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Larvicidal Activity and Phytochemical Analysis of Some Selected Plant Extracts Against Filarial Vector Culex Quinquefasciatus Say (Diptera: Culicidae)

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Abstract

Mosquitoes are the well-known vectors responsible for spreading several diseases all over the world. It is recognized that the diseases transmitted by mosquitoes has a global impact on public health. Species belonging to genera *Aedes, Anopheles* and *Culex* are vectors for the causative agent of various diseases like Dengue fever, Dengue hemorrhagic fever, Malaria, Japanese Encephalitis and Filariasis [1]. Insecticides and chemical pesticides were found to be effective in controlling mosquitoes. At the same time, it has several adverse effects and the mosquitoes tend to gain resistance. Hence it is high time we introduce extracts of plant origin to produce biopesticides which can be effective to control mosquitoes. As far as resistance by vectors and pests against botanicals has not been reported biopesticides can be considered pest or target specific. These are also readily biodegradable, target specific, has lower bioaccumulation, environmentally safe and lack toxicity to higher animals, can be produced with low cost and can be used by individuals and communities in specific situations [2]. The present attempt was to identify the larvicidal efficiency of some selected plants in aqueous and methanol extract against larvae of *Culex quinquefasciatus*, reared under laboratory conditions. The conduction of preliminary phytochemical analysis gives us an idea of the possible constituent in a plant responsible for its larvicidal activity. Statistical Analysis of data such as standard deviation, test of significance, LC50 and LC90 calculation were done. From the experimental results we could analyse that *Chromollaena odorata* and *Ricinus communis* are the most effective plants.

Keywords

Culex quinquefasciatus, Preliminary phytochemical analysis, standard deviation, LC50 and LC90

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1.Introduction

Mosquitoes (Diptera: Culicidae) are vectors which cause diseases. Today, even a slight hike in body temperature brings us fear. Targeting larvae with organophosphate and insect growth regulators is adopted now to control mosquitoes thereby diseases. But these may lead to disappearance of many useful organisms and may bring harmful effects in future. Biopesticides can act as substitute in this context. The plant extracts can be analyzed to

find its larvicidal or anti-mosquito activity. Preliminary phytochemical analysis can reveal the possible constituents in a plant responsible for its larvicidal activity whose extraction in future can lead to efficient production of biopesticides.

2. MATERIALS AND METHODS

The present study was conducted to test the larvicidal activity of aqueous extracts of twelve plants belonging to varied taxonomic groups (Table 1). Based on the activity noticed plants with higher activity were selected for methanol extract preparation and preliminary phytochemical analysis (Table 4).

Table 1: Larvicidal Activity of Plant Extracts in Distilled Water

Sl No	Name of the plant	Family	The mortality rate in 10% fresh extract
1	*Alternanthera sessilis*	Amaranthaceae	50
2	*Asteracantha longifolia*	Acanthaceae	0
3	*Chromolaena odorata*	Asteraceae	100
4	*Cuscuta reflexa*	Convolvulaceae	0
5	*Hyptis suaveolens*	Lamiaceae	40
6	*Loranthus falcatus*	Loranthaceae	20
7	*Mikania scandens*	Asteraceae	0
8	*Pistia stratiotes*	Araceae	0
9	*Ricinus communis*	Euphorbiaceae	100
10	*Tridax procumbens*	Asteraceae	0
11	*Cissus vitigenia*	Vitaceae	0
12	*Wattakaka volubilus*	Asclepiadaceae	10
13	control- water		0

Table 2: Larvicidal Activity of Plant Extracts in Methanol Against *Culex Quinquefasciatus* in 24 Hrs

