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Phytochemical composition and ovicidal efficacy of *Catharanthus roseus* leaf extract against the mosquito *Culex quinquefasciatus* (Diptera: Culicidae)

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Abstract
Under the Integrated Mosquito Management (IMM), emphasis was given on the application of alternative strategies in mosquito control because of the ill effects of chemical insecticides. The prime focus of this study was to discover non-toxic, easily available and biodegradable mosquito control agent of botanical origin. In the present study the phytochemical composition and mosquito (*Cx. quinquefasciatus*) ovicidal potential of leaf extracts of *Catharanthus roseus* were tested. The results of the study revealed that ethanol extract of *C. roseus* leaves exhibited 100% ovicidal activity against the eggs of *Cx. quinquefasciatus* followed by chloroform and petroleum ether. Phytochemical analysis of the ethanol extract of *C. roseus*, showed the presence of phytochemicals such as alkaloids, tannins, phenols, flavonoids, terpenoids, protein and quinones. The presence of various phytochemicals might have contributed to the ovicidal efficacy of selected leaf extracts. The findings of the current study emphasized the potential of *C. roseus* leaves for controlling the mosquito population

Keywords: Mosquito, Plants, Vector control, *Culex quinquefasciatus*, *Catharanthus roseus*, Ovicidal activity, Phytochemical analysis

1. Introduction
Arthropods are dangerous vectors of deadly pathogens and parasites, which may spread as epidemics or pandemics in the increasing world population of humans and animals [1]. Among them, mosquitoes are one of the most medically significant transmitters as they extend variety of parasites and pathogens, which persist to have destructive effects on human beings [2, 3]. *Cx. quinquefasciatus* is a vector of lymphatic filariasis, which is a widely distributed in tropical region with around 120 million people infected worldwide, and 44 million people have common chronic manifestations [4]. Insufficient water supply and inadequate waste management systems has also consequently led to a rise in mosquito breeding sites. Several other factors has also led to an explosion in the mosquito borne diseases infecting over 70 crore people globally and 4 crore of the Indian population every year [5].
Mosquito borne diseases have an economic impact, including loss in commercial and labour outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector borne disease [6]. In the present time, over use of chemical insecticides leads mosquito to develop resistance towards chemical insecticides. Continued applications of synthetic compounds have some drawbacks, including the widespread development of insecticide resistance [7].
Another drawback with the use of insecticide is that these are non-selective and could be harmful to other organisms in the environment. The toxicity problem, together with the growing insecticides, has revived interest on more detailed studies of naturally occurring insecticides [8]. Plant constitutes a rich source of bioactive compound which might act deadly on the insect physiological system and kills them [9]. Several plant extracts and isolated compounds from different plant families have been reported for their promising ovicidal activities [10]. In all perspectives the plants were used to control insects by the presence of phytochemicals that were predominantly secondary compounds produced by plants to protect themselves against herbivorous insects [11].
The toxicological properties of phytochemicals reflect the potential of plants as a source of insecticidal agents. Prospection for new larvicidal molecules based on rich plant biodiversity is

appreciable as compounds of plant derivatives are safer to use and leaves no residue in the environment [12]. Plant derived substances have recently become a matter of great interest owing to their versatile applications [13]. Thus, the research is focused on finding newer insecticides of plant origin with high potency, safety and easy availability at low cost. Secondary metabolites are present in plant as key candidate with insecticidal properties and can be explored to develop the natural compounds to control mosquito population.

2. Materials and Method

2.1 Origin and laboratory maintenance of the mosquito colonies

Mosquitoes used in study were *Culex quinquefasciatus*. Individuals were reared for several generations during October 2016 – February 2017 in the Department of Zoology, Nirmala College for Women, Coimbatore by Hay infusion method under laboratory conditions.

Collection and preparation of leaf powder and extracts

Fully developed fresh leaves of the plant *C. roseus* were collected during October 2016 – November 2016 from natural habitat of Coimbatore locale, Tamil Nadu, India. They were, washed in water 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 each of the leaf powder were weighed using an electronic balance and were subjected to extraction [14, 15]. Petroleum ether extraction was followed by chloroform and ethanol extraction in their increasing order of polarity. The leaf extract thus obtained were concentrated by distillation and dried by evaporation in a water bath. The residue thus obtained was used for further bioassays.

