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Mosquitocidal and repellent activity of the leaf extract of *Citrullus vulgaris* (cucurbitaceae) against the malarial vector, *Anopheles stephensi* liston (diptera culicidae)

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Abstract. – The efficacy of the Cucurbitaceous plant *Citrullus vulgaris* against the *Anopheles stephensi* was assessed in the present study. The leaf extract of *Citrullus vulgaris* with different solvents viz, benzene, petroleum ether, ethyl acetate and methanol were tested for larvicidial, ovicidal, repellent and insect growth regulatory activities against *Anopheles stephensi*. The larval mortality was observed after 24 h exposure. The LC50 values are 18.56, 48.51, 49.57 and 50.32 ppm respectively. The mean percent hatchability of the egg of *Anopheles stephensi* were observed after 48 h. 100 per cent mortality was exerted at 250 ppm with benzene extract and the other extracts exerted 100 percent mortality at 300 ppm. Skin repellent test at 1.0, 2.5 and 5.0 mg per cm^2 concentration gave the mean complete protection time ranged from 119.17 to 387.83 minutes with the four different extracts tested. The *Citrullus vulgaris* plant extract have shown insect growth regulatory activity against *Anopheles stephensi* at five different test concentrations ranging from 10 to 150 ppm with different solvents and they exhibit the following EI50 values 28.99, 70.02, 106.33 and 84.25 ppm respectively.

Key Words:

Larvicidal, Ovicidal, Repellent, Insect Growth Regulatory activity, *Citrullus vulgaris, Anopheles stephensi.*

Introduction

Vector-borne diseases are among the most important public health problems and obstacles to socio economic development of developing countries, particularly in the tropics, with malaria alone causing an estimated 1.5-2.7 million deaths and 300-500 million[1] cases per year. Insect pests have mainly been controlled with synthetic insecticides in the last fifty years. In recent years interests in the plant based products have been revived because of the development of resistance, cross-resistance and possible toxicity hazards associated with synthetic insecticides and their rising cost. Botanicals are a promising source of pest control compounds. The pool of plants possessing insecticidal substances is enormous[2]. The first insecticides to be used by a man were from plants, the biological activities of which were known from the earliest recorded times[3]. Some phytochemicals act as general toxicants (insecticide/larvicides) both against adult as well as larval stages of mosquitoes. While others interfere with growth and development (growth inhibitors) or with reproduction (chemosterilant) or produce olfactory stimuli thus acting as repellent or attractant.

The aim of the study was to determine the effect of mosquitocidal activities of the cucurbitaceous plant *Citrullus vulgaris* against the malarial vector *Anopheles stephensi* and to examine the repellent properties of *Citrullus vulgaris* against mosquito bites.

Materials and Methods

The leaves of *Citrullus vulgaris* were collected from Kothattai, Cuddalore district, Tamilnadu, India. They were washed with tapwater, shade dried and powdered. The dried powder was then subjected to extraction in various solvents viz, benzene, ethylacetate, petroleum ether and methanol using soxhlet apparatus and solvent

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evaporation by vacuum evaporator. The plant material was reduced to a viscous dark green residue and crude extracts were further concentrated to pastes and they were covered by aluminium foil sheet and stored in a freezer until assayed. One g of plant residue was dissolved in Dimethyl sulphoxide (DMSO) universal solvent and 1.0 percent stock solution was prepared. From the stock solution various concentrations were prepared.

The eggs were obtained from vector control research center, Puducherry, and maintained at 27 ± 2°C, 75-85% relative humidity, and a photoperiod of L: D 14:10. Larvae were fed with 3:1 mixture of dog biscuits and yeast powder. Adults were provided with a 10%. Sucrose solution and were periodically blood fed on restrained 5-7 weeks – old chicks. Eggs, larvae and adult females were continuously available for the experiments.

Larvicidal Bioassay

For the larvicidal bioassay, the WHO standard protocols (WHO, 1996) with slight modifications were followed. The five different test concentrations were prepared by adding different range of the stock solution to 250 ml of water in a 500 ml capacity of glass beaker. Similarly other test concentrations were prepared by using different extracts. Twenty five larvae were exposed to the prepared 250 ml of test concentration. Symptoms of treated larvae were observed and the mortality counts were made after 24 h of the exposure period. The experiments were replicated six times and the data were subjected to Probit analysis[4].

