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Research Article

Evaluation of Mosquito Larvicidal Activity of Ethanolic Extract of *Grewia flavescens* Juss. Whole Plant (Family: Tiliaceae) Against *Culex quinquefasciatus*

Dr. G.N. Pramodini^{1*}, Dr. Parwez Alam², Dr. Mohd Rafiq³, Dr. Shaik mohd Khasim⁴¹ Associate professor, Department of pharmacognosy, Shadan college of pharmacy, Peerancheeru, Hyderabad-500091, Telangana, India.² Associate professor, Department of pharmacognosy, Shadan college of pharmacy, Peerancheeru, Hyderabad-500091, Telangana, India.³ Associate professor, Department of pharmacognosy, Shadan college of pharmacy, Peerancheeru, Hyderabad-500091, Telangana, India.⁴ Director, Shadan College of pharmacy, peerancheeru, Hyderabad-500091, Telangana, India.

ABSTRACT

To evaluate the mosquito larvicidal activity on the ethanolic extract of *Grewia flavescens* juss (EEGF). Whole plant against *Culex quinquefasciatus* larvae. The larval mortality of 4th instar larvae of *Culex quinquefasciatus* after 24h and 48h of treatment which were tested separately in control and five test concentrations of 125 ppm, 250 ppm, 500 ppm, 750 ppm, 1000 ppm concentration. The plant extract was screened to identify phytochemical bioactive compounds like alkaloids, flavonoids, saponins, tannins and glycosides. Different concentration of crude ethanolic extract of *Grewia flavescens* juss. whole plant effectively showed larvicidal activity on *Culex quinquefasciatus* larvae. Based on the probit analysis values for 24h and 48h ethanolic extract of *Grewia flavescens* shows pronounced larvicidal activity. The LC₅₀ and LC₉₀ values of EEGF against mosquito 4th instar larvae of *Culex quinquefasciatus* was found 223.87 ppm and 724.43 ppm at 24hrs and for 48hrs the LC₅₀ and LC₉₀ values was found to be 181.97ppm and 374.76 ppm respectively. The preliminary phytochemical was performed in search of plants secondary metabolites which might be responsible for the larvicidal activity. The bioactive phytochemical classes which were identified and confirmed as alkaloids, flavonoids, saponins, tannins and glycosides. The ethanolic extract of this plant showed potent larvicidal activity and can be considered for further investigation. This experimental study was a pioneer attempt to establish *Grewia flavescens* juss. as a valuable resource of effective target specific natural mosquito larvicide.

Keywords: *Culex quinquefasciatus*, larvicidal activity, ethanolic ext., phytochemical.

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*Address for Correspondence:

Dr. G.N. Pramodini, Associate professor, Department of pharmacognosy, Shadan college of pharmacy, Peerancheeru, Hyderabad-500091, Telangana, India.

1) INTRODUCTION:

Mosquitoes are the carriers of many diseases such as malaria, dengue fever, yellow fever, filariasis etc. They are responsible for the death and illness of millions of people through the transmission of diseases.¹ In most developing countries of the tropical and subtropical regions of the world, mosquitoes constitute as the foremost vectors of several debilitating diseases affecting human and domestic animals.²

Culex quinquefasciatus mosquitoes, belonging to one of the disease transmitting mosquito genera.³ *Culex quinquefasciatus* is a vector of lymphatic filariasis that is endemic in many countries in Africa, South Asia, The pacific

island and the american worldwide, an estimated 20 million peoples are affected by lymphatic filariasis, with above one third of their suffering from hydrocele or lymphedema.⁴ Breeding in polluted water such as blocked drains, damages septic tanks, soak age ponds close to human habitations.⁵

