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Original paper

Larvicidal potency of *Spatholobus parviflorus* (DC.) Kuntze against *Aedes albopictus* (Diptera: Culicidae)

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ABSTRACT. *Aedes albopictus* (Diptera: Culicidae) is one well-established vector of a series of mosquito-borne diseases. The larval stage of their life cycle is best suited to control a large population of mosquitoes easily. Controlling the larval stage of such vectors can also control the spread of the pandemic disease through the vector. Larvicides, which are synthetic, is a promising solution now. This kind of synthetic larvicides affects humans and animals equally and damages the environment through unscientific and widespread use. Beyond that, their continued use could create selection pressure and, thus, a resistant population. Plant-based larvicide is a way to overcome the shortcomings of synthetic larvicides. This study infers the larvicidal potency of *Spatholobus parviflorus* (DC.) Kuntze solvent extracts (SPSE) and crude water extracts (SPWE) against *Aedes albopictus*, using the dose-response larvicidal assay. The assay conducted using different concentrations of extract (0.1, 0.2, 0.3, 0.4, and 0.5%) and standard (bleaching powder), with the concentrations 0.01, 0.02, 0.03, 0.04 and 0.05%. The nourishment of the larvae is maintained by supplementing each of the Petri plates with 10 mg of larval food. The mortality of the nascent is recorded in a successive time interval of 12 h, up to 72 h. A significant (P<0.05) mortality was observed in SPWE of the leaf. The observed data analyses using Log probit analysis, and the highest LC_{50} (lethal concentration to kill 50% of the population), 0.57 mg/ml, is observed at the 24^{th} h of the larvicidal assay. The lowest LC_{50}, 0.35 mg/ml is observed at the 60^{th} h of the larvicidal assay. In conclusion, the results show that *S. parviflorus* leaf water extract (SLWE) has significant larvicidal activity against *A. albopictus*, and this is the first account for the larvicidal potency of *S. parviflorus*. The study concludes that *S. parviflorus* is an excellent candidate plant for the development of a plant-based larvicide. This possibly influences a reduction in the use of typical household bleach and conventional chemical larvicides.

Keywords: *Spatholobus parviflorus*, *Aedes albopictus*, larvicide, dose-response, mosquito, bleaching powder

Introduction

Blood-feeding mosquitoes play a significant role in the transmission of pathogens. Climatic changes, ecological imbalance, and globalization of international trade are the leading reasons behind their cosmopolitan distribution. In the last decades, various vector mosquito species, in particular *Aedes albopictus* have spread from their tropical origin and subsequently colonized the temperate zones [1].

Aedes albopictus (Asian tiger mosquito) is one of the well-established vectors of a minimum of 22 arboviruses, including DENV, CHIKV, ZIKV and YFV, responsible for lethal diseases like dengue, chikungunya, Zika virus disease, and yellow fever, respectively [2–5]. Eradication of such diseases is possible by reducing the vector density to a level that is not sufficient for the easy transmission of the disease that might be epidemic [2]. Control of vectors in the larval stage is more accessible than that of the adult stage. The breeding of mosquitoes is through water, and the high population of mosquitoes in the small water body can be managed easily using pesticides; besides that, this stage of mosquitoes is favorable for pesticides to act upon [6,7].

The *Aedes albopictus* mosquitoes originate from the forest of South Asia; from there, they spread to Europe, Africa, the Middle East, North and South America, and the Caribbean simultaneously there was an evolutionary process in their habit and host. This kind of flexibility to cope with different environmental conditions is due to their strong physiological and ecological plasticity [3,8]. The

adaptability and successive evolution to new land cause disturbance in native vector-virus interaction and results in the emergence of new viral strains with altered epidemiology [3].

