Ovicidal, larvicidal and pupicidal activity of *Pterolobium hexapetalum* ((Roth) Santapau & Wagh) extracts against the dengue vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae)

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Abstract

The present study was aimed to determine the ovicidal, larvicidal and pupicidal activity of hexane, dichloromethane and ethanol extracts of *Pterolobium hexapetalum* (leaf) against the dengue vector- *Aedes aegypti*. The leaves of the plant *P. hexapetalum* was extracted with increasing polarity of organic solvents and the extracts were ascertained for their ovicidal, larvicidal and pupicidal activity against the freshly laid eggs, 3rd instar larvae and pupae of *A. aegypti* at various concentrations ranging from 200-800 mg/l under the laboratory conditions. After 72h of exposure, at the concentration of 800 mg/l the ethanol extract exhibited the highest ovicidal, larvicidal and pupicidal activity. Whereas hexane and dichloromethane extracts showed considerably lesser activity when compared to ethanol. The phytochemical test also proved that the insecticidal secondary metabolites were present in the ethanol extract. Therefore, from this investigation it could be suggested that the leaf extracts of *P. hexapetalum* could be used as a best novel and ecofriendly approach to control the population of *A.aegypti*.

Key words: Plant extracts, ovicidal, larvicidal, pupicidal, *Aedes aegypti* and *Pterolobium hexapetalum*

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1. Introduction:

Mosquitoes are the important components because they are being served as a food for number of animals. So, they are ecologically important in aquatic and terrestrial food chain. But, these mosquitoes are considered as a great menace to human's life because of their bites which causes either allergy or the vectors transmits numerous life threatening diseases like malaria, dengue, chikungunya, zika virus, filariasis, West Nile virus, Japanese encephalitis etc (Jang *et al*., 2002; WHO, 2006 and Krishnapa *et al*., 2012). Because, of these diseases, it has been recorded that millions of death takes place every year. It is one of the common insect found everywhere around the world and 3500 species of mosquitoes have been identified throughout (Deepa *et al*., 2015; WHO, 2005; Morisson *et al*., 2008 and James, 1992). It is an endemic disease in more

than 100 developing countries and it is one of the important goals to improve worldwide health (Pancharoen *et al*., 2002). *Aedes* mosquito originated initially in Africa and it was seen especially in a tree hole forest, but later on due to its adaptation in the man-made containers vide urban environment its population has become prevalent in tropical and sub-tropical regions throughout (Powell and Tabachnick, 2013). This mosquito is identified by its white markings on its leg and a lyre type of marking on the upper surface of the thorax. *Aedes aegypti* is an important species for arboviruses and is also known as a yellow fever mosquito which causes yellow dengue fever, Zika fever, yellow fever, mayaro and chikungunya. It has a close association with humans which provides an easy mode of transmission of virus to humans (Morisson *et al*., 2008). This primarily bites during the day and especially two hours after the sun rise and two hours before the sun set and it mostly bites on ankles and elbows.

Usually this species does not move away from the houses where they have been initially released and incase if they move, their maximum distance will be 200 meters (Harrington *et al*., 2005). An increase in the adult population of this species, it indicates that there is an increase in the incident of arboviral disease (Higa *et al*., 2015 and da Cruz Ferreira *et al*., 2017). *A. aegypti vector* exhibit a preference for feeding on blood meal, egg dormancy period, preference for oviposition or larval sites, time in aquatic development and their proficiency towards vector viruses (Powell *et al*., 2013)

Since, more than 120 countries are suffering from Dengue and 2.5 million infections was caused by another type of virus termed as chikungunya (arthropod-borne virus) there has been an increasing interest in global public health towards controlling the population of *Aedes aegypti* (Brady *et al*., 2012; Staples and Fischer, 2014; Sharp *et al*., 2014; Powers, 2014 and Schaffner *et al*., 2013). Accordingly, to accomplish this, various steps have been taken to make the *A. aegypti* population below the threshold level because there is no successful vaccine against this vector. The various approaches like mechanical, biological and chemical steps had been carried out with each having its own pros and cons. In spite of all other alternatives, this vector population continues to increase (Maciel-de-Freitas *et al*., 2014). But, there was a rapid decline in the vector population after the usage of synthetic organic chemical insecticides. Though it was successful it was not in continuous usage for controlling the population because the vector started developing an insecticidal resistance and this was observed in the medically important vectors of malaria, filariasis and dengue (Singh *et al*., 2002; WHO, 1992; Kumar and Pillai, 2010 and Kumar and Pillai, 2011). This insecticide resistance changes includes vector's enzyme system which results in detoxification of the insecticide or there was also a mutation in the target site which prevents the interaction between the insecticide and the target site of the vector (Hemingway *et al*., 2004). Inorder to alleviate these issues, the major prominence on the usage of natural products has a safe and better way to bring down the vector population and it is also a secured alternate to organic synthetic chemical insecticides (Zhu *et al*., 2008; Dhanasekaran *et al*., 2013 and Gokulakrishnan *et al*., 2013). From the time immemorial a number of plant products were in use for controlling the insects because of its rich phytochemical components present in it. These phytochemicals

