International Journal of Research and Review
www.ijrrjournal.com E-ISSN: 2349-9788; P-ISSN: 2454-2237

Original Research Article

Phytochemical Screening and Larvicidal Efficacy of Solvent Extracts of *Delonix regia* Leaf and Flower against Vector Mosquito *Culex quinquefasciatus*

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ABSTRACT

Background: Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of outrageous diseases. The management of these disease vectors using synthetic insecticides has failed in part due to their efficiency in attaining physiological resistance.
Methods: In view of an increasing interest in developing plant based insecticides as an alternative to chemical insecticides, the present study was undertaken to assess the larvicidal potential of the solvent extracts of *Delonix regia* leaf and flower extracts against the fourth instar larvae of mosquito *Culex quinquefasciatus* at 100, 150, 200, 250 and 300 ppm concentrations. Phytochemical analysis was also carried out using standard procedures.
Results: Results revealed that chloroform and acetone extracts of *D. regia* leaf and chloroform and ethanol extracts of *D. regia* flower displayed maximum and moderate larvicidal activity. A number of phytochemicals were also revealed when the extracts were tested.
Conclusion: Our data suggest that chloroform and acetone extracts of *D. regia* leaf and chloroform and ethanol extracts of *D. regia* flower have the potential to be used as an ecofriendly approach for the control of the fourth instar larvae of mosquito *Culex quinquefasciatus*.

Keywords: Larvicidal; Plant extracts; Phytochemicals; Solvents; *Delonix regia*; Mosquito larvae, *Culex quinquefasciatus*

1. INTRODUCTION

Mosquitoes are one of the most medicinal significant vectors and they transmit parasites and pathogens which continue to have a devastating impact on human beings. [1] The vector borne diseases caused by mosquitoes are one of the major health problems in many countries. Several numbers of species belonging to genera Culex, Anopheles, Aedes, are vectors for the pathogens of various diseases like Filariasis, Malaria, Dengue, Yellow fever, Japanese encephalitis, Chikungunya are some of the deadly diseases spread by mosquitoes. *Culex quinquefasciatus* is an important vector of Bancroftian filariasis in tropical and sub tropical regions. According to WHO [2] about 90 million people worldwide one infected with *Wuchereria bancrofti*, the lymphatic dwelling parasite and ten time more people are at the risk of being infected. In India alone 25 million people suffer from filarial diseases manifestations. [1] Thus, one of approaches for the control of these mosquitoes borne

diseases in the interruption of transmission by killing or preventing mosquito bite. [3]

Current strategies based on the elimination of breeding sites and applications of chemical insecticides for larval and adult mosquito control have resulted in development of resistance without eliminating the constant risk of epidemics. [4] However the discovery, development and uses of synthetic organic insecticidal chemicals with persistent residual action not only over shadowed the use of herbal products as insecticides of choice against mosquitoes but also become the major weapon for mosquito control. [5] This has necessitated the need for research and development of environmentally safe, biodegradable low cost indigenous method for vector control, which can be used with minimum care by individual and communities in specific situation. [6] Herbal insecticides of plant origin become a priority in this search. Several laboratory and field based studies have already been carried out in this area and some potentially active larvicides of plant origin like octacosane, falcarinol, geranial, azadirachtin, piper non alineplumbagin, β-sitosterol, etc. have been isolated so far. [4]

Chemicals larvicides could be carcinogenic, mutagenic and teratogenic for humans. The nonstop use of chemical larvicides has often led to the disorder of the natural biological control system. [6] There are several native reports about crude solvent extracts of different parts of plants, essential oils or their chromatographic fractions that showed various levels of bioactivity against different developmental stages of mosquito vectors. [8] Some plants have phytochemicals constituents for the control of mosquitoes. One of the earliest reports of the use of plant extracts against mosquito larvae is extraction of plants' alkaloids like nicotine, anabasine, methyl anabasine and lupinine from the Russian weed in 1933. [9] Scientists have begun to look toward traditional botanicals as an alternative for managing insects. [10-11] Plants contain a wide range of potential phytochemicals that are target specific, rapidly biodegradable, ecofriendly, and less toxic to human health. Thus, attention has been steadily diverted toward plant-based chemicals for insect control.

2. MATERIALS AND METHODS

Origin and laboratory maintenance of the mosquito colonies

Mosquitoes used in study were fourth instar larvae of *Culex quinquefasciatus*. Individuals were reared for several generations under laboratory conditions by Hay infusion method.

Collection of test materials

Leaves and flowers of the selected tree namely *Delonix regia* were collected from natural habitat of our College Campus. The specimens were identified, certified and the voucher specimen number (BSI/SRC/5/23/2018/Tech/2474) was deposited at the Botanical Survey of India.

