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Arpita Gope
Ph.D., Scholar, Laboratory of Parasitology, Vector biology and Nanotechnology, Department of Zoology The University of Gour Banga, Mokdumpur, Malda, West Bengal, India

Dr. Anjali Rawani
Assistant Professor, Laboratory of Parasitology, Vector biology and Nanotechnology, Department of Zoology The University of Gour Banga, Mokdumpur, Malda, West Bengal, India

Corresponding Author:
Dr. Anjali Rawani
Assistant Professor, Laboratory of Parasitology, Vector biology and Nanotechnology, Department of Zoology The University of Gour Banga, Mokdumpur, Malda, West Bengal, India

Evaluation of mosquitocidal potency of leaves and fruits extracts of *Phyllanthus acidus* L. against filarial vector *Culex quinquefasciatus* Say

Arpita Gope and Anjali Rawani

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Abstract
The present piece of work was done to carry out larvicidal, pupicidal, adulticidal, and repellent activities of crude extracts of mature leaf and fruit extract of *Phyllanthus acidus* against filarial vector *Culex quinquefasciatus*. The percentage mortality of 1st instar stages of *Cx. quinquefasciatus* at 0.7% concentration was considerably higher ($p < 0.05$), followed by second, third and fourth instars larvae. Whereas crude extract of fruits showed 100% mortality at 0.9% concentration in case of first instar larvae of *Cx. quinquefasciatus* after 24 h of contact. The lowest LC_{50} value observed was 0.26 ml and 0.34 ml against the first instar larval stages of *Cx. quinquefasciatus* after exposure of 24 hours in crude leaf and fruit extract of *P. acidus*, respectively. In pupicidal bioassay, the crude leaves extract of *P. acidus* exhibited pupicidal potency with LC_{50} values of 7.463% after 24 h. The extract from fresh leaves of *P. acidus* revealed repellency against the mature females of *Cx. quinquefasciatus* with 94% protection. In adulticidal activity, after 24 h of exposure there is 93.3% death of adult mosquitoes of *Cx. quinquefasciatus* observed. ANOVA (three-way factorial) was done for both the crude extracts of plant parts and it showed a substantial variance in larval mortality ($p<0.05$). Qualitative phytochemical investigation showed the phytochemical components of both the crude extracts. The analysis indicated the occurrence of many secondary metabolites such as flavonoid, tannins, steroids, terpenes, alkaloids, saponins etc. in both the crude extracts of *P. acidus*. From the study, it is concluded that the crude extract of fruit and leaves of *P. acidus* showed an excellent possibility to be adopt as an idyllic environment friendly method for controlling the population of *Cx. quinquefasciatus*.

Keywords: *Phyllanthus acidus*, *culex quinquefasciatus*, larvicidal activity, pupicidal activity, repellent, adulticidal activity, phytochemical analysis

Introduction

Mosquitoes cause nuisance as well as they are the chief vector of several vector-borne diseases affecting human beings and animals (Panneerselvam *et al*., 2012) [19]. Numerous diseases, such as malaria, filariasis, dengue, Japanese encephalitis, Chikungunya, and yellow fever spread by this primary sole group of public health insect. These vector-borne diseases cause millions of deaths yearly in almost all tropical and subtropical countries (Ghosh *et al*., 2012) [9]. Bancroftian filariasis transmitted mainly by *Culex quinquefasciatus* is broadly dispersed in tropical counties. The disease is accounted around 120 million infections yearly and the common chronic signs devoured by approximately 44 million people worldwide (Bernhard *et al*., 2003) [3]. Right now 863 million people in 47 countries globally continue susceptible by Bancroftian filariasis and need precautionary chemotherapy to break the chain of spread of this parasitic infection (WHO, 2022) [39]. Mainly two filarial nematodes, *Wuchereria bancrofti* and *Brugia malayi* are pathogenic agent of lymphatic filariasis. *Cx. Quinquefasciatus* transmitted the nocturnally periodic *Wuchereria bancrofti*, which is carried by definitive host i.e. infected people (Murugan *et al*., 2012) [18]. *Cx. quinquefasciatus* is a domestic mosquito and breeds in habitats such as eutrophic waterbodies which is rich in organic matter *viz* drain, that contains standing water filthy with human or animal waste, where it can reach very high larval densities (Harvey-Samuel *et al*., 2021) [11].