obtained either from the whole plant or any parts of the plant by using different solvents based on the polarity and it could act as a suitable ovicidal, larvicidal and pupicidal agent against the targeted vector – *Aedes aegypti* (Amer and Mehlhorn, 2006; Rajkumar and Jebanesan, 2007 and Govindarajan *et al*., 2011).

In that way, the plant *Pterolobium hexapetalum* was chosen after having thorough knowledge on it. It was observed that this plant has the efficiency role in herbal usage and the different parts of the plant plays a significant role in day to day life. Few roles like the leaves are used on delivery pains; flowers were used for curing constipation problem, ulcer, cough etc; stem bark were used for curing chest pain, heat boils, diarrhea etc. The leaf extract was also tested for its antibacterial efficiency on the few selected pathogens and it was found that hot water and methanol leaf extract showed effective inhibition (Kavitha Bommana *et al*., 2012). The leaves of the plant also tested for antimicrobial activity by using three different extracts and among the extracts tested ethanol extract showed the highest inhibition activity (Anantharaj and Tangavelou, 2015).

In view of this, in the present investigation, an attempt was made to study the mosquitocidal activity of the *Pterolobium hexapetalum* (leaves) by using the different solvents like hexane, dichloromethane and ethanol against the dengue vector, *Aedes aegypti*.

2. Materials and Methods:

2.1. Collection of plant material: Fresh leaves of the plant *Pterolobium hexapetalum* was collected from Javadhi hills (12° 40' N, 78° 40' E), Vellore district, Tamil Nadu. The collected plant was stored in a zip lock cover to prevent the effect of humidity and evaporation. The collected leaves were washed thoroughly with tap water, shade dried and ground to a fine powder with an electric blender.

2.2. Preparation of plant extracts: The extraction of plant sample was done with three different solvents: Hexane, dichloromethane and ethanol (Plate 1). About 50g of sample was taken and soaked with 250 ml of Hexane solvent for 24 hours in a brown glass bottle and kept in a rotator shaker for continuous shaking. After the completion of 24hours, the filtration was carried out in a Whatman No.1 filter paper. The filtrates were then placed in a rotary evaporator with a hexane boiling point of 69°C; with a rotary speed of 3-6 rpm for 2 hours and the crude obtained from the evaporator is again air dried to remove traces of hexane solvent. Then, the crude was stored in a brown vial for further study. Now, the residue left in the filter paper was shade dried for complete evaporation of the hexane solvent and then it is allowed to extract with dichloromethane and then followed by ethanol. The crude of all the extracts were stored in brown vials and kept in a refrigerator.

2.3. The qualitative analysis on leaves of the plant *P.hexapetalum* was performed according to Harborne and Kokate method (Harborne, 1998 and Kokate, 1997) (Plate 3).

2.4. Mosquito rearing: Eggs of *Aedes aegypti* was collected within the college campus by placing a water-filled plastic trays (23×15× 6.5 cm) with a lining of partially immersed filter paper. The eggs were placed in

plastic trays (30×24×10 cm) each containing 2 l of tap water and kept at room temperature (27±2°C) with a photoperiod of 12:12 h (L: D) for larval hatching. The larvae of different mosquito species were maintained in individual trays under the identical laboratory conditions and fed with yeast powder. The trays with pupae of each mosquito species were maintained in separate mosquito cages at 27 ± 2°C and relative humidity of 75±5% for adult emergence. Cotton soaked with aqueous sucrose (10%) solution in a petri dish to feed adult mosquitoes was placed individually in each mosquito cage. An immobilized young chick was placed for 3 h inside the cage in order to provide a blood meal especially for female mosquitoes. A plastic tray of (11× 10×4 cm) possessing partly immersed wrapped filter paper and filled with tap water was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions as described above. Only the newly hatched specific instars of larvae or the pupae of different mosquito species were used in all bioassays.