Preparation of leaf and flower powder and extracts

Leaves and flowers were collected and dried under shade at room temperature for 2 to 3 weeks and were powdered using an electric pulverizer. Fine powder was obtained by sieving. 10g of the leaf and flower powder was weighed using an electronic balance and were subjected to extraction. [12-13] Chloroform extraction was followed by acetone and ethanol extraction, so that powders were subjected to extraction with solvents in the order of increasing polarity. The leaf and flower extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further bioassays.

Larvicidal Bioassay

A pilot study was carried out to find out the effective doses of both leaf and flower extracts that produced mortality in larvae of *Cx. quinquefasciatus*. After determining the effective doses, the detailed investigations were undertaken. *Delonix regia* leaf and flower extract were tested for larvicidal

activity at 5 concentration viz., 100, 150, 200, 250 and 300 ppm and a control treatment was also maintained. Twenty newly emerged fourth instars larvae of *Cx. quinquefasciatus* were introduced into the 250 ml beakers for the bioassay studies. Control with three replications was also maintained simultaneously. The experimental setup consisted to six treatments; each with three replications one set for leaf extract and another for flower extract. The following parameters were observed to assess the effective dose:

i. Larval mortality at 24 h, 48 h, 72 h and 96 h of treatment
ii. Pupal mortality
iii. Adult emergence

Test for larvicidal activity

The larval mortality in both treatment and control was recorded at 24 h of treatment and percentage of mortality was calculated using Abbott's formula. [14]

$$\% \text{ Mortality} = \frac{\text{Mortality in treatment (\%)} - \text{Mortality in control (\%)}}{100 - \text{Mortality in control (\%)}}$$

Statistical Analysis

Standard deviation was calculated for the data which was obtained from the test for larvicidal activity against *Cx. quinquefasciatus* mosquito. Each value (Mean ± SD) represents average of three replications.

Phytochemical Screening

The preliminary qualitative phytochemical analysis has been attempted in the effective leaf and flower extracts of *D. regia* to find out the presence or absence of certain bioactive compounds. The preliminary screening was carried out by using standard procedures.

Test for Alkaloids

- **Mayer's test:** [15] One ml of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test:** [16] One ml of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.
- **Hager's test:** [17] One ml of extract was treated with 1 or 2 ml of Hager's reagent and observed for the formation of prominent yellow precipitate.

Test for Tannins

- **Ferric chloride test:** [18] 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate.

Test for Phenols

- **Ferric chloride test:** [19] The extract (50mg) was dissolved in 5 ml of distilled water and treated with few drops of 5% ferric chloride and observed for the formation of dark green colour
- **Lead acetate test**: [20-21] The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

Test for Flavonoids

- **NaOH test:** [18] 1 mg of extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.
- **Lead acetate test:** [20-21] Test extract (50 mg) was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

Test for Sterols

- **Liebermann-Burchard test:** [22] The extract (50 mg) is dissolved in 2 ml of acetic anhydride. To this one or two drops of Conc. $H_2SO_4$is added along the

side of the test tube and observed for an array of colour changes.

Test for Terpenoids

- **Liebermann-Burchard test:** [23] 50 mg of extract was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of Conc. H_2SO_4. A change in colour from pink to violet showed the presence of terpenoids.

Test for Saponins

- **Foam Test:** The extract (50 mg) or dry powder was diluted with distilled water and made up to 20 ml. The suspension is vigorously shaken in a graduated cylinder for 15 minutes and observed for the formation of 2 cm layer thick foam.

Test for Anthraquinones

- **Borntrager's test:** [23] 0.2 g of extract to be tested was shaken with 10 ml of benzene and then filtered. 5 ml of the 10% ammonia solution was then added to the filtrate and thereafter shaken and observed for the appearance of a pink, red or violet colour in the ammoniacal (lower) phase.

Test for Proteins

- **Ninhydrin test:** [24] Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate and observed for the presence of characteristic purple colour.
- **Biuret test:** [25] An aliquot of 2 ml of filtrate is treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added followed by excess of potassium hydroxide pellets and observed for the formation of pink ethanolic layer.

