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Research Article

Larvicidal Activity of *Ageratum Conyzoidz* against *Anophels Stephensi*

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ABSTRACT

Mosquitoes are the vectors of various diseases. The extensive uses of synthetic organic insecticides during the last five decade have resulted in environmental pollution and also in the development of physiological resistance in major vector species. The present problem was proposed to be undertaken to provide suitable alternatives to control vector mosquito using biological control methods. *Ageratum conyzoides* whole plant of family Asteraceae was collected locally from Vidisha district of M.P. The crude extract were isolated and purified using column chromatography, TLC, acid hydrolysis, methylation and other chemical methods. Alkaloid isolated from *Ageratum conyzoides* have shown larvicidal activity against *Anopheles stephensi* at concentration of 100 to 500 ppm. The LC₅₀ value for second and fourth instar larvae were 181.97 and 165.96 ppm respectively. The results were quite dose dependents. The results obtained suggest that bioactive compound of *Ageratum conyzoides* could be used in search for new larvicidal compound of plant origin.

Keywords: Larvicidal, Alkaloid, Phytochemical, Bioactive, *Anopheles stephensi*, *Ageratum conyzoides*

1. INTRODUCTION

Mosquito are well known as vector of serious diseases like malaria, filaria, dengue, encephalitis and other viral diseases. *Anopheles stephensi* is a vector of malaria. It is established that repeated use of synthetic larvicide results in the development of resistance in mosquitoes and hence effective ecofriendly phytochemicals are the need of the day. Plant world comprises a store house of biochemical that could be tapped for use as insecticides and they are the richest source of renewable bioactive organic chemicals. Total no. of plant chemicals may exceed 40,000 of these 10,000 are secondary metabolites whose major role in the plant is reportedly defensive¹. The naturally occurring pesticides thus appear to have prominent role in the development of future commercial pesticides not only for agriculture productivity but also for safety of environment and public health².

Ageratum conyzoides Linn. (Asteraceae) commonly known as Kubhi in hindi and distributed throughout India. This plant is quite useful in fever and the root of this plant posse's antihelmintic and anti- dysenteric properties. Isolation of new alkaloid compound from this plant have been tested against larvicidal activity against second and fourth instar larvae of *Anopheles stephensi*. The present paper reports the analysis of statistical data of *Ageratum conyzoides* chloroform extract against second and fourth instar larvae of *Anopheles stephensi*.

2. MATERIAL AND METHODS

2.1 Plant material

The whole plant of *Ageratum conyzoides* Linn of family Asteraceae was collected during pre-monsoon month from the campus of S.S.L Jain College Vidisha (M.P.) India. The collected plant seeds were shade dried at room temperature in laboratory keeping for one day at 100° c in the oven. A voucher specimen of the plant has been preserved in our herbarium

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record in Pest control and Ayurvedic Drug Research Laboratory Vidisha for future reference.

2.2 Extraction method

The extraction of shade dried powder material of *Ageratum conyzoides* (40-60) mesh size was carried out separately by soxhlet apparatus in the laboratory using different solvents in increasing order of polarity. The extraction procedure adopted as given by Harborne (1984)³. The plant material was extracted in n-hexane, benzene, chloroform, acetone and methanol.

2.3 Experimental bioassay

Laboratory colonized *Anopheles stephensi* second and fourth instar larvae were used for the experimental bioassay. Larval study was conducted according to standard of procedure WHO (1981)⁴. The crude was made 1% stock solution and serial dilution from 100 to 500 ppm concentration was made, which

was used against 2nd and 4th instar larvae of *Anopheles stephensi*. The mosquito larvae was reared in the insectory maintained at temperature 25±2^oc, RH 75 ±5%, under a L: D, 14:10 photoperiod cycle.

For experimental bioassay, 25 second and fourth instar larvae of *Anopheles stephensi* were kept in 500 ml of the test compound. Acetone was used as solvent to dilute the compound to an appropriate test concentration. The treatments were replicated three times. Each replicate set contains one control, which received 1 ml of 50% acetone and 249 ml of distilled water and one untreated, which contained only 250 ml of distilled water. The number of dead larvae, pupae and adults were recorded. Mortality was corrected according to Abbott formula (1925)⁵. Statistical evaluation of data was carried out by probit analysis of Finney⁶ and level of significance Duncan's ⁷ multiple range test.