Indoor residual spraying (IRS) or insecticide-treated bed nets (ITN) is a mode of insecticidal application targeted to adult mosquitoes, and through this productive mosquito population gets controlled [9]. Broad and unscientific application of such chemical pesticides will harmfully affect the health of humans as well as other fauna by polluting the water bodies and distressing the environment. Besides that, due to continuous and extensive use, its efficacy gives way to the evolution of a population having resistance to insecticides [9]. The study of Li et al. [10] concluded that *Aedes albopictus* population in the urban parts of China is resistant against DDT and deltamethrin, Guangzhou is one of the urban areas with a continuous outbreak of dengue fever occurred for the last 40 years. Diflubenzuron, methoprene, and pyrethroid are a few of the widely used chemical pesticides against mosquito larvae. Due to unscientific practice, pyrethroid resistance is developed in some populations of *A. albopictus* in Papua New Guinea [11–13]. Records of larvicidal resistance of *A. albopictus* in the continent of Africa and its islands from 1990 also proves that they are resistant to DDT, pyrethroid, carbamate, and organophosphates, as well as have a metabolic resistance to mixed-function oxidases, piperonyl butoxide, diethyl maleate, and glutathione S-transferase [14,15]. Studies of Kushwah et al. [14] proclaim that, on a light scale, *A. albopictus* from Kerala and Delhi (India) is resistant to 0.75% of permethrin and 0.05% of deltamethrin. The study of Marcombe et al. [16] reveals that *A. albopictus* larval population of the US has acquired resistance to spinosad and malathion, which are two pervasively using commercial products both as larvicide as well as insecticide. Insecticide resistance acquired in the larval stage due to adverse effects of gene mutation will retain in the adult stage and is likely to transfer to the succeeding generations.

Chlorine (organochlorines) is another chemical that has been used since ancient times to control the mosquito population in its pre-mature stage. The larvicidal property of chlorine was identified about a century ago [17]. Sherman et al. [18] developed a protocol „La Untadita", which mentions the combined application of chlorine and alkaline detergent to control the larval population of *Aedes aegypti* in washbasins and drums. The active ingredient of household bleach is sodium hypochlorite, which could also be used as a larvicide against mosquitoes [17]. The organochlorines, which are used as a larvicide, not only control the larval population but also, after the completion of the imposed task on the larvicide, it became part of the water cycle and came back to humans, and other animals rely on water to sustain their life. The organochlorines have a series of effects on life forms. They can damage DNA and initiate cancer, organochlorines can act as neurotoxins that can affect the normal function of the neural as well as brain system and, in high dose, lead to dysarthria, euphoria, excitement, nervousness, irritability, depression, anxiety, mental confusion, and memory disorders [19].

The conventional use of pesticides involves its direct application to the water source. However, such a mode of direct application of chemical pesticides to water can cause several risks to people in particular and the environment in general. Plant-based natural pesticides will be more promising in this kind of application [6]. In this work, chlorine is considered as a standard in order to compare the activity of *Spatholobus parviflorus*.

As with traditional medical knowledge, plants were used by people for non-medicinal purposes. They relied on plants to repel insects as well as to control their population [20,21]. This type of biological control is also effective in mosquitoes that are capable of spreading diseases. They are also an alternative to conventional agents, which affords a cheap, easy-to-use, and environment-friendly method [7,13]. There are numerous plants, which belong to the family Fabaceae, that have potent larvicidal properties against different mosquito species. *Delonix elata*, a tree from the same family, has a notable larvicidal activity against *Anopheles stephensi* and *Aedes aegypti* [22], while tree species *Erythrina indica* has a larvicidal property against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* [23]. *Pithecellobium dulce* and *Tephrosia purpurea* from the family Fabaceae and has a larvicidal property against *Anopheles stephensi* and *Aedes aegypti* [24,25]. This study intended to evaluate the larvicidal property of the *Spatholobus parviflorus* and thereby to recommend the plant as a source for the development of plant based larvicide.

Materials and Methods

Plant material

Leaves and bark of *Spatholobus parviflorus* are collected from the Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala (8°34'03.2"N 76°53'15.8"E). The plant material was identified and authenticated by experts, and a voucher specimen was deposited in Kerala University Botany Herbarium under reference number KUBH 10115. Shade dried, powdered plant samples were used for extract in different non-polar and polar solvents. The crude extract was prepared with the aid of freshly collected plant material at the time of assay.

