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The efficacy of *Pajanelia longifolia* (Willd.) K. Schum leaf extracts against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae)

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Abstract

Pajanelia longifolia (Willd.) K. Schum is a medicinal deciduous tree with a history of traditional use. The use of biologically active plant materials with mosquitocidal characteristics has piqued the curiosity of scientists worldwide. The goal of this study was to see if *P. longifolia* crude extracts (in petroleum ether, chloroform, and methanol) had any ovipositional deterrent, ovicidal, larvicidal, or pupicidal activity against *Anopheles stephensi* Liston. The LC_{50} and LC_{90} values were determined by varying the concentrations of the leaf extracts. The highest oviditerrence activity (98.58% at 900 ppm), 100% ovicidal activity at 1700 ppm, 100% larvicidal activity at 1300 ppm, and pupicidal activity at 2100 ppm were found in the methanol extract. This study's findings suggest that the methanol extract of *P. longifolia* could be used to control mosquitoes. These discoveries could aid in the development of a potential alternate source of mosquito control. These kinds of biological insect control methods reduce the need for synthetic pesticides. The study proves that controlling mosquitoes in their infancy is easier and more effective than controlling the adult mosquitoes.

Keywords

Pajanelia longifolia; *Anopheles stephensi*; ovicidal activity; larvicidal activity; pupicidal activity

Introduction

Many of the deadliest diseases in the world are transmitted by mosquito populations. Malaria is a potentially fatal mosquito-borne disease that remains a major cause of morbidity and mortality on a global and regional scale. According to World Health Organization (WHO) estimates, there were 229 million malaria cases in 2019 and more than 400,000 deaths (1). In tropical countries, children are more vulnerable to malaria than adults. The WHO's Global Malaria Program (GMP) aims to control and eliminate malaria by 2030 (2). To decrease the transmission of infectious malaria and accomplish the objective of GMP, it is imperative to control the mosquito population.

The deadly disease malaria is caused by active female Anopheline bloodsuckers. Warm environments are required for these mosquitos to breed and transmit the disease (3). *An. stephensi* Liston's efficient 'type form' is known to be a major urban malaria vector in the Indian subcontinent and the Middle East (4). Rapid urbanisation in developing nations lead to a rise in the population of the endophilic and endophagic *An. stephensi*

mosquitoes. *An. stephensi* is regarded by WHO as a highly competent vector of *Plasmodium falciparum* and *Plasmodium vivax* (5), and its rapid spread in urban areas poses a threat to malaria elimination. However, disease control is now threatened by rapidly increasing insecticide resistance in insect populations, highlighting the need for newer initiation of specific strategies that can reduce vector-mediated transmission (6).

Plants are rich in bioactive molecules that are extremely useful in everyday life. Botanical preparations were historically used as insecticides by ancient people (7). Thus, plant exploration will assist the human population in overcoming the challenges of an integrated vector control strategy, sustaining malaria elimination, and preventing its reintroduction.

P. longifolia is a small to medium tree that is either evergreen or deciduous and belongs to the Bignoniaceae family. They can be found at heights of up to 1000 meters above sea level in the deciduous and semi-evergreen highland rainforests of India, Sri Lanka, Myanmar, Thailand, Malaysia, the Peninsula, Sumatra, and Borneo (8). The plant is high in secondary metabolites that have medicinal value. It is well-known for its medicinal properties as a hepato-protective agent (9). In folk medicine, the young tender leaves of the plants are used to treat nail infections and skin rashes (10). Leaf extracts containing polar components were found to have antibacterial activity (10, 11). The plant's bark powder extract has antioxidant and antimicrobial properties (8, 12). As a result, no experimental data on the validation of *P. longifolia*'s anti-mosquito properties are available.

The current study intends to use *P. longifolia* leaf extracts against different developmental stages of *An. stephensi* mosquitoes, one of the most important malaria vectors. This is motivated by the understandable hope that reducing the early stages of mosquitoes will benefit malaria control and public health more than only controlling adult mosquitoes.