Concentration ppm	Plant Names						Control	
	Alternanthera sessilis	***Chromolaena odorata***	***Hyptis suaveolens***	***Loranthus falcatus***	***Ricinus communis***	***Wattakaka volubilis***	**Distilled water**	**DMSO**
50 (0.005%)	0±0.00	1±0.00	0±0.00	0±0.00	0.67±0.58	0±0.00	0±0.00	0±0.00
75 (0.0075%)	0±0.00	4.67±0.58	0±0.00	0±0.00	1.67±0.58	0±0.00	0±0.00	0±0.00
100 (0.01%)	1.33±0.58	9.67±0.58	2.67±0.58	0.67±0.58	4.67±0.58	0±0.00	0±0.00	0±0.00
200 (0.02%)	2.00±0.00	10±0.00	5±0.00	1.33±0.58	7.67±0.58	0±0.00	0±0.00	0±0.00
300 (0.03%)	3.67±0.58	10±0.00	7.67±0.58	2.67±0.58	9.67±0.58	0±0.00	0±0.00	0±0.00
400 (0.04%)	4.67±0.58	10±0.00	9.00±0.00	3.33±0.58	10±0.00	0±0.00	0±0.00	0±0.00
500 (0.05%)	9.00±1.00	10±0.00	10±0.00	4.67±0.58	10±0.00	0±0.00	0±0.00	0±0.00
800 (0.08%)	10±0.00	10±0.00	10±0.00	7.67±0.58	10±0.00	1.00±0.00	0±0.00	0±0.00
1000 (0.1%)	10±0.00	10±0.00	10±0.00	9.67±0.58	10±0.00	2.33±0.58	0±0.00	0±0.00
1250 (0.125%)	10±0.00	10±0.00	10±0.00	10±0.00	10±0.00	4.33±0.58	0±0.00	0±0.00
1500 (0.15%)	10±0.00	10±0.00	10±0.00	10±0.00	10±0.00	9.67±0.58	0±0.00	0±0.00

***Each value represents the mean ± S.D of three replicates of larvae dead at each concentration of the treated substance. Means in a column followed by S.D are not significantly different (P<0.002). Control mortality nil.**

Table 3: LC50 and LC90 Values of Methanol Extract of Plants

Plant Name	LC 50 (ppm)	LC 90 (ppm)
Alternanthera sessilis	305.96	648.33
Chromolaena odorata	72.51	113.87
Hyptis suaveolens	184.68	372.82
Loranthus falcatus	437.81	996.04
Ricinus communis	116.63	233.89
Wattakaka volubilis	1180.57	1930.71

Table 4: Phytochemical Analysis of Plant Extracts

SL NO	COMPOUND	TEST	EXTRACT	*Alternanthera sessilis*	*Chromolaena Odorata*	*Hyptis suaveolens*	*Loranthus falcatus*	*Ricinus communis*	*Wattakaka volubilis*
1	ALKALOIDS	Dragendruff's test	Fresh	-	-	-	-	-	-
			Methanol	+	-	-	+	+	-
		Haeger's test	Fresh	-	-	-	-	-	-
			Methanol	-	-	-	+	+	-
2	PHENOLS	$FeCl_3$	Fresh	+	+	-	+	+	+
			Methanol	+	+	+	+	+	+
3	STEROIDS AND TERPENOIDS	Salkowski test	Fresh	+	+	+	+	+	+
			Methanol	+	+	+	+	+	+
		Acetic anhydride	Fresh	+	+	+	+	+	+
			Methanol	+	+	+	+	+	+
4	FLAVONOIDS	NaOH	Fresh	+	+	+	+	+	+
			Methanol	-	+	+	+	+	+
		Lead acetate	Fresh	+	+	+	-	-	-
			Methanol	+	+	+	-	-	-
5	COUMARINS	$FeCl_3$	Fresh	+	+	+	+	+	+
			Methanol	+	+	+	+	+	+
6	CARDIACGLYCOSIDES	Keller Killani	Fresh	+	+	+	+	+	+
			Methanol	+	+	-	+	+	+
7	TANNINS	Potassium Dichromate	Fresh	-	-	+	+	-	-
			Methanol	+	-	-	+	-	+
		Lead acetate	Fresh	-	-	-	+	+	+
			Methanol	-	-	-	+	+	+
8	SAPONINS	Froth test	Fresh	-	-	-	+	-	-
			Methanol	-	-	-	+	-	-
9	QUINONES	HCl	Fresh	-	-	-	-	-	-
			Methanol	-	-	-	-	-	-

The experiment was carried out in the following steps.