Mosquito control is necessary to check the propagation of mosquito-borne diseases that in turn mend the quality of the environment and public health (Ghosh *et al*., 2012) [9]. Immatures of mosquito are mainly aimed for pesticides application because they confined to water bodies and, thus, are easily locate and control (Amer *et al*., 2006) [2]. However, since the discovery of synthetic pyrethroids as mosquitocidal insecticides, very limited new efficient and cost-effective insecticides have been developed (Shaalan *et al*., 2005) [30]. The application of synthetic insecticides brings out some problems, such as increase of resistant in insect, disruption of ecosystems, and harm to other non-target organisms (NTOs) (Maheswaran *et al*., 2008) [14]. Frequent use of synthetic chemical insecticides also responsible for disruption of natural biological control systems and resurrections in vector mosquito populations (Das *et al*., 2006) [6]. The insecticides of herbal origin are excellent substitutes of chemical insecticides for control of vector mosquitoes (Ghosh *et al*., 2012, Rawani *et al*., 2014, 2017) [9, 27, 28]. Botanical insecticides are generally favored due to their innate biodegradability and harmless to other NTOs (Prabakar *et al*., 2003) [20]. Plant phytochemicals have potency to act as larvicide, pupicide, adulticide, repellency, and ovicide (Panneerselvam *et al*., 2012) [19]. Floral extracts may be better choice to control mosquito population because they harbor a varieties of phytochemicals that are easily degradable and suitable for application in their natural breeding places (Kamaraj *et al*., 2009, Rawani *et al*., 2010, 2012, 2013) [13, 24, 26].

Phyllanthus acidus (L) from the family Phyllanthaceae is a transitional among a shrub and tree, reach a height up to 2 to 9 m (Center for New Crops & Plants Products, 2011) [4]. The plant is distributed throughout Central and South America, Asia, and the Caribbean region (Janick *et al*., 2008) [12]. There are many medicinal properties of plant parts. The leaves are used for the treatment of sciatica, lumbago, and rheumatism, while the fruits are eaten as a blood enhancer for the liver (Janick *et al*., 2008) [12]. Few authors reported phytochemical constituents extracted from plants such as 4-hydroxybenzoic acid, caffeic acid, adenosine, kaempferol, and hypogallic acid (Sousa *et al*., 2006) [32].

The main objective of this piece of work was to study the larvicidal, pupicidal, adulticidal, and repellent activity of the mature leaves and fruit extracts of *Phyllanthus acidus* against different life form stages of filarial vector *Cx. quinquefasciatus*. Qualitative analysis of phytochemical was also executed to get the pilot data about phytochemical constituents of both the plant part extracts.

2. Materials and Methods

2.1 Collection of plant parts

From different regions of village-Nalagola, Malda district, West Bengal, India, developed leaves and fruits of the *Phyllanthus acidus* plant have been collected.

2.2 Preparation of sample material

The present study was conducted at the parasitology, vector biology and Nanotechnology laboratory, University of Gour Banga, Malda, from Jan to Mar 2022. Fresh, mature green leave and fruits of *P. acidus* were randomly collected from plants. Firstly, the mature leaves and fruits were cleaned with distilled water and then dried out on a paper towel. By crushing both the plant materials separately in a mechanical grinder, the raw crude extract of both the plant parts was extracted. Then the crushed material filtered over Whatman No. 1 filter paper.