Materials and Methods

Plant collection and extraction

P. longifolia fresh leaves were collected between April and June in Kasaragod, a district in the tropical Indian state of Kerala. The samples were delivered to the Department of Applied Botany (Mangalore University, Karnataka, India). The samples were further examined at the university to eliminate any faulty samples. The final specimen's twigs were pressed, dried, and treated with mercuric chloride ($HgCl_2$). The voucher specimen, 'SSK-RKC-0013,' is stored in the department herbarium. The freshly collected leaves were thoroughly washed under running tap water and shade dried for one week at room temperature (27 ± 30 °C). The dried (200 gm) leaves were mechanically powdered with an electrical stainless-steel blender and extracted in a soxhlet apparatus separately with Petroleum ether (900 ml, CDH), Chloroform (1500 ml, EMPLURA), and Methanol (1900 ml, EMPLURA) until exhaustion. The extracts were concentrated further using a rotary evaporator, and the resulting residue was stored at 4°C. The following formula was used to calculate the percentage yield of the extract,

$$\% \text{ yield} = \frac{WE}{WP} \quad \text{……….……………….(Eqn.1)}$$

Where, WE is for Weight of plant extract (g), WP stands for Weight of plant material (g).

The extracts were screened for the presence of phytochemical components using standard techniques (13, 14).

The 1% stock solutions were prepared by dissolving 200 mg of the crude extract in 20 ml of ethanol. The desired test solutions were made from the stock solutions using ethanol or distilled water. Polysorbate 80 (Qualigens) was used as an emulsifier in the final test solution at a concentration of 0.05%.

Mosquitoes rearing technique

The eggs of *An. stephensi* were obtained from the National Institute of Malaria Research in Bangalore (Karnataka, India). The eggs were kept for hatching in a white tray containing 3 cm of reverse osmosis (RO) water for 24 to 48 hours at a temperature of (27 ± 2) °C, refractive humidity of (75% – 85%), and photoperiod cycles of 14 L: 10 D. For the first two days after the eggs hatch, the larvae were fed 50 – 60 mg of the finely ground yeast slurry. After the third day, 50 – 60 mg of finely powdered yeast was added as a feed to the tray containing approximately 300 2nd instar larvae. On the fifth day, take 120 – 200 mg of finely ground and powdered yeast and dog biscuits (40:60) were fed on the 3rd and 4th instar larvae. The pupal stage lasts 7-8 days. Pupae were removed from the trays, placed in labelled plastic bowls, and placed in a freshly prepared cage for emergence.

After emergence, the cotton swabs were prepared for mosquito feeding by soaking them in a 10% glucose solution and placing them inside the cage. After 3-4 days of emergence, the mosquitoes begin to feed on blood. The mosquitos were starved for 3-4 hours during the day before being fed blood. For 2-3 hours, mosquitos were fed a blood meal via membrane feeding. Later, the mosquitos were given a 10% glucose solution in cotton swabs. Water filled Ovi-traps were placed in the cage. After three days of a blood meal, the eggs were laid in the Ovi-trap (15).

Oviposition deterrence activity

For this study, 30 male and female pupae from well-maintained stock colonies were separated based on the presence or absence of terminalia using a stereomicroscope. Pupae were placed in the test cage (45 X 45 X 40 cm) and allowed to emerge. A cotton swab was used to administer 10% glucose to newly emerged mosquitos. A blood meal was provided for egg formation after the fifth day of emergence (after the mating period). Females who had been blood-fed were transferred to a new cage using an aspiration tube. Oval test cups (ovitraps) were filled with

100 ml of distilled water and solvent extracts at concentrations of 100, 300, 500, 700, and 900 ppm for oviposition. The cage was filled with test cups. The Control cage is kept separate. To eliminate any effect of position on oviposition, the test bowl positions were alternated between replicates. Three replicates of each concentration were run for each bioassay, with cages set side by side. After 24 hours, thenumber of eggs laid in treated and control bowls was counted using a stereomicroscope (16, 17). The following formula was used to compute the % ovipositional deterrent for each concentration,

$$OD = \frac{NC - NT}{NC} \times 100 \qquad \text{.....(.Eqn. 2)}$$

Where, OD is for Effective Deterrence, NC stands for Number of Eggs in Control, and NT stands for Number of Eggs in Treatment.

The following formula was used to determine the oviposition activity index (OAI),

$$OAI = \frac{NT - NC}{NT + NC} \qquad \text{.....(Eqn. 3)}$$

Where NT represents the total number of eggs in the test solution and NC represents the total number of eggs in the Control solution. (18).

Ovicidal activity

For the ovicidal activity, 100 gravid female mosquitoes were introduced into the cage, and 10 ovi-traps were set up for oviposition two days following a blood meal. Once the eggs were removed from the cage, they were placed in cups with varying concentrations of petroleum ether (300, 700, 1100, 1500, 1900, and 2300 ppm), chloroform (500, 900, 1300, 1700, 2100, 2500, and 2900 ppm), and methanol (100, 300, 500, 700, and 900 ppm) extract containing 100ml of distilled water. After 24 hours of treatment with the test solution, the eggs were carefully removed from the test cups with a brush. Following that, eggs were placed in 100 ml of fresh distilled water with plastic bows and labelled according to concentrations. Experiments were conducted in triplicate. The percent ovicidal activity was calculated 48 hours after treatment using the formula (19, 20),

$$\%OA = \frac{EHC}{EHT} \times 100 \qquad \text{.....Eqn. 4)}$$

Where, %OA is represents Percent ovicidal activity, EHC stands for % of eggs hatched in control and, EHT stands for % eggs hatched in treated.

Larvicidal Activity

An. stephensi third instar larvae were used in the bioassay. The stock solution was used to make test solutions of various concentrations. 25 healthy larvae were placed in plastic cups containing 199 ml of distilled water (the depth of the water in the cups was kept between 5 and 10 cm; as deeper levels may cause undue mortality) and 1 ml of test solutions. The activity was measured using the standard WHO method with minor modifications at (27 ± 2) °C, (75 % - 85 %) RH, and 14 L: 10 D photoperiod cycles (21-23). Ethanol was used as a control. The LC_{50} and LC_{90} were calculated using probit analysis after 24 and 48 hours of exposure (24). The test was repeated if pupation occurred during the exposure period and more than 10% of the larvae died in the control group (21-23). Abbott's formula (25) was used to correct the control mortalities.

$$CM = \frac{MT - MC}{MC} \times 100 \qquad \text{.....(Eqn. 5)}$$

Where, CM stands for Corrected Mortality, MT stands for Mortality In treatment and, MC stands for Mortality in control.

$$PM = \frac{NDL}{NLI} \times 100 \qquad \text{..... (Eqn. 6)}$$

Where, PM represents the Percentage mortality, NDL stands for Number of dead larvae and NLI stands for Number of larvae introduced.

Pupal toxicity

The bioassay was carried out on freshly emerged pupae at 25 pupae per concentration. Pupae were placed in a 500 ml plastic bowl containing 199 ml of distilled water and 1ml of each concentration of test sample. Control was ethanol (26, 27). Abbott's formula (25) was used to correct the control mortality. Using probit analysis, the LC_{50} and LC_{90} were calculated from the data (24).

$$CM = \frac{MT - MC}{MC} \times 100 \qquad \text{.....(Eqn. 7)}$$

Where, CM stands for Corrected Mortality, MT stands for Mortality in treatment and, MC stands for Mortality in control.

$$PM = \frac{NDP}{NPI} \times 100 \qquad \text{.....(Eqn. 8)}$$

Where, PM represents the Percentage mortality, NDP stands for Number of dead Pupae and NPI stands for Number of Pupae introduced.

Statistical analysis

Data were expressed as mean ± SEM and one-way ANOVA, which was followed by Tukey's test. To determine LC_{50} and LC_{90}, the average larval mortality data were subjected to probit analysis using the SPSS 16.0 version. The data were statistically significant at $^{*}p < 0.05$ (26).

Results

The crude leaf extract percentage yield ranged from 9.4 to 32.4 gm. It was discovered that methanol extract (16.2 gm) had the highest yield, followed by chloroform (9.2 gm) and petroleum ether (4.7 gm) extracts (Table 1). The prelimi-

nary qualitative phytochemical analysis reveals that the extracts of *P. longifolia* contain a wide range of phytochemical constituents, including alkaloids, carbohydrates, starch, glycosides, saponins, steroids, phenols, tannins, flavonoids, proteins, and resins (Table 2).

Table 1. The percentage yield of crude leaf extracts of *P. longifolia*.

Extract	% of yield
Petroleum ether (PE)	**4.7**
Chloroform (CH)	**9.2**
Methanol (ME)	**16.2**

Table 2. The preliminary qualitative phytochemical analysis of crude leaf extracts of *P. longifolia*.

Phytochemicals	Crude extracts		
	Petroleum ether	Chloroform	Methanol
Alkaloids	-	-	-
Carbohydrates	-	-	+
Starch	-	-	+
Glycosides	-	+	-
Saponins	-	-	+
Steroids	-	+	+
Phenols	+	+	+
Tannins	+	-	+
Flavonoids	+	+	+
Proteins	-	-	+
Resins	-	-	+

Where, **+** indicates presence and **–** indicates the absence of phytochemicals.

Oviposition deterrence activity

The oviposition deterrent activity of *P. longifolia* extracts differed. According to the findings, the methanol leaf extract is the most effective oviposition deterrent, followed by chloroform and petroleum ether in decreasing order of efficacy. Methanol extract at 900 ppm demonstrated 98.58 % oviposition deterrent activity with a negative OAI of 0.97 against *An. stephensi* (Table 3). The oviposition deterrent activity of chloroform extract was 50.27% with a negative OAI of 0.31 and petroleum ether extract was 41.07% with a negative OAI of 0.26 against *An. stephensi* (Table 4). The experiment results clearly show that as the concentration of the extract increases, the polar solvent exhibits greater activity than the nonpolar solvent.

Ovicidal activity

During the ovicidal action study, methanol leaf extract showed 100% mortality at 900 ppm, with LC_{50} and LC_{90} values of 722.683 ppm and 1223.950 ppm, respectively. At higher concentrations, petroleum ether and chloroform extracts were lethal. At 2300 ppm, petroleum ether extract showed 99.17% mortality with LC_{50} of 1172.495 ppm and LC_{90} of 2437.433 ppm, respectively. With 99.58% mortality at 2900 ppm, chloroform extract outperformed the other two extracts. Chloroform extracts have LC_{50} and LC_{90} values of 1054.343 ppm and 2849.878 ppm, respectively (Table 5).

Larvicidal activity

The larvicidal activity of three different *P. longifolia* extracts (methanol, chloroform, and petroleum ether) against *An. stephensi* was calculated (Table 6). The bioassay was performed in triplicate, with mortality counts taken after 24 and 48 hours of exposure. Bioassay tests that revealed more than 20% control mortality were discarded and repeated. The current study's findings clearly showed that methanol extract had the highest larvicidal activity, followed by petroleum ether and chloroform extract. In 24 hours, the most potent methanol extract of *P. longifolia* showed 100% mortality at 1300 ppm, with LC_{50} and LC_{90} values of 446.56 and 750.65 ppm, respectively. The mortality rate grows with increasing exposure time. After 48 hours of exposure at 950 ppm, these values are clearly visible, with LC_{50} and LC_{90} values of 453.94 and 685.02 ppm, respectively. Petroleum ether and chloroform extracts demonstrated minimal activity, with LC_{50} values of 2458.72 and 2118.56 ppm at 24 and 48 hours, respectively, and LC_{90} values of 7653.88 and 7536.79 ppm at 24 and 48 hours. The findings clearly show that mortality increases with time spent exposed to leaf extracts.

Pupicidal activity

An. stephensi pupal mortality was observed after treatment with methanol, chloroform, and petroleum ether extract (Table 7). *P. longifolia* methanol extract exhibited 100% pupal mortality at 2100 ppm in 24 hours, with LC_{50} and LC_{90} values of 1210.62 and 2010.02 ppm, respectively. With increasing exposure time, the mortality rate rises. After 48 hours of exposure at 1900 ppm, these values are clearly visible, with LC_{50} and LC_{90} values of 998.32 and 1620.26 ppm, respectively. Petroleum ether and chloroform extracts demonstrated minimal activity, with LC_{50} values of 7205.86 and ppm 6376.38 at 24 hours and 6641.34 and 6361.86 ppm at 48 hours, respectively.

Table 3. Percent oviposition deterrent (OD) activity of the crude extracts against *An. stephensi* females.

Mosquito species	Plant	Plant part	Treated extracts	Concentration (ppm)				
				100	300	500	700	900
Anopheles stephensi	*Pajanelia longifolia*	Leaf	Petroleum ether*	15.91 ± 0.31	22.4± 0.24	29.78 ± 0.24	34.22 ± 0.35	41.07 ± 0.34
			Chloroform*	07.24 ± 0.18	22.53 ± 0.54	36.31 ± 0.50	46.40 ± 0.35	50.27 ± 0.35
			Methanol*	41.24 ± 0.46	60.80 ± 0.15	85.38 ± 0.19	89.42 ± 0.31	98.58 ± 0.35

Data are the mean ± standard error (SE). Means are separated by Tukey's test of multiple comparisons, one-way analysis of variance (ANOVA). *p ≤ 0.05, level of significance. **ppm** = parts per million.

Table 4. Oviposition activity index (OAI) of the crude extracts against *An. stephensi* females.

Mosquito species	Plant	Plant part	Treated extracts	Concentration (ppm)				
				100	300	500	700	900
Anopheles stephensi	*Pajanelia longifolia*	Leaf	Petroleum ether*	- 0.09	- 0.13	- 0.17	- 0.21	- 0.26
			Chloroform*	- 0.04	- 0.13	- 0.22	- 0.30	- 0.34
			Methanol*	- 0.26	- 0.44	- 0.74	- 0.81	- 0.97

Increased negative value in the table indicates the decreased oviposition deterrence. *p ≤ 0.05, level of significance. **ppm** = parts per million.

Table 5. Percent Ovicidal activity of the leaf crude extracts against *An. stephensi* eggs.

Plant	Treated extracts	Concentration (ppm)	Ovicidal activity (%) ± SE	Lethal concentration (ppm)	
				LC_{50} (LCL –UCL)	LC_{90} (LCL-UCL)
Pajanelia longifolia	Petroleum ether	100	23.33 ± 0.88	1172.495* (1006.488 – 1305.352)	2437.433* (2280.242 – 2646.200)
		500	44.67 ± 1.20		
		900	57.67 ± 0.58		
		1300	69.33 ± 0.88		
		1700	78.67 ± 0.88		
		2100	87.33 ± 1.76		
		2500	97.67 ± 0.67		
		2900	98.33 ± 0.33		
		Control	0.0 ± 0.0		
	Chloroform	100	7.33 ± 1.45	1054.343* (858.755 – 1214.074)	2849.878* (2566.674- 3278.330)
		500	32.33 ± 1.76		
		900	50.67 ± 0.88		
		1300	60.67 ± 0.33		
		1700	68.33 ± 0.33		
		2100	77.67 ± 0.88		
		2500	90.67 ± 0.33		
		2900	100.00 ±0.00		
		Control	0.0 ± 0.0		
	Methanol	100	43.33 ± 1.20	722.683* (644.184 – 788.596)	1223.950* (1158.872 – 1294.211)
		300	55.67 ± 1.76		
		500	65.00 ± 1.53		
		700	72.00 ± 1.53		
		900	84.00 ± 0.58		
		1300	95.00 ± 0.58		
		1700	100.00 ± 0.0		
		2100	100.00 ± 0.0		
		2500	100.00 ± 0.0		
		2900	100.00 ± 0.0		
		Control	0.0 ± 0.0		

Data are the mean ± standard error (SE). Means are separated by Tukey's test of multiple comparisons, and one-way analysis of variance (ANOVA). *p ≤ 0.05, level of significance. **ppm** = parts per million. **LCL** =Lower control limit, **UCL**= Upper control limit.

Discussion

Mosquitoes are nuisance insects that have a higher potential for disease transmission than any other cohort of arthropods. *An. stephensi*, a common urban malaria vector, prefers to breed in artificial breeding grounds. Synthetic chemicals have been applied to control mosquitos for numerous decades. These hazardous chemicals residues in the environment have a global impact on biodiversity and are directly or indirectly responsible for global warming. For decades, the worldwide utilization of synthetic chemicals such as organochlorines, organophosphates, carbamates, and pyrethroids led to vector resistance (27).

Resistance to DDT, Dieldrin, Malathion, and Pyrethroid insecticides has been reported in malaria-endemic India (28 – 38). The rapid increase in insect populations resistant to synthetic insecticides could be attributed to a

Table 6. Larvicidal activity of the leaf crude extracts against 3rd instar larvae of *An. stephensi* mosquitoes.

(Extract	Concen-tration (ppm)	Larval Mortality (24 hrs) (%)	95% Confidence Limits (ppm) (24hrs)		Larval Mortality (48 hrs) (%)	95% Confidence Limits (ppm) (48hrs)	
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)		LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)
Petroleum ether	100	0 ± 0.0			0 ± 0.0		
	500	4 ± 2.31			5 ± 1.33		
	900	11 ± 1.33			23 ± 2.67		
	1300	20 ± 2.31	2458.72[ace]	7653.88[ace]	33 ± 1.33	2118.56[bdf]	7536.79[bdf]
	1700	40 ± 2.31	(2094.06 – 2869.24)	(5446.16 – 16296.30)	40 ± 2.31	(1648.54 – 2561.37)	(5572.01 – 13059.38)
	2100	49 ± 2.67			48 ± 2.31		
	2500	49 ± 3.53			57 ± 1.33		
	2900	52 ± 2.31			61 ± 1.33		
Chloroform	100	0 ± 0.0			1 ± 1.33		
	500	0 ± 1.33			7 ± 1.33		
	900	3 ± 1.33			9 ± 1.33		
	1300	9 ± 1.33	4029.56[cae]	12057.35[cae]	13 ± 1.33	5901.49[dbf]	33687.71[dbf]
	1700	21 ± 1.33	(3243.07 – 5726.32)	(7703.62 – 31359.83)	21 ±1.33	(3974.05 – 21948.42)	(12456.05 – 1296057.47
	2100	25 ± 1.33			27 ± 1.33		
	2500	28 ± 0.00			29 ± 1.33		
	2900	29 ± 1.33			31 ± 1.33		
Methanol	100	7 ± 1.33			21 ± 3.53		
	500	69 ± 3.53			77 ± 2.67		
	900	97 ± 1.33			100 ± 0.00		
	1300	100 ± 0.00	446.56[eac]	750.65[eac]	100 ± 0.00	453.94[fbd]	685.02[fbd]
	1700	100 ± 0.00	(403.28 – 483.03)	(701.70 – 814.07)	100 ± 0.00	(398.00 -495.90)	(639.97 -743.28)
	2100	100 ± 0.00			100 ± 0.00		
	2500	100 ± 0.00			100 ± 0.00		
	2900	100 ± 0.00			100 ± 0.00		

Data are the mean ± standard error (SE). Means are separated by Tukey's test of multiple comparisons, one-way analysis of variance (ANOVA). $p \leq 0.05$ [a,b,c,d,e,f] considered as significant. [a]indicates compared to petroleum ether extract at 24 hour, [b]indicates compared to petroleum ether extract at 48hour, [c]indicates compared to chloroform extract at 24hour, [d]indicates compared to Chloroform extract at 48hour, [e]indicates compared to methanol extract at 24hour, [f]indicates compared to methanol extract at 24hour, **ppm** = parts per million. **LCL** =Lower control limit, **UCL**= Upper control limit.

variety of physiological mechanisms, including target site insensitivity and increased production of detoxifying enzyme (35). Recent research shows that the distribution of *An. stephensi* has spread to Sri Lanka, Djibouti, Ethiopia, and Sudan (27, 39 - 41). Long-term use of synthetic insecticides can be hazardous to one's health and the environment. They disrupt the food chain through bio-magnification and cause ecosystem imbalance by destroying non-target organisms. To meet the global goal of eliminating malaria by 2030, researchers need to find solutions to techniques to combat malaria vectors. This knowledge sparked the idea of employing bioactive plants to establish an environmentally acceptable vector control technique. Much research has shown promising plant extracts as

Table 7. Pupicidal activity of the leaf crude extracts on *An. stephensi* mosquitoes.

Extract	Concentra-tion (ppm)	Pupal Mortality (24 hrs) (%)	95% Confidence Limits (ppm) (24hrs)		Pupal Mortali-ty (48 hrs) (%)	95% Confidence Limits (ppm) (48hrs)	
			LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)		LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)
Petrole-um ether	100	0 ± 0			0 ± 0		
	500	0 ± 0			1 ± 1.33		
	900	5 ± 1.33			13 ± 1.33		
	1300	11 ± 1.33	7205.86[ace]	34651.02[ace]	17 ± 1.33	6641.34[bdf]	46989.35[bdf]
	1700	13 ± 1.33	(4702.72 – 21461. 69)	(14272.15 – 604866.76)	21 ± 1.33	(4166.80 – 14933.88)	(19080.79 – 411697.81)
	2100	15 ± 1.33			23 ± 1.33		
	2500	19 ± 1.33			24 ± 2.31		
	2900	21 ± 1.33			25 ± 1.33		

Chloro-form	100	0 ± 0.0			0 ± 0		
	500	0 ± 0.0			1 ± 1.33		
	900	3 ± 1.33			7 ± 1.33		
	1300	5 ± 1.33	6376.381^{cae}	24341.29^{cae}	9 ± 1.33	6361.86^{dbf}	32235.41^{dbf}
	1700	16 ±2.31	(4418.33 – 16083.08)	(11384.22 – 234788.78)	19 ±1.33	(4392.51 - 17624. 45)	(13391.38 - 554979.31
	2100	16 ± 2.31			21 ± 1.33		
	2500	17 ± 1.33			23 ± 1.33		
	2900	19 ± 1.33			23 ± 1.33		
Methanol	100	13 ± 1.33			23 ± 1.33		
	500	19 ± 1.33			45 ± 1.33		
	900	35 ± 2.67			59 ± 1.33		
	1300	59 ± 1.33	1210.62^{eca}	2010.02^{eca}	75 ± 1.33	998.32^{fdb}	1620.26^{fdb}
	1700	79 ± 3.53	(1108.25 -1295.97)	(1855.28 – 2250.57)	99 ± 1.33	(769.96 – 1127.53)	(1482.89 – 1805.45)
	2100	100 ± 0.00			100 ± 0.00		
	2500	100 ± 0.00			100 ± 0.00		
	2900	100 ± 0.00			100 ± 0.00		

Data are the mean ± standard error (SE). Means are separated by Tukey's test of multiple comparisons, one-way analysis of variance (ANOVA). $p \leq 0.05^{a,b,c,d,e,f}$ considered as significant. [a]indicates compared to petroleum ether extract at 24hour, [b]indicates compared to petroleum ether extract at 48hour, [c]indicates compared to chloroform extract at 24hour, [d]indicates compared to Chloroform extract at 48hour, [e]indicates compared to methanol extract at 24hour, [f]indicates compared to methanol extract at 24hour, **ppm** = parts per million. **LCL** =Lower control limit, **UCL**= Upper control limit.

alternatives to synthetic pesticides against the rising number of mosquito-borne diseases. Environmentally friendly crude extracts from plants with a significant number of phytochemicals have synergistic effects on target species. This lessens the detrimental impact on the environment.

In this study, methanol leaf extract of *P. longifolia* was found to have significant oviposition deterrence, ovicidal, larvicidal, and pupicidal properties against the malaria vector *An. stephensi* mosquitoes. This result is also comparable to earlier reports by Prathibha *et al.* (2013), who reported that *Spilanthus mauritiana* exhibited higher oviposition deterrence activity, followed by *Solidago canadensis*, *Euodia ridleyi*, and *Eugenia jambolana* (42). Veni *et al.* (2016) studied the oviposition-deterrent activity of the seaweed *Lobophora variegata* extracts, viz., hexane, benzene, chloroform, ethyl acetate, and methanol, which were tested against gravid *An. stephensi* mosquitoes (43). Wangrawa *et al.* (2016) observed the oviposition deterrence activity of crude acetone, ethanol, and hexane leaf extracts of *Lantana camara* L., *Ocimum canum*, *Hyptis suaveolens*, and *Hyptis spicigera* against *Anopheles gambiae* Lam (44). Elango *et al.* (2009) studied the oviposition deterrence activity of *Aegle marmelos*, *Andrographis lineata*, and *Cocculus hirsutus* extracts against malarial vector *Anopheles subpictus* mosquitoes (17).

Ali *et al*. (2020) investigated the larvicidal activity of *Saussurea costus* root and leaf extracts against *An. stephensi* mosquitos. The roots' methanol extract had the strongest larvicidal activity, with LC_{50} and LC_{90} values of 7.96 and 34.39 ppm, respectively. (45). Muhammed *et al.* (2022) studied the larvcidal activity of *Calpurnia aurea*, *Momordica foetida*, and *Zehneria scabra* leaf extracts, finding the lowest LC_{50} values in aqueous extracts of *M. foetida,* followed by *Z. scabra* extract and *C. aurea* leaves at 34.61, 35.85, and 38.69 ppm, respectively, against the larvae (46). Veni *et al.* (2016) observed the ovicidal and larvicidal activities of *Lobophora variegata* extracts. The methanol extract was notable for its remarkable ovicidal activity, whereas a larvicidal response was observed with a LC_{50} value of 61.63 ppm (43). Veni *et al.* (2017) assessed the larvicidal and avicidal efficacy of *Terminalia chebula* against *An. stephensi* mosquitoes; the highest larval mortality was recorded in the methanol extract of *T. chebula* against the larvae with an LC_{50} of 87.13 ppm (47). Prabhu *et al.* (2011) evaluated and proved the larvicidal activity of *Moringa oleifera* against *An. stephensi* 3rd instar larvae with a LC_{50} value of 78.93 ppm (22); Kovendan et al. (2012) observed the larvicidal and pupicidal activity of *Leucas aspera* with 10.823% larval mortality and 0.073% pupal mortality (24). Krishnappa *et al.* (2012) investigated the ovicidal, larvicidal, and pupicidal effects of *Gliricidia sepium* (Jacq.) (Leguminosae) against the *An. stephensi* mosquito, with LC_{50} values of 250 ppm, 121.79 ppm, and 75 ppm, respectively (48).

After screening natural bioactive plants with anti-mosquito activity, leaf extracts were identified as general toxicants against the early stages of the malarial vector *An. stephensi*. In this study, multiple crude extracts of *P. longifolia* were used against the early stages of *An. stephensi* mosquitoes, and it was discovered that the methanol extract was more effective against larvicidal activity than other early stages of mosquitoes. These findings may motivate the search for novel, active natural chemicals that can be used as an alternative to synthetic pesticides derived from other medicinal plants. Further research into the mode of action of the constituents, their impacts on non-target organisms, and field evaluation are required.

Conclusion

P. longifolia plant extracts were the most effective against all early stages of *An. stephensi* mosquitoes. According to the findings, a methanol extract of the plant may be used to kill the *An. stephensi* mosquito, and the active ingredient responsible for the bioactivities could be isolated further. These findings will aid in the hunt for more selective, biodegradable, and naturally occurring mosquitocidal phytochemicals.

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Authors contributions

Sowmyashree K: The selection of the objectives and methodology for this study, performed all practical investigations, and wrote the complete original article draft. Raju Krishna Chalannavar: The selection of the topic and finalising the objectives for the study, supervision of overall the experiment methods, and reviewing of the manuscript. Nityasree B R: The statistical analysis, reviewing, and editing of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interests.

Ethical issues: None.

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