2.1 Collection of Plant Material

Fresh, mature and green twigs of twelve common plants belonging to varied taxonomic groups (Table 1) were collected during November- December 2018, from College campus (geographical coordinates 11.25 °N and 75.78°E), Palakkad, Kerala, India. The selection of plants was carried out based on their local availability and reported medicinal properties. The materials were taken from healthy plants, free from dust, dirt and other impurities and were brought to the laboratory for subsequent processing.

2.2 Preparation of fresh plant extracts

The washed and air-dried plant materials (Table 1) were chopped properly and kept in clean trays. For the preparation of extracts, approximately ten grams of plant material (leaf) were taken and ground in a homogenizer using distilled water. The extracts were filtered, and the filtrate was made up to 100 ml with distilled water and retained as a stock solution for further experimentation. Serial dilutions of the stock solution (2%, 4%, 6%, 8% & 10% (w/v)) in water were carried out for assessing treatment efficiencies. Based on the mortality rate at 10% solution, plants showing high mortality were selected for methanol extract preparation.

2.3 Preparation of methanol extracts of plants

Plants having higher larvicidal activity in fresh extracts were selected for the preparation of methanol extracts. These six plants (Table 2) were taken, shade dried and grind into fine powder. Ten gram of the dried powder was weighed and extracted with 100 ml of 100% methanol in a magnetic stirrer apparatus for 12 h. The extracts were filtered through Whatman No.1 filter paper. After complete evaporation of the solvent, the concentrated extracts were stored in closed amber coloured glass bottles and kept under refrigeration till use. For experimental treatment, one mg of each of the extracts was dissolved in one mL of Dimethyl sulphoxide (DMSO) and shaken gently to form a homogenous stock solution. Graded series of test solutions of different concentrations (50ppm, 75 ppm, 100 ppm, 200 ppm, 300 ppm, 400ppm, 500 ppm, 800 ppm, 1000 ppm, 1250 ppm and 1500 ppm) were prepared from the stock solutions by serial dilution with distilled water.

2.4 Culture of Mosquito larvae

The experiment was carried out using laboratory reared mosquito larvae. The larval colonies were

grown in open plastic trays and dishes containing tap water and fed a diet of decaying leaves and yeast granules. The larvae were identified as *Culex quinquefasciatus* Say. The early fourth instar larvae were used for the study.

2.5 Mosquito Larvicidal Bioassays

All bioassays were performed essentially following the standard protocols [3]. The fourth instar larvae (10 each) were tested for different concentrations of fresh extracts, methanol extracts as well as controls. Two control tests were set up in parallel for comparison at a time; one consisted of distilled water alone, another of distilled water and DMSO (Dimethyl sulfoxide). Three replicates were made per concentration. Mortality and survival of the larvae were recorded after 24 h of exposure and the larvae were starved during this period. The larvae were considered dead if they were not responsive to a gentle prodding with a fine needle. The mortality rate of the larvae was reported from the average of three replicates. Toxicity and effect were reported as LC50 and LC90, representing the concentrations in ppm with 50% and 90% larval mortality rate in 24 h., respectively.

2.6 Statistical Analysis

Standard deviations with means of larval mortalities and t- test were calculated. $P < 0.05$ was considered to be significant. Mortality data obtained were analyzed by probit analysis to obtain LC50 and LC90 values [4]. All statistical analyses were performed by using the computer software Microsoft Excel and SPSS 21.0 for Windows.