Ovicidal Bioassay

For the ovicidal activity the method of Su and Mulla[5], was slightly modified and followed. From the stock solution the various concentrations were prepared (50 to 300 ppm). The freshly laid eggs (100 nos.) were exposed to each dose of leaf extract until they hatched or dried. Eggs exposed to DMSO in water served as control. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. The test was replicated six times. The hatch rate were assessed 48 h post treatment by the following formula:

$$\frac{\text{Number of hatched larvae}}{\text{Total number of eggs in the egg rafts}} \times 100$$

Repellency Test

The percentage of protection in relation to dose method was used to evaluate the repellency test[6]. 3-4 days old blood starved female *Anopheles stephensi* mosquitoes (100, 250, 500 density) were kept in a net cage (45-30-45 cm^2). Two cages with hungry mosquitoes, for test and control were kept aside. The various ranges of stock solutions (1.0, 2.5 and 5.0 mg cm^2) were prepared by dissolving the residues in ethanol. The arms of the volunteer skin were washed and cleaned with ethanol and ethanol served as control. After air drying, the arms of the volunteer, only 25 cm^2 dorsal side of the skin on the each arm was exposed and the remaining area being covered by rubber gloves. The extracts of the plants were applied at 1.0, 2.5 and 5.0 mg/cm^2 separately[6]. The control and treated arm were introduced simultaneously into the cage. The first bite was noted over 5 min every 30 min from 18.00 h to 06.00 h. The test was over after the confirmation of mosquito bite in the extract to be tested. The experiment was replicated five times. It was observed that there was no skin irritation from the leaf extract tested.

Insect Growth Regulatory (IGR) Activity

Insect growth regulatory (IGR) activity of different extract of *Citrullus vulgaris* was also tested against *Anopheles stephensi.* Twenty five larvae were introduced into 500ml enamel bowls containing 249 ml of water. The five different test concentrations ranging from 10-150 ppm was tested against *Anopheles stephensi* and each test concentration was replicated six times. The control experiments were run parallel with each replicate[7]. Mortality of the larvae, pupae, larval pupal intermediate and adult mortality was recorded at regular intervals. Observation was continued in both treated and control bowls until the last immature pupates. Morphological abnormalities were also noted. The dead larvae and pupae removed daily and counted. The percentage emergences at different concentrations were recorded. Growth index was assessed by the following formula:

$$GI = \frac{\text{Total emergence}}{\text{Average developmental period (days)}}$$

Results and Discussion

The LC_{50} of *Citrullus vulgaris* ranged from 18.56 to 50.32 ppm with four different solvents.

Table I. Larvicidal activity of *Citrullus vulgaris* leaf extracts against *Anopheles stephensi.*

Solvent used	LC_{50} (ppm)	LC_{90} (ppm)	95% Confidence limits (ppm)		Regression equation	X^2 (df)
			LCL *LC50 (LC90)*	*UCL* *LC50 (LC90)*		
Benzene	18.56 ± 2.20	39.08 ± 2.63	8.77 ± 0.92 (30.45 ± 1.71)	26.22 ± 1.18 (60.88 ± 1.13)	y = 15.77 + 1.79X	27.46*(4)
Petroleum ether	48.51 ± 1.36	99.36 ± 1.22	33.04 ± 1.58 (82.63 ± 0.58)	61.93 ± 0.12 (130.45 ± 1.56)	y = 13.45 + 0.73X	14.72*(4)
Ethyl acetate	49.57 ± 0.77	91.35 ± 1.18	40.12 ± 1.43 (78.61 ± 2.04)	59.00 ± 1.01 (113.18 ± 0.27)	Y = 4.00 + 0.93X	10.54*(4)
Methanol	50.32 ± 0.87	95.39 ± 1.67	39.30 ± 1.97 (80.51 ± 1.04)	61.38 ± 0.92 (123.15 ± 1.11)	Y = 5.49 + 0.89X	12.57*(4)

$\chi 2$ Values are significant at $P < 0.05$ level; Each value (X ± SD) represents mean of six values. LCL: Lower confidence limit; UCL: Upper confidence limit.