2.5. Ovicidal assay: The ovicidal assay was performed by placing batches of 100 mosquito eggs in 100 ml of each test medium in a plastic bowl containing a specific concentration of the *P. hexapetalum* extract. In control, the same number of eggs was maintained at 50 ml of dechlorinated tap water containing appropriate volume of 0.9% saline. All containers were maintained at room temperature (27±2°C) with naturally prevailing photoperiod (12:12 hrs L:D) in the laboratory.

Water lost through evaporation was compensated by the periodic addition of dechlorinated tap water. All the test media were carefully examined for every 24 h up to 72 h for the number of intact (unhatched) eggs as well as the appearance of the number of first-instar larvae, and the latter indicated the successful egg hatchability. Besides, the unhatched eggs remaining in the test media after 72 h of exposure were transferred to tap water and maintained up to 24 h in order to ascertain the mortality of these eggs. The eggs that failed to hatch out even under this ideal condition were considered to be dead due to their previous exposure to a particular test medium. Percentage of ovicidal activity was calculated according to Su and Mulla method (Su and Mulla, 1998).

$$\%\ of\ ovicidal\ activity = \frac{Number\ of\ eggs\ hatched}{Total\ number\ of\ eggs\ treated}\ X\ 100 \quad (2.1)$$

2.6. Larvicidal bioassay: The Larvicidal activity of the extract was determined by following the standard procedure (Plate 2). Mosquito larvae were exposed to 200, 400, 600 and 800 mg/l concentrations and were used to determine the lethal concentration of 50% (LC_{50}) and the lethal concentration of 90% (LC_{90}) values. DMSO (emulsifier) in water served as a control. The 3rd instar larvae of these mosquito species (25 nos.) were introduced in 500-ml plastic cups containing 250 ml of aqueous medium (249 ml of dechlorinated water + 1ml of emulsifier) and the required amount of plant extract were added. Five replicates were kept for each test concentration as stated earlier. In each replicate 25 larvae were used, with five replicates of control. The experiment was performed under laboratory conditions at 27 ± 2°C. The mortality in the treated

groups should be corrected, if 5% & 20% of the control mortality was seen according to corrected Abbott's formula (Abbott, 1925). The LC_{50}, LC_{90}, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), chi-square values and the degrees of freedom were calculated by using Probit analysis with Statistical Package for Social Sciences (SPSS) 17.0 Version in MS-Excel, 2007.

$$Percented\ Mortality = \frac{Number\ of\ dead\ larvae}{Number\ of\ larvae\ introduced}\ X\ 100 \quad (2.2)$$

$$\text{Corrected Mortality} = \frac{\%\ \text{of mortality in treated group} - \%\ \text{of } mortality \text{ in control group}}{100 - \%\ \text{of mortality in control group}} \text{X100} \quad (2.3)$$

2.7. Pupicidal assay: Batches of ten pupae were introduced into 50 ml of the test medium (tap water) in a 100ml plastic bowl containing a particular concentration of the selected solvent extracts of the plants with the same concentrations as mentioned in the previous experiments. In control, the same number of pupae was maintained at 50 ml of dechlorinated tap water containing appropriate volume of 0.9% saline. All containers were maintained at room temperature (27±2°C). Any pupa was considered to be dead if its appendages did not move when prodded repeatedly with a soft brush. Mortality of pupae was recorded after 24 h of exposure to the extract. The percentage of pupicidal activity was calculated by using the corrected Abbott's formula (Abbott, 1925).

$$Percented\ Mortality = \frac{Number\ of\ dead\ pupae}{Number\ of\ pupae\ introduced}\ X\ 100 \quad (2.4)$$

$$\text{Corrected Mortality} = \frac{\%\ \text{of mortality in treated group} - \%\ \text{of } mortality \text{ in control group}}{100 - \%\ \text{of mortality in control group}} \text{X100} \quad (2.5)$$

3.0 Results:

3.1 Phytochemical screening of solvent extracts of *Pterolobium hexapetalum***.**

The phytochemical screening of *P. hexapetalum* of different solvent extracts was assessed and the results pertaining to the experiments are shown in table 3.1. Hexane extract showed the presence of alkaloid, terpenoids, carbohydrate, Anthoquinone and coumarins. Similarly, DCM exhibited the presence of alkaloid, phenols, flavonoids, tannins, terpenoids, Carbohydrate, Anthoquinone, coumarins and protein. Apart from the secondary metabolites like tannins, saponins and proteins all other metabolites are presence for ethanol extract.