Mosquito control is a difficult task, due to a variety of factors including the development of insecticide resistant in target population. The high cost of new insecticides and concern over environmental pollution. While it is likely that chemical insecticides will continue to be required for mosquito control so, an increased emphasis is being placed all over the world on the development of suitable alternatives to control vector borne diseases⁶. Many plant based products are

widely used for their insecticidal and repellent properties for the control of mosquitos.^{7,8} *Grewia flavescens* (Tiliaceae) popularly known as "donkeys berry", is a shrub or small tree, often seen in groups along the edges of roads, river banks and dry rivers, growing in large uniform groups. The plant parts are being used in Indian folk medicine. The leaves were reported to be useful in ulcerated tongue, colic pain, wounds, cholera and dysentery. *Grewia flavescens* is a multi-stemmed shrub or small tree, up to 5 m high. Its bark is dark grey-brown belongs to Tiliaceae family. The plant is used as Anthelmintic⁹, CNS depressant, anti-inflammatory¹⁰, antimalarial, antidiabetic¹¹ and analgesic in Indian traditional system of medicine. The berries of *Grewia flavescens* are soaked in water for two or three days to make a refreshing drink. Based on the traditional claim as in olden days the plant is used as an antimalarial agent so, we tried to expedite to cheek for the larvicidal activity of *Grewia flavescens* juss whole plant against *Culex quinquefasciatus* mosquito larvae.

2) MATERIALS AND METHODS:

2.1). Plant collection and authentication:

The crude *Grewia flavescens* juss. whole plant was collected from Sri Venkateshwara univesity, Titupathi, Andhra pradesh, India. The plant was authenticated with plant voucher specimen no. 1397, by plant taxonomist Dr. K. Madhava chetty, Assisstant professor, Department of botany, Sri Venkasteshwara university, Tirupathi, Andhra pradesh, India.

2.2) Preparation of plant extract:

The fresh whole plant of *Grewia flavescens* juss. were collected in the month of december from Tirupathi, India. The whole plant *Grewia flavescens* juss was washed with tap water, shade dried for two weeks, and pulverized. Then passed through sieve number 60 and stored in an air tight container.

About 1000g of powered drug extracted with 80% ethanol by using maceration method of extraction. The method is followed until the phytoconstituents were completely exhausted. The ethanolic extract was evaporated through rotary evaporator under reduced pressure at 40°C and labeled as EEGF 6.2% yield and preserved at 5°C in airtight container until further use.

2.3) Phytochemical screening:

The phytochemical screening was carried out as described by harbone¹². By this analysis, the prescence of several phytochemical listed in Table 3 was tested.

2.4) Selection of mosquito species and cultures:

The mosquito species selected for the present study was culex quinquefasciatus , its larval form were collected from mosquito genic region of chandrayangutta, Hyderabad. This larvae were collected in the month of march 2019 on the filter paper and kept in the specimen box. Collected larvae were numbered, recorded and identified by Dr. Deepa jaiswal scientist D zoologist, zoological survey of india. They identified it as larvae of culex quinquefasciatus say, 1823 (commonly known as the southern house mosquito). The larvae were fed on larval food powdered dog biscuit and yeast in the ratio 3:1.¹³

2.5). Larvicidal Bioassay:

The plant extrat was established in ethanol and diluted with distilled water to give 2000 ppm stock solution with tween 80 kept at concentration of 1%. Different concentration of 125, 250, 500, 750, 1000 ppm were prepared from the stock solution. The bioassay tests were conducted according to a standard WHO procedure (1981) with slight modifications. The bioassay was carrid on 4th instar stage larvae, six groups were made including controlled solution with each group containing 25 larvae. The larvae were placed in 100ml beaker of test solution and fed on powdered cat food during all testing. Larvae were considered dead if they were unarousable even when gently prodded. The dead larvae were counted after 24h and 48h and percentage mortality for each concentration were calculated. The control solution used in this experiment is distilled water.

2.6) Larval Susceptibility test:

The larval susceptibility tests were carried according to standard WHO procedure. The extract solutions of different concentrations were prepared and larvae of *Culex quinquefasciatus* were placed in each test solution to observe the larvicidal property as per the following procedure.