2.7 Preliminary phytochemical analysis

The methanol extracts of plants which exhibited higher larvicidal activity were selected for preliminary qualitative analysis of phytochemicals, to find out the components responsible for its larvicidal activity. The powdered plant parts were weighed to 1 g and mixed with 14 ml of water and centrifuged for 15 minutes. This was done to analyze the phytochemicals present in the fresh extract of the plants. Preliminary phytochemical test of the methanol extracts of the plants was conducted by taking approximately 1 ml of the extract as a test solution. The various qualitative phytochemical analysis of the extract was conducted using a standard procedure [5, 6, 7].

2.7.1 TESTS FOR ALKALOIDS

Dragendorff's test: To 1 ml of each of the sample solution taken in a test tube a few drops of Dragendorff's Reagent (potassium bismuth iodide solution) was added. An orange-red precipitate was observed indicating the presence of alkaloids.

2.7.2 TESTS FOR TANNINS AND PHENOLIC COMPOUNDS

Ferric chloride test: When three drops of 5% ferric chloride were added to 5 drops of sample solution a dark green/ brown color precipitate appears.

2.7.3 A **TEST FOR STEROIDS AND TRITERPENOIDS**

Acetic anhydride test:

Three drops of acetic anhydride were added to the sample solution and boiled. The solution was then cooled, and conc. sulphuric acid was added along the sides of the tube. Brown ring was observed at the junction. The green colour in the upper region indicated the presence of steroids and deep red colour in the lower region indicates the presence of triterpenoids.

Salkowski test:

To 1 ml of the sample solution few drops of chloroform were added and then few drops of conc. sulphuric acid were added along the sides of the test tube. Reddish brown colour at the interphase indicating the presence of steroids and triterpenoids.

2.7.4 TESTS FOR FLAVONOIDS

NaOH test:

3 ml of dilute NaOH was added to 1 ml of the sample solution. A yellow coloured precipitate was formed, of which the yellow colour disappeared on addition of dilute HCl indicating the presence of flavonoids.

Lead acetate test:

Added 1 ml of lead acetate to sample solution. White turbid precipitate indicates the presence of flavonoids.

2.7.5 TEST FOR COUMARINS

$FeCl_3$ test:

To 1 ml of the sample in a test tube, I ml of $FeCl_3$ was added. The appearance of a dark green colour which turns yellow upon addition of five drops of Conc.HNO_3 indicates the presence of coumarins.

2.7.6 A TEST FOR CARDIAC GLYCOSIDES

Keller Killani test: To 5 ml of extract in a test tube added 2 ml of acetic acid along with one drop of $FeCl_3$ solution and one ml of Conc. H_2SO_4 solution. The appearance of brown ring indicates the presence of cardiac glycosides.

2.7.8 TEST FOR TANNINS

Potassium dichromate test: To a few drops of sample, strong potassium dichromate solution is added. Yellow colour precipitate indicates the presence of tannins.

Lead acetate test:
Added 1 ml of lead acetate to sample solution. Yellow precipitate indicates the presence of tannins.

2.7.9 TEST FOR SAPONINS

Froth test: To 1 ml of the sample solution in a test tube added 2 ml of distilled water and shaken well. The appearance of froth on shaking the mixture shows the presence of saponins.

2.7.8 TEST FOR QUINONES

A small amount of extract was treated with concentrated HCl and observed for the formation of the yellow colour precipitate

3. RESULT

The study was conducted to find the larvicidal activity of selected plant extracts and also to identify the possible phytochemicals present in them which may be responsible for the corresponding larvicidal activity. Among the twelve fresh extracts of plants, six plants such as *Alternanthera sessilis, Hyptis suaveolens, Loranthus falcatus, Wattakaka volubilis, Ricinus communis* and *Chromolaena odorata* showed larvicidal activity at 10 % concentration (Table 1). *Chromolaena odorata* and *Ricinus communis* exhibited maximum larvicidal activity, causing 100% mortality to the larvae at 10% concentration. The plants *Asteracantha longifolia, Cuscuta reflexa, Pistia stratiotes, Mikania scandens, Tridax procumbens* and *Cissus vitiginea* showed no larvicidal activity in the same concentration (Table 1). Considering this, the six plants, which exhibited larvicidal activity, were selected for methanol extract analysis.

