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Larvicidal Efficacy Of *Pergularia Daemia* Leaf Extract Against *Aedes Aegypti*, And *Culex Quinquefasciatus.*

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Abstract:

Mosquitoes pose a significant threat to public health by transmitting various diseases worldwide. In South-East Asian countries like India, the vector-borne diseases have become a pressing issue. *Aedes aegypti* and *Culex quinquefasciatus* are prominent mosquito species responsible for spreading viral infections like dengue and West Nile virus. Traditional vector control methods involving synthetic insecticides present environmental and health hazards, prompting the search for eco-friendly alternatives. This study focuses on *Pergularia daemia*, a plant with known medicinal properties, as a potential source of bioactive compounds for mosquito control. Crude extracts from *P. daemia* leaves were prepared using different solvents, and phytochemical screening revealed the presence of alkaloids, steroids, tannins, saponins, flavonoids, and phenolic compounds. Ethanol extracts exhibited the highest larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* larvae, with mortality rates proportional to concentration. The results suggest that *P. daemia* extracts, particularly those in ethanol, hold promise as effective and environmentally friendly mosquito larvicides.

Keywords: Mosquito control, Pergularia daemia, Larvicidal activity, Eco-friendly insecticides, Vector-borne diseases

1. Introduction

Mosquitoes can spread more diseases than any other group of arthropods and affect millions of people entire the world. World Health Organization has declared the mosquitoes as 'Human's first enemy'(Anoopkumar and Aneesh, 2021). They

act as the vector for most of the life threatening human diseases. In recent years vector-borne diseases (VBD) have emerged as a serious public health problem in South-East Asian countries, including India (WHO, 2020). A mosquito species *Aedes aegypti* is a significant vector causing viral infections like Dengue, Dengue hemorrhagic fever, Yellow fever, etc. in tropical nations (Shajahan *et al.,*2022). The female mosquito lays eggs on damp surfaces just above or close to the water bodies like ponds, lack, rivers, water logging areas etc. (Chen and Wilson, 2020). It only hatches when inundated and it can withstand desiccation for several months. *Ae. aegypti* primarily reproduces in domestic settings; which prefers roof gutters, leaf axils, bamboo stumps, and temporary containers like jars, drums, used car tires, tin cans, bottles, plant pots, coconut shells, abandoned kitchen utensils, etc. due to the presence of clean water these ecosystems (Ansari *et al.,*2023) and (Faraone *et al.,* 2021). *Culex quinquefasciatus*, commonly called as the southern house mosquito, is a widespread mosquito species with global distribution. Which can be identified by its brownish coloration and a prominent white abdominal band, this mosquito is predominantly active during the evening and night. *Cx. quinquefasciatus* is a vector for various vector borne diseases, including West Nile virus and lymphatic filariasis. It frequently breeds in stagnant water sources, such as urban drainage systems, and is adaptable to both rural and urban environments. Effective mosquito control measures have been undertaken to eliminate stagnant water, using insecticides, and employing preventive strategies to reduce the risk of disease transmission by *Cx. quinquefasciatus*. (Nakasen *et al*., 2021).Controlling these vectors is an essential need to prevent such a diseases. The synthetic insecticides has resulted in environmental hazards results in the development of insecticide resistance in vector populations, persistence and accumulation of non-biodegradable chemicals in the ecosystem, and also affects the biological magnification through the food chains and finally, its toxicity affect human health and also non-target organisms (Muhammed *et al*., 2022). These problems have accelerated the researchers to find new and safe mosquito repellent and control agents as alternatives to synthetic chemicals. So, it is necessary to find out and explore alternative eco-friendly methods to control programs, biologically active plant extracts perform well and environmentally acceptable for mosquito control programs. (Ogunah *et al.,* 2020).*Pergularia daemia* is a perennial herbaceous plant in the Apocynaceae family, widely referred to as the Wild Snake Root or Adam's Globe Amaranth. This plant is frequently found beside roadsides, wastelands, and open fields. Twining vines, elliptical leaves, and clusters of petite, tubular flowers with a characteristic five-lobed corolla are the characteristics of *P.daemia*. Parts of *P.daemia* have been used in traditional medicine systems throughout history because of its antipyretic and anti-inflammatory actions.(Iyekowa *et al*.,2023)In recent years, bioactive compounds used as insecticides which are considered to be more environmentally safe and biodegradable materials. The plant extracts and their novel bioactive compounds can be used as an effective and alternative source over synthetic insecticides. It does not affects any other organisms and leads to new strategies for selective mosquito larval control. Hence the present study aims to assess the larvicidal activity of *Ae.aegypti*, and *Cx. quinquefasciatus* through *P.daemia* plant leaf extract