Test mosquitoes

Larvae of *Aedes albopictus* was collected from an obsolete water tank in the campus of the University of Kerala, Kariavattom, Thiruvananthapuram, Kerala (8°34'02.5"N 76°53'14.2"E), India and from the rubber plantation of the ninth ward, Chirakkadavu village, Kottayam, Kerala (9°31'57.8"N 76°45'51.0"E). It was ensured that the collecting sites were free from contaminations by any chemicals before starting the collection. In addition, the larvae were identified by the experts of the Department of Zoology, Madappally College, Kozhikode, Kerala. Collected larvae were reared in a transparent glass bottle containing well water, free of chlorine, and were maintained in existing environmental conditions (atmospheric temperature 26±2°C and relative humidity 74–91%) without any control. The larvae were fed by larval feed. The feed is a combination of powdered dog biscuit, and dried yeast powder in the ratio of 3:1 [26].

Preparation of solvent extract

Solvent extracts of the *Spatholobus parviflorus* was extracted using Soxhlet extraction (Continuous Hot Percolation). Shade dried plant sample was powdered using an ordinary sterile blender. The powdered sample (20 g) was extracted using the Soxhlet extraction apparatus having 100 ml extractor capacity. Serial extraction was initiated with petroleum ether (Merk, emparta boiling range 40–60°C), and the same sample was exposed to chloroform (Merk, emparta), acetone (Himedia AR grade), methanol (Himedia AR grade), and sterile distilled water one followed by the other for further extraction, by increasing polarity. In extraction, siphoning was continued for 6–8 h until a colorless solvent was observed in the siphon tube. The Soxhlet extraction was carried out in an order from a non-polar solvent to polar solvent (petroleum ether, chloroform, acetone, methanol, and water) for eluting most of the phytoconstituents. The mixture of solvent and extract were separated using a rotary vacuum evaporator (Superfit).

Preparation of crude water extract

The crude water extract of leaves and bark were prepared separately according to the procedure of Kamaraj et al. [27]. Tri-foliar leaf from 4–8 nodes at the apex of a branch was used as leaf material. Freshly collected leaves and bark of *Spatholobus parviflorus* were washed in distilled water to remove dust and dirt particles, and the traces of water were removed by using a towel. Leaf and bark sample, each of 50 g, weighed out and grounded individually using mortar and pestle. By using Whatman No. 1 filter paper, the slurry filtered and the subsequent supernatant formed can be regarded as 100% stock solution of the crude. The required concentrations, 0.1, 0.2, 0.3, 0.4, and 0.5%, were prepared from the stock solution using sterile distilled water.

Preparation of standard

The larvicidal activity of plant extracts was compared with the activity of bleaching powder. Required concentrations (0.01, 0.02, 0.03, 0.04 and 0.05%) were prepared from the stock solution (10 mg/ml) using sterile distilled water.

Dose-response larvicidal bioassay

The larvicidal bioassay was carried out with the procedure of Rawani et al. [26] a modified procedure of WHO [28]. For dose-response assay, both the solvent and crude extract were prepared in different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5%). Sterile distilled water was used to make up different concentrations of all solvent and crude water extracts from its stock. For the assay of standard (bleaching powder), a required concentration of (0.01, 0.02, 0.03, 0.04, and 0.05%) was prepared. Each of the extracts prepared was transferred to sterile glass Petri plates. Ten collected larvae of *Aedes albopictus* were placed into each of the Petri plates containing different concentrations of the extract. Ten mg of larval food was added to each of the Petri plates. The mortality rate of larvae was recorded from the 12^{th} hour after a period of incubation, and subsequently, data was collected with a period of 12

Table 1. Potency of different concentrations of *S. parviflorus* leaf crude water extract (SLWE) of and bleaching powder against *Aedes albopictus* larvae

Concentration of crude extract (%)		Mortality rate (%), Mean ± Standard error					
		12 h	24 h	36 h	48 h	60 h	72 h
Leaf crude water extract	0.1	3.33±5.77	3.33±5.77	6.66±5.77	10.00±0.00	10.00±0.00	10.00±0.00
	0.2	0.00±0.00	3.33±5.77	13.33±11.54	13.33±11.54	20.00±10.00	20.00±10.0
	0.3	13.33±15.27	20.0±17.32	20.0±17.32	23.33±15.27	23.33±15.27	23.33±15.27
	0.4	30.00±36.05	40.0±26.45	43.33±32.14	50.00±36.05	60.00±36.05	60.00±36.05
	0.5	0.00±0.00	3.33±5.77	10.00±10.00	16.66±20.81	20.00±26.45	20.00±26.45
Bleaching powder	0.01	3.33±5.77	23.33±5.77	36.66±5.77	53.33±5.77	83.33±5.77	90.00±10.0
	0.02	3.33±5.77	26.66±5.77	43.33±11.54	66.66±5.77	80.00±0.00	93.33±5.77
	0.03	6.66±5.77	26.66±5.77	53.33±5.77	73.33±5.77	90.00±0.00	96.66±5.77
	0.04	13.33±5.77	33.33±5.77	73.33±5.77	90.00±10.00	100.0±0.00	100.0±0.00
	0.05	20.00±5.77	46.66±5.77	73.33±5.77	93.33±5.77	100.0±0.00	100.0±0.00

h intervals. The larvae that failed to move while probing in the siphon or the cervical region were considered dead larvae. The experiment was conducted at an atmospheric temperature of 26±2°C and relative humidity of 74–91%. The experiment was conducted in 3 successive days (n=3).

Statistical analysis

To prove the correlation of concentration and period of incubation with mortality rate, data from dose-response larvicidal assay were subjected to two-way ANOVA (analysis of variance) after Levene's test in SPSS Statistics for homogeneity of variances. The mortality rate of larvae was also subjected to probit analysis to calculate the LC_{50} value according to the procedure of Currell [29], using MS EXCEL 2013. A plot of LC_{50} for bleaching powder and the extract was illustrated using GraphPad Prism 8.4.1.676 trial version for Windows, GraphPad Software, La Jolla California USA.

Results

The assay conducted to access the larvicidal property revealed that *Spatholobus parviflorus* leaf crude water extract (SLWE) has a promising larvicidal activity. The result of the assay on SLWE

Table 2. Regression analysis of the larvicidal activity of SLWE and bleaching powder against *Aedes albopictus* larvae

Model		Unstandardized coefficients		Standardized coefficients	t	Sig	R
		B	SE	Beta			
Leaf	Constant	5.429	4.911		1.105	0.272	0.246
	concentration	38.095	14.806	0.246	2.573	0.012	
Bleaching powder	Constant	34.810	8.421		4.134	0.000	0.208
	concentration	547.619	253.903	0.208	2.157	.033	

Explanations: dependent variable: mortality; B: unstandardized beta; SE: standard error; Sig: significance probability; R: correlation coefficient

Table 3. Levene's test for homogeneity conducted using the concentration and time variances

Levene statistic	Test of homogeneity of variances		
	df1	df2	Sig.
Bleaching powder	4	100	1.000
Leaf	6	98	1.000

Explanations: df: degree of freedom; Sig: significance probability

shows that 60%, which is the highest mortality was observed at a concentration of 0.4% in a period of 60th and 72nd h incubation, among all the concentrations (Tab. 1). Assay carried out with bleaching powder showed a 100% death at a concentration of 0.04% and 0.05% for a period of 60th and 72nd h (Tab. 1).

The data of SLWE larvicidal assay on linear regression analysis in between the variables (mortality and concentration) confirmed that, there is a positive correlation, with an R-value of 0.246 (Tab. 2). Regression analysis on bleaching powder also affirms the positive correlation between mortality and concentration, an R-value of 0.208 (Tab. 2).