2.3 Collection and maintenance of larvae

Larvae of *Culex quinquefasciatus* were collected from their natural breeding places surrounding the campus of University of Gour Banga, Malda. These larvae were brought to the laboratory and shifted to 18cm * 13cm * 4cm plastic trays and maintained following the protocol of Kamaraj *et al*., 2009 [13]. The experiments were conducted at 27±2° C and 75-85% RH underneath a 14:10 h light and dark cycle.

2.4 Maintenance of pupae and adults

The transformed pupae were separated manually from the culture tray through the glass dropper taken into a 500 ml glass beaker containing tap water. The jars were kept in a 90cm*90cm*90cm mosquito cage for adult emergence. 10% starch solution soaked in a cotton ball was used for the feed of adult mosquitoes.

2.5 Mosquito larvicidal bioassay

WHO standard protocols (WHO, 2005) followed for the larvicidal bioassay with minor modifications. 25 First to fourth instar larval stages of *Cx. quinquefasciatus* were taken in 3 sets in 100 ml of water. 0.5 to 1 ml (0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml) concentration of the leave and fruit crude extract were added. A control was also set with 25 larvae in 100 ml of water for each experiment. Dead larvae were counted after exposure of 24 h, 48 h, and 72 h. Abbott's formula applied for the correction of percentage mortality which was fixed by means of the average of three replicates. The LC_{50} value was determined by probit analysis (Finney, 1979) [8].

2.6 Pupal toxicity test

The pupicidal bioassay done by the WHO (1975) standard protocols with slight modification. Pupal stages of mosquitoes were used for the pupicidal bioassay. 100 ml of water taken into each 500 ml glass beaker and twenty-five pupae were introduced. 0.4% - 1% concentration of crude extract of leaves of *P. acidus* was added. At each tested concentration, three replicates were made. 25 pupae in 100 ml of dechlorinated water functioned as control. The pupal death was assessed after exposure of 24 h.

A larva or pupa was declared dead if it did not show any movement while gently touched with the pipette's tip or found immobile at the container's bottom and when it did not show any diving response when water was disturbed.

$$\text{Percent mortality} = \frac{\text{(Number of larva/pupae introduced - Number of dead larvae/pupae)}}{\text{Number of larva/pupae introduced}} \text{ X } 100$$

2.7 Adulticidal Bioassay

The bioassay was performed using adult stages of *Cx. quinquefasciatus* following the protocol of WHO (1981) [38] with slight modifications. Different concentrations such as 1ml, 2 ml, 3 ml, 4 ml, and 5 ml of mature leaves crude extract used for the adulticidal activity. According to Dua *et al*., 2008

[7], mentioned dose were spread on filter papers (size 12 cm * 15 cm). In control set up, only distilled water applied on filter papers were used. Twenty-five adult female mosquitoes (blood starving 2-5 days old mosquitoes and glucose fed) were used for the bioassay. At first, they were smoothly moved into an elastic holding tube. Inside the tube they were kept for an hour for the acclimatization and after that they exposed to the treated paper (filter paper) for an hour. After the contact hour, adult female mosquitoes remain positioned inside the elastic holding tube and seized for 24 hours to recover. On the mesh screen a cotton plug drenched with 10% starch solution remained positioned for the feeding purpose. Mortality of mosquitoes was observed after the 24 h recovery period. Abbott's formula (Abbott, 1925) used for the correction of the percent mortality.

2.8 Repellent activity
Test of repellency of crude extract of leaves of plant was tested by the author itself. Repellent activity was implemented by using the methodology of Murugan *et al*., 2007 [17]. 3-5 days old blood starving female *Cx. quinquefasciatus* (100) were introduced in a mesh cage having dimension 45 cm× 30 cm ×45 cm. The hands were properly cleaned with water. 25 cm^2 area on the dorsal side of the skin on each arm used for the experiment, and the rest of the part of the skin was covered with rubber gloves. The crude leaves extract was applied with a concentration of 0.8 g/cm^2 in the uncovered part of the hand. For control, ethanol was used. At afternoon from 13:00 to 18:00 repellency against *Cx. quinquefasciatus* has been tested. Both the control arms and tested arms were entered inside the mesh cage. The assessment was carried out by placing the processed arms and control arms in the similar cage for a minute of each five minutes. On the hand, the number of mosquitoes sat was noted and before sucking the blood, they were knocked off. The following formula calculated the percentage of repellency.

$$\% \text{ Repellency} = [(Ta-Tb) / Ta] \times 100$$

here Ta denotes the number of mosquito bites in the control set, and Tb indicates the number of mosquito bites in the tested set.

Qualitative phytochemical analysis of the plant extracts
Qualitative analysis of phytochemical of crude extract of both the plant parts of *P. acidus* were evaluated according to the procedures of Harborne (1984) [10], Trease (1989) [34] and Sofowora (1983) [31]. The presence of following phytochemicals were studied flavonoid, steroids, alkaloid, saponins, terpenoids, tannin, flavonoids, phenolics, glycoside phlobatanins and anthraquinones. This preliminary study gives an idea regarding the kinds of active ingredient liable for larval mortality.

2.9 Statistical analysis
Abbott's formula was used for the correction of percentage of larval mortality (Abbott, 1925). Statplus 2009 computer software and MS EXCEL 2010 has been used for the analysis of experimental data. Probit analysis and regression coefficient values were calculated by Statplus 2009 software. SPSS 11.0 software used for the analysis of three-way factorial ANOVA.

3. Result
The study revealed 100% mortality of first instar larvae of *Cx. quinquefasciatus* at 0.7 ml in the mature crude leaf extract of *P. acidus* after 72 h, while second and third larval instar showed 100% mortality at 0.9 ml afterward 48 h and 72 h, respectively (Table 1). Whereas, fourth instar larvae showed 100% mortality at 0.9 ml of crude extract of mature leaf of *P. acidus* after 72 hours of contact (Table 1). Table 2 shows the percentage mortality of first to fourth larval instar of Cx. *quinquefasciatus* at 0.5 to 1 ml of raw extract of fruits of *P. acidus* after 24h, 48h, and 72h of exposure. 100% mortality observed in first to fourth immature stages of *Cx. quinquefasciatus* at 0.9 ml concentration after 24 h, 48 h, and 72 h. Regression analysis exhibited a positive correlation between percentage mortality and concentration of extracts of mature leaves and fruits of *P. acidus*, and regression coefficient (R) value found to be close to 1 in each case. Probit analysis (95% confidence level) carried out to determine the values of LC_{50} and LC_{90} of leave as well as fruit crude extract against larvae, pupae and adults of *Cx. quinquefasciatus* which revealed lowest value after exposure of 72 h which is followed by 48 h and 24 h. (Table 3 and table 4). The lowest LC_{50} value observed is 0.26 ml and 0.34 ml against the first immature stage of *Cx. quinquefasciatus* after exposure of 24 hours in crude leaf and fruit extract of *P. acidus*, respectively. Table 5 displays the pupicidal efficacy of crude leaves extract of *P. acidus*. The highest pupicidal activity has been observed at 1 ml concentration (73.3% mortality) after exposure of 24 h, having an LC_{50} value of 7.463 ml afterward 24 h. Adults of *Cx. quinquefasciatus* showed the highest mortality at 5 ml concentration with an LC_{50} value of 2.869 ml after contact of 24 h (Table 6). Biting repellency of mature *Cx. quinquefasciatus* observed at a concentration of 0.8 gm/cm^2 (Table 7) which showed the repellent activity of crude extract of mature leaves of *P. acidus*. After 15 minutes, only six bites were observed in the processed hand and thus showed 94% repellency. Outcome of the three-way factorial ANOVA (Table 8 and Table 9) presented a significant variance in larval mortality ($p<0.05$), where different larval instars, various concentrations of leaves and fruit crude extracts and different hours of exposure used as variables. The qualitative analysis of phytochemical of leaves and fruit crude extract provides the preliminary information about the phytochemical constituents. The presence of these secondary metabolites may account for their biocontrol potential (Table 10).

4. Discussion
Resistance of vector mosquitoes to conventional chemical insecticides paves the way towards the development of new insecticides. Plant extract exerts varieties of physiological activity on pests, including larvicidal, pupicidal, adulticidal, ovicidal, repellent, etc. (Murugan & Babu, 1996; Venkatachalam & Jebanesan, 2001; Rajkumar and Jebanesan, 2004, Rawani *et al*., 2010, 2012, 2013) [16, 21, 24, 25, 35]. This may be due to a variety of phytochemicals in plants working synergistically to produce such responses. Several bioactive compound such as phenolics, alkaloids, phytosteroids, flavonoids, tannins, glycosides, and terpenoids from a wide range of plants were described previously by Shaalan *et al*., (2005) [30] for their pesticide or insecticide properties. Pesticides or insecticides of plant origin are eco-friendly and hardly develop pest resistance due to the various combination

of bioactive compounds, which attenuates the longstanding environmental effects of the use of synthetic pesticides (Maurya *et al*., 2012) [15]. In vector mosquito control management, potency of plant extracts can be defined compared to single or synthesized chemical pesticides due to their cost effectiveness, easy to apply and pollution free properties (Rahuman & Venktesan, 2008) [22].

Nowadays, the mosquito control program focuses more on eliminating mosquitoes at the larval stage with plant extract. Approximately 1,200 plant species were defined by Roark (1947) [29], while 344 plant species that showed anti-mosquito activity were listed and described by Sukumar *et al*. (1991) [33]. Rawani *et al*. 2009 [23] stated the efficacy of the raw extracts from three plants namely *Carica papaya*, *Murraya paniculata*, and *Cleistanthus collinus* against larvae of *Cx. quinquefasciatus*. The comparative efficiency of the extracts of plant parts as larvicide was as follows: seed extract of *C. papaya* > fruit extract of *M. paniculata* > leaf extract of *M. paniculata* > leaf extract of *C. collinus*. Maximum mortality observed in 0.5% crude extract of each plant after exposure of 72 h bioassay followed by 24 and 48 h. During the study it was also observed that 3rd instar stage larvae are typically more sensitive than 1st, 2nd and 4th larval stages to most plant extracts. The potency of the raw extracts of following plants *Alternanthera sessilis*, *Trema orientalis*, *Gardenia carinata*, and *Ruellia tuberosa* against larval stages of *Culex quinquefasciatus* has also been studied by Rawani *et al*. 2014 [27]. Efficacy of raw extracts of all these plant parts were studied up to 72 h of exposure. The laboratory bioassay revealed maximum mortality in crude extract of *A. sessilis* at 1.5 percent concentration having an LC_{50} value of 0.35 percent, followed by *R. tuberosa* with LC_{50} value of 1.84%, *G. carinata* with LC_{50} value of 2.11% and *T. orientalis* with LC_{50} value of 2.95%. Leaf extract of *P. acidus* has the efficacy to be employed in vector control programs because of the richness of the phytochemicals it contains.

During this study, the biological control potential of crude *P. acidus* leaf and fruit extracts against *Cx. quinquefasciatus* have been well established under experimental conditions. During the study it was noted that, highest mortality found in crude leave extract, followed by fruit extract. Thus the lowest LC_{50} value calculated was 0.26 ml and 0.35 ml of crude leaves and fruit extract of *P. acidus* against the first larval instar of *Cx. quinquefasciatus* after exposure of 72 h. In pupicidal bioassay, highest mortality observed in crude extract of leaves at 1 ml concentration after 24 hours, having an LC_{50} value of 7.463 ml. The investigation on adulticidal activity showed an LC_{50} vale of 2.869 ml after 24 h of contact. The study on repellent activity of extract of leaves showed 94% repellency from biting of *Cx. quinquefasciatus* when tested at concentration of 0.8 g/ cm^2 applied on the uppermost surface of the hand. Qualitative analysis of phytochemical of two crude extracts affirms the occurrence of several bioactive compounds that, alone or in combination, can be accountable for larval death.