The methanol extracts of all the six plants showed remarkable mosquito larvicidal activity (Table 2). Among the different plants, *Chromolaena odorata* and *Ricinus communis* showed maximum mortality (100%) at 200 ppm and 400 ppm respectively. These plants exhibited the lowest activity at 50 ppm.

The LC50 value of *Chromolaena odorata* and *Ricinus communis* were found to be 72.51ppm and 116.63ppm. Their LC 90 value was found to be 113.87ppm and 233.89 ppm respectively. Least activity was exhibited by *Wattakaka volubilis*. It showed 10% activity at 800 ppm and 100% activity at 1500 ppm. The LC 50 and LC 90 values were 1180.57ppm, 1980.71ppm. The other plants like *Alternanthera sessilis, Hyptis suaveolens, Loranthus falcatus,* showed 100% mortality at 800 ppm, 500 ppm, 1250 ppm, respectively and least mortality at 100 ppm. Their LC 50 values were 305.96ppm, 184.68ppm and 437.81ppm and their LC90 values were 648.33ppm, 372.82ppm and 996.04ppm respectively (Table 3). Hence on the basis of these LC 50 and LC 90 values, we can arrange these plants according to their larvicidal activity in the order *Chromolaena odorata* > *Ricinus communis* > *Hyptis suaveolens* > *Alternanthera sessilis* > *Loranthus falcatus* > *Wattakaka volubilis* (Figure 1). Mean and standard deviation of larval mortality was calculated using Microsoft Excel (Table 2).

From these data, we could infer that remarkably higher larvicidal activity was shown by *Chromolaena odorata and Ricinus communis* in both fresh extracts as well as in methanol extracts.

Preliminary phytochemical analysis of both fresh extracts, as well as methanol extracts of plants having larvicidal activity, was conducted to find out the different phytochemicals present in the plant extracts. The results of the analysis are summarised in Table 4. From the data, it is clear that compounds such as steroids, coumarins and terpenoids were present in all the fresh and methanol extracts of all the plants. Quinones was absent in all the plants. A compound such as saponin was present only in *Loranthus falcatus*. Phenols were present in methanol extracts of all the plants. From the results, we can conclude that though *Chromolaena odorata* and *Ricinus communis* show greater activity, the constitution of phytochemicals qualitatively is not the same. Phenols, steroids, terpenoids, coumarins and cardiac glycosides were present in both and saponins and Quinones were absent in both. But the occurrence of alkaloids, flavonoids, and tannins varied in both.

4. DISCUSSION

Mosquitoes are pestiferous vectors which are responsible for the transmission of various dreadful diseases, causing millions of deaths every year. Indiscriminate use of chemical insecticides has resulted in the development of resistance by these organisms, resulting in rebounding vectorial capacity. Moreover, such chemicals have given rise to serious environmental issues. This has led to the search for phytochemicals, which are having several advantages over the chemical insecticides in the control of vectors. The freshwater and methanol extracts of the plants showed significant larvicidal activity. *Chromolaena odorata* and *Ricinus communis* were the plants which exhibited an incredibly higher range of larvicidal activity. The LC50 value of *Chromolaena odorata* and *Ricinus communis* were found to be 72.51 ppm and 116.63 ppm. Their LC 90 value was found to be 113.87ppm and 233.89 ppm respectively. Least activity was exhibited by *Wattakaka volubilis*. It showed least activity at 800 ppm and maximum activity at 1500 ppm. The LC 50 and LC 90 values were 1180.57 ppm and 1930.71

ppm. The standard deviation of the data we gained was very low indicating that the data align with the mean or is concentrated around the mean. Hence there is no doubt that in the experiment done as part of the project work the amount of variation in the group studied is very low. This serves as an aid supporting the validity of the study conducted thereby supporting that the analysis made is true and can be used for further studies. The driving force behind the larvicidal activity of plant extracts may be due to the presence of certain phytochemical constituents in them. Hence the preliminary qualitative phytochemical screening of the plant extracts helped us to find few of the compounds present in them.