Table 3.1: Qualitative analysis of phytochemical in different solvent extracts of *Pterolobium hexapetalum*

S.No	Phytochemical groups	Extracts tested		
		Hexane extract	Dichloromethane	Ethanol extract

			extract	
1.	Alkaloids	Mayer's Test: Formation of yellow coloured ppt. Indicates the presence of alkaloids.	Wagner's Test: Formation of brown reddish ppt. Indicates the presence of alkaloids.	Dragendroff's Test: Formation of red white ppt. Indicates the presence of alkaloids.
2.	Phenols	Ferric Chloride Test Absence of bluish black colour formation. Indicates the absence of alkaloids.	Ferric Chloride Test: Presence of bluish black colour formation. Indicates the presence of alkaloids.	Ferric Chloride Test: Presence of bluish black colour formation. Indicates the presence of alkaloids.
3.	Flavonoids	Lead Acetate Test: No intense yellow colour formation so flavanoids are absent	Lead Acetate Test: Intense yellow colour formation. Indicates the presence of flavanoids.	Lead Acetate Test: Intense yellow colour formation. Indicates the presence of flavanoids.
4.	Tannins	Absence of blue or green colour shows tannins are absence.	Presence of blue or green colour shows tannins are present.	Absence of blue or green colour shows tannins are absence
5.	Terpenoids	Copper Acetate Test Presence of emerald green colour . Indicates the presence of terpenoids.	Copper Acetate Test: Presence of emerald green colour. Indicates the presence of terpenoids.	Copper Acetate Test: Presence of emerald green colour. Indicates the presence of terpenoids.
6.	Saponins	Foam Test: Absence of foam formation. No saponins are present.	Foam Test: Absence of foam formation. No saponins are present.	Foam Test: Absence of foam formation. No saponins are present.
7.	Carbohydrate	Molisch's Test: Presence of violet coloured ring formation. Indicates the presence of carbohydrate.	Benedict's Test: Presence of orange red ppt. Indicates the presence of carbohydrate.	Fehling's Test: Presence of red ppt. Indicates the presence of carbohydrate.
8.	Glycosides	Legal's Test: Presence of glycosides are confirmed by the appearance of pink color.	Legal's Test: Presence of glycosides are confirmed by the appearance of pink color.	Legal's Test: Presence of glycosides are confirmed by the appearance of pink color.
9.	Anthoquinone	Appearance of colouration. Presence of anthoquinons.	Appearance of colouration. Presence of anthoquinons.	Appearance of colouration. Presence of anthoquinons.
10.	Coumarins	Indicates the presence of yellow colour. Indicates that coumarins are present.	Indicates the presence of yellow colour. Indicates that coumarins are present.	Indicates the presence of yellow colour. Indicates that coumarins are present.
11.	Steroids	Libermann Burchard's Test: No appearance of brown ring formation. Steroids are absence.	Libermann Burchard's Test: No appearance of brown ring formation. Steroids are absence.	Libermann Burchard's Test: Appearance of brown ring formation. Steroids are presence.
12.	Protein	Ninhydrin Test: Absence of blue colour	Ninhydrin Test: Appearance of blue	Ninhydrin Test: Absence of appearance of

		denotes protein are absent.	colour denotes protein are present.	blue colour denotes protein are absent.
13.	Acid	Absence of effervescence. Indicates absence of acid.	Absence of effervescence. Indicates absence of acid.	Presence of effervescence. Indicates presence of acid.

3.2: Ovicidal, Larvicidal and Pupicidal activity of hexane, DCM and ethanol extracts of *P. hexapetalum* against the freshly laid eggs, third instar larvae and pupae of dengue vector *A. aegypti*

Among the different extracts treated on the eggs of *A. aegypti* for different concentrations and readings were recorded continuously for 3 days. The highest significant activity of 83.8±0.45, 85.6±0.55 and 87.0±0.00% was portrayed for 72 hours at the highest concentration of 800 mg/l and the data pertaining to the experiment are shown in table 3.2.