In recent years, plant-based and environmentally friendly insecticides have become increasingly important. They are nontoxic to other aquatic organisms because of their target specificity and readily biodegradable properties. Outcome of the present study indicate that leaves and fruits of *P. acidus* can serve as an effective larvicide, pupicide, and adulticide against *Cx. quinquefasciatus*. It will be profitable since it has efficacy at a low concentration. It is native, easily degradable and safe in comparison to synthetic chemical insecticides, which are unsafe to the environment and also toxic. This study paves the way to future studies on the solvent extraction of used plant parts and identifying active ingredients which can be utilized for a broad variety of species of mosquito in the mosquito control program.

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Conflicts of interest/Competing interests

Authors are stated to have no competing interests.

Availability of data and material

Entire data produced and analyzed for this study are presented in the manuscript.

Ethics approval

Not applicable

Consent for publication

Authors hereby approve to publish this document. The data has not been published incompletely or as a whole in any other journal.

Table 1: Efficacy of different concentrations of crude extract of mature leaf of *P. acidus* on different larval instars of *Culex quinquefasciatus*

Larval stage	Concentration (%)	% Mean mortality 24 hrs. 48 hrs. 72 hrs.		
1st instar	0.5	66.7±3.3	88.3±3.3	90.0±5.8
	0.6	80.0±5.8	88.3±3.3	93.3±3.3
	0.7	83.3±3.3	90.0±0.0	93.3±3.3
	0.8	93.3±3.3	96.7±3.3	100.00±0.00
	0.9	100.00±0.00	100.00±0.00	100.00±0.00
	1.0	100.00±0.00	100.00±0.00	100.00±0.00
	Control	0.57 ± 0.15	0.93±0.02	0.96±0.02
2nd instar	0.5	46.7±3.3	60.0±0.0	70.0±0.0
	0.6	63.3±3.3	66.7±8.67	70.0±0.0
	0.7	73.3±3.3	86.7±3.3	90.0±0.0
	0.8	83.3±3.3	90.0±0.0	96.7±3.3
	0.9	96.7±3.3	100±0.0	100±0.0
	1.0	100.00±0.00	100.00±0.00	100.00±0.00

	Control	0.76±0.08	0.83±0.06	0.87±0.05
3rd instar	0.5	53.3±3.3	60.0±5.8	70±5.80
	0.6	56.7±3.3	66.7±6.7	76.7±8.80
	0.7	63.3±3.3	80.0±5.8	90.0±0.00
	0.8	83.3±3.3	90.0±5.8	90.0±0.00
	0.9	90.0±5.8	96.7±3.3	100.00±0.00
	1.0	96.0±0.0	100.00±0.00	100.00±0.00
	Control	0.73±0.07	0.82±0.06	0.87±0.05
4th instar	0.5	43.3±3.3	46.70±3.3	66.7±8.8
	0.6	53.3±3.3	66.7±3.3	83.3±3.3
	0.7	60.0±5.8	73.3±3.3	86.7±3.3
	0.8	70.0±5.8	76.3±3.3	90.0±5.8
	0.9	83.3±3.3	90.0±0.0	93.3±3.3
	1.0	93.3±3.3	96.7±3.3	100.00±0.00
	Control	0.67±0.07	0.74±0.07	0.86±0.04

Table 2: Efficacy of different concentrations of crude extract of mature fruit of *P. acidus* on different larval instars of *Culex quinquefasciatus*