Table 1: Percent loss in weight after shade drying

Plant	Wet weight of the plant in gm.	Dry weight of the plant in gm.	Total weight loss in after drying in gm.	Percent of weight loss
<i>Ageratum conyzoides</i>	1200	960	240	20.00%

Table 2: Percentage yield of *Ageratum conyzoides* by soxhlet apparatus in different solvents

S. No.	Solvent used	Weight of plant material powder in gram	Temperature	Weight of extract in gram	Percentage of yield
1.	n-haxane	100	40 ^o C	3.5	3.5
2.	Benzene	100	-"-	2.1	2.1
3.	Chloroform	100	-"-	6.17	6.17
4.	Acetone	100	-"-	5.4	5.4
5.	Methanol	100	-"-	6.5	6.5

Table 3: Statistical data of *Ageratum conyzoides* chloroform extract treated on second and fourth instar larvae of *Anopheles stephensi*

Larval stage	Concn. (ppm)	Larval mortality (%)	Regretion eq. (y=a+bx)	x2=(n-1) Heterogeneity	LC ₅₀ (ppm)	LC ₉₀ (ppm)	Log LC ₉₀ ± S.D.	95%Fiducial limits (ppm)
II nd instar	100	38						
	200	52						M1=139.05
	300	64	1.55+1.52x	1.75(6-1)	181.97	452.01	2.26+0.08	
	400	72						M2=286.28
	500	100						
IV th instar	Control	04						
	100	45						
	200	56						M1=127.05
	300	65	2.32+1.2 x	0.18(6-1)	165.95	489.58	2.22+ 0.10	
	400	68						M2=313.32
	500	100						
	Control	04						

Twenty five second and fourth instar larvae were taken at each concentration in average of three replicates. The value were significantly different (p<0.01) from the control at df (n-1) = 5.

3. OBSERVATIONS AND RESULTS

When whole plant of *Ageratum conyzoides* was shade dried the percent loss in weight was observed 20% as shown in table-1.

After shade drying the powdered material of *Ageratum conyzoides*, when Soxhleted in different solvent of increasing order of polarity gave maximum yield in methanol extract, which accounted 6.5% and 3.5 in n-hexane, 2.1% in benzene, 6.17% in chloroform, 5.4% in acetone as shown in Table-2.

In present study larvicidal activity observed for malaria vector *Anopheles stephensi* as indicated in table-3.

Table (20) reports the results obtained on biostatistic analysis of experimental data for II and IV instar larvae of *Anopheles stephensi*. When chloroform extract of *Ageratum conyzoides* was used it gave 181.97 and 165.95 ppm, LC50 value for II and IV instar larvae respectively. The results also indicate that 100% ppm concentration was a lethal concentration giving 100% mortality within 24 hr duration. The results were found quite significant ($p < 0.01$) at ($n-1 = 5$ df).

4. DISCUSSION

In the present study methanol extract gave highest 6.5% yield as indicated in Table 1. Innocent et al.⁸ have also isolated a bioactive component for controlling *Anopheles* larvae from Lantana plant extracts. The % yield of each fraction recorded by them was 1.5g and 6.4 g respectively.

In the present study 5 different concentration of *Ageratum conyzoides* chloroform extract were tested against second and fourth instar larvae of *Anopheles stephensi* a dose dependent larvicidal activity was noticed. Similar, dose dependent larvicidal effect by *Lantana camara* against mosquito species *Aedes aegypti* and *Culex quinquefasciatus* was noticed by Kumar and Maneemegalai⁹. Wandscheer et al. (2004)¹⁰ have also reported the larvicidal activity of two plants of family Maleaceae against *Aedes aegypti*.

5. CONCLUSION

The findings of present studies, therefore suggest the use of *Azadirachta indica* chloroform extract as a local resource in controlling mosquito larvae.

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