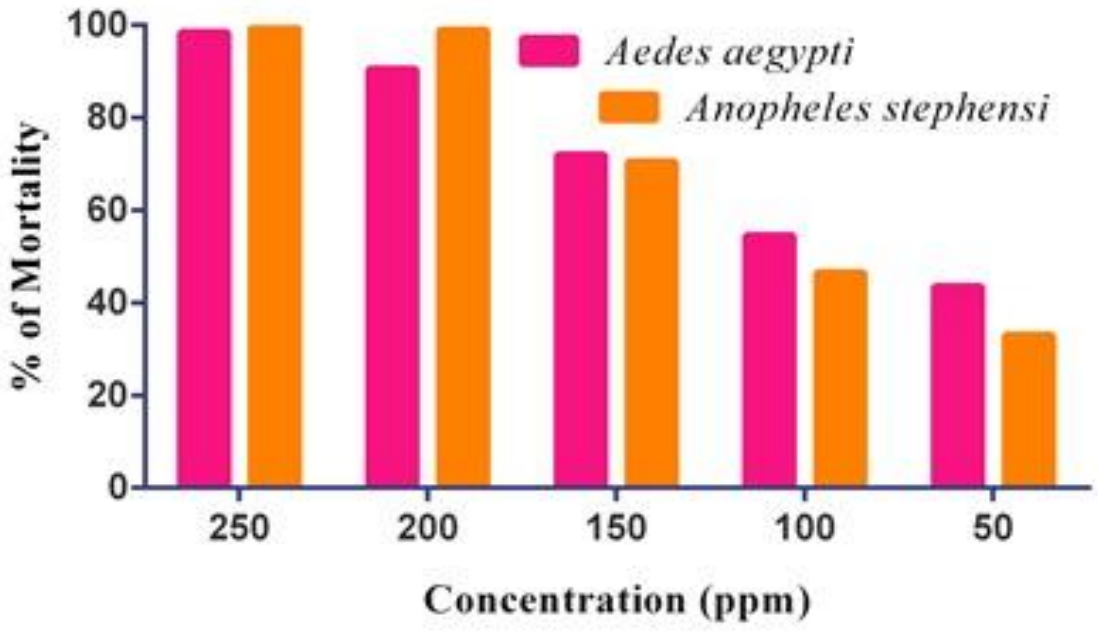
S. No	Test	Inference
1	Alkaloids	+
2	Anthraquinones	-
3	Carbohydrates	++
4	Coumarin	++
5	Flavonoids	++
6	Glycosides	++
7	Phenol	+++
8	Proteins	+
9	Quinones	++
10	Saponins	-
11	Steroids	-
12	Tannins	++
13	Total Amino acids	++
14	Total fatty acids	+
15	Triterpenes	++

+++ - **Strongly Positive**
++ - **Positive**
+ - **Trace**
- - **Not detected**

Table 1. Lethal concentration methanolic extract of *A. bidentata* against 4th instar larvae of *A. aegypti* and *A. stephensi*

Species	Concentrations	% [a] Mortality SD±SE	LC50 (UCL-LCL)	LC90 (UCL-LCL)	Slope	r^2
Aedes Aegypti	250	100±0.00				
	200	92±0.43	110.68	330.09	0.54	0.827
	150	74±2.34	(127.71-95.91)	(349.42-312.82)		
	100	56±4.31				
	50	45±0.54				
Anopheles Stephensi	250	100±0.00				
	200	100±0.00	123.37	369.42	0.57	0.882
	150	72±3.43	(133.61-13.61)	(412.14-341.01)		
	100	48±4.52				
	50	36±1.58				

Figure 1. Percentage Mortality of *Aedes aegypti* and *Anopheles stephensi* larvae treated with the different concentrations of the plant extract



A survey of literature on control of different species of mosquito revealed that assessment of the efficacy of different phytochemicals obtained from various plants has been carried out by a number of researchers in the field of vector control [33].

Plants could be an alternative source for mosquito larvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Phytochemicals derived from plant sources act as larvicides, insect growth regulators, repellent and ovipositor attractant and have different activities which have been observed by many researchers. Triterpenoids are generally credited with mosquito larvicidal activities [34].

The potent larvicidal activity of *A. bidentata* could be attributed to the strong presences of terpenoids, triterpenoids and alkaloids. Glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins in *J. curcas*, with analoids in *W. somnifera*, seeds of *C. colocynthis* constituted phytosterols such as elaterin, citrullol, hentriacontane and a mixture of fatty acids showed toxic effects against mosquito species [35]. The results of the study revealed that the methanol extract of *A. bidentata* was effective against the 4th instar larvae of mosquito when compared with *A. stephenesi* and *A. aegypti*. The high mortality rate of the extract at both high and low concentrations is in line with the findings of earlier researchers that most plant extracts showed high larvicidal potential against mosquitoes at relatively high and low concentration [36]. Those larvae that were able to emerge into pupae could be due to level of internal resistance threshold developed by the pupae that survive. All these reports emphasize that the members of the genus, *Achranthus* are generally having lethal effects against various mosquitoe species.

CONCLUSION

It is evident from the present study that herbal extract from *A. bidentata* might have promising larvicidal efficacy. The product of these plants can be well utilized for preparing biocides or phytochemicals from which all the non–target organisms cab be rescued from harmful vectors. These plants would be eco- friendly and may serve as suitable alternative to synthetic insecticides. Hence the large biomass of the *A. bidentata* available in the wastelands of Southern India can be used as a bio resource to commercially produce mosquito larvicides.

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