Test for Quinones

- **H_2SO_4 test:** [21] To 1 ml of extract, 1 ml of Conc. H_2SO_4 was added and was observed for the formation of red colour.
- **HCl test:** [26-27] To 1 ml of the extract 5 ml of HCl was added and observed for the presence of yellow colour precipitate

3. RESULTS AND DISCUSSION

The efficacy of plants may vary from one another on the basis of their toxic effects. This study was completely focused on the biological control of *Cx. quinquefasciatus* using the solvent extracts of *D. regia* leaves and flowers. The larvae of *Cx. quinquefasciatus* were treated with selected doses of plant extracts at different concentrations. In all treatments the larvicidal activity was concentration dependent. Generally, as the concentration increases the rate of larval mortality increases. Throughout the experiment larval mortality was found to be 0 % in the control.

Larvicidal activity of leaf extract of *Delonix regia*

The results of larvicidal activity of *D. regia* leaf against the fourth instar larvae of *Cx. quinquefasciatus* is presented in Table 1. The maximum larvicidal activity was noted in chloroform extract of *D. regia* leaf. Larval mortality was found to be 100% in the concentration ranging from 100-250 ppm in 48 h itself and was 100% in 24 h at the concentration of 300 ppm. Total larval mortality of 100% was recorded in all the test concentration. The control treatment did not provide any larval mortality. Medicinal plant extracts can be effective as mosquito larvicides and may also greatly reduce the risk of adverse ecological effects as they do not induce any known or recorded insecticide resistance in mosquito; they are also expected to have low human toxicity and a high level of biodegradation. [28] Earlier Govindarajan *et al* [29] has reported that the methanol extract of *Cassia fistula* when tested for larvicidal activity against *Cx. quinquefasciatus* and *An. stephensi* showed significant larvicidal activity. Efficiency of any mosquito control intervention should be measured by its selectivity for the target organism.

However, the moderate larvicidal activity was exhibited by acetone extract of *D. regia* leaf. The higher concentration of 250 to 300 ppm showed 100% mortality at 72 h of the experiment. Even the lowest concentrations of 100 ppm and 150 ppm recorded total larval mortality of 92% and 97% respectively. Similar study was

conducted by Mathew *et al* [30] that reported that the seed methanol extract of *Clitoria ternata* was effective against the larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*. The leaf extract of *Cassia obtusifolia* had significant larvicidal effect against *An. stephensi* according to the study of Rajkumar and Jebanesan. [31] Saravanan *et al* [32] has revealed that 100% mortality was observed in 1% concentration of petroleum ether and ethanol extract of *Caesalpinia bonduc*, whereas it was 55% in 2.5% of aqueous extract and 92.6% in 2.5% of fixed oil against the fourth instar larvae of *Cx. quinquefasciatus*.

Table 1: Larvicidal activity of solvent extracts of *D. regia* leaf against *Cx. quinquefasciatus*

Solvent	Conc. in (ppm)	Larval mortality %				Pupal mortality	Total mortality	Adult emergence
		24 h	48 h	72 h	96 h			
AC	Cont	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	100±0.0
	100	40±4.08	66.66±2.35	86.66±10.27	90±5.0	2±1.0	92±2.05	8±0.816
	150	50±8.16	71.66±6.24	88.33±2.35	96.66±2.35	1±0.0	97±2.35	3±0.47
	200	60±8.16	76.66±6.23	93.33±2.35	100±0.0	-	100±0	-
	250	65±8.16	85±7.07	100±0.0	-	-	100±0	-
	300	71.66±6.26	91.66±6.24	100±0.0	-	-	100±0	-
EH	Cont	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	100±0.0
	100	38.33±6.26	56.66±2.35	73.33±2.35	83.33±2.36	12±1.63	95.33±4.08	4.67±0.47
	150	43.33±6.23	65±4.08	78.333±2.36	88.33±2.35	6±0.47	94.33±2.62	5.67±0.47
	200	53.33±6.29	73.33±2.35	88.33±2.35	96.66±2.35	1±0.0	97.66±2.16	2.34±1.5
	250	65±4.08	81.66±2.35	95±4.08	100±0.0	-	100±0	-
	300	70±4.08	90±4.08	100±0.0	-	-	100±0	-
CH	Cont	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	100±0.0
	100	70±4.08	100±0.0	-	-	-	100±0.0	-
	150	76.66±2.35	100±0.0	-	-	-	100±0.0	-
	200	86.66±2.46	100±0.0	-	-	-	100±0.0	-
	250	95±0	100±0.0	-	-	-	100±0.0	-
	300	100±0.0	-	-	-	-	100±0.0	-

Mean ± Standard Deviation of three replications AC – Acetone; EH – Ethanol; CH – Chloroform; Cont - Control

