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Efficacy of larvicidal and pupicidal activity of *Catharanthus roseus* aqueous and solvent extracts against *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae)

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ABSTRACT

Objective: To investigate the larvicidal and pupicidal activities of aqueous, ethyl acetate and methanol extracts of *Catharanthus roseus* (*C. roseus*) against malaria and filariasis vectors. **Methods:** The larvicidal and pupicidal activities of *C. roseus* leaf extracts were tested against the fourth instar larvae and pupae of *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*). The mortality was observed after 24 and 48 h post the treatment. The data were subjected to probit analysis to determine the lethal concentrations (LC_{50} and LC_{90}) at which 50% and 90% of the treated larvae or pupae of the tested species were killed. **Results:** The larval and pupal mortality were observed after 24 and 48 h of exposure of aqueous, ethyl acetate and methanol extracts of *C. roseus*; no mortality was observed in the control group. The LC_{50} values against the fourth-instar larvae of *An. stephensi* were 68.62 and 72.04 mg/mL for the aqueous extract, 82.47 mg/mL for the ethyl acetate extract, and 78.80 and 86.64 mg/mL for the methanol extract, while the aqueous, ethyl acetate and methanol extracts had LC_{50} values of 85.21, 76.84 and 94.20 mg/mL against the fourth-instar larvae of *Cx. quinquefasciatus*. The aqueous, ethyl acetate and methanol extracts had LC_{50} values of 118.08, 182.47 and 143.80 mg/mL against the pupae of *An. stephensi* and 146.20, 226.84 and 156.62 mg/mL against the pupae of *Cx. quinquefasciatus*, respectively. **Conclusions:** The aqueous and methanol extracts of *C. roseus* leaves had an excellent potential to control the malarial vector *An. stephensi* and filariasis vector *Cx. quinquefasciatus*.

1. Introduction

Mosquitoes constitute a major public health problem as vectors of serious human diseases like malaria, filariasis, Japanese encephalitis, dengue fever, chikungunya, and yellow fever. Mosquitoes alone transmit disease to more than 700 million people annually[1,2]. *Anopheles stephensi* (*An. stephensi*) transmits malaria in the plains of rural and urban areas of India. Malaria afflicts 36% of the world population, i.e., 2020 million in 107 countries and territories situated in the tropical and subtropical regions. In the South East Asian Region of WHO, out of about 1.4 billion people living in 11 countries, 1.2 billion (85.7%) are exposed to the risk of malaria, most of whom live in India. Of the 2.5 million reported cases in South East Asia, India alone contributes about 70% of the total cases[3].

Culex quinquefasciatus (*Cx. quinquefasciatus*) is the vector of lymphatic filariasis which is a widely distributed tropical disease with around 120 million people being infected worldwide and 44 million people having common chronic manifestation[4]. Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pests and vectors. Plants are considered as a rich source of bioactive chemicals and they may be an alternative source of mosquito control agents. Natural products are generally preferred because of their less harmful nature to non-target organisms and due to

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their innate biodegradability[5].

Today, synthetic insecticides are at the forefront of mosquito-controlling agents. However, the environmental threat that these chemicals pose, effects on non-target organisms and the resistance of mosquitoes to insecticides have all increased during the last five decades[6]. These limitations therefore necessitate the search for new control method which may replace these synthetic insecticides for controlling mosquitoes which are more environmentally safe and also biodegradable and target specific against the mosquitoes. In fact, many researchers have reported the effectiveness of plant extracts or essential oils against mosquito larvae[7].

Several plants have been demonstrated toxic effects on mosquito larvae. Saxena *et al*. discovered growth inhibitory and juvenile hormone mimicing activity in the larvae of *Cx. quinquefasciatus* treated with acetone extracts of *Ageratum conyzoides*, *Cleome icosandra* and *Tridax procumbens*[8]. *Annona squamosa* and *Lansium domesticum* showed larvicidal potential against *Cx. quinquefasciatus*[9]. Leaf acetone, chloroform, ethyl acetate, hexane, and methanol extracts of *Aegle marmelos*, *Andrographis lineata*, *Andrographis paniculata*, *Eclipta prostrata* and *Tagetes erecta* showed good larvicidal activity against the fourth-instar larvae of *Cx. tritaeniorhynchus*[10]. Ethyl acetate and methanol extracts of *Acacia concinna*, *Cassia siamea*, *Coriandrum sativum*, *Cuminum cyminum*, *Lantana camara*, *Nelumbo nucifera*, *Phyllanthus amarus*, *Piper nigrum* and *Trachyspermum ammi* exhibited good larvicidal and repellent activities against *An. stephensi* and *Cx. quinquefasciatus*[6].

Catharanthus roseus (L.) G. Don (*C. roseus*; formerly *Vinca rosea* L.) is an important medicinal plant that accumulates dimeric terpenoid indole alkaloids like vinblastine and vincristine in its leaves. The dried leaf powder of *C. roseus* was used to assess the bioefficacy on *Aedes aegypti* (*Ae. aegypti*)[11].

In the light of earlier literature, it is known that larvicidal and pupicidal agents play a vital role in controlling mosquitoes in their breeding sites, but vector resistance still remains unanswered. In addition, they show a negative impact in areas of beneficial and non-target organisms. In view of the recently increased interest in developing plant-origin insecticides as an alternative to chemical insecticides, this study was undertaken to assess the larvicidal and pupicidal potential of the extracts from *C. roseus* against two medically important species, *An. stephensi* (malaria vector) and *Cx. quinquefasciatus* (filarial vector).

2. Materials and methods

2.1. Plant collection

Leaves of *C. roseus* (Apocynaceae) were collected from Cuddalore District, Tamil Nadu, India. It was authenticated by a plant taxonomist from Department of Botany, Annamalai University. Voucher specimen has been deposited in the laboratory of Zoology, Annamalai University.

2.2. Mosquito culture

Larvae of *An. stephensi* and *Cx. quinquefasciatus* were collected from pond and stagnant water areas of Annamalainagar and identified in Zonal Entomological Research Centre (Cuddalore, Tamil Nadu, India). The colony and larvae were kept in plastic and enamel trays containing tap water. They were maintained and all the experiments were carried out at (27±2) °C and 75%-85% relative humidity under 14 h/10 h light and dark cycles. The larvae were fed on a diet composed of Brewer's yeast, dog biscuits and algae collected from ponds in a ratio of 3:1:1[12]. The feeding was continued until the larvae transformed into the pupal stage.

2.3. Maintenance of pupae

Pupae were transferred from the trays to a cup containing tap water and were maintained in an insectary (45 cm×45 cm×40 cm) where adults emerged. Adults were maintained in glass cages and were continuously provided with 100 g/L sucrose solution in a jar with a cotton wick. On day 5, the adults were given a blood meal from a pigeon placed in resting cages overnight for blood-feeding by females. Glass Petri dishes with 50 mL of tap water lined with filter paper were kept inside the cage for oviposition[12].

2.4. Preparation of solvent extract of C. roseus leaves

The dried leaves of *C. roseus* (650 g) were powdered mechanically using a commercial electrical stainless steel blender and extracted with 1 000 mL of aethyl acetate and 1 000 mL of methanol (Qualigens, Mumbai, India) in a Soxhlet apparatus separately until exhaustion. The extract was concentrated under reduced pressure 22-26 mm Hg (1 mmHg=0.133 kPa) at 45 °C and the residue obtained was stored at 4 °C.

2.5. Preparation of aqueous extract of C. roseus leaves

Around 500 g air-dried *C. roseus* leaf powder was soaked in 2 000 mL of distilled water for about 24 h. The aqueous extract was then evaporated under reduced pressure in a rotary evaporator, and the residue was dissolved in a small quantity of water and subjected to freeze-drying. Freeze-dried extracts was collected in small glass bottles and kept at 0 °C for further evaluation.

2.6. Larval toxicity test

During preliminary screening with the laboratory trial, the mosquito larvae were collected from the insect-rearing cage. One gram of the crude extract was first dissolved in 100 mL of respective solvent and distilled water (stock solution). From the stock solution, 50 mg/mL was prepared with dechlorinated tap water. Polysorbate 80 (Qualigens, Mumbai, India) was added as an emulsifier at a volume ratio of 0.05%

in the final test solution. The larvicidal activity was assessed by the procedure of WHO[13]. For bioassay test, the larvae were taken in five batches of 20 in 249 mL of water and 1.0 mL of the desired plant extracts concentration (10, 20, 30, 40 and 50 mg/mL). The control was set up with respective solvent, distilled water and polysorbate 80. The numbers of dead larvae were counted after 24 and 48 h of exposure, and the percentage of mortality was reported from the average of three replicates. The experimental media in which 100% mortality of larvae occurred alone were selected for dose-response bioassay.

2.7. Pupal toxicity test

A laboratory colony of the mosquito pupae was used to observe the pupicidal activity of *C. roseus*. Twenty-five individuals of freshly emerged pupae were kept in a 500-mL glass beaker containing 249 mL of dechlorinated water and 1 mL of desired plant extract concentration (10, 20, 30, 40 and 50 mg/mL). At each tested concentration, 2-5 trials were made and each trial consisted of three replicates. The control was set up with respective solvent, distilled water and polysorbate 80. The numbers of dead pupae were counted after 24 and 48 h of exposure, and the percentage of mortality was reported from the average of three replicates.

2.8. Dose-response bioassay

From the stock solution, different concentrations ranging from 10 to 50 mg/mL were prepared. Based on the preliminary screening results, the crude solvent and aqueous extracts of *C. roseus* leaves were subjected to dose-response bioassay for larvicidal and pupicidal activity against the larvae and pupae of *An. stephensi* and *Cx. quinquefasciatus*. The numbers of dead larvae and pupae were counted after 24 and 48 h of exposure, and the percentage of mortality was reported from the average of three replicates.

2.9. Statistical analysis

The data of average larval and pupal mortality were subjected to probit analysis for calculating lethal concentration of 50% and 90% (LC_{50} and LC_{90}) and other statistics at 95% fiducial limits of the upper confidence limit and lower confidence limit, and chi-square values were calculated by using the FORTRAN program[14] for rapid determination of lethal concentration. Results with $P<0.05$ were considered to be statistically significant.

3. Results

3.1. Larvicidal activity of C. roseus leaf extract against An. stephensi and Cx. quinquefasciatus

The mortality rates of the fourth-instar larvae of *An. stephensi* and *Cx. quinquefasciatus* exposed to the crude extracts of *C. roseus* leaves are presented in Table 1. Among the different treatment groups, 100% mortality in the larvae of *An. stephensi* was observed when they were exposed to 50 mg/mL aqueous or methanol extracts for 24 and 48 h or to the 50 mg/mL ethyl acetate extract for 48 h. For *Cx. quinquefasciatus*, 100% mortality in the larvae was observed when they were exposed to 50 mg/mL aqueous, methanol or ethyl acetate extracts for 48 h. The LC_{50} and LC_{90} values of the extracts against the larvae of *An. stephensi* and *Cx. quinquefasciatus* at 48 h are shown in Table 1. The chi-square values were significant at $P<0.05$ level (Table 1).

3.2. Pupicidal activity of C. roseus leaf extract against An. stephensi and Cx. quinquefasciatus

Table 2 illustrates the considerable pupal mortality after the treatment with the aqueous, ethyl acetate and methanol extracts of *C. roseus* leaves. After 48 h of exposure to the extracts at the concentration of 50 mg/mL, 100% mortality was observed in the pupae, except that 93% mortality was observed in the pupae treated with the ethyl acetate extract. The LC_{50} and LC_{90} values of the extracts against the pupae of *An. stephensi* and *Cx. quinquefasciatus* at 48 h are represented in Table 2.

4. Discussion

In this study, the aqueous, methanol and ethyl acetate extracts of *C. roseus* leaves showed potential larvicidal and pupicidal activities against *An. stephensi* and *Cx. quinquefasciatus*. The biological activity of the experimental plant extracts may be attributed to the presence of various compounds, including phenolics, terpenoides, flavonoids and alkaloids[15], which may jointly or independently contribute to produce larvicidal and pupicidal activity against *An. stephensi* and *Cx. quinquefasciatus*. Alam *et al.* has demonstrated the petroleum ether fraction of *C. roseus* possesses good larvicidal properties against *An. stephensi* (LC_{50}=150 mg/L)[16]. Other extracts of several plants have also been proved to have larvicidal activity against *An. stephensi* and *Cx. tritaeniorhynchus*. The methanol extract of *Ervatamia coronaria* (Family: Apocynaceae) leaves showed good larvicidal activity against larvae of *Cx. quinquefasciatus* (LC_{50}=72.41 mg/L; LC_{90}=65.67 mg/L at 24 h), *Ae. aegypti* (LC_{50}=62.08 mg/L; LC_{90}=136.55 mg/L at 24 h) and *An. stephensi* (LC_{50}=127.24 mg/L; LC_{90}=120.86 mg/L at 24 h)[17]. The methanol extract of *Ervatamia coronaria* also showed potential larvicidal activity against the early third instar of *An. subpictus* (LC_{50}=86.47 mg/L; LC_{90}=159.59 mg/L) and *Cx. tritaeniorhynchus* (LC_{50}=131.53 mg/L; LC_{90}=245.00 mg/L)[18]. The petroleum ether extract of *Rauvolfia serpentine* (Family: Apocynaceae) seeds showed strong activity against the third instar larvae of *Cx. quinquefasciatus* after 24 h of exposure[19].

Our results agree with those obtained from some previous studies. The leaf methanol extract of *Cassia fistula* was tested for larvicidal activity against *Cx. quinquefasciatus* and *An. stephensi*, respectively with the LC_{50} values of 17.97 and 20.57 mg/L[20-24]. The aqueous extract of *Calotropis*

Table 1
Larval toxicity of the extracts of *C. roseus* leaves against *An. stephensi* and *Cx. quinquefasciatus*.

Extract	Mosquito	Extract concentration (mg/mL)	Mortality (%)		LC_{50} (LCL-UCL) (mg/mL)	LC_{90} (LCL-UCL) (mg/mL)	Chi square value (*df*=4)
			24 h of exposure	48 h of exposure			
Aqueous extract	*An. stephensi*	50	100±1.20		68.62±8.42 (28.39-46.26)	184.85±16.50 (84.26-116.19)	12.86
		40	72±2.06				
		30	52±1.82				
		20	28±1.40				
		10	17±1.82				
Aqueous extract	*An. stephensi*	50		100±0.00	72.04±10.64 (36.28-52.08)	236.36±24.20 (120.50-168.46)	16.06
		40		86±2.18			
		30		68±1.20			
		20		46±1.42			
		10		23±1.68			
Aqueous extract	*Cx. quinquefasciatus*	50	89±2.60	100±0.00	85.21±0.84 (18.64-28.75)	262.70±26.82 (52.92-79.31)	10.52
		40	68±2.81	84±1.82			
		30	41±1.06	60±4.20			
		20	36±1.28	42±1.87			
		10	28±1.10	38±2.69			
Ethyl acetate extract	*An. stephensi*	50	79±2.60	100±0.00	82.47±10.89 (92.72-144.22)	254.33±17.58 (126.44-187.22)	10.79
		40	58±2.81	86±0.82			
		30	42±1.06	68±1.60			
		20	26±1.28	43±1.48			
		10	18±1.10	24±2.04			
Ethyl acetate extract	*Cx. quinquefasciatus*	50	86±3.02	100±0.00	76.84±10.04 (142.46-72.81)	248.62±17.89 (184.09-129.14)	14.78
		40	74±0.82	86±2.40			
		30	52±4.24	74±1.64			
		20	40±1.27	62±1.32			
		10	32±4.60	48±2.04			
Methanol extract	*An. stephensi*	50	100±0.00		78.80±8.78 (42.34-77.27)	220.54±12.68 (96.78-157.95)	10.75
		40	76±2.82				
		30	62±4.20				
		20	42±1.87				
		10	22±2.69				
Methanol extract	*An. stephensi*	50		100±0.00	86.64±12.86 (48.62-96.14)	268.60±18.56 (128.16-186.62)	16.84
		40		84±2.60			
		30		68±3.24			
		20		46±2.08			
		10		28±4.20			
Methanol extract	*Cx. quinquefasciatus*	50	86±1.87	100±0.00	94.20±14.06 (38.43-86.46)	286.50±22.60 (132.62-178.40)	14.80
		40	64±2.02	84±1.82			
		30	52±3.80	60±4.20			
		20	30±2.67	42±1.87			
		10	26±2.06	38±2.69			

The mortality (Mean±SD) is the mean value of three replicates. Nil mortality was observed in the control. The LC_{50} and LC_{90} (Mean±SE) is the lethal concentration at which 50% and 90% of the exposed larvae are killed, respectively. LCL: lower confidence limit; UCL: upper confidence limit; df: degree of freedom. All the chi-square values were significant at $P<0.05$ level.

Table 2
Pupal toxicity of the extracts of *C. roseus* leaves against *An. stephensi* and *Cx. quinquefasciatus*.