The benzene extract of *Citrullus vulgaris* found to be more effective than the other extracts (Table I). Table II shows the mean per cent hatchability of *Anopheles stephensi*. The benzene extract exerted zero hatchability (100% mortality) at 250 ppm, while the other extracts exerted 100 per cent mortality at 300 ppm.

The results from the skin repellent activity of *Citrullus vulgaris* leaf extract against *Anopheles stephensi* are given in Table III. The repellent activity was very high at the initial stage of exposure. Increase in the exposure period showed reduction in repellent activity and it depends upon the concentration of the extract and density of mosquito. The methanolic extract of *Citrullus vulgaris* gave 100 per cent protection time at 5 mg/cm^2 concentration (387.83 min) where as the ethyl acetate extract gave 290.83 at 5 mg per cm^2 concentration.

Insect growth regulatory activity of *Citrullus vulgaris* against *Anopheles stephensi* was studied and presented in Table IV. The total mortality with different concentrations ranged from 13.15 to 92.82%, 9.16 to 81.81%, 11.49 to 98.50% and 8.82 to 88.15% with benzene, ethyl acetate, petroleum ether and methanolic extracts and the plant extracts affect the length of the larval developmental period. EI_{50} values ranged from 28.99 to 106.33 ppm (Table IV).

The results are comparable with early reports of Mullai et al[8]; who observed the larvicidal and ovicidal activity of *Luffa acutangula* against *Aedes aegypti.* Singh et al[9] reported the mosquito larvicidal properties of the leaf extract of the herbaceous plant *Ocimum canum with* the 2nd, 3rd and 4th larval stages with *Anopheles stephensi, Aedes aegypti* and *Culex quinquefasciatus*.

The complete ovicidal activity (100 per cent mortality) was attained at 300 ppm for all the four extracts of *Citrullus pubescens* against *Culex quinquefasciatus*[10]. The bioactive com-

Table II. Ovicidal activity of *Citrullus vulgaris* leaf extracts against *Anopheles stephensi*.

Solvent used	Egg hatchability (%) Concentration (ppm)						
	Control	*50*	*100*	*150*	*200*	*250*	*300*
Ethyl acetate	99.6 ± 0.94	73.8 ± 0.83	62.0 ± 1.22	39.6 ± 0.94	21.6 ± 0.55	10.5 ± 0.59	NH
Benzene	100.0 ± 0.0	70.0 ± 0.40	59.3 ± 1.46	37.8 ± 0.65	11.8 ± 0.57	NH	NH
Petroleum ether	99.2 ± 0.90	77.5 ± 1.01	64.0 ± 0.81	41.3 ± 0.71	25.6 ± 0.40	12.3 ± 1.27	NH
Methanol	100.0 ± 0.0	81.2 ± 1.29	64.2 ± 0.59	45.1 ± 1.01	29.3 ± 1.63	17.5 ± 1.30	NH

NH: No hatchability (100% mortality); Each value (X ± SD) represents mean of six values.

Table III. Repellent activity of *Citrullus vulgaris* leaf extract against *Anopheles stephensi*.