Levene's test for homogeneity conducted using the concentration and time variances (Tab. 3) shows that there is no significance (P>0.05) in the variation between the variables, with a 95% confidence level. The magnitude of variation in each of the concentrations with its mortality is also promising.

It is clear that the result of the two-way factorial ANOVA (Tab. 4 and 5) of SLWE and bleaching powder carried out at different concentrations and different time intervals reveals a significant difference in larval mortality (P<0.05). Partial eta squared value indicates that 42.2 and 76.5% of the variability in mortality, respectively, in SLWE and bleaching powder is admitted on the variation in the concentration. As well, 28.2 and 98.5% of mortality is an account for the time of exposure in the assay using SLWE and bleaching powder, respectively.

Log probit analysis conducted for the analysis of SPWE and bleaching powder showed that the LC_{50} is decreasing with increasing the time of exposure (95% confidence level). For SPWE, the highest and lowest LC_{50} observed is 0.576 and 0.350 mg/ml for 24 and 60 h of exposure, respectively. Bleaching powder exhibited the lowest LC_{50} at a concentration of 0.023 mg/ml in the 48 h of the exposure period.

Discussion

The dose-response larvicidal assay was carried out with solvent extracts and crude water extracts of leaf and bark of *Spatholobus parviflorus* and results showed only *S. parviflorus* crude water extract of the leaf (SLWE) has the satisfying larvicidal activity. Based on the regression analysis of SLWE and bleaching powder, standardized beta coefficients centered on the t-distribution is significant (P<0.05),

Table 4. Two-way factorial ANOVA of dose-response larvicidal assay of SPWE using different concentrations at different periods of exposure

Source of variation	SS	df	MS	F	Sig.	PES
Corrected Model	28396.190[a]	34	835.182	2.649	0.000	0.563
Intercept	29837.143	1	29837.143	94.650	0.000	0.575
Concentration (C)	16091.429	4	4022.857	12.761	0.000	0.422
Time (T)	8676.190	6	1446.032	4.587	0.001	0.282
C×T	3628.571	24	151.190	0.480	0.977	0.141
Error	22066.667	70	315.238			
Total	80300.000	105				
Corrected Total	50462.857	104				

Explanations: [a] R-squared = 0.563 (adjusted R-squared = 0.350); SS: sum of squares; df: degree of freedom; MS: mean square; F: F-distribution; Sig: significance probability; PES: partial eta squared

Table 5. Two-way factorial ANOVA of dose-response larvicidal assay of bleaching powder using different concentrations at different periods of exposure

Source of variation	SS	df	MS	F	Sig.	PES
Corrected Model	143739.048[a]	34	4227.619	147.967	0.000	0.986
Intercept	275660.952	1	275660.952	9648.133	0.000	0.993
Concentration (C)	6481.905	4	1620.476	56.717	0.000	0.764
Time (T)	134085.714	6	22347.619	782.167	0.000	0.985
C×T	3171.429	24	132.143	4.625	0.000	0.613
Error	2000.000	70	28.571			
Total	421400.000	105				
Corrected Total	145739.048	104				

Explanations: [a]·R-squared = 0.986 (adjusted R-squared = 0.980); SS: sum of squares; df: degree of freedom; MS: mean square; F: F-distribution; Sig: significance probability; PTS: partial eta squared

and confirm the correlation between the concentration and morality. The regression equation (95% confidence interval of upper and lower confidence limit) is formulated based on the unstandardized coefficient hence, mortality on SLWE = 5.429+38.095(X), where X is the concentration of SLWE, as well the mortality on bleaching powder = 34.810+547.619(Y), where Y is the concentration of bleaching powder. The test for homogeneity of variance and successive two-way fractional ANOVA (P<0.05) proves that the concentration and period of exposure to the extract are directly proportional to the mortality rate. The higher mortality rate of SLWE is found in 0.4% than 0.5%, which is an exception. A higher value of standard deviation (SD) due to a wide range of data was observed. It is because of the reason that the crude extract contains the compounds interrupting the activity. The log probit analysis is conducted to substantiate the relationship between the concentration of extract and the number of larval death. The LC_{50} graph gives a dose-response correlation that the LC_{50} is inversely proportional to the period of exposure (Fig. 1).