2.2 Ovicidal bioassay

Ovicidal activity was assessed by the slightly modified method of Su and Mulla [16]. The egg raft/eggs of *Cx. quinquefasciatus* were collected from Department of Zoology, Nirmala College for Women, Coimbatore. The *C. roseus* leaf extracts were diluted in the appropriate solvents to achieve various concentrations ranging from 100 to 300 ppm. Eggs of the mosquito species (100 nos.) were exposed to each concentration of *C. roseus* leaf extracts. After treatment the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope.

Each experiment was replicated three times along with appropriate control. The hatch rates were assessed 48h after treatment and counts were made every 24h after exposure until the test was terminated. The hatch rates were assessed by the following formula.

$$\% \text{ of egg mortality} = \frac{\text{Mortality at treatment} - \text{Mortality at control}}{100 - \text{Mortality at control}} \times 100$$

Statistical analysis

The data on bioassay studies were also subjected to One Way Analysis of Variance (ANOVA) as described by Panse and Sukahtme [17]. The egg mortality data were subjected to probit analysis [18].

2.3 Phytochemical screening

Qualitative analysis

Preliminary phytochemical screening of leaf extract of selected plant was carried out using the standard procedures.

Test for Alkaloids

- **Mayer's test** [19]: A fraction 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** [20]: A fraction 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** [21]: A few 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** [22]: About 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** [23]: 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** [24, 25]: 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** [22]**:** Few quantity of the 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** [24, 25]**:** 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** [26]**:** 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** [27]: A little of extract (50 mg) 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** [27]: About 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** [28]: Two drops of ninhydrin solution (10 mg of ninhydrine in 200 ml of acetone) are added to 2 ml of aqueous filtrate and observed for the present of characteristic purple colour.

- **Biuret test** [29]: 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.

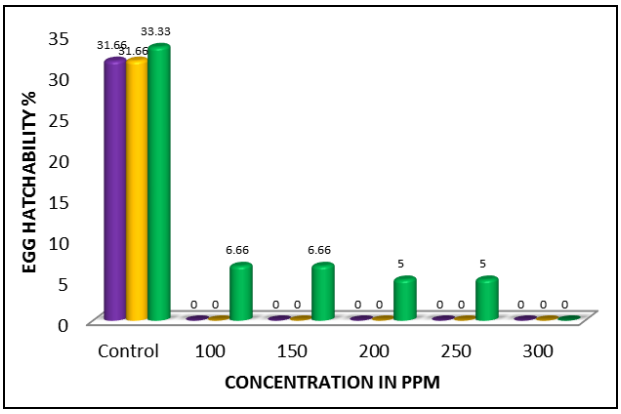
Test for Quinones

- **H_2SO_4 test** [25]: To 1 ml of extract add 1 ml of Conc. H_2SO_4 and observed for the formation of red colour.
- **HCl test** [30, 31]: To 1 ml of the extract 5 ml of HCl and observed for the presence of yellow colour precipitate.

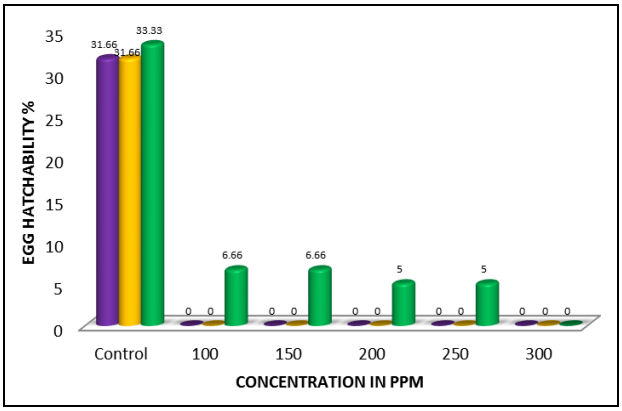
3. Result and Discussion

In this study, petroleum ether, chloroform and ethanol extract of *C. roseus* leaves was examined for its ovicidal activity against the eggs of *Cx. quinquefasciatus* mosquito. The maximum ovicidal activity was found in ethanol extract in which egg hatchability was totally inhibited at concentration ranging from 150 – 300 ppm. At 100 ppm zero percentage egg hatchability was recorded at 72 h and 96 h (Figure 1 a,b,c).