Concentration (mg/cm^2)	Solvent used	Mosquito density	Range (min)	Treatmean (min)	Variance ratio	Tabulated F-Value	
						1% level	*5% level*
1.0	Methanol	100	264-270	266.83	2449.98	6.36	3.68
		250	215-221	216.50			
		500	184-186	185.67			
	Benzene	100	232-236	234.00	1273.45	6.36	3.68
		250	189-193	190.50			
		500	166-175	169.00			
	Petroleum Ether	100	198-208	203.67	224.27	6.36	3.68
		250	167-182	174.83			
		500	141-150	145.67			
	Ethyl acetate	100	188-198	193.00	480.27	6.36	3.68
		250	163-174	167.00			
		500	113-125	119.17			
2.5	Methanol	100	346-353	349.00	2357.58	6.36	3.68
		250	305-310	308.17			
		500	268-272	269.50			
	Benzene	100	310-317	312.17	1532.81	6.36	3.68
		250	275-282	277.50			
		500	230-235	233.33			
	Petroleum Ether	100	259-265	261.67	777.36	6.36	3.68
		250	212-223	216.17			
		500	180-189	184.67			
	Ethyl acetate	100	223-228	225.67	1727.52	6.36	3.68
		250	186-190	187.50			
		500	142-150	145.00			
5.0	Methanol	100	385-390	387.83	2562.95	6.36	3.68
		250	323-330	326.00			
		500	268-278	273.17			
	Benzene	100	346-350	348.17	795.14	6.36	3.68
		250	288-296	293.83			
		500	259-272	266.00			
	Petroleum Ether	100	322-330	325.67	777.18	6.36	3.68
		250	282-289	286.33			
		500	237-251	244.67			
	Ethyl acetate	100	288-295	290.83	2714.56	6.36	3.68
		250	260-266	263.67			
		500	197-202	200.33			

Significant at 1 and 5% levels.

pound Azadirachtin (*Azadiracta indica*) showed complete ovicidal activity in the egg of *Culex tarsalis* and *Culex quinquefasciatus* exposed to 100 ppm concentration[5].

The skin repellent activities of *Solanum triolbatum* leaf extract against *Anopheles stephensi* with higher concentration provided over 100 minutes protection against mosquito bites. Lower concentrations provided 70 to 90 minutes of protection[11]. *Cymbopogan citratus* had repellency activity against the adult mosquito *Culex quinquefasciatus*. Maximum of 100 per cent protection time was obtained at the concentration[12] of 5.0 mg per cm^2.

The IGR activity of the methanolic extract of A*tlantia monophylla* against *Aedes aegypti* and *Culex quinquefasciatus* were 325 times and 162.5 times more sensitive than *Anopheles*

Table IV. Insect growth regulatory activity of *Citrullus vulgaris* leaf extract against *Anopheles stephensi.*