Likewise, the larval mortality was recorded for 24hours, 48hours and 72 hours and the maximum dead larvae were enumerated in 72 hours. The readings were demonstrated in the table 3.3 and as follows for hexane extract, the LC_{50} value was found to be 358.80 and their confidence limits ranged from 279.72 (LCL) to 419.31 (UCL). Furthermore, the LC_{90} value was found to be 934.32 and their confidence limits ranged from 823.29 (LCL) to 1118.26 (UCL). The calculated chi-square value was 0.392. Similarly, for DCM extract the calculated lethal concentrations of LC_{50} were 361.16 and their confidence limits ranged from 290.66 (LCL) to 416.73 (UCL). In addition to it, the LC_{90} value was found to be 884.69 and their confidence limits ranged from 788.39 (LCL) to 1036.96 (UCL). The calculated chi-square value was 0.811. Finally, the calculated readings for ethanol extract were 267.70 (LC_{50}) value and their confidence limits ranged from 181.19 (LCL) to 329.14 (UCL). Moreover, the LC_{90} value for the data was 767.77 and their confidence limits ranged from 686.54 (LCL) to 893.74 (UCL). The calculated chi-square value was 0.988.

Finally, 91.4% of pupicidal activity was seen in the ethanol extract for increased exposure time period of 72 hours and for increased concentration of 800 mg/l and displayed in the fig 3. Figure 1 and 2 also clearly depicts that even for hexane and DCM extract the immense activity was accounted for 72 hours at the highest concentration. Therefore, in generally as the concentration increases the activity also increases. But, among the extracts treated specifically, ethanol extract showed greatest activity as the exposure period and the concentration increases.

4. Discussion:

The mosquito-borne diseases can be controlled by either killing or preventing mosquito bite to human beings or also by causing egg, larval and pupal mortality in the breeding centers like stagnated water, sewage leekage etc. Initially, the mortality of the vector *A. aegypti* was achieved by using the synthetic insecticides in the last five decades. But due to their physiological resistance development it resulted in environmental hazards. So, there was an urgent need to control the vector naturally because these naturally synthesized products will be easily biodegradable, environmental safe, low cost and will be more specific resulting in a drastic reduction in the population of *A. aegypti* vector (Sarita Kumar *et al*., 2012; Anupam Ghosh *et al*., 2012; Anjali Rawani *et al*., 2017; Paoletti and Pimentel, 2000; Roberts and Karr, 2012; Pavela *et al*., 2016; Krishnappa *et al*., 2013; Krishnappa and Elumalai, 2013; Dhanasekaran et asl., 2012). The phytochemicals extracted from the different aromatic medicinal plant extracts and also from different parts of the plant by using the different solvents have revealed the efficacy of ovicidal, larvicidal and pupicidal activity against the dengue vector (Velu *et al*., 2015; Alagarmalai Jeyasankar *et al*., 2012; Krishnappa and Elumalai, 2012; Jayapal Subramaniam *et al*., 2012; Danga *et al*., 2014; Anupam Ghosh *et al*., 2012 and Gaurav Kumar *et al*., 2012). In this experiment hexane extract showed the presence of alkaloid, terpenoids, carbohydrate, Anthoquinone and coumarins. DCM exhibited the presence of these secondary metabolites phenols, flavonoids, tannins and protein inaddition to the phytochemicals present in the hexane extract. Whereas for ethanol, all other metabolites were presence apart from the secondary metabolites like tannins, saponins and proteins. Plants are the promising botanicals in integrated mosquito management (Kanika Tehri and Naresh Singh, 20152).

In the current study, on examining the crude hexane, dichloromethane and ethanol extracts of the plant *P. hexapetalum* exhibited the promising activity against the vector for ethanol extract. The eggs of this vector have a potency to enter into a diapauses condition for several months and this vector lays the eggs separately on the damp places. Unlike the other vector it hatches out irregularly at extended period of time because hatching out is possible only when the eggs are submerged with water. So, this makes difficult to kill the dengue vector's egg together. Therefore, it may be achieved successfully by collecting the *Aedes* eggs which are laid on the damp surfaces or on the water's edge, tree hole, man-made containers like tyres, discarded bottles or in general the places where the rain water stagnates and treating the eggs with ethanol crude extract. By doing so, it showed 90% ovicidal activity against the egg. Whereas, the similar mortality was also observed in the methanol extract of *R. cordifolia* root against the eggs of *Cx. quinquefasciatus* and *Ae. Aegypti* with 82.40% and 70.40% respectively (Rajiv Gandhi Munusamy). The percentage of egg hatch was greatly reduced to 49% in the petroleum ether extract (Radhika Warikoo and Sarita Kumar, 2014). The methanol extract of *Ervatamia coronaria* showed 100% mortality against the eggs of *C. quiquefasciatus, A. aegypti and A. stephensi* (Govindarajan *et al*., 2011). The butanol extract of the