Larval stage	Concentration (%)	% Mean mortality		
		24 hrs.	48 hrs.	72 hrs.
1st instar	0.5	63.3±3.3	76.7±3.3	86.7±3.3
	0.6	73.3±3.3	80.0±5.8	80.0±5.8
	0.7	73.3±8.8	80.0±5.8	90.0±5.8
	0.8	90.0±5.8	93.3±3.3	93.3±3.3
	0.9	100.00±0.00	100.00±0.00	100.00±0.00
	1.0	100.00±0.00	100.00±0.00	100.00±0.00
	Control	0.83±0.06	0.84±0.06	0.91±0.03
2nd instar	0.5	56.7±3.3	60.0±5.8	76.7±0.0
	0.6	66.7±3.3	76.7±3.3	86.7±3.3
	0.7	76.7±3.3	80.0±5.8	93.3±3.3
	0.8	86.7±3.3	90.0±0.0	96.7±3.3
	0.9	93.3±3.3	100.00±0.00	100.00±0.00
	1.0	100.00±0.00	100.00±0.00	100.00±0.00
	Control	0.79 ±0.06	0.84±0.06	0.91±0.03
3rd instar	0.5	43.3±3.3	60.0±5.8	70±5.80
	0.6	60.0±5.8	66.7±6.7	76.7±8.80
	0.7	73.3±3.3	80.0±5.8	90.0±0.00
	0.8	86.7±3.3	90.0±5.8	90.0±0.00
	0.9	96.7±5.8	96.7±3.3	100.00±0.00
	1.0	100.00±0.00	100.00±0.00	100.00±0.00
	Control	0.76±0.08	0.82±0.06	0.87±0.05
4th instar	0.5	50.0±5.8	76.70±3.3	86.7±6.7
	0.6	56.7±8.8	76.7±8.8	90.0±5.8
	0.7	56.7±3.3	83.3±3.3	93.3±3.3
	0.8	80.0±5.8	93.3±3.3	100.00±0.00
	0.9	93.3±3.3	100.00±0.00	100.00±0.00
	1.0	100.00±0.00	100.00±0.00	100.00±0.00
	Control	0.72±0.08	0.88±0.04	0.95±0.02

Table 3: Log probit analysis and regression analysis of larvicidal activity of mature leaves of *P. acidus* against different instar larval forms of *Cx. quinquefasciatus*

Larval instar	Larval exposure duration	LC_{50} value (ml)	LC_{90} value (ml)	Regression equation	R value	Chi-square value
1st	24	0.44	0.70	Y=6.76X+3.60	0.90	0.22
	48	0.32	0.66	Y=3.62X+6.39	0.84	0.14
	72	0.26	0.53	Y=2.09X+7.98	0.80	0.04
2nd	24	0.54	0.82	Y=10.76X+0.35	0.96	0.42
	48	0.48	0.76	Y=8.38X+2.05	0.92	0.39
	72	0.45	0.71	Y=7.04X+3.38	0.90	0.71
3rd	24	0.57	0.91	Y=11.33X+1.33	0.94	0.69
	48	0.48	0.79	Y=8.82X+1.95	0.88	0.34
	72	0.42	0.71	Y=6.19X+4.08	0.83	0.15
4th	24	0.58	1.06	Y=10.80X+0.78	0.94	0.59
	48	0.54	0.87	Y=10.57X+0.43	0.89	1.02
	72	0.39	0.69	Y=3.42X+6.93	0.90	0.12

Table 4: Log probit analysis and regression analysis of larvicidal activity of mature fruits of *P. acidus* against different instar larval forms of *Cx. quinquefasciatus*

Larval instar	Larval exposure duration	LC_{50} value (ml)	LC_{90} value (ml)	Regression equation	R value	Chi-square value
1st	24	0.47	0.76	Y=8.00X+2.33	0.87	0.85
	48	0.39	0.70	Y=5.43X+4.76	0.83	0.58
	72	0.35	0.63	Y=3.81X+6.36	0.71	0.52
2nd	24	0.49	0.82	Y=8.76X+1.42	0.96	0.31
	48	0.47	0.74	Y=8.00X+2.45	0.91	0.31
	72	0.39	0.62	Y=4.37X+5.79	0.85	0.03
3rd	24	0.54	0.81	Y=11.62X+1.05	0.95	0.38
	48	0.47	0.71	Y=7.91X+2.68	0.93	0.09
	72	0.41	0.62	Y=4.76X+5.65	0.83	0.14
4th	24	0.55	0..90	Y=10.95X+0.94	0.91	2.15
	48	0.41	0.70	Y=5.62X+4.62	0.82	0.56
	72	0.37	0.58	Y=2.95X+7.29	0.81	0.08

