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MOSQUTIO LARVICIDAL ACTIVITY OF SAPONIN ISOLATED FORM *EUPHORBIA HIRTA* LINN OF EUFORBIACEAE

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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 isolation of mosquito larvicidal bioactive saponin form an indigenous plant found in India –*Euphorbia hirta* of family –Euforbiaceae.

The mosquito larvicidal activity of bioactive saponin isolated from *Euphorbia hirta* have been tested against *Culex quinquefasciatus* by exposing IInd and IV instar larvae to four different concentration of the compound. Three trials were performed for each concentration along with control and untreated. 24 hours LC_{50} and LC_{90} values were determined using probit analysis method. It was noticed that IVth instar larvae are more susceptible than IInd instar larvae. The results obtained suggest that bioactive compound of *Euphorbia hirta* could be used in the search for new larvicidal compound of plant origin.

Key words: Bioactive saponin, *Euphorbia hirta*, Larvicidal, *Culex quinquefasciatus*.

INTRODUCTION

Mosquitoes are the vector for various disease including malaria, filarial Japanies encephalitis, yellow fever and chikungunya. Mosquito have developed resistance to the chemical pesticides. Further, the mosquito pesticidal compound caused several ill effect to the human beings. This is the reason that new control methods have been tried during the last one decade all over the world. In this contest, the present problem was proposed to be undertaken to provide suitable alternatives to control vector mosquito using biological control methods.

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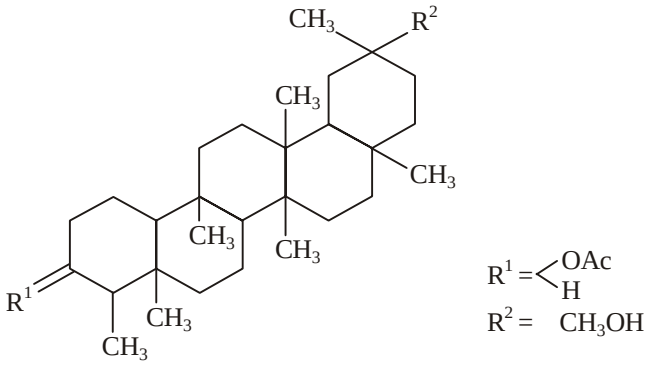
Among these mosquito borne disease, lymphatic filariasis caused by *Wucheria bancrofti* is transmitted by Culex spp. Especially *Culex quinquefasciatus* and is of prime importance throughout India. The filariasis is a widely distributed of infection affectiong people residing both in the urban and rural areas. Sharma[1] reported that in 1985, it was estimated that 342 million population is exposed to the risk of filarial and 252 million in rural areas.

Many plant based products are widely used for their insecticidal and repellent properties for the control of mosquitoes. By reviewing the literature, it is quite apparent that plants have been a good sources of secondary metabolites to be used against malaria and filarial vectors. Therefore, present paper reports isolation of mosquito larvicidal bioactive saponin form an indigenous plant found in India, *Euphorbia hirta* of family euphorbiaceae.

EXPERIMENTAL

Material and methods

Plant material: *Euphorbia hirta* a berb belonging to the family Euphorbiaceae is found distributed through out the country. The plant after proper identification was procured in the harberium data sheet of the laboratory as a bouchar specimen. The shade dried powered material was soxhlated in 90% alchohol and water, respectively. Saponins are much more polar compounds and hence, they are extracted in polar solvents. The saponin were isolated form the crude extract using TLC plates coated with silica gel (E. Marck, Germany) with n-hexane,



Euphorbin - A

benzene and ethyl acetate as solvent. Water soluble fraction of n-hexane extract was chromographed from glass coloumn and purified till white needles are obtained. Upon methylation, the white crystalline substance afforded a compound with molecular formula $C_{34}H_{54}O_3$ and melting point 260^oC.

Experimental bioassay methods

For experiment on mortality, WHO 1971 guidelines were followed. For detail bioassay 25 IInd and IVth instar larvae of *Culex quinquefaciatus* from laboratory stock were used. Each experiment was conducted in 500 mL beaker in 3 replicates having one control and one untreated group of insects. 24 hour LC_{50} value was determined using biostatical method of Finney[2]. Mortality including moribund larvae were removed time to time and development and behavioural changes were also noticed.

The results are reported in Table 1-3.