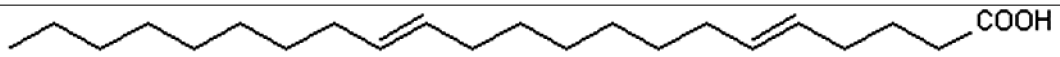
Solvent used	Conc. (ppm)	Larval mortality (%)	Larval pupal intermediate mortality (%)	Pupal mortality (%)	Adult mortality (%)	Total mortality (%)	Total emergence (%)	Average developmental period (Days)	Growth index	EI_{50} (ppm)
Benzene	10	7.16 ± 0.83	3.33 ± 0.41	2.66 ± 0.54	–	13.15 ± 0.59	86.85 ± 1.14	14.5	5.98 ± 0.91	**28.99**
	20	12.50 ± 1.23	7.00 ± 1.28	4.33 ± 0.66	2.16 ± 0.48	25.99 ± 1.11	74.01 ± 2.03	14.0	5.28 ± 0.59	
	30	20.66 ± 1.46	18.66 ± 1.26	9.16 ± 1.94	3.50 ± 0.67	51.98 ± 1.62	48.02 ± 1.77	14.0	3.43 ± 0.22	
	40	34.33 ± 1.22	22.00 ± 1.94	12.33 ± 0.59	3.66 ± 1.69	74.66 ± 0.71	25.34 ± 1.97	13.5	1.87 ± 0.82	
	50	47.16 ± 0.69	27.33 ± 2.04	13.50 ± 0.92	4.83 ± 1.23	92.82 ± 1.45	7.18 ± 0.71	13.5	0.53 ± 0.61	
	Control	1.00	1.00	–	1.00	3.00	97.00	13.0	7.46 ± 1.11	
Ethyl acetate	30	2.83 ± 1.01	3.50 ± 0.80	1.33 ± 0.60	1.50 ± 1.04	9.16 ± 0.49	90.84 ± 1.94	15.5	6.26 ± 0.67	**106.3**
	60	11.16 ± 0.81	7.66 ± 1.61	2.50 ± 1.30	–	21.32 ± 1.18	78.68 ± 1.18	14.0	5.62 ± 0.71	
	90	20.16 ± 0.97	11.83 ± 1.42	4.66 ± 1.43	2.66 ± 1.56	39.31 ± 1.48	60.69 ± 1.40	14.0	4.33 ± 0.50	
	120	26.00 ± 1.27	17.33 ± 1.38	7.00 ± 0.33	3.16 ± 0.97	53.49 ± 1.37	46.51 ± 1.22	13.5	3.44 ± 1.16	
	150	43.66 ± 1.00	20.33 ± 1.72	12.66 ± 1.67	5.16 ± 1.10	81.81 ± 1.19	18.19 ± 1.05	13.5	1.38 ± 0.54	
	Control	1.00	–	1.00	1.00	3.00	97.00	13.0	7.46 ± 0.49	
Petroleum ether	25	6.33 ± 1.29	3.83 ± 0.74	–	1.33 ± 0.82	11.49 ± 1.32	88.51 ± 1.09	14.5	6.10 ± 0.92	**70.02**
	50	15.16 ± 0.85	6.66 ± 1.20	3.33 ± 1.09	1.50 ± 0.47	26.65 ± 1.81	73.35 ± 1.55	14.0	5.23 ± 1.04	
	75	26.50 ± 0.94	18.66 ± 0.78	7.16 ± 1.71	2.66 ± 0.31	54.98 ± 0.92	45.02 ± 1.22	14.0	3.21 ± 0.25	
	100	43.33 ± 0.59	21.16 ± 1.37	8.00 ± 0.97	3.33 ± 1.01	75.82 ± 1.04	24.18 ± 1.67	13.5	1.79 ± 0.77	
	125	57.00 ± 1.63	26.00 ± 2.04	12.00 ± 1.40	3.50 ± 0.51	98.50 ± 1.45	1.50 ± 0.52	13.5	0.11 ± 0.50	
	Control	1.00	1.00	–	–	2.00	98.00	13.0	7.53 ± 043	
Methanol	25	4.33 ± 1.71	2.83 ± 0.87	1.66 ± 0.29	–	8.82 ± 0.80	91.18 ± 1.33	14.5	6.43 ± 1.04	**84.25**
	50	12.00 ± 1.00	3.83 ± 1.43	3.33 ± 0.95	–	19.16 ± 1.22	80.84 ± 1.12	14.0	5.77 ± 0.54	
	75	20.50 ± 0.85	5.00 ± 0.59	4.66 ± 1.18	3.50 ± 1.12	33.66 ± 0.83	66.34 ± 1.04	14.0	4.74 ± 1.19	
	100	36.50 ± 1.94	16.17 ± 1.42	7.33 ± 1.50	5.00 ± 1.00	65.00 ± 1.59	35.00 ± 1.10	13.5	2.59 ± 0.33	
	125	48.66 ± 1.20	21.16 ± 1.14	12.00 ± 0.82	6.33 ± 0.95	88.15 ± 1.46	11.85 ± 0.48	13.5	0.88 ± 0.49	
	Control	1.00	–	1.00	1.00	3.00	97.00	13.0	7.46 ± 0.71	

Significant at 1 and 5% levels.

Table V. Spectral analysis of compound.

^{1}H NMR, 200 MHz, $CDCl_3$		^{13}C NMR, 200 MHz, $CDCl_3$	
δ, ppm, values	Group	δ, ppm, values	Group
4.51 – 5.35	2 – CH = CH -, m	174.61	– C – OH ‖ O
1.50 – 1.76	16 CH_2, m		
0.89 – 1.26	CH_3, m	125.08 – 136.14	2 – CH = CH -
		24.24 – 38.21	16 – CH_2
		14.88 – 18.45	- CH_3

(m – multiplet)
Mass: 336 (m/z)
IR (cm^{-1}): 2955, 2851, 1733, 1543, 1459, 1161, 1070, 962,776.
UV: 260, 320 nm.
Molecular formula: C_{22} H_{40} O_2
Molecular weight: 336



5, 13 – Docosadienoic acid.