2.Materials and Methods:

2.1. Procurement of Plants

In the present study, plants were selected namely *P.daemia* which was collected from in and around Tirunelveli, Tamilnadu, India. The plants were identified and authenticated by the botanist, Dr.M.Syde Ali Fathima Department of Botany, Sadakathullah Appa college, Tirunelveli, Tamilnadu, India

2.2. Preparation of Crude Solvent Extracts

The selected plants leaves were washed in running tap water and rinsed with double-distilled water for removing dust. After being thoroughly cleaned, the plant leaves were allowed to shadow air dry for roughly 20 days at room

temperature. The dried plant leaves were made into fine powder using an electric blender then it was sieved to get fine powder. The plant powder was extracted with water, acetone, chloroform, ethanol, hexane, petroleum ether and aqueous using a soxhlet extractor over a period of 8-10 hours. By using a rotary flash evaporator, all of the extracts were concentrated, then placed in sealed bottles and kept at 5°C for further use.

2.3. Preliminary Phytochemical analysis

The phytochemical screening such as alkaloids, Cardiac glycosides, phenol, essential oil, saponin, Terpenoids, Amino acids flavonoids, steroids, and protein were analyzed in the plant extract (Harborne, 1998).

2.4. Laboratory colonization of mosquitoes

The egg rafts and eggs of two mosquito species (i.e. *Culex quinquefasciatus* and *Aedes aegypti*) were obtained from ICMR- Vector Control Research Centre, Central Research Medical Entomology Institute Madurai, Tamilnadu, India. In the laboratory condition the larvae was maintained at 70-85% RH, 28±2º C temperature and 12; 12 light and dark photoperiod cycle. The larvae were fed on powdered mixture of dog biscuits and yeast powder in 3:1 ratio. The adults were provided with 5% glucose solution and honey was given to male and female. Larvae were used for the bioassays from these laboratory colonized mosquitoes. (Azarudeen*et al*., 2018)

2.5. Larvicidal bioassay

The larvicidal activities of the selected plant extracts were evaluated as per the method recommended by World Health Organization (2005). All the plant extracts were tested for larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus*. Initially, mosquito larvae were exposed to a wide range of test concentrations with a control to find out the activity range of the plant extracts. After determining the mortality of larvae in a wide range of concentrations, a narrow range of concentrations was used to determine the lethal concentration of 50% (LC_{50}) mortality and the lethal concentration of 90% (LC_{90}) mortality values. One gram of plant crude dissolved in 100 ml of Acetone (1%stock solution). Twenty early fourth instar larvae were exposed 500 ml glass beaker containing 249 ml dechlorinated water and 1 ml of plant extracts were added for the desired concentrations. From the stock solution larvae were treated with different concentrations (60 to 300) ppm was prepared with dechlorinated water respectively. At each test concentration for five replications. A corresponding control was maintained as 1 ml of acetone with 249 ml of dechlorinated water. The larval mortality of fourth instar of *Ae. aegypti*and *Cx. quinquefasciatus* was observed. The numbers of larvae mortality were noted at the end of 24 hours and the percentage mortality was calculated. The corrected mortality number was accounted by Abbot'ts formula. The percentage of mortality was calculated by

$$\text{Percentage of mortality} = \frac{\text{No. of larva dead}}{\text{No. of larvae introduced}} * 100$$

$$\text{Corrected Mortality} = \frac{\text{observed mortality in treatment} - \text{observed mortality in control}}{100 - \text{Control mortality}}$$

2.6. Statistical Analysis

The average mortality data were subjected one way ANOVA after to probit analysis for calculating LC_{50}, LC_{90} and 95% confidence of upper confidence limit and lower confidence limit, chi-square were calculated by using the software using SPSS-20 version, results with $p< 0.01$ were considered to be statistically significant.

3. Results:

3.1. Qualitative phytochemical screening of *Pergularia daemia*

The qualitative phytochemical components analysis in the *P.daemia* leaf extract was done in acetone, hexane, chloroform, ethanol, petroleum ether, and aqueous extract. Table 1. showed the results of the qualitative analysis of plant extracts. All extracts contained alkaloids, steroids, tannins, saponins, flavonoids, and phenolic compounds. The terpenoid were present in all extracts except acetone. The presence of Cardiac glycosides was found in the ethanol extract. Essential oils were found in Acetone, Chloroform and Aqueous extract. Carbohydrates occurred in the Acetone, hexane and aqueous extract. Acetone ,chloroform and petroleum ether had reducing sugar. Based on the preliminary analysis the phytocompound maximum bioactive compounds were present in aqueous and ethanol extracts.

Table 1. Qualitative phytochemical analysis of *Pergularia daemia* leaf extracts

Phytocompounds	Solvents					
	Acetone	Hexane	Chloroform	Ethanol	Petroleum Ether	Aqueous Extract
Alkaloids	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Terpenoids	+	-	+	+	-	-
Cardiac glycosides	+	-	-	+	-	-
Phenolic Compound	+	+	+	+	+	+
Amino Acid	-	+	-	+	+	-
Essential oil	+	-	+	-	-	+
Carbohydrates	-	+	-	-	-	+
Reducing sugar	+	-	+	-	+	-

+ Present - absent

3.2. Results of larvicidal bioassay:

The results showed the larvicidal activity of *P. daemia* crude leaf extract in different solvents against the *Ae. aegypti* and *Cx. quinquefasciatus* 4th instar mosquito larvae are shown in (Tables 2 and 3). The results reveal the mortality of mosquito larvae in direct proportion to concentration. The maximum mortality was noticed in 97% ethanol extract against *Ae. aegypti* at 300 ppm concentration. The LC_{50} and LC_{90} values of *Ae.aegypti* and *Cx.quinquefasciatus* ethanol extract was (118.53, 261.95) and (126.01, 283.82) ppm respectively. The minimum mortality was found to be 80 % against *Cx.quinquefasciatus* larvae at 60 ppm concentration and their LC_{50} values of 177.03 respectively. The data are statistically significant at $p<0.01$. Beyond that, there is no mortality was observed in Control after treatment of 24 hours.

Table 2.The larvicidal activity of *P.daemia* different extract against *Ae.agypti*

Name of the Extract	Concentration (ppm)	%Mortality ± SD	LC_{50}(ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2 (df=3)
Ethanol	Control	0.0±0.0	118.53(101.05-133.28)	261.95(240.93-290.48)	1.91
	60	31±0.83			
	120	51±0.44			
	180	70±0.70			
	240	83±0.54			
	300	97±0.89			
Acetone	Control	0.0±0.0	125.68 (108.00-140.73)	276.68 (253.98-307.85)	1.46
	60	29±1.30			
	120	49±1.30			
	180	68±1.57			
	240	80±0.70			
	300	95±1.00			
Chloroform	Control	0.0±0.0	131.64 (113.64-147.08)	290.12 (265.68-324.07)	1.05
	60	27±1.30			
	120	47±1.14			
	180	68±0.84			
	240	78±1.14			
	300	92±1.51			
Petroleum ether	Control	0.0±0.0	141.44 (124.60-156.37)	297.85 (273.15-332.03)	0.41
	60	25±1.22			
	120	43±0.54			
	180	64±0.83			
	240	77±1.54			
	300	91±1.30			
Hexane	Control	0.0±0.0	149.34 (132.54-164.54)	311.41 (284.88-348.55)	0.15
	60	24±1.92			
	120	41±1.64			
	180	60±1.58			
	240	75±0.70			
	300	89±0.83			
Aqueous extract	Control	0.0±0.0	159.92(144.00-174.82)	319.67(292.86-357.10)	0.24
	60	20±0.87			
	120	39±1.30			
	180	57±1.14			
	240	73±0.85			
	300	87±1.0			

Value represents mean±S.D. of five replications*

Table 2. The larvicidal activity of *P.daemia* different extract against *Cx. quinquefasciatus*

Name of the Extract	Concentration (ppm)	%Mortality ± SD	LC_{50}(ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	χ^2 (df=3)
Ethanol	Control	0.0±0.0	126.01 (107.52-141.64)	283.82(259.85-317.10)	0.56
	60	29±0.83			
	120	49±1.30			
	180	68±1.51			
	240	80±0.70			
	300	93±0.51			
Acetone	Control	0.0±0.0	136.16 (117.83-151.99)	301.44(275.26-338.28)	0.67
	60	26±1.64			
	120	47±1.14			
	180	65±1.87			
	240	77±1.57			
	300	90±1.22			
Chloroform	Control	0.0±0.0	138.48 (119.45-154.87)	311.55 (283.56-351.49)	1.04
	60	25±1.30			
	120	45±1.67			
	180	69±1.22			
	240	75±1.34			
	300	87±1.34			
	Control	0.0±0.0	154.64 (137.46-170.38)	324.67 (295.73-365.90)	1.47
	60	23±0.54			
	120	42±1.94			
	180	61±1.78			
	240	74±1.81			
	300	85±1.00			
Hexane	Control	0.0±0.0	156.46 (138.94-172.51)	330.52 (300.63-373.51)	0.56
	60	22±1.81			
	120	41±0.82			
	180	58±1.09			
	240	74±1.67			
	300	84±0.44			
Aqueous extract	Control	0.0±0.0	177.03 (160.53-193.29)	351.92(319.74-398.34)	0.99
	60	17±0.54			
	120	36±1.30			
	180	53±1.14			
	240	70±0.89			
	300	80±1.41			

Value represents mean±S.D. of five replications* *mortality of the larvae observed after 24hrs of exposure period

LC_{50}=Lethal Concentration brings out 50% Mortality ,LC_{90} = Lethal Concentration brings out 90% mortality. ,LCL = Lower Confidence Limit UCL = Upper Confidence Limit; ,Statistically significant at $P<0.01$.

4.Discussion:

Mosquito control is a crucial technique for preventing and spreading mosquito borne disease. However, a high level of insecticide resistance has developed from synthetic pesticides as faces several problems in vector control strategies. Finding different vector control plans is important to solve these issues. A search for more environmentally acceptable methods for managing vectors has been prompted by the public by growing concerns about safe food and a healthy environment and the management of the vectors. Bio-pesticides provide an alternative to synthetic pesticides because of their lower impact on environmental pollution, low toxicity to humans and some other advantages (Kumar *et al*., 2021).The Present investigations were identify the qualitative and larvicidal activity of the locally available plant of *P.daemia* as in the control of *Ae. aegypti* and *Cx. quinquefasciatus* larvae. *P.daemia* screened with different solvent extracts based on polarity basis (ethanol, acetone, chloroform, petroleum ether, hexane and aqueous extract) exhibited great larvicidal activity.Among the six solvents ethanol extracts had high phytocompounds like alkaloids, steroids, flavonoids, saponin, tannins, phenol, cardiac glycosides and amino acids.Similar results were found in (Nithyatharani and

Kavitha., 2018) reported that the qualitative analysis of the ethanol leaves showed the presence of alkaloids, steroids, terpenoids, flavanoids, saponins, phenols, tannins and amino acids. The highest mortality was observed in 97% of *P.daemia* ethanol extracts against *Ae. aegypti* at 300 ppm concentration which also had a low LC_{50} value of 118.53 and LC_{90} value of 261.95 when compared with other solvents. The lowest mortality was observed at 17 % in aqueous extract against fourth instar larvae of *Cx. quinquefasciatus* at 60ppm concentration. The results are line with Nikkon *et al*., 2009 reported ethanol, and methanol extracts of *Duranta repens* were found to be potent against fourth instar larvae of *Ae. agypti* and *C. quinquefasciatus* species of mosquito when compared with aqueous, acetone and chloroform extracts. The numerous bioactive organic compounds found in plants act as defense mechanisms against pest and disease invasion. Botanical pesticides offer an advantage over synthetic pesticides and are less toxic, less prone to the development of resistance and they can be degraded easily. Varieties of plant species have been used all over the world to control mosquito populations (Haldhar *et al*., 2017). During the present investigation bio control potential of crude *P.daemia* extracts has been done in laboratory conditions. The maximum mortality was found in *P.daemia* ethanol extracts. The extracts had varieties of phytocompound which act as separately or combined to control the respective mosquito larvae. In conclusion, the ethanol extracts of *P.daemia* can be recommended for field study and further study is needed to know about the compound's response against the control of target organisms.

5.Reference

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