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PHYTOCHEMICAL SCREENING AND LARVICIDAL ACTIVITY ON THE WHOLE PLANT OF *BARLERIA CUSPIDATA* ETHANOLIC EXTRACT

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Mosquitoes, *Barleria cuspidata*, Larvicidal activity, Mortality, and *Aedes aegypti*

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ABSTRACT: Introduction: *Aedes* mosquitoes are responsible for transmitting several life-threatening diseases worldwide, which poses a significant societal burden. It is crucial to control these mosquito vectors to prevent the transmission of diseases. In this regard the present work focused on phytochemical screening and larvicidal activity on the whole plant of *Barleria cuspidate* ethanolic extract. **Methods:** Ethanolic extract of *Barleria cuspidata* was prepared by successive extraction using the maceration method. The larvicidal activity of different concentrations (20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml, and 100 mg/ml) was assessed against 4th instar *Aedes aegypti*. Mortality rates were recorded after 24 hrs and 48 hrs to evaluate the larvicidal activity against *Aedes aegypti*. **Results:** The phytochemical screening of *Barleria cuspidata* ethanolic extracts revealed the presence of Alkaloids, Saponins, Flavonoids, Steroids, and Tannins. At a concentration of 100 mg/ml, the extract demonstrated the highest inhibitory effect, with mortality rates of84% and 93% observed at 24 and 48 hours respectively. In contrast, no mortality was observed in the control group, indicating that the observed effects were specifically due to the extract. The LC50 values, representing the concentration at which 50% of larvae were killed, were found to be 68 mg/ml at 24 hrs and 54 mg/ml at 48 hrs. These results indicate a time dependent decrease in LC50, suggesting an increase in potency of the extract over time. **Conclusion:** The results therefore clearly indicate ethanolic extract of *Barleria cuspidata* that possesses larvicidal activity against *Aedes aegypti*.

INTRODUCTION: Mosquitoes play a crucial role as vectors in disease transmission, with diseases such as dengue, malaria, filariasis, yellow fever, and Japanese encephalitis being among those they spread. In recent years, dengue viruses transmitted by infected female *Culicidae mosquitoes*, particularly *Aedes aegypti* and *Aedes albopictus*, have become a serious global public health concern.

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However, the effectiveness of various medicinal plant preparations as larvicidal agents or mosquito repellents remains uncertain. There has been increasing interest in plant-based insecticides and environmentally friendly alternatives to traditional pesticides. Natural substances are being considered as ideal alternatives because they are eco-friendly, bio-sourced, and safe to use, causing less harm to the ecosystem and non-target species [1-3].

Barleria is the third largest genus in the family Acanthaceae, known for its large and attractive flowers that come in various colors such as purple, pink, white, and yellow. It's a popular traditional flower crop in South India, with a chromosome number (2n) of 40.

The origin of Barleria is from Southern China to India and Myanmar. This genus comprises around 300 species, including *Barleria cristata, Barleria buxiflora*, *Barleria cuspidata*, *Barleria tomentosa, Barleria grandiflora, Barleria montana*, *etc*., found mostly in tropical and sub-tropical regions, especially in Africa. Traditionally, the roots and leaves of Barleria were used for various medicinal purposes like treating stomach aches, acting as a tonic, febrifuge, and relieving cough, bronchitis, and inflammation. Barleria cuspidate, in particular, has been found to have wound-healing properties and hepatoprotective activity [4]. More than 21 species of Barleria are native to India and widely distributed in the lower hills of West Bengal, the Deccan Plateau, Karnataka, Andhra Pradesh, Madhya Pradesh, and the North Eastern Himalayan Region. Locally known as "Mancat-gemmule, Vellaimuli, or spiny Barleria", this plant has been cultivated in tropical and subtropical regions worldwide since ancient times. Various nutritionally important compounds such as alkaloids, phytosterols, terpenoids, glycosides, flavonoids, and other metabolites have been isolated from different parts of *Barleria cuspidata*, showing various pharmacological activities. The medicinal properties of spiny Barleria include antioxidant, antibacterial, anti-diabetic, anti-hyperlipidemic, wound healing, hepatoprotective, and anti-parkinson activities [5-6].

MATERIALS AND METHODS:

Plant Collection and Identification: The whole plant of *Barleria cuspidata* was collected from their natural habitats in Sanankaadu thottam, Periya senguttai, Avinasi (TK), Tiruppur (DT), Tamil Nadu. In January 2024. It was authenticated by Dr. D. Stephen, Ph.D., Assistant Professor in Botany, The American College, Madurai- 625002, Tamil Nadu, India.

Preparation of Plant Extracts [7]: For small-scale extraction, maceration typically involves several steps. Initially, the plant materials are ground into small particles to increase the surface area for proper mixing with the solvent. In this process, 500 grams of the whole plant of *Barleria cuspidata* was coarsely powdered, then placed in a closed vessel with ethanol, and allowed to stand at room temperature for at least three days with frequent agitation until the soluble matter was dissolved. The mixture is then strained, and the damp solid material is pressed. The combined liquids are clarified by filtration or decantation after standing.