From the above details ^{1}H, ^{13}C NMR, IR and Mass spectroscopy techniques the possible structure of the compound is predicted as 5, 13-Docosadienoic acid.

stephensi respectively[13]. The active fraction of *Moschosma polystachyum* showed IGR activity against 3rd instar larvae of *Culex quinquefasciatus*, the EI_{50} value was 13.19 ppm[14,15].

The results of the current investigation revealed that the *Citrullus vulgaris* plant extract was more effective against *Anopheles stephensi* with maximum larvicidal, ovicidal, repellent and insect growth regulatory activity and it can be used to control the most important vector mosquito *Anopheles Stephensi* The biological activity of the plant extract might be due to a variety of compounds in this plant. These compounds may jointly or independently contribute to cause the mosquitocidal activities against *Anopheles stephensi*. One of the compound is identified as 5, 13-Docosadienoic acid by the spectral analysis viz; ^{1}H, ^{13}C NMR, IR and Mass spectroscopy techniques (Table V).

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Anopheles stephensi Liston is the primary vector of malaria in India and other West Asian countries, Malaria remains one of the most prevalent diseases in the tropical world. With 200 million to 450 million infections annually worldwide, it causes up to 2.7 million deaths.Â The ovicidal activity was determined against An. stephensi to various concentrations ranging from 50-350ppm under laboratory conditions.Â This work focus towards the action of the plant extracts against many diseases and disorders. Many more researches are being done in this field to come to know that the plant is more active and less in causing side reactions in humans and animals. View. Keywords: Adulticidal activity Repellent activity Anopheles stephensi Eclipta alba Andrographis paniculata. ABSTRACT.Â the leaf methanol, benzene, and acetone extracts of Cassia fistula were studied for the larvicidal, ovicidal, and repellent activities against Ae. aegypti. Rohani et al.[28], has reported the efficacy of few Malaysian essential oils such as Litsea elliptica, Polygonum minus and Piper aduncum as potential mosquito adulticides while Sulaiman et al.[29] has reported the essential oils of Melaleuca cajuputi and Cymbopogon nardus have adulticidal effects on Aedes mosquito at high-rise flats in Kuala Lumpur. Mosquitocidal and Repellent activity â€“ A study conducted to evaluate mosquitocidal and repellent activity of the leaf extract of Citrullus vulgaris against the malarial vector, Anopheles stephensi liston (diptera culicidae) have shown that Citrullus vulgaris plant extract have significant insect growth regulatory activity against Anopheles stephensi. Anti Diabetic Activity â€“ A research done on Anti-diabetic effect of Watermelon (Citrullus vulgaris Schrad) on Streptozotocin-induced Diabetic Mice have proved its beneficial effect on diabetes. Anti diarrhoea Activity â€“ A study was conducted to ev Mosquito adulticidal and repellent activities of botanical extracts against malarial vector, Anopheles stephensi Liston (Diptera: Culicidae). Asian Pac J Trop Med.Â Objective: To determine the adulticidal and repellent activities of different solvent leaf extracts of Eclipta alba (E. alba) and Andrographis paniculata (A. paniculata) against malarial vector, Anopheles stephensi (An. stephensi). Methods: Adulticidal efficacy of the crude leaf extracts of E. alba and A. paniculata with five different solvents like benzene, hexane, ethyl acetate, methanol and chloroform was tested against the five to six day old adult female mosquitoes of An. stephensi. The adult mortality was observed after 24 h under the laboratory conditions. Biological Malarial Vector Mosquito Anopheles stephensi control of malaria vectors. Indian Journal of Liston (Diptera:Culicidae). Int J Interdis Res Medical Research 106, 174â€“197.Â Mosquitocidal and repellent activity of and repellent activity of selected indigenous the leaf extract of Citrullus vulgaris medicinal plants against malarial vector (cucurbitaceae) against the malarial vector, Anopheles stephensi (Liston), dengue vector Anopheles stephensi liston (dipteraculicidae).