Table 5: Pupicidal activity of *P. acidus* crude leaf extract on pupa of *Cx. quinquefasciatus*

Concentration(ml)	% Mean mortality after 24 hrs.	LC_{50} value (ml)
0.4	6.7±0.33	7.463ml
0.5	13.3±3.3	
0.6	23.3±3.3	
0.7	46.7±3.3	
0.8	56.7±3.3	
0.9	70±5.8	
1.0 ml	73.3±3.3	
Control	0.41±0.10	

Table 6: Adulticidal activity of *P. acidus* crude leaf extract on adults of *Cx. quinquefasciatus*

Concentration(ml)	% Mean mortality after 24 hrs.	LC_{50} value (ml)
1.0	13.3±0.33	2.869 ml
2.0	16.7±3.3	
3.0	43.3±3.3	
4.0	66.7±3.3	
5.0	93.3±3.3	
Control	0.46±0.15	

Table 7: Repellent activity of *P. acidus* mature leave crude extract against female *Culex quinquifasciatus*

Mosquito repellent product	Concentration	Observation time (4 pm- 6 pm)	Total no. of mosquitoes	No. of bites in the treated arm	% Repellancy
Mature leaves of *P. acidus*	$0.8gm/cm^2$	15 min	100	06	94
		30 min		11	89
		60 min		23	77
		90 min		36	64
		120 min		47	53

Table 8: Completely randomized three-way factorial ANOVA related to mortality of larval forms (instars) of *Culex quinquefasciatus* using different larval instars, different concentrations of crude leaf extract and different hours of exposure as three variables-

Source of variation	Sum of squares	DF	Mean squares	F value	P value
Instar	42.718	3	14.239	33.072	<0.001
Hour	72.528	2	36.264	84.226	<0.001
Concentration	412.931	5	82.586	191.813	<0.001
Instars * Hour	15.102	6	2.517	5.846	<0.001
Instar*Conc.	18.421	15	1.228	2.852	0.001
hour*Conc.	25.417	10	2.542	5.903	<0.001
Instars*Hour *Conc.	13.843	30	0.461	1.072	O.379*
Residual	62.000	144	0.431		
Total	662.958	215			

* denotes non-significant

Table 9: Completely randomized three-way factorial ANOVA related to mortality of larval forms (instars) of *Culex quinquefasciatus* using different larval instars, different concentrations of crude fruit extract and different hours of exposure as three variables

Source of variation	Sum of squares	DF	Mean squares	F value	P value
Instar	2.940	3	0.980	2.301	0.001
Hour	78.778	2	39.389	92.478	0.001
Concentration	306.153	5	61.231	143.759	0.001
Instars * Hour	11.074	6	1.846	4.333	0.001
Instar*Conc.	13.310	15	0.887	2.083	0.014
hour*Conc.	37.278	10	3.728	8.752	0.001

Instars*Hour *Conc.	9.093	30	0.303	0.712	O.862*
Residual	61.333	144	0.426		
Total	519.958	215			

* denotes non-significant

Table 10: Result of qualitative phytochemical analysis of the crude extract of leaves and fruits of *P. acidus*

Phytochemicals	Present/Absent	
	Leaves	**Fruits**
Tannin	+	+
Terpenoid	+	+
Glycoside	-	+
Phenolics	+	+
Flavonoid	+	+
Steroid	+	-
Anthraquinone	-	-
Saponin	-	+
Alkaloid	+	+
Phlobatanins	-	+

+ denotes present; - denotes absent

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