As synthetic insecticides are hazardous for both aquatic and terrestrial ecosystem it is also responsible for biological magnification through the food chain. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified. The secondary phytochemicals of plants are a vast repository of compounds with a wide range of biological activities [8]. Hence these phytochemicals can be extrtacted in future to prepare effective biopesticides. Mathivanana *et al.* [9] reported the remarkable larvicidal activity of alkaloids, saponins, tannins, flavonoids and steroids against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* . Among the six plants, the methanol extract of *Alternanthera sessilis* (L), *Loranthus falcatus* (L) and *Ricinus communis* (L) showed positive in the preliminary test. Hence the larvicidal activity exhibited by the plants during the course of study may be due to the presence of certain phytochemicals in them or due to the combined acvtivity of those phytochemicals.

5. CONCLUSION

In short, it can be concluded that *Chromolaena odorata, Ricinus communis, Alternanthera sessilis, Hyptis suaveolens, Loranthus falcatus and Wattakaka volubilis* are the most promising plants in future, which can be used as a mosquito repellent. The extreme larvicidal activity of these plants may be due to phytochemicals present in the plant extracts. The preliminary qualitative phytochemical analysis revealed the presence of chemicals such as alkaloids, phenols, steroids, flavonoids, coumarins and cardiac glycosides. All these investigations justify the larvicidal activity of different plant extracts. All the objectives of the present investigation were done successfully. The results reported in the present study will open up the possibility of further investigations on evaluation, identification and isolation of the bioactive components of these plant extracts and its systematic effects on target mosquitoes, which would eventually facilitate the application of the extract as larvicidal, adult emergence inhibition and ovicidal agent in small-volume aquatic habitats or breeding sites of limited size in and around human dwellings.

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7. REFERENCES

[1] WHO. Malaria fact sheets No. 94. WHO Report. Geneva: WHO media center, (2010).

[2] Wariko, R., Ray, A., Sandhu, J. K., Samal, R., Wahab, N., Kumar, S. Larvicidal and irritant activities of hexane leaf extracts of *Citrus sinensis* against dengue vector *Aedes aegypti* L. Asian Pac J trop Biomed, 2(2): 152-155, (2012).

[3] WHO. Mineographed Document, WHO, (1985).

[4] Finney, D. J. Probit Analysis, 3rd ed. Cambridge University Press, London, (1971).

[5] Evans,W.C., Trease . Trease and Evans Pharmacognosy, 177-179, (2009).

[6] Kokate, C. K. Practical Pharmacognosy. Vallabh Prakashan Publication. New Delhi, India, 115, (1999).

[7] Siddiqui, A.A., Ali, M. Practical Pharmaceutical chemistry. 1st Edition. CBS Publishers and distributors, New Delhi, 126-131, (1997).

[8] Hossain, E., Rawani, A., Chandra, G., Mandal, S. C., Gupta, J. K. Larvicidal activity of Dregea Volubilis and Bombax malabaricum leaf extracts against the filarial vector Culex quinquefasciatus. Asian Pac J Trop Med, 4(6): 436-441, (2011).

[9] Mathivanana, T., Govindarajan, M., Elumalai, K., Krishnappa, K., Ananthan, A. Mosquito larvicidal and phytochemical properties of Ervatamia coronaria Stapf . (Family: Apocynaceae). J Vector Borne Dis, 47(3) : 178-181, (2010).