plant showed highest egg mortality of 86% (Rajasingh Raveen *et al*., 2017). At 200 mg/l concentration of *Polygala arvensis* explained the highest ovicidal activity against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Deepa *et al*., 2014). The significant ovicidal activity was observed in all the four solvent extracts of *Cleistanthus collinus, Leucas aspera, Hydrocotyle javanica, Zanthoxylum limonella, Murraya koeingii* and *Sphaeranthus indicus* (Samuel Tennyson *et al*., 2011). The increased amount of mortality is observed in the ethanol crude extract is due to the strong toxic nature which disturbs the *Aedes aegyti* eggs (Krishnappa and Elumalai, 2012 and Deepa *et al*., 2014).

Whereas, the dichloromethane extract and hexane extract showed 83.8% and 85.6% of ovicidal activity at 72 hours. 60% of Ovicidal activity was recorded in the hexane extracts of *Limonia acidissima* (Appadurai Daniel Reegan *et al*., 2015).

The larvicidal activity was seen abundantly in the 72 hrs of the ethanol extract where the larvae showed no dive movement towards the bottom of the water container even after disturbing the containers in which it was present. This may be due to the malfunction of the spiracles which is situated in the 8^{th} abdominal segments or the malfunction in the 8^{th} abdominal segments itself which were brought about by the strong aromatic phytochemicals constituents present in the *P. hexapetalum*. Therefore, the larva does not pass through different stages or instars stage or pupae or adult.

Table 3.2: Ovicidal activity of different extracts of *P. hexapetalum* tested against eggs of *A. aegypti*.

Concentrations tested (mg/l)	Exposure periods (in Hrs)		
	24	48	**72**
	Hexane extract		
200	12.0 ± 1.00[a] (20.27)	26.6 ± 0.55[a] (31.05)	35.0 ± 1.00[a] (36.27)
400	31.8 ± 0.84[b] (34.33)	45.0 ± 0.7[b] (42.13)	57.4 ± 0.89[b] (49.26)
600	48.0 ± 0.71[c] (43.85)	54.4 ± 0.55[c] (47.52)	68.8 ± 0.84[c] (56.04)
800	69.6 ± 0.55[d] (56.54)	77.2 ± 0.45[d] (61.48)	83.8 ± 0.45[d] (66.77)
Dichloromethane extract			
200	11.2 ± 1.30 [a] (19.55)	25.8 ± 1.10 [a] (30.53)	31.8 ± 0.45 [a] (34.33)
400	27.6 ± 0.89[b] (31.69)	42.0 ± 0.71[b] (40.4)	56.2 ± 0.45[b] (48.56)
600	51.0 ± 1.00 [c] (45.57)	56.4 ± 0.55[c] (48.68)	70.2 ± 0.45[c] (56.9)
800	70.4 ± 0.55 [d] (57.04)	80.8 ± 0.45[d] (64.01)	85.6 ± 0.55[d] (67.7)
Ethanol extract			
200	22.0 ± 1.41 [a] (27.97)	34.2 ± 1.10 [a] (35.79)	42.0 ± 1.00 [a] (40.4)
400	34.6 ± 1.34 [b] (36.03)	45.6 ± 0.89 [b] (42.48)	56.6 ± 0.89 [b] (48.79)
600	52.0 ± 0.71 [c] (46.14)	53.2 ± 0.84 [c] (46.83)	69.2 ± 0.45 [c] (56.29)
800	72.2 ± 0.45 [d] (58.18)	84.2 ± 0.45 [d] (66.58)	87.0 ± 0.00 [d] (68.87)
Neem azal	74.4±0.84[d] (59.6)	80.4±0.45[d] (63.72)	89.6±0.77[d] (71.19)

Values expressed are mean mortality ± standard deviations of five replications (n=20). Values with different alphabet in the column shows statistical significance at $p<0.05\%$ level; DMRT.

Table 3.5: Determined lethal concentrations values of different extracts of *Pterolobium hexapetalum* tested against the vector – *Aedes aegypti*.