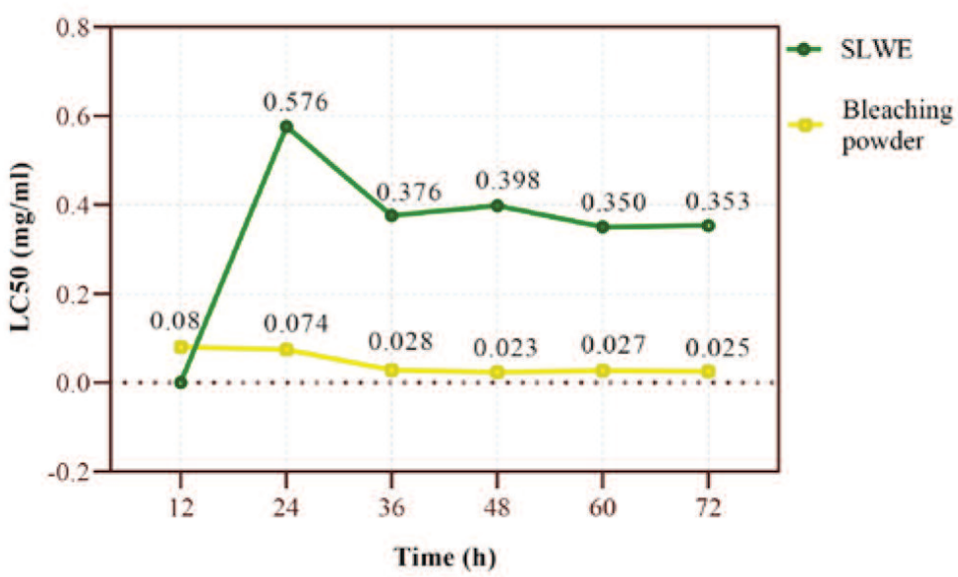

Figure 1. LC_{50} (mg/ml) values of SPWE and bleaching powder at different periods of exposure in the dose-response larvicidal assay
Explanations: SLWE: *S. parviflorus* leaf water extract; LC_{50}: lethal concentration to kill 50% of the population

Studies related to the larvicidal efficacy of different plants are there, but the report on the efficacy of plants, especially to *Aedes albopictus* is limited, and this study is the first account for the larvicidal potency of *S. parviflorus*. By comparing the larvicidal activity of individual compounds, dimethyl trisulfide (LC_{50} 36.36 µg/ml) and methyl propyl disulfide LC_{50} 86.16 µg/ml) [29] against *A. albopictus*, the activity of the SLWE is relevant. *Citrus reticulate* and *Citrus sinensis* are two species with larvicidal potency against *A. albopictus* [30].

It is unknown at this time how the whole extract or the compounds in it works on mosquito larvae as a larvicide [31,32]. Nevertheless, certain insecticidal property evaluation studies give a peripheral illustration of the activity legume lectin in the insecticidal mid-gut [33].

The larvicidal activity of *Spatholobus parviflorus* can only be found in SLWE; hence the larvicidal potency will be due to a volatile compound or by the comprehensive activity of water-soluble compounds like proteins because the activity of solvent extracts is not evident. However, the *S. parviflorus* is a rich source of micro and macromolecules or proteins, like lectins with insecticidal properties [33–35].

In conclusion, apart from the conventional larvicidal and insecticidal use, possible and eco-friendly practices like plant-based larvicides and insecticides are a promising solutions to control many epidemics. The current study concludes that the *Spatholobus parviflorus* is a potent source responsible for the control of *Aedes albopictus* population. Further investigation is necessary to identify and isolate the compound or its group responsible for the production of a commercial plant-based larvicide instead of typical household bleach and conventional chemical larvicides.

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