Extract	Mosquito	Extract concentration (mg/mL)	Mortality (%)		LC_{50} (LCL-UCL) (mg/mL)	LC_{90} (LCL-UCL) (mg/mL)	Chi square value (*df*=4)
			24 h of exposure	48 h of exposure			
Aqueous extract	*An. stephensi*	50	73±1.26	100±0.00	118.08±10.26 (68.43-96.14)	314.18±26.82 (184.36-268.29)	16.28
		40	69±2.06	89±2.18			
		30	56±1.82	68±1.20			
		20	34±1.40	45±1.42			
		10	15±1.82	26±1.68			
Aqueous extract	*Cx. quinquefasciatus*	50	89±1.60	100±0.00	146.20±12.24 (86.14-108.26)	328.30±28.02 (196.09-279.40)	14.42
		40	65±2.56	76±1.56			
		30	32±1.46	58±3.20			
		20	29±1.03	32±2.23			
		10	17±1.76	26±2.59			
Ethyl acetate extract	*An. stephensi*	50	68±2.60	100±0.00	182.47±16.80 (110.62-146.40)	364.38±27.48 (186.44-277.29)	18.70
		40	58±3.81	88±0.82			
		30	32±1.26	66±1.72			
		20	29±1.85	43±1.52			
		10	12±1.05	24±1.04			
Ethyl acetate extract	*Cx. quinquefasciatus*	50	72±3.02	93±2.00	226.84±16.04 (125.84-188.06)	368.09±27.82 (219.14-284.09)	15.08
		40	64±1.82	78±1.56			
		30	32±2.36	64±1.64			
		20	20±1.07	42±1.65			
		10	12±2.60	38±1.09			
Methanol extract	*An. stephensi*	50	84±1.80	100±0.00	143.80±12.78 (96.30-127.27)	346.54±24.68 (192.78-276.95)	18.05
		40	68±1.40	64±1.82			
		30	46±2.68	52±3.20			
		20	24±2.82	36±1.86			
		10	16±3.54	26±4.04			
Methanol extract	*Cx. quinquefasciatus*	50	86±1.10	100±0.00	156.62±18.37 (116.30-142.60)	362.48±24.12 (214.80-294.20)	17.24
		40	60±3.06	78±1.11			
		30	45±1.34	55±2.23			
		20	26±1.07	36±1.23			
		10	13±1.10	22±2.79			

The mortality (Mean±SD) is the mean value of three replicates. Nil mortality was observed in the control. The LC_{50} and LC_{90} (Mean±SE) is the lethal concentration at which 50% and 90% of the exposed larvae are killed, respectively. LCL: lower confidence limit; UCL: upper confidence limit; df: degree of freedom. All the chi-square values were significant at $P<0.05$ level.

procera leaves had 50% of adult emergence inhibition of 277.90 and 183.65 mg/L respectively for *An. arabiensis* and *Cx. quinquefasciatus*, and the pupal stage was not affected till a concentration of 5 000 mg/L[25]. The larvicidal, growth inhibiting and repellent actions of *Dalbergia sissoo* oil was evaluated against *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* under laboratory conditions and no adult emergence was observed at a concentration of 4 mL/m^2[26].

The pure limonoids of neem seed were tested for biological, larvicidal, pupicidal, adulticidal, and antiovipositional activities against *An. stephensi*, and the results showed that the larval mortality was dose-dependent with the highest dose of 1 mg/L azadirachtin evoking almost 100% mortality, affecting pupicidal and adulticidal activities and significantly decreased fecundity and longevity of *An. stephensi*[27]. In the study of Kamaraj *et al*., the petroleum ether extract of *Cassia auriculata* leaves and methanol extract of its flowers caused the highest mortality against the larvae of *An. subpictus* and *Cx. tritaeniorhynchus*[12].

All the toxins used in vector control pose some hazards to the user and also to the aquatic environment. Hence, this research is mainly focused on finding newer insecticides which are more effective, biodegradable and easily available at low cost. In our observation, the aqueous, ethyl acetate and methanol extracts of *C. roseus* leaves possessed higher larvicidal and pupicidal activities against the malaria and filariasis vectors.

In conclusion, an attempt has been made to evaluate the possible role of medicinal plant extracts in the control of mosquitoes. Natural products are generally preferred in vector control measures due to their less deleterious effect on non-target organisms and their innate biodegradability. In the context of resistance developed by the mosquito larvae against chemical insecticides, it is worthwhile to identify new active compounds from natural products against mosquitoes. Hence, the results reported here open the possibility of further investigations on the larvicidal and pupicidal efficacy of medicinal plant extracts, and they may be exploited against mosquitoes. The bioassay-guided fractionation, purification and isolation of pure compounds

from the crude aqueous and methanol extract of *C. roseus* leaves are in progress.

Conflict of interest statement

We declare that we have no conflict of interest.

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