Table 1: Percentage loss in weight

Wet weight of the plant (g)	Dry weight of the plant (g)	Total weight loss in after drying (g)	Percentage of weight loss (%)
1550	1240	310	20.00

Table 2: Percentage yield of *euphorbia hirta* by Soxhlet apparatus in different solvents

Solvent used	Weight of plant material powder (g)	Weight of extract (g)	Percentage of yield (%)
n-Haxane	200	5.62	2.62
Chloroform	200	6.42	3.21
Ethyl acetate	200	2.85	1.42
90% Alchohol	200	5.30	2.65
Water	200	15.3	7.66

Table 3: Different fractions isolated form 90% alcohol of *Euphorbia hirta*
Amount of Crude 90% alcohol extract = 500 mg; Amount of silica gel packed in glass column = 11.875 g

Solvent	Fraction code	Weight of fraction (mg)	Characteristics and biological active fraction
MeOH-H_2O (13 : 7)	A	55.3	On TLC, four spots were rechromatographed. Fraction E1-light yellow green (2 mg) R_f = 0.60; found effective against the mosquito
H_2O-n-BuOH (1 : 1)	B	25.4	Negligible amount was obtained on rechromatography. Hence, not tested
EtOAc-Acetone (4 : 1)	C	60.2	On TLC, three spots were obtained, out of which fraction E3 –yellow (6.2 mg) R_f = 0.60, found much effective on the mosquito.
Haxane-Me_2O (4 : 1)	D	12.5	Negligible amount was obtained on rechromatography. Hence, not tested.
n-BuOH-H_2O	E	20.5	On TLC, three spots were obtained, rechromatographed. Fraction E5-light green, R_f = 0.55 showed some biological activity as compared to fraction E1 and E3

RESULTS AND DISCUSSION

The treatment of IInd and IVth instar larvae to five different concentrations of crude extract of *Euphorbia hirta* have been mentioned in Table 4 and 5. The maximum LC_{50} value was found to be 596.76 and 446.4 ppm for the plant extract. From the results, it appeared that IVth instar are more susceptible than the IInd instar larvae.

Lakshmana Kumar[3] also reported that IVth instar are more susceptible than IInd instar larvae in all the six extracts. The present findings are quite comparable with earlier reports of Saxena and Yadav[4] and Prasad et al.[5], who have reported the high larvicidal activity in aqueous and alcoholic extract. Similar views have been observed by Singara et al.[6] who have noticed the larvicidal activity in water extract against three species of mosquito larvae. The activity was found to be dose dependent. As the dose was increased, the mortality was also found to be increased. On the basis of the present results, it may be concluded that water and alcoholic extracts contain potent larvicidal compound, which may be further purified to have its synthetic analogues.

Table 4: Larvicidal activity of n-hexane extract of *Euphorbia hirta*against *Culex quinquefasciatus*

Larval state	Conc. (ppm)	24 hrs larval mortality (%)	Regression equation (v = a + bx)	Heterogeniety Chi-square [x^2(n-2)]	LC_{50} (ppm)	LC_{90} (ppm)	Variance (v)	S.E.	S.D.	Fiducial limits (ppm)
IInd Instar	100	20	1.719 + 82x	2.337 (5-2)	596.765	725.250	0.0109	0.068	0.10481	M1 = 313 M2 = 80.317
	200	26								
	300	34								
	400	42								
	500	48								
	Control	04								
IVth Instar	100	30	2.541 + 0.928x	0.25 (5-2)	446.461	680.267	0.01572	0.01572	0.05608	M1 = 22.039 M2 = 70.157
	200	36								
	300	42								
	400	48								
	500	65								
	Control	04								

25 Each second and fourth instar larvae were taken in average of three replicates. Values are significantly different than the control ($p < 0.01$)

Table 5: Larvicidal activity of chloroform extract of *Euphorbia hirta* against *Culex quinquefasciatus*

Larval state	Conc. (ppm)	24 hrs larval mortality (%)	Regression equation (v = a + bx)	Heterogeniety Chi-square [x^2(n-2)]	LC_{50} (ppm)	LC_{90} (ppm)	Variance (v)	S.E.	S.D.	Fiducial limits (ppm)
IInd Instar	100	32	2.513 + 1.0x	0.16 (5-2)	306.902	725.75	0.01448	0.5382	0.12034	M1 = 145.926 M2 = 432.380
	200	42								
	300	48								
	400	54								
	500	60								
	Control	04								
IVth Instar	100	36	1.892 + 1.327x	0.651 (5-2)	219.849	670.35	0.007346	0.03833	0.0857	M1 = 191.956 M2 = 313.413
	200	44								
	300	54								
	400	62								
	500	72								
	Control	04								

25 Each second and fourth instar larvae were taken in average of three replicates. Values are significantly different than the control ($p < 0.01$)

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