Exposure Periods (hrs)	Concentrations	Mortality (%)	LC_{50}	LC_{90}	χ^2
Hexane extract					
24	200	13.6 ± 0.89[a]	606.20 (557.32-663.81)	1072.57 (962.11-1242.91)	0.579
	400	29.4 ± 0.55[b]			
	600	46.2 ± 0.45[c]			
	800	72.0 ± 0.00[d]			
48	200	27.6 ± 1.34[a]	508.10 (447.64-569.92)	1096.23 (958.71-1328.17)	1.563
	400	38.6 ± 0.89[b]			
	600	54.2 ± 0.84[c]			
	800	76.8 ± 0.45[d]			
72	200	35.4 ± 1.14[a]	358.80 (279.72-419.31)	934.32 (823.29-1118.26)	0.392
	400	55.8 ± 0.84[b]			
	600	68.6 ± 0.55[c]			
	800	84.2 ± 0.45[d]			
Dichloromethane extract					
24	200	12.8 ± 1.10[a]	580.69 (534.67-632.40)	1022.75 (924.17-1170.98)	0.108
	400	31.0 ± 0.71[b]			
	600	52.6 ± 0.55[c]			
	800	73.2 ± 0.45[d]			
48	200	27.4 ± 1.14[a]	462.62 (402.54-518.54)	1013.98 (896.07-1205.99)	1.822
	400	46.4 ± 0.89[b]			
	600	57.2 ± 0.84[c]			
	800	81.0 ± 0.71[d]			
72	200	33.6 ± 1.34[a]	361.16 (290.66-416.73)	884.69 (788.39-1036.96)	0.811
	400	56.8 ± 1.10[b]			
	600	69.4 ± 0.89[c]			
	800	86.6 ± 0.55[d]			
Ethanol extract					
24	200	18.2 ± 1.30[a]	500.51 (453.36-547.69)	947.41 (857.13-1082.12)	1.686
	400	42.8 ± 0.84[b]			
	600	57.0 ± 0.71[c]			
	800	81.8 ± 0.45[d]			
48	200	33.8 ± 1.48[a]	378.06 (305.73-435.67)	934.42 (826.41-1110.57)	1.564
	400	54.8 ± 0.84[b]			
	600	65.0 ± 0.71[c]			
	800	85.4 ± 0.55[d]			
72	200	42.8 ±0.84[a]	267.70 (181.19-329.14)	767.77 (686.54-893.74)	0.988
	400	65.4 ± 0.89[b]			
	600	77.2 ± 0.45[c]			
	800	92.6 ± 0.55[d]			

The value represents mean ±S. D. of five replications. *mortality of the larvae observed after 24h, 48h & 72h of the exposure period, WHO (2005). LC50=Lethal Concentration brings out 50% Mortality and LC90 = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with different superscript alphabets are significantly different at $P < 0.05$; LSD -Duncan Multiple Range Test).

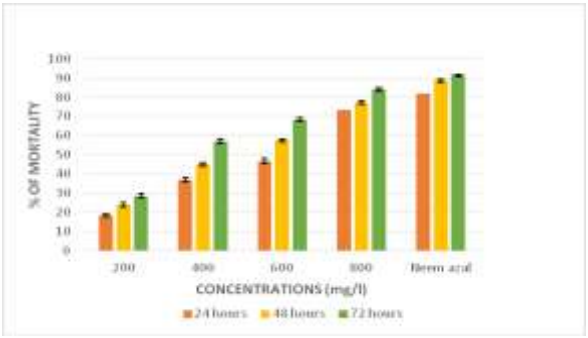


Figure 1: Pupicidal activity of hexane extract of *P. hexapetalum* tested against the pupae of *A. aegypti*.

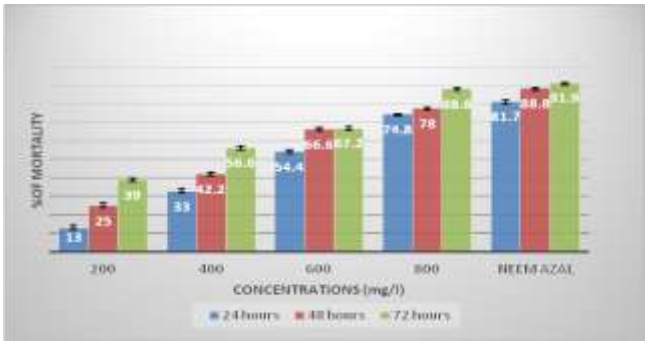


Figure 2: Pupicidal activity of dichloromethane extract of *P. hexapetalum* tested against the pupae of *A. aegypti*.