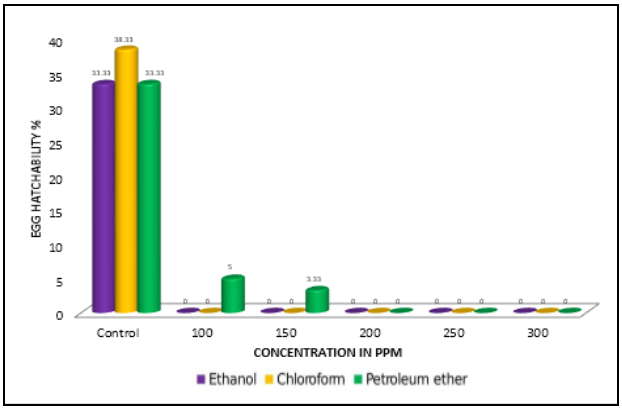
Moderate ovicidal activity was recorded in chloroform extract of *C. roseus* leaf. Egg hatchability was found to be totally inhibited at concentrations ranging from 200 – 300 ppm (Figure 2a). At 100 and 150 ppm zero percentage egg hatchability was recorded at 72h and 96h. LC_{50} and LC_{90} value for chloroform extract were noted as 32.68 and 123.79 respectively.



a) 48 h

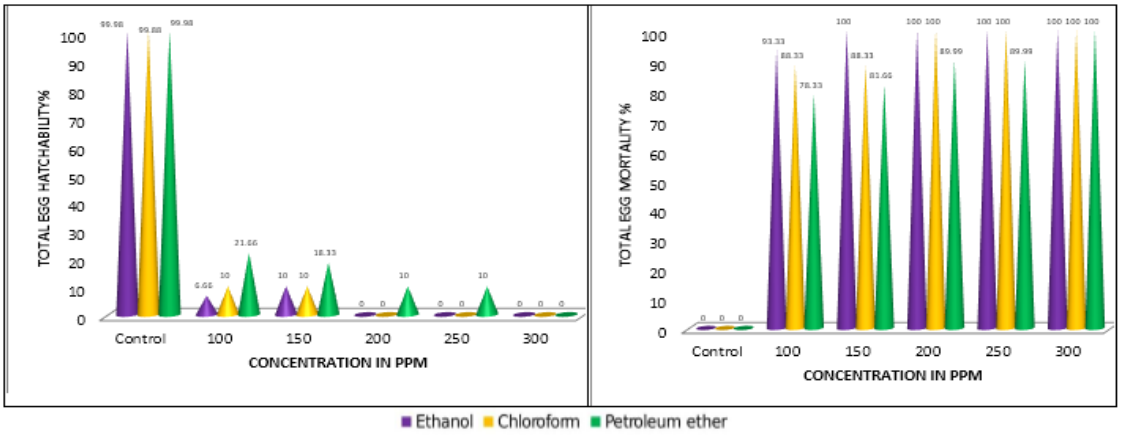


b) 72 h



c) 96 h

Fig 1: Effect of *C. roseus* leaf extracts on egg hatchability of *Cx. quinquefasciatus*



a) Total egg hatchability **b)** Total egg mortality

Fig 2: Effect of *C.roseus* leaf extracts on total egg hatchability and mortality of *Cx.quinquefasciatus*

Table 1: Lethal concentration of *C. roseus* leaf extracts against eggs of *Cx.quinquefasciatus*

Solvents Used	Log LC_{50}	Log LC_{70}	Log LC_{90}	LC50 (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression Equation	95% Confidence Limits				χ^2	SE
								UCL (ppm)		LCL (ppm)			
								LC_{50}	LC_{90}	LC_{50}	LC_{90}		
Petroleum ether	1.65	1.92	2.29	45.68	83.34	198.49	Y=1.66+2.00X	218.7	341.9	9.54	115.2	8.1	1.47
Chloroform	1.51	1.75	2.09	32.68	56.37	123.79	Y=1.64+2.21X	1.48	308.4	7.19	1.64	8.33	12.4
Ethanol	-	-	-	-	-	-	-	-	-	-	-	-	-

In the present study, it has been noticed that in the higher concentration, ethanol extract of *C. roseus* possessed strong ovicidal activity against *Cx. quinquefasciatus*. Similar report was given by Jeyasankar and Ramar [32] in which petroleum ether extract of *Andrographis paniculata* when tested for its ovicidal activity against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* showed strong ovicidal activity at higher concentration of 250 ppm.

In a study Pravin *et al* [33] reported that the hatchability of *Ae. aegypti* eggs were decreased when placed in media of acetone leaf extract. The reduction in hatchability was inversely proportional to the concentration of acetone leaf extract. Similar observation were noted in the present study, in which hatchability was inversely proportional to the concentration ie., petroleum ether showed 10%, 8.33%, and 5% egg hatchability at 48h in 100, 150 and 200 ppm concentrations respectively.

Followed by chloroform extract, petroleum ether showed minimum ovicidal activity (Figure 2a). At 250ppm zero percentage egg hatchability was recorded at 96h. Maximum egg mortality was shown in ethanol extract which showed 100% egg mortality in the concentrations ranging from 150–300 ppm. Ethanol extract was followed by chloroform extract which recorded 100% egg mortality at concentrations ranging from 200–300 ppm (Figure 2b).

Roni *et al* [34] carried out ovicidal activity with ethyl acetate, aqueous solution and ethanol leaf extracts of *Nerium oleander* against *An. stephensi* at 100-300 ppm. At a concentration of 100 ppm, the percentage of hatchability was very high and nil hatchability was recorded when the concentration of extract was increased to 300 ppm. Similar findings were reported in the present as the study also showed dose dependent ovicidal activity. Petroleum ether extract showed minimum ovicidal activity in which 100% egg mortality was observed in higher concentration of 300 ppm. LC_{50} and LC_{90} value for petroleum ether extract was noted as 45.68 and 198.49 respectively.

Similar works that is parallel to the present study was carried out by many researchers. Dhanasekaran *et al* [35] tested ovicidal activity of ethanol leaf extracts of *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solenaamplexi caulis* and *Srermacoce hispida* which showed 100% ovicidal activity against *An. stephensi, Ae. aegypti* and *Cx. tritaeniorhynchus* at 300 ppm. Pannerselvam and Murugan [36] reported that among the extracts tested for ovicidal activity against *An. stephensi*, the leaf methanol extract of *A. paniculata* caused 100% mortality at 150 - 300 ppm.

Petroleum ether, chloroform and ethanol extract of the *C. roseus* leaves were screened for the presence of major phytochemical groups. Highest ovicidal activity was shown by ethanol extract of *C. roseus*, which when tested showed the presence of phytochemicals such as alkaloids, tannins, phenols, flavonoids, terpenoids, protein and quinones. Secondary metabolites like alkaloids, phenols, terpenoids, antraquinone, protein and quinones were present in chloroform extract that exhibited moderate ovicidal activity (Table 2). These phytochemical compounds may be the key candidates in the medicinal value of this plant.

Table 2: Phytochemical constituents present in *Catharanthus roseus* leaf extracts

Sl. No.	Constituents	*C. roseus leaf*		
		Ethanol ether extract	Chloroform extract	Petroleum ether extract
1	Alkaloids	+	+	-
2	Tannins	+	–	-
3	Phenols	+	+	-
4	Flavonoids	+	–	+
5	Sterols	-	-	+
6	Terpenoids	+	+	-
7	Saponins	-	-	+
8	Anthroquinones	–	+	-
9	Proteins	+	+	-
10	Quinones	+	+	+

In the present study, the leaf extracts of *C. roseus* showed almost all the tested phytochemicals in different extracts. Similar observation were recorded by Bai *et al* [37] in which phytochemical screening of *Lagerstremia speciosa* yielded phenolic compounds, flavonoids, saponins, tannins such as corosolic acid, ellagitannin, lagerstroemin, gallotannins and penta-O- galloyl-glucopyranase.

Usta *et al* [38] reported that phytocompounds such as flavonoids acts as an effective ovicide when treated at the early stages of egg development and higher concentration of these compounds cause maximum egg mortality. Similar observation were found in the present study in which ovicidal activity of *C. roseus* leaf extract may be due to the presence of many phytochemicals in them. Rajkumar and Jebanesan [39] studied ovicidal activity of flavonoids compounds from *Poncircus trifoliate* against *Ae. aegypti.* The results confirmed the plant to be effective as an ovicide in the early stage of egg development. Phenols are generally known to be important sources of potent insecticides, fungicides, bactericides and herbicides for pest control. Triterpenoids are also credited with mosquito activities as reported by Gbolade [40].

These observations establish extract of *C. roseus* leaves as a potent source of mosquito controlling agent and its possible use for mosquito control. However, mosquito controlling potential of the plant may vary according to the parts of the plant used, solvent choice, season of plant collection, geographical location where the plants were grown, resistant level of mosquito and the application method.

5. Conclusion

The flora of India has rich aromatic plant diversity with potential for development of natural insecticides for control of mosquito and other pests. Therefore, the researchers today are emphasizing on evaluation and characterization of various plants and plant constituents against a number of diseases. It is now believed that nature has given the cure of every disease in one way or another. From the results of the present study it may be confirmed that the leaf extracts of *Catharanthus roseus* have a great potential to be developed in the mosquito control programme. Therefore, this study has proposed new alternative potential biopesticides from local flora, which is

easily available with low technology and can easily be integrated into the ongoing mosquito management.

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