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

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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 LC50 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 LC50 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) [19]. 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 larvicde, 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, lumbergo, 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 leaves 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 LC50 value was determined by probit analysis (Finney, 1979) [9].

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.

Percent mortality = \[
\frac{\text{Number of larva/pupae introduced} - \text{Number of dead larva/pupae}}{\text{Number of larva/pupae introduced}} \times 100
\]
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 cmx 30 cm x45 cm. The hands were properly cleaned with water. 25 cm² 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/cm² 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.

% Repellency = [(Ta-Tb) / Ta] × 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 Harbore (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 phlobatans 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 after 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 LC50 and LC90 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 LC50 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 LC90 value of 7.463 ml after 24 h. Adults of Cx. quinquefasciatus showed the highest mortality at 5 ml concentration with an LC50 value of 2.869 ml after contact of 24 h (Table 4). Biting repellency of mature Cx. quinquefasciatus observed at a concentration of 0.8 gm/cm² (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, 55]. 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 mosquito larvae 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 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 LC50 value of 0.35 percent, followed by R. tuberosa with LC50 value of 1.84%. G. carinata with LC50 value of 2.11% and T. orientalis with LC50 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 LC50 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 pupcidal bioassay, highest mortality observed in crude extract of leaves at 1 ml concentration after 24 hours, having an LC50 value of 7.463 ml. The investigation on adulticidal activity showed an LC50 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/cm² 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 phytochemicals, which are unapplied 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.

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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 |                                           | 2nd 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  | 100.0±0.0 |
| 0.9          |                   | 100.0±0.0  | 100.0±0.0 | 100.0±0.0 |
| 1.0          |                   | 100.0±0.0  | 100.0±0.0 | 100.0±0.0 |
| Control      |                   | 0.57±0.15  | 0.93±0.02 | 0.96±0.02 |
|              |                   | 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        | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 |
### Table 3: Log probit analysis and regression analysis of larvicidal activity of mature leaves of *P. acida* against different instar larval forms of *Cx. quinquefasciatus*

| Larval instar | Larval exposure duration | LC₅₀ value (ml) | LC₉₀ 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+2.03        | 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<sub>50</sub> value (ml) | LC<sub>90</sub> value (ml) | Regression equation | R value | Chi-square value |
|---------------|-------------------------|-----------------------------|-----------------------------|---------------------|---------|------------------|
| 1<sup>st</sup> | 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             |
| 2<sup>nd</sup>| 24                      | 0.49                        | 0.82                        | Y=8.76X+1.42        | 0.96    | 0.31             |
|               | 48                      | 0.39                        | 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             |
| 3<sup>rd</sup>| 24                      | 0.47                        | 0.81                        | Y=7.91X+2.68        | 0.93    | 0.38             |
|               | 48                      | 0.47                        | 0.71                        | Y=5.62X+4.62        | 0.91    | 0.09             |
|               | 72                      | 0.41                        | 0.62                        | Y=4.76X+5.65        | 0.83    | 0.14             |
| 4<sup>th</sup>| 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<sub>50</sub> 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<sub>50</sub> 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 leaf 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<sup>2</sup> | 15 min                         | 100                     | 94                             |              |
|                            |               | 30 min                         |                         | 11                             |              |
|                            |               | 60 min                         |                         | 23                             |              |
|                            |               | 90 min                         |                         | 36                             |              |
|                            |               | 120 min                        |                         | 47                             |              |

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  |
| Instar* Hour        | 11.074         | 6  | 1.841        | 4.333   | <0.001  |
| Instar* Concentration | 13.843       | 15 | 0.887        | 2.083   | 0.014   |
| Hour* Concentration | 25.417         | 10 | 2.542        | 5.903   | <0.001  |
| Total               | 662.958        | 215|              |         |         |

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   |
| Instar * Hour       | 11.074         | 6  | 1.846        | 4.333   | 0.001   |
| Instar* Concentration | 13.310        | 15 | 0.887        | 2.083   | 0.014   |
| Hour* Concentration | 37.278         | 10 | 3.728        | 8.752   | 0.001   |

* 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       | +             | +      |        |
| Phlobatannins  | -             |        | -      |

+ denotes present; - denotes absent

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