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EVALUATION OF MOSQUITO LARVICIDAL ACTIVITY OF BIOACTIVE SAPONIN ISOLATED FROM *TRIDEX PROCUMBENS* LINN. (FAMILY : ASTERACEAE) AGAINST *AEDES AEGYPTI*

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ABSTRACT

Many plant based products are widely used for their insecticidal and repellent properties for the control of mosquitoes. The present paper reports an isolation of mosquito larvicidal bioactive saponin from an indigenous plant found in Indian sub continent as a common weed t*ridex procumbens* of Family -Asteraceae.

The mosquito larvicidal activity of bioactive saponin isolated from *tridex procumbens* have been tested against *Aedes aegypti* Linn. by exposing second and fouth instar larvae to four different concentrations of the compound. Three trials were performed for each concentration along with control and untreated. 24 hours LCso and LCw values were determined using probit analysis method.

The compound gave LC_{50} value to be 150.79 ppm for fourth instar larvae and 240.10 ppm. for second instar larvae. It was noticed that fourth instar larvae are more susceptible than second instar larvae. The results obtained suggest that the bioactive compound of *tridex procumbens* could be useful in the search for new larvicidal compound of plant origin.

Key words : Bioactive saponin, *Tridex procumbens*, Larvicidal, Ecofriendly.

INTRODUCTION

Mosquito borne diseases are among the world's leading causes of illness and death today. W. H. O. estimates that more than three hundred million clinical cases each year are attributed to the mosquito borne diseases such as malaria, filaria, japanies encephalitis, yellow fever and chikungunya. Mosquito control is a difficult task due to a variety of factors including the development of insecticides resistant in target population, the high

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cost of new insecticides and concern over environmental pollution. While it is likely that chemical insecticides will continue to be required for mosquito control, an increased emphasis is being placed all over the world on the development of suitable alternatives to control vector borne diseases.

Secondary metabolites are diverse natural products synthesized by the plants for their defence. In our laboratory, more than a dozen indigenous plants have been tested against three prominent mosquito vector species, which are the causative of malaria, filaria and dengue fever viz. *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti*[1-3]. Effect of Annona squamosa on mosquito larvicidal and growth disrupting activity and the loss of fecundity and fertility in *Culex quiquefasciatus* by *Spheranthus indicus* plant extract were noted[4, 5]. Ansari et al[6] have reported the mosquito larvicidal and repellent activity by *Pinus longifolia* oil. Similarly, many plant based products are widely used for their insecticidal and repellent properties for the control of mosquitoes[7, 8]. Ciccia et al[9] have reported the insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants.

The present paper reports an isolation of mosquito larvicidal bioactive saponin from an indigenous plant found in Indian subcontinent as a common weed *Tridex procumbens* of Family -Asteraceae.

EXPERIMENTAL

Material and methods

Plant material

Tridex procumbens Linn. is a common weed found distributed in tropical climate throughout the country. The plant was authenticated by a taxanomist of Botany Department and a voucher specimen was procured in the herbarium record of pest control laboratory at S. No. 19.The whole herb after collection from the forest of the Vidisha was dried in shed after washing with tap water. Leaves of this plant were used for curing bronchial catarch. This plant has been reported to posses glycoside saponin[10].

Extraction, isolation and characterization of larvicidal principle

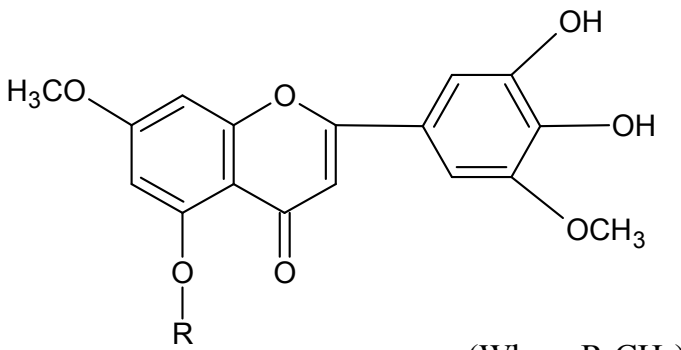
The powdered material of 40-60 mesh size was extracted in Soxhlet apparatus in 90% alcohol and water separately. The weight of the powder was 600 g, which yielded greenish semi-solid crude 3.83 g, after vacuum evaporation in water extract and 16.5 g, in alcoholic extract. The purification of the crude was carried out using TLC and column

chromatography using chloroform : acetic acid : water (90 : 45 : 6) as per phytochemical methods[11] .

Structure elucidation

The purified fraction of alcoholic extract, which showed larvicidal potential was analysed for spectrum to get IR, UV, CNMR and mass spectrum, which yielded a molecular formula $C_{16}H_{16}O_7$ with a melting point 220^0C. The result of the spectrum showed maximum peak at 240 mm, m. w. 344, IR (KBr, Cm^{-1}) 2984 (C-H arom.), 1542(C=O), 1277 (C-O-C), 1244 (C-O-C asym.), 1071(C-O). ^{1}H NMR : s, 5.29, (1H), s, 4.93-4.70 (2H), s 4.28-4.20 (4H).

Thus, the preliminary structure of the compound elucidated as 5, 3', 4'-trihydroxy-7-5'-dimethoxy-5-O-α-L-methyl pyranoside.



(Where R:CH_3)

Flavone glycoside-5, 3', 4'-trihydroxy-7-5' dimethoxy-5-O-α-L-methyl pyranoside

Experimental bioassay

Laboratory colonized *Aedes aegypti* used in present study were maintained at 2.72^0C. 75-85% RH, under a L : D, 14 : 10 photoperiod cycle. The larvae were fed with a diet of finely ground brewer's yeast and dog biscuit (3 : 1). Adults were fed adiibitum on 10% sucrose solution. Females were allowed to blood-feed from a rabbit placed in a restraining cage 3 days after emergence.

For experimental bioassay, 25 second and fourth instar larvae of *Aedes aegypti* 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[12].

Statistical evaluation of data was carried out by probit analysis[13] and level of significance[14] multiple range test.

RESULTS AND DISCUSSION

Flavon glycoside isolated from *Tridex procumbens* weed found distributed throughout India from 90% alcoholic extract showed larvicidal potential against *Aedes aegypti larvae.*

Laboratory colonized early second and fourth instar larvae were exposed to four different concentrations of the test compound, which gave 24 -h LC_{50} and LC_{90} value as 242.10 and 410.56 ppm for second instar and 150.79 and 378.5 ppm for fourth instar larvae respectively. Ultimately, the larvae die either due to failure of ecdysis or by delayed metamorphosis.

Further studies of the plant as possible agent for mosquito control is required. It was noticed that the 500 ppm concentration caused 100% mortality to both the instar larvae of *Aedes aegypti*. Mortality of larvae in different concentrations during the experiment ranged from 30% to 95% as compared with 5% in the control and untreated group (Table 1).

These results are quite comparable to our previous reports on indigenous plant extract against *Anopheles culicifascius* and *Anopheles Stephensi* in which crude extracts of two plants *Premina integnfolia* and *Ageratum conyzoides* were found toxic causing 100% mortality to second instar larvae of 500 ppm. Concentration[1, 2]. Larvicidal activity of various plant extract such as *Pedolium murax, Cllome icosandra* and *Dictyola dichotoma* have been found to be promising against *Culex quinquefasciatus* showing 24 hours LC_{50} values of 23, 11 and 2 ppm. respectively[15].

There is a renewed interest in recent years to control mosquitoes at their larval stages, which remains in water by plant products. Some recent reports of mosquito

larvicidal activity of essential oils of eleven aromatic medicinal plants against early fourth stage larvae of *Aedes aegypti* and *Culex pipiens* have shown 100% mortality at 25 ppm[16].Similarly, Geogre and Vincent[17] have reported the synergistic activity of three plant extracts against mosquito larvae. The mortality of second and fourth instar larvae may be attributed due to increase titre of juvenile hormone in their body, which delayed metamorphosis.

Table 1. Larvicidal activity of bioactive saponin of *Tridex procumbens* to second and fourth instar larvae of *Aedes aegypti* Linn.

Treated larvae	Concentration (ppm)	24 hours larval mortality (%)	Regression equation (y = a + bx)	Chi Square (χ^2)	LC_{50} (ppm)	LC_{90} (ppm)	90% Fduciol limit (ppm)
Second instar	100	30	Y = 1.128 + 1.56X	0.22 (2)*	242.10*	410.56	231.63 Lower 500.00 Upper
	200	38					
	300	68					
	400	89					
	Control	05					
	Untreated	Nil					
Fourth instar	100	39	Y = 3.647 + 0.621X	0.22 (2)*	150.79*	378.5	68.5 Lower
	200	52					
	300	63					
	400	95					
	Control	05					
	Untreated	Nil					

*Values are significantly different from control and untreated (Duncan's multiple range $P < 0.05$) 25 second and fourth instar larvae of *Aedes aegypti* were used in each set with three replicates.

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