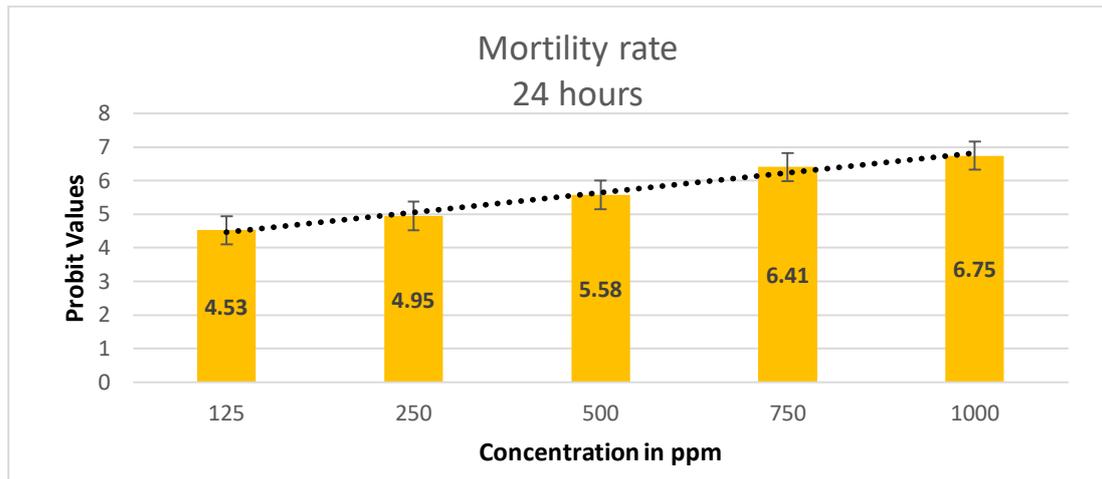
Group of 25 larvae were placed in 200ml of the extract solution. Control experiments without extract were run in parallel. The larvae in each solution were then left for 24h and 48h and the number of dead larvae were counted after 24h and 48h of exposure, and the percentage mortality was reported from the average of five replicates. Mortality was recorded when control mortality ranged from 5-20 percent, it was corrected by using Abbott's formula.¹⁴

3) Data Analysis

All the collected data is analysed using probit analysis and LC₅₀ and LC₉₀ were calculated. A graph

TABLE 1: Effect of EEGF whole plant on *Culex quinquefasciatus* 4th instar larvae (24 h).

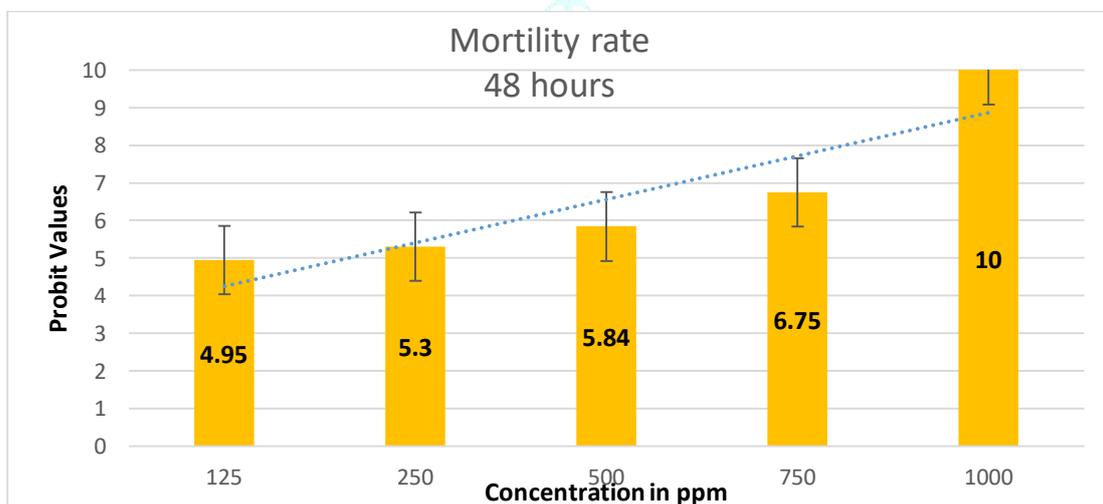
Exposure time in hours	Conc. (PPM)	Log Conc.	Number of larvae exposed	Number of deaths	% death	Probit
24hrs	C (00)	-	25	-	-	-
24hrs	125	2.09	25	8	32	4.53
24hrs	250	2.39	25	12	48	4.95
24hrs	500	2.69	25	18	72	5.58
24hrs	750	2.87	25	23	92	6.41
24hrs	1000	3	25	24	96	6.75