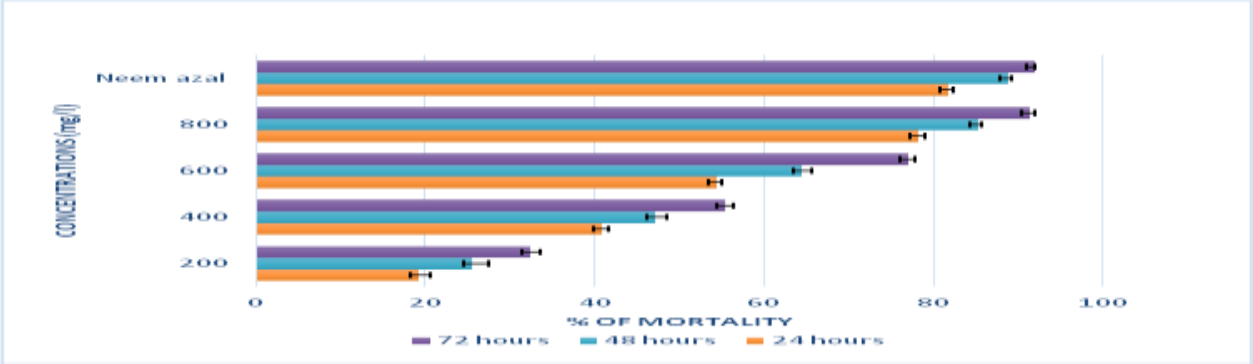


Figure 3: Pupicidal activity of ethanol extract of *P. hexapetalum* tested against *Aedes aegypti*.

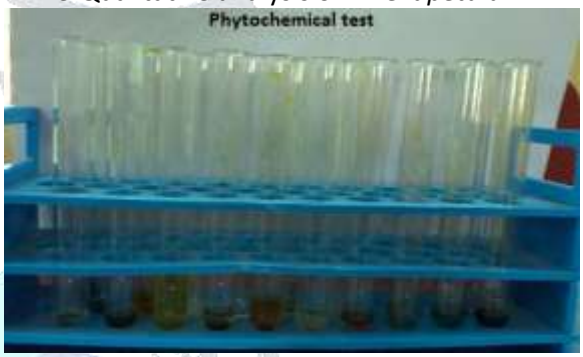
The maximum larvicidal activity exhibited by the ethanol extract of the plant *P. hexapetalum* and our result was in accordance with the various inventors as follows: *Piper nigrum* fruit exhibited 100% larval mortality in *A. aegypti* and *Ochlerotatus togoiI (Aedes togoi)* (Young-Cheol Yang *et al*., 2004). Similarly 84% of larvicidal activity were exhibited by the ethanolic endocarp *Dracaena loureiri* (Damrongpan Thongwat *et al*., 2017). The acetone extract of *Pinus caribaea* exhibited the maximum larvicidal activity (Luiz Alberto Kanis *et al*., 2009). The ethanolic extracts of *Cymbopogon citratus, Ixora coccinea, Murraya koenigii, Euphorbia hirta, Gliricidia sepium, Eucalyptus globules, Jatropha curcas* and *Capsicum frutescens* demonstrated the larvicidal activity *A. aegypti* and *A. albopictus* (Michael Russelle *et al*., 2015). *Annona reticulata* showed strong larvicidal efficacy against *A. aegypti* (Bavani Govindarajulu *et al*., 2015). The ethanol and hexane extracts of *Anacardium occidentale* exhibited promising

PLATES

1. Hexane , DCM and ethanol extract of *P.hexapetalum*

2. Larvicidal activity of *P.hexapetalum*

3.Qualitative analysis of *P.hexapetalum*



larvicidal activity against *Aedes aegypti* (Rosalinda Torres *et al*., 2015). The vectors *A. aegypti and C.quinquefasciatus* were greatly reduced by the efficacy of *Gmelina asiatica* leaf extract (Augustian Rajam Florence and Jeeva Solomon, 2016). On comparing the larvicidal activity of ethanol extracts with hexane and dichloromethane extract which showed only 84-86% of larvicidal activity.

The pupae do not feed and mostly hang on the surface of the water through respiratory trumpets. So, at this stage treating them with the different extracts of the plant *P. hexapetalum* may reaches into the cephalothorax region at the time of breathing and results in difficulty in further breathing. This makes impossible for the pupa's dorsal surface of the cephalothroax to splits and adult vector do not emerge out. Among the three different extracts, the highest pupicidal activity was displayed by the ethanol extract (91.4%) followed by DCM (88.6%) > Hexane (84%) extract respectively. The analogous results were also recorded by the other investigators. The acