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Phytochemical screening of *Barleria longiflora* (L.) and its Mosquitocidal properties against *Culex quinquefasciatus*

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Abstract
Mosquitoes are responsible for transmitting various human diseases and contribute to millions of deaths each year. This study examines the effectiveness of leaf extracts from *Barleria longiflora* on larvae of the filarial vector, *Culex quinquefasciatus*. The preparation of extracts were using petroleum ether, ethyl acetate, and ethanol. Larvicidal activity assays were conducted on the *B. longiflora* extracts at different concentrations of 0.625%, 1.25%, 2.5%, and 5%. The larval mortality in percentage was assessed after 48 and 72 hours. The ethyl acetate extract of *B. longiflora* revealed the highest efficacy on Cx. quinquefasciatus larvae at the 5% concentration, with a lethal concentration (LC50) value of 6.439. The crude extracts of *B. longiflora* displayed significant potential for vector control. These extracts may contain active phytochemicals that require further investigation. Additionally, plant-derived compounds offer a positive, eco-friendly method for mosquito vector control programs.

Keywords: *Barleria longiflora, Culex quinquefasciatus,* larvicidal, leaf extracts.

Introduction
Mosquito cause most of the deadly illnesses, such as Malaria, Chikungunya, and Yellow fever (Mittal, 2003) [14]. In most countries, mosquito-borne diseases cause 70,000,000 persons globally, and 40,000,000 of these persons are Indians. The endless use of these artificial insecticides has led to concerns about their resistance. 40% of the world's population mostly lives in the poorest countries. 2.5 million People are at risk of malaria; more than 50 million become severely ill with malaria each year, and over one million die from the disease (WHO, 2007). The best way to control mosquito infestations is by preventing their breeding, particularly through the use of larvae prevention methods. Controlling the mosquito population is undeniably more effective at the larval stage compared to the adult stage (Naik *et al.*, 2005) [16]. The excessive usage of synthetic chemical pesticides leads to the prevention of vector populations, resulting in the perseverance and increase of non-ecological toxic compounds in ecosystems. This adversely affects humans and non-target organisms while contributing to natural reduction in the food chain (Jeyasankar *et al.*, 2012) [10]. To prevent the spread of several mosquito diseases and maintain the environment's quality for human health. Mosquito control is essential for humans around the world (Jamal *et al.*, 2021) [7]. More than several studies have controlled the mosquito larvae to use plant extracts all over the globe (Bagavan and Rahuman, 2011) [3].

Generally identified as known Kattukanakambaram or *Barleria longiflora* L. is a member of Acanthaceae. In traditional medicine, it is regarded as one of the most significant medicinal plants (Baskaran *et al.*, 2016) [4]. The specific plant parts were used to extract compounds for mosquito control. The purpose of phytochemical screening is to identify the secondary metabolites used in mosquito control. They used a lot of methods to control the mosquitoes, including larvicidal, pupicidal, ovicidal, adulticidal, and repellent activities. Secondary metabolites are used to prevent important medical issues. Plant utilization is safe, avoids the side effects, is degradable in soil naturally, and increases mosquito vector species' resistance (Jeyasankar and Ramar, 2016) [10]. To enhance knowledge, avoid chemical pesticides, use botanical pesticides instead. The present study aimed the *Barleria longiflora* to assess the preliminary phytochemical analyses and larvicidal properties with using extracts of different solvents on Culex quinquefasciatus.

Materials and Methods
Collection of plant materials
Barleria longiflora leaves were collected in August 2023 from Theerthamalai in the Dharmapuri district of Tamil Nadu, India. The plant was identified by Dr. S. John Britto, Director of The Rapinat Herbarium and Centre for Molecular Systematics at St. Joseph's College in Tiruchirapalli, Tamil Nadu, India. *B. longiflora* specimen of voucher (IPH No. 3) was arranged and placed at the PG and Research Department of Zoology at Government Arts College (Autonomous) in Coimbatore, Tamil Nadu, India.

Soxhlet extraction method
The leaf was shade dried at room temperature (27.0±2°C and 75 ± 5% RH). After shade drying, the plant leaf was powdered by utilizing an electric blender. 100 grams of powdered leaf sample of *Barleria longiflora* was extracted in 250 mL using the soaking method with different solvents of Petroleum ether, Ethyl acetate, and Ethanol. Samples were left at room temperature for 72 hours. The sample was filtered using Whatman's No. 1 filter paper and concentrated using a vacuum rotary evaporator (Yamato Scientific Co., Ltd RE 600, Japan) and then air dried. Crude extracts were collected until a thick and viscous paste or powder of extract was visible. The concentrates were gathered in clean borosilicate vials and stored in the refrigerator for experiments against important mosquito vectors.

Qualitative Phytochemical Screening
The *Barleria longiflora* plant leaf extracts were examined for the presence of major phytochemicals, and examinations

for secondary metabolites were performed for all the centrifuged filtrates (successive extracts) according to regular procedures.

Mosquito species & Vector rearing

The larvae of *C. quinquefasciatus* were collected from the NCDC (National Centre for Disease Control) located in Mettupalayam, Coimbatore District, India. The larvae were stored in plastic trays full of tap water and were provided with dog biscuits and yeast once a day during the later stages of development. For oviposition, an oviposition cage measuring 44x44x43 cm was used. In the cage pupae were transferred to a cup containing tap water and placed. Once the adults emerged, a 10% sucrose solution was provided for feeding. On the third day after emergence, broiler chicken (*Gallus gallus domesticus*) was used to provide a blood meal to the adult mosquitoes. Small plastic bowls filled with tap water were placed in the cage to facilitate oviposition. The temperature was maintained at 28 ± 2°C with a relative humidity of 70-80%, under a light and dark cycle of 14:10 hours (Kamaraj *et al*., 2009) [11].

Larvicidal activity

The freshly moulted 4th instar *C. quinquefasciatus* larvae were tested against plant extracts, with the dissolving of 2 drops of Tween 20 added and diluted with 100 mL of dechlorinated water to obtain desired concentrations. The control was prepared in 100 mL of dechlorinated water by adding 2 to 3 drops of Polysorbate 20 (Tween 20). The bioassay is prepared, and five replications are maintained in 250-ml transparent cups. The number of freshly moulted 4th instar larvae of mosquitoes was added in respective concentrations of plant extracts. The results were noted and reported after the treatment of 48 hours and 72 hours. The probit analysis was used for LC50 calculations (Finney, 1971) [6]. The statistical package of social science (SPSS) version 16.0 for Windows software was used to calculate LC50, LC90, and other statistics. Chi-square values were calculated.

Correctedmortality

$$\frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \text{ X } 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \text{ X}100$$

Statistical analysis

A profit analysis was conducted using MS Excel 2007. The term LC50 refers to the concentration of a test substance that results in a 50% mortality rate of the test organisms within a specified exposure time. By showing mosquitoes at various developmental stages to different concentrations of the extract, a suitable concentration was identified. SPSS 16.0 software was used for probit analysis and the LC50 and LC90 values, along with their 95% confidence interval limits, based on the mortality rates observed during the bioassays with the test organisms.

Results

Phytochemical analysis

Qualitative phytochemical screening

The *Barleria longiflora* was identified as the major secondary metabolite using preliminary phytochemical screening and the results are presented in Table 1. The results indicated that the secondary metabolites, including alkaloids, flavonoids, tannins, steroids, triterpenoids, and glycosides, tested positive in all extracts examined.

In the present study, we tested the larvicidal activity of *Barleria longiflora* extracts of petroleum ether, ethyl acetate, and ethanol against *C. quinquefasciatus*. The tables 2 and 3 revealed the results of larval mortality observed at 48 and 72 hours and including the LC50 and LC90 values, are presented.

This study of results indicated the exposure time increased, the mortality rate also increased. After 72 hours, the highest larvicidal activity was observed and compared to 48 hours, suggesting that plant extracts had a gradual effect on the developmental system of the larvae. Extracts with a lower concentration of 0.625% demonstrated a steady increase in larval mortality, reaching a maximum mortality at 5% higher concentrations.

The extract of ethyl acetate was more susceptible to larvae of *Culex quinquefasciatus*, showing that 50% mortality was recorded in 5% at 48 hours, and 60% mortality was found in 72 hours.

Table 1: Preliminary phytochemical screening of crude extracts of *Barleria longiflora*

S. No.	Chemical Constituent	Tests	Organic solvents		
			PE	EA	Et
1	Alkaloids	a) Dragendorff's test	_	+	+
		b) Mayer's test	-	-	+
		c) Picric acid test	-	-	+
2	Flavonoids	10% HCl& 5% NaOH test	+	-	+
3	Tannins	5% $FeCl_3$ test	-	-	+
4	Steroids	Liebermann-Burchard's test	+	+	+
5	Triterpenoids	a) Liebermann-Burchard's test	_	+	+
		b) Salkowski's test	-	-	-
6	Saponins	Foam test	-	-	-
7	Glycosides	Keller - Kiliani test	+	+	-
8	Gum &Mucilages	Whistler &BeMiller test	-	-	-
9	Fixed oils	Spot test	-	-	-
10	Anthraquinones	Sanker and Nahar test	_	_	_

PE – Pertroleum ether, EA – Ethyl acetate, E – Ethanol, (+ present, - absent)

Table 2: Larvicidal activity of *Barleria longiflora* leaf extracts against *Culex quinquefasciatus* at 48 hrs

Solvents	Concentrations Tested (ppm)	Larval Mortality (%)	LC_{50} (ppm) 95%Confidence Limits (ppm) (LCL – UCL)	LC_{90}(ppm) 95%Confidence Limits (ppm) (LCL – UCL)	χ^2 (df=4)
Petroleum ether	Control	0	6.036 (1.25 – 10.11)	224.76 (218.70 – 245.80)	0.955
	0.625	10			
	1.25	30			
	2.5	30			
	5	40			
Ethyl acetate	Control	0	5.439 (2.60 – 11.75)	217.72 (198.55 – 233.33)	0.996
	0.625	20			
	1.25	30			
	2.5	30			
	5	50			
Ethanol	Control	0	7.677 (2.55 – 13.42)	233.56 (221.70 – 241.60)	0.294
	0.625	10			
	1.25	20			
	2.5	30			
	5	40			

LC_{50} = Lethal Concentration 50; LC_{90} = Lethal Concentration 90.
LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table 3: Larvicidal activity of *Barleria longiflora* leaf extracts against *Culex quinquefasciatus* at 72 hrs

Solvents	Concentrations Tested (ppm)	Larval Mortality (%)	LC_{50} (ppm) 95%Confidence Limits (ppm) (LCL – UCL)	LC_{90}(ppm) 95%Confidence Limits (ppm) (LCL – UCL)	χ^2 (df=4)
Petroleum ether	Control	0	4.442 (1.70 – 8.40)	150.12 (134.60 – 165.90)	0.185
	0.625	20			
	1.25	40			
	2.5	40			
	5	50			
Ethyl acetate	Control	0	3.554 (1.25 – 7.10)	110.73 (96.70 – 122.40)	0.169
	0.625	30			
	1.25	30			
	2.5	40			
	5	60			
Ethanol	Control	0	4.805 (1.40 – 7.90)	115.55 (99.26 – 129.66)	0.202
	0.625	20			
	1.25	30			
	2.5	40			
	5	50			

LC_{50} = Lethal Concentration 50; LC_{90} = Lethal Concentration 90.
LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

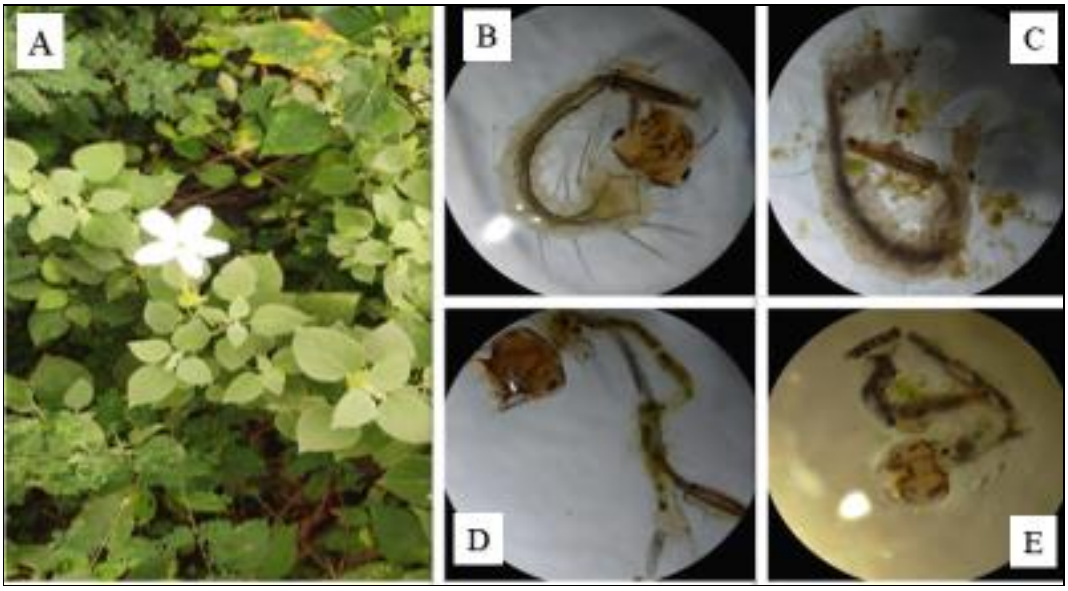

Fig 1: A- *Barleria longiflora*. B- Control larvae of *C. quinquefasciatus,* head, thorax, midgut, and anal gill parts. C to E- *C. quinquefasciatus* treated with 5% of ethyl acetate extract of *B. longiflora* showing toxic effects on different body parts (thorax, midguts, anal gills) as well as losing external hairs, crumbling outer cuticle of the epithelial layer, abdominal breakage and larvae shrinkage.

Discussion

The biologically active plants are biodegradable, with no side effects on animals, humans, and non-target organisms in the environment. So the scientists involved in the plant-based products are to apply the insecticidal properties all over the world. Plant extract compounds to control the human vector mosquitoes to thereby avoiding the use of chemical pesticides. The recent investigations reported that the plant compounds control the insecticidal properties to resistance of vector mosquitoes. Several authors have already described similar activities.

In this investigation, we tested the larvicidal activity of *B. longiflora* leaf extracts using petroleum ether, ethyl acetate, and ethanol against *C. quinquefasciatus*. The results revealed that the larval mortality rate increased with longer exposure to the extracts. By reaching 72 hours, the larval mortality was at a maximum level. So, finally, *Culex* showed much susceptibility to the extracts. The results obtained were similar to those obtained in earlier reports. Larvicidal activity of several Euphorbiaceae plants, such as *J. curcas (Jatropha curcas), P. tithymaloides (Pedilanthus tithymaloides), P. amarus (Phyllanthus amarus), E. hirta (Euphorbia hirta),* and *E. tirucalli* (*Euphorbia tirucalli),* was assessed in various solvents of ethyl acetate, butanol, and petroleum ether to assess their efficacy against *A. aegypti* and *C. quinquefasciatus*. Crude extracts were evaluated on freshly moulted 4th instar larvae of *A. aegypti* and *C. quinquefasciatus*. They confidently recorded larval mortality after 24 hours, with petroleum ether extracts showcasing the most effective larvicidal properties. The *Jatropha curcas* and *Euphorbia tirucalli* petroleum ether extracts achieved notable larval mortality rates for *A. aegypti* (LC50 = 8.79 and 4.25 ppm, respectively) and *C. quinquefasciatus* (LC50 = 11.34 and 5.52 ppm, respectively). These findings demonstrate the significant potential of these extracts in controlling mosquito larvae. (Rahuman et *al.,* 2008) [17].

Maheshwaran *et al.*, (2008) worked with *C. phlomidis* plant crude extracts tested against larvicidal activity of *C. quinquefasciatus* and observed 72% larval mortality in the chloroform extracts against Culex. The fraction 5 values of LC_{50} were 5.02 and 32.86 ppm, respectively.

Ethyl acetate extracts of *Ocimum sanctum* leaves tested against *A. aegypti* and *C. quinquefasciatus.* LC50 values are 425.94 ppm and 592.60 ppm, respectively (Anees, 2008) [2]. Elango *et al.* (2009) [5] studied the LC50 values of ethyl acetate extracts from Aegle marmelos leaves were found to be 167.00 and 99.03 ppm against *Culex* mosquitoes. Rawani *et al.*, (2010) [20] investigated the *Solanum nigrum* leaves of ethyl acetate extracts on 4th instar larvae of *C. quimquefasciatus*. The LC50 value of 17.04 ppm after 24 hours of exposure.

Kamaraj *et al.*, (2011) [11] tested the larvicidal activity of *T. procumbens* leaf crude ethyl acetate extracts on *Cx. tritaeniorhynchus* and showed the highest mortality. The *Phyllanthus emblica* Linn ethyl acetate extracts exhibited a significant larvicidal effect, achieving a 90% larval mortality at 250 ppm when applied to *C. quinquefasciatus* (Jeyasankar *et al.,* 2012) [10]. *S. mahagoni* leaf Ethyl acetate extract exhibited promising mortality against *Culex* with LC50 values of 51.45, 45.65 and 40.55 ppm at 72h, respectively (Adhikari and Chandra, 2014) [1].

The *Melia azedarach* leaf extracts, specifically those extracted with acetone, ethyl acetate, butanol, and chloroform, were tested for larvicidal activity on 3rd instar larvae of *C. quinquefasciatus* and *A. aegypti*. The ethyl acetate extract demonstrated the highest larvicidal activity and effectiveness on mosquito vectors (Ravichandran and Kanayairam, 2014).

Jeyasankar and Ramar (2014) [8] reported that hexane extracts from the leaves of *Tragia involucrata* demonstrated larvicidal activity. After 24 hours, an LC50 value of 153.51 ppm (Ramar and Jeyasankar, 2014) [18]. The Phyllanthaceae family of *Breynia Vitis-idea* leaf extracts was established for toxicity on 3rd instar larvae of *A. aegypti*, *C. quinquefasciatus* and *A. stephensi*, showing respectively LC50 values of 98.2, 107.79, and 115.8 ppm. Ramar and Jeyasankar, (2018) reported, the results revealed that the lowest and highest mortality rates were observed at 25 ppm (23.32, 26.33 and 22.46) and 400 ppm (97.20, 99.00 and 99.24) and respectively for *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. Similarly, Elango *et al.,* (2009) [5] revealed that the *E. prostrata* ethyl acetate extract exhibited an LC50 value of 78.28 and an LC90 value of 360.75 ppm on *A. subpictus*, and an LC50 of 119.89 and an LC90 of 564.85 ppm against *Cx. tritaeniorhynchus.* Mondal *et al.*, (2022) [15], many researchers throughout the globe evaluated the larvicidal properties of various plant parts on various vector mosquitoes.

The findings of the current study indicate that *B. longiflora* leaves of ethyl acetate extract possess significant mosquitocidal properties against vector mosquitoes. Generally, phytochemicals are ecological and harmless to living and non-living organisms. Thorough screening of particular plant phytochemicals leads to the rapid elimination of various vector mosquito species. In conclusion, established on these results, plant-derived compounds hold considerable potential for incorporation into mosquito vector control programs. The diverse applications of these compounds can greatly contribute to human health across the globe. Therefore, upcoming research must focus on the useful application of these bioactive compounds.

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