Phytochemical Analysis [8-9]: The Ethanolic extract of the seed of*Barleria cuspidata* was subjected to preliminary phytochemical screening to reveal the presence of various phytoconstituents according to the methods outlined in Kokate C. K 2007 and Khandelwal 2008.

Larvicidal Activity [10-13]:

Selection of Experimental Model: The eggs of Aedes aegypti were collected from the "National Centre for Disease Control field station of Mettupalayam, Tamil Nadu, India". These egg rafts were then placed in trays containing tap water (as a culture medium) under laboratory conditions (at 29±1°C). After 24 hours of incubation, the eggs hatched into first instar larvae. To promote larval growth, an appropriate amount of nutrients (sterilized yeast powder and dog biscuit in a 1:1 ratio) were added. The study utilized 4th instar larvae.

Experimental Design: The plant extracts were dissolved in 10µl of DMSO to enhance their solubility in water. The larvicidal activity was evaluated following the WHO procedure with some modifications. Twenty healthy larvae were placed into each 250 ml glass beaker containing 200 ml of the test concentration. Mortality was observed at 24 and 48 hours after treatment. Larvae were considered dead when they showed no movement upon probing with a needle. Three trials with three replicates per trial for each concentration were conducted. Simultaneous controls were run, with distilled water serving as the control.

The larval percentage mortality was calculated by using following formula:

Percentage of Mortality = (Number of dead larvae) / (Number of larvae introduced) × 100

RESULTS AND DISCUSSION:

Phytochemical Analysis: Ethanolic extracts of *Barleria cuspidate* whole plant was subjected to various chemical tests for detection of phytoconstituents and results obtained are illustrated in **Table 1.**

TABLE 1: PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACTS OF *BARLERIA CUSPIDATE* WHOLE PLANT

S. no.	Phytochemical Test	Ethanolic extract
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Phenols	+
5.	Terpenoids	+
6.	Anthraquinones	+
7.	Saponins	+
8.	Mucilage	-
9.	Carbohydrates	+

Note: (+) Present (-) Absent

Larvicidal Screening: From the phytochemical screening, the ethanol extract showed the presence of alkaloids, saponins, steroids, flavonoids, tannins. So, we selected the ethanol extract of *Barleria cuspidate* for larvicidal screening. Various concentrations of ethanol extract (20, 40, 60, 80, 100mg/ml) were used for testing the larvicidal activity. The results were shown in the **Table 3.**

TABLE 2: LARVICIDAL ACTIVITY OF ETHANOLIC EXTRACTS OF *BARLERIA CUSPIDATE* WHOLE PLANT

Plant Name	Concentration (mg/ ml)	Percentage Mortality			
		Ethanol Extract			
		24hrs	LC_{50}	48hrs	LC_{50}
Barleria cuspidata	Control	0±0.11	68	0±0.25	54
	20	18±0.61		28±0.42	
	40	27±0.75		39±0.65	
	60	43±0.85		54±0.78	
	80	68±0.42		81±0.57	
	100	84±0.63		93±0.82	

Values are expressed as Mean ± SEM. n=3,

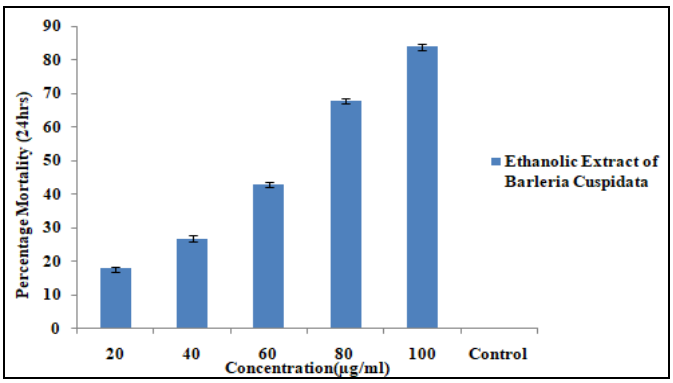

FIG. 1: PERCENTAGE MORTALITY OF ETHANOL EXTRACT OF *BARLERIA CUSPIDATA* (24 HRS)

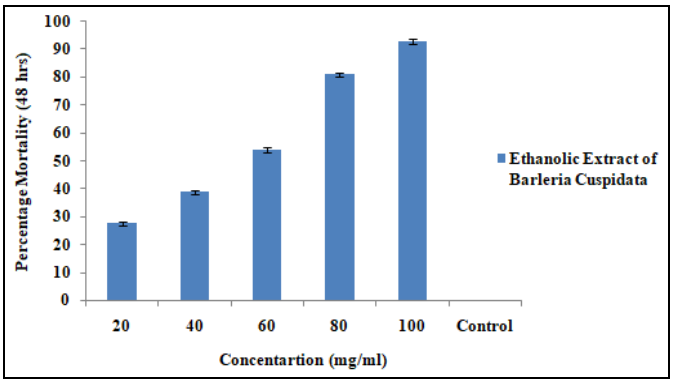

FIG. 2: PERCENTAGE MORTALITY OF ETHANOL EXTRACT OF *BARLERIA CUSPIDATA* (48 HRS)

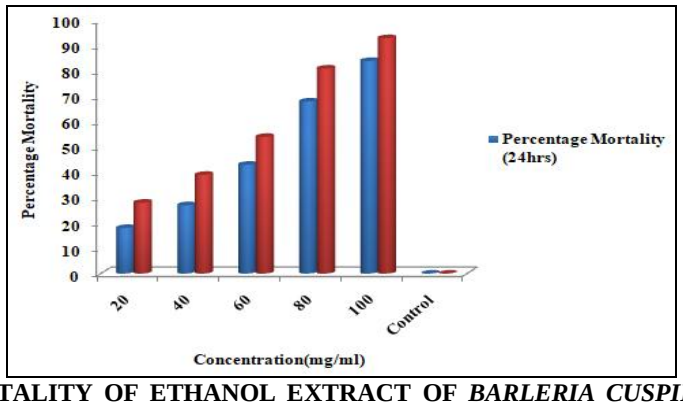

FIG. 3: PERCENTAGE MORTALITY OF ETHANOL EXTRACT OF *BARLERIA CUSPIDATE* (24 HRS AND 48 HRS)

The ethanolic extract of *Barleria cuspidata* exhibited significant mortality in the 4th instar larvae of *Aedes aegypti*, as evidenced by the results presented in **Table 2** and **Fig. 1, 2** and **3**. Notably, at a concentration of 100 mg/ml, the extract demonstrated the highest inhibitory effect, with mortality rates of 84% and 93% observed at 24 and 48 hours respectively. In contrast, no mortality was observed in the control group, indicating that the observed effects were specifically due to the extract.

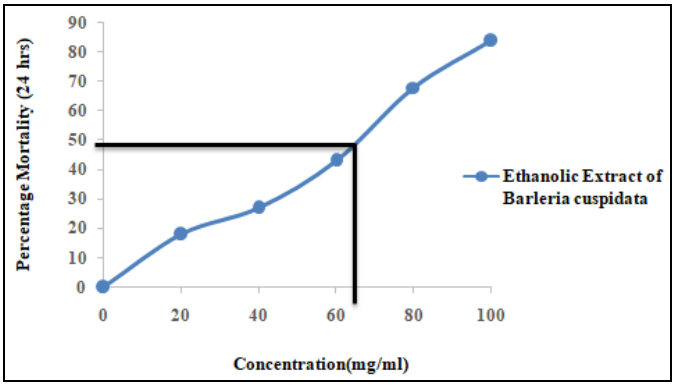

FIG. 4: LC_{50} CURVE OF ETHANOLIC EXTRACT OF *BARLERIA CUSPIDATA* (24 HRS)

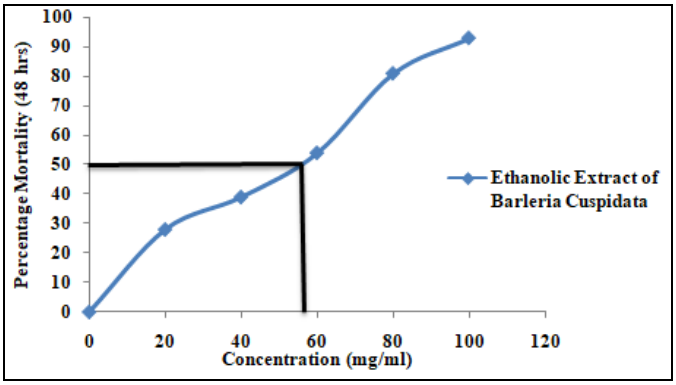

FIG. 5: LC_{50} CURVE OF ETHANOLIC EXTRACT OF *BARLERIA CUSPIDATA* (48 HRS)

From **Table 2** and **Fig. 4** and **Fig. 5** the ethanolic extract of *Barleria cuspidata* demonstrated significant larvicidal activity against *Aedes aegypti* larvae. The LC_{50} values, representing the concentration at which 50% of larvae were killed, were found to be 68 mg/ml at 24 hours and 54 mg/ml at 48 hours. These results indicate a time-dependent decrease in LC_{50}, suggesting an increase in potency of the extract over time.

CONCLUSION: From the present study we conclude that the preliminary phytochemical analysis of ethanolic extracts of *Barleria cuspidate* whole plant indicated the presence of Alkaloids, Saponins, Flavonoids, Steroids and Tannins which showed combination effects in terms larvicidal action to mosquito larvae. Phytochemicals derived from plant sources can act as larvicides, insect growth regulators and repellent. The ethanolic extracts of *Barleria cuspidata* shows 93% mortality after 48hrs of incubation. Moreover, behavioural changes were observed in the movement of the larvae. These effects may be due to the presence of neurotoxin compounds in plant extracts. No behavioural changes were obtained in control group. Further analysis is required to isolate the active principles and elucidate the mechanism of action of ethanolic extracts of *Barleria cuspidate* whole plant. The phytochemicals of *Barleria cuspidata* extracts can be well utilized for preparing biocides or insecticidal formulation.

The overall comparative studies showed that the ethanol extract of *Barleria cuspidata* showed highest degree of larvicidal activity.

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CONFLICTS OF INTEREST: Nil

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