The minimum larvicidal activity was recorded in ethanol in which 100% larval mortality was exhibited at 72 h of the treatment in the higher concentration of 300 ppm. 250 ppm concentration showed 100% larval mortality at 96 h of the treatment. Total larval mortality in the concentration ranging from 100-200 ppm was noted as 95.33 %, 94.33 % and 97.66 % respectively. The larval mortality of *Culex* sp when tested with plant extracts were in agreement with results of various other studies of Cetin *et al;* [33] Dharmshaktu *et al;* [34] Hamouda *et al.* [35]

Larvicidal activity of flower extract of *Delonix regia*

The results of larvicidal activity of *D. regia* leaf against the fourth instar larvae of *Cx. quinquefasciatus* is presented in Table 2. The maximum larvicidal activity was noted in chloroform extract of *D. regia* flower. Larval mortality was found to be 100 % in the concentration ranging from 100-250 ppm in 48 h itself and was 100 % in 24h at the concentration of 300 ppm. Total larval mortality of 100% was recorded in all the test concentration. The control treatment did not provide any larval mortality. In parallel to this Mehra and Hiradhar [36] reported the larvicidal potential of crude acetone extract of the seed of *Annona squamosa* on the larvae of *Cx. quinquefaciatus*. Susan and Vincent [37] reported comparative efficiency of *Annona squamosa*, *Pongamia glabra* and *Azadirachta indica* against mosquito vectors

However, the moderate larvicidal activity was exhibited by ethanol extract of *D. regia* flower. The higher concentration of 300 ppm showed 100% mortality at 72 h of the test period. 100% larval mortality at 250 ppm concentration accorded at 96h of the experiment. Even the lowest concentration of 100 ppm and 200 ppm recorded, total larval mortality of 91.66 %, 93.33 % and 96 % respectively. Minjas and Sarda [38] (1986) reported variations in toxicological efficacy with three mosquito species to crude aqueous extract of fruit pods of *Swartzia madagascariensis*.

Table 2: Larvicidal activity of solvent extracts of *D. regia* flower against *Cx. quinquefasciatus*

Solvent	Conc. in (ppm)	Larval mortality %				Pupal mortality	Total mortality	Adult emergence
		24 h	48 h	72 h	96 h			
AC	Cont	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	100±0.0
	100	30±4.082	50±4.24	66.66±4.71	80±7.071	12±1.63	92±2.05	8±0.81
	150	41.66±4.71	56.66±2.35	73.33±2.35	86.66±4.73	6±0.81	92±2.05	8±0.81
	200	56.66±6.23	68.33±6.23	81.66±4.72	93.33±6.24	3±0.471	96.33±2.36	4±0.47
	250	73.33±4.71	85±4.082	93.33±2.35	97.5±2.50	-	100±0	-
	300	83.33±2.35	91.66±2.36	100±0.0	-	-	100±0	-
EH	Cont	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	100±0.0
	100	30±4.082	45±4.23	65±4.56	76.66±2.36	15±0.47	91.66±2.35	8±0.81
	150	41.66±2.35	56.66±2.36	76.66±2.42	88.33±4.71	5±0.81	93.33±2.36	7±2.16
	200	56.66±2.36	70±4.082	85±4.13	95±4.53	1±0.0	96±2.36	4±0.47
	250	68.33±2.35	86.66±4.08	95±4.13	100±0.0	-	100±0.0	-
	300	80±4.082	93.33±2.36	100±0	-	-	100±0.0	-
CH	Cont	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	100±0.0
	100	70±4.082	100±0.0	-	-	-	100±0.0	-
	150	80±4.085	100±0.0	-	-	-	100±0.0	-
	200	86.66±2.36	100±0.0	-	-	-	100±0.0	-
	250	96.66±2.31	100±0.0	-	-	-	100±0.0	-
	300	100±0.0	-	-	-	-	100±0.0	-

Mean ± Standard Deviation of three replications AC – Acetone; EH – Ethanol; CH – Chloroform; Cont - Control

The minimum larvicidal activity was recorded in acetone extract in which 100% larval mortality was exhibited at 72 h of the treatment in the higher concentration of 300 ppm. Concentration of 250 ppm showed 100% larval mortality at 96 h of the treatment. Pushpanathan *et al* [39] has reported larvicidal properties of *Cymbopogon citratus* on *Cx. quinquefasciatus* after 72 h exposure time. Results have shown that the aqueous extracts of the test plants had less larvicidal effect compared to the ethanol extracts. Similarly in the present study total larval mortality in the concentration ranging from 100-200 ppm of acetone extract of *D. regia* flower was noted as 92 %, 92 % and 96.33 % which was comparatively less larvicidal than the other extracts.

The solvents extracts ie., chloroform and acetone extracts of *D. regia* leaf and chloroform and ethanol extracts of *D. regia* flower that displayed maximum and moderate larvicidal activity against fourth instar larvae of *Cx, quinquefasciatus* was carried forward for further phytochemical analysis.