GRAPH 1: Graphical presentation of probabilities of larvicidal activity of EEGF at different concentrations (24h)

TABLE 2: Effect of EEGF whole plant on *Culex quinquefasciatus* 4th instar larvae (48 h).

Exposure time in hours	Conc. (PPM)	Log Conc.	Number of Larvae Exposed	Number of deaths	% deaths	Probit
48hrs	C(00)	-	25	-	-	-
48hrs	125	2.09	25	12	48	4.95
48hrs	250	2.39	25	16	64	5.3
48hrs	500	2.69	25	20	80	5.84
48hrs	750	2.87	25	24	96	6.75
48hrs	1000	3	25	25	100	10



GRAPH 2: Graphical presentation of probabilities of larvicidal activity of EEGF at different concentrations (48h).

3.1 Statistical Analysis

The concentration at which mortality observed (ppm) was corrected using Abbott's formula.¹⁴ Statistical analysis of the

experimental data was performed with MS-Excel 2007 to find the standard deviation and LC₅₀ and LC₉₀ using Probit analysis.

4) RESULTS AND DISCUSSION:

4.1 LETHAL CONCENTRATIONS (LC₅₀ & LC₉₀)

TBALE 4: Lethal concentrations of whole plant extract of *Grewia flavescens* Juss. against fourth instar larvae of *Culex quinquefasciatus*.

Plant Extract	Time (hours)	LC ₅₀ value (ppm)	LC ₉₀ value (ppm)	Regression Equation Y = ax + b	95% Confidence limit				Chi-Square	P-Value
					UCL(ppm)		LCL(ppm)			
					LC ₅₀ ppm	LC ₉₀ ppm	LC ₅₀ ppm	LC ₉₀ ppm		
<i>Grewia flavescens</i> Juss.	24 Hours	223.87	724.43	$y = 2.50x + (-0.89)$	246.94	747.5	212.95	713.51	0.99	0.36
<i>Grewia flavescens</i> Juss.	48 Hours	181.97	374.76	$y = 4.5x + (-5.18)$	206.15	398.94	167.36	360.15	1.13	0.41

The qualitative phytochemical screening of *Grewia flavescens* juss. were done to detect the presence of various chemical constituents, the ethanolic extract of *Grewia flavescens* gave positive test for various phytoconstituents such as Steroids, Flavonoids, Alkaloids, Tannins, Saponins etc.

The LC₅₀ and LC₉₀ for ethanol extract of *Grewia flavescens* juss. against 4th instarlarvae of *Culex quinquefasciatus* after 24 h post treatment were 223.87 ppm and 724.43 ppm respectively and after 48 h exposure were 181.7897 ppm and 374.76ppm (Table 4).

5) CONCLUSION:

The *Grewia flavescens* whole plant ethanolic extract had showed significant larvicidal activity that was carried out on fourth instar stagelarvae of *Culex quinquefasciatus*, the LC₅₀ and LC₉₀ values were calculated for time period of 24h and 48h thereby, increased in the larval mortality was observed. Hence, we confirmed that the plant under study *Grewia flavescens* juss. whole plant is a herb having an alternate source of herbal larvicidal agent, it is considered to be safe, easily available, economic and most importantly an effective against larvae of *Culex quinquefasciatus*. The above findings justified the traditional claim for the larvicidal activity of this plant *Grewia flavescens* juss. possess a good larvicidal activity which is proven scientifically in a well systemic manner.

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