Phytochemical analysis of leaf extracts of *Delonix regia*

The results of phytochemical analysis of *D. regia* flower extract are presented in Table 3. The chloroform extract of *D. regia* leaf that exhibited high larvicidal activity when tested for the presence of pytochemicals showed the presence of tannins, phenols, sterols, terpenoids, anthroquinones and quinones. An insecticide does not have to cause high mortality on target organisms in order to be acceptable, [40] but it should prevent breeding. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, in expensive and are readily available throughout the world. [41] The biological activity observed in the present study might be due to the various compounds, including phenols, terpenoids and alkaloids existing in plants. Park *et al* [42] has also reported that the bioactivity of plants might be due to its secondary metabolites.

Table 3: Phytochemicals present *Delonix regia* leaf extracts

Sl. No.	Constituents	*Delonix regia* leaf	
		Acetone extract	Chloroform extract
1	Alkaloids	+	-
2	Flavonoids	+	-
3	Sterols	+	+
4	Terpenoids	-	+
5	Anthroquinones	-	+
6	Phenols	-	+
7	Saponins	-	-
8	Tannins	-	+
9	Proteins	+	-
10	Quinones	-	+

"+" Presence "-" Absence

In the present study acetone extract of *D. regia* leaf that exhibited moderate larvicidal activity when analysed revealed the presence of alkaloids, flavonoids, sterols

and proteins. Saxena *et al* [43] noted that the degenerating effect of the extract on the mosquito influenced the overall failure in adult emergence, maybe due to interference in chitinous cuticle formation. Pupation followed by prolonged larval stage in *An. stephensi* when treated with acetone extract of *O. sanctum* along with abnormal larvae, intermediate pupae and several morphogenetic aberrations were also noted.

Phytochemical analysis of flower extracts of *Delonix regia*

The results of phytochemical analysis of *D. regia* flower extract are presented in Table 4. The chloroform extract of *D. regia* flower, that which exhibited high larvicidal efficacy when tested for phytochemicals showed the presence of alkaloids, tannins, phenols, flavonoids, terpenoids and proteins. In the present study the different solvent extracts of *D. regia* flower showed varying larvicidal effects. However, highest mortality was observed in chloroform and ethnol flower extracts when compared to the other extract. These results are in accordance with the observations of Elumalai *et al* [44] who reported the larvicidal potential of the whole plant extracts of *Tridax procumbens* against the larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The larvicidal property of the plant extracts may be due to the presence of alkaloids, flavonoids and tannins and phenolic compounds according to Anees. [45]

Table 4: Phytochemicals present in *Delonix regia* flower extracts

Sl. No.	Constituents	*Delonix regia* flower	
		Chloroform extract	Ethanol extract
1	Alkaloids	-	+
2	Flavonoids	-	+
3	Sterols	+	-
4	Terpenoids	+	+
5	Anthroquinones	-	-
6	Phenols	-	+
7	Saponins	-	-
8	Tannins	-	+
9	Proteins	-	+
10	Quinones	-	-

"+" Presence "-" Absence

The ethanol extract of *D. regia* flower, in the present study showed moderate larvicidal activity and displayed the presence of phytoconstituents such as sterols, saponins, anthraquinones, terpenoids and quinones. The present study reveals that even relatively short term exposure of larvae to the leaf and flower extracts of *D. regia* extracts was found to markedly increase the mortality of the larvae over time and thus reduce the total number of viable adults leading to a possible reduction in the total population dynamics of vectors. Observations in accordance to the present study were also reported by Ansari *et al.* [46]

4. CONCLUSION

Mosquitoes are not only the most important vectors for the transmission of malaria, filariasis, and viral diseases, but are also an important pest to humans, causing allergic responses that include local skin reaction and systemic reactions. Effective, repeated use of controlling agents has disrupted natural biological control systems and led to outbreaks of insect species showing pesticide resistance. It has also provoked unwanted effects, including toxicity to non-target organisms and fostered environmental and human health concerns. These problems have highlighted the need for the development of new strategies for selective mosquito larval control. Plants may be a source of alternative agents for control of mosquitoes, because they are rich in bioactive chemicals, and are active against a limited number of species including specific target insects, and are bio-degradable. In this context, the findings of the present study might be useful.

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How to cite this article: Sudhapriya. A, Dhivya R. Phytochemical screening and larvicidal efficacy of solvent extracts of *delonix regia* leaf and flower against vector mosquito *culex quinquefasciatus*. International Journal of Research and Review. 2019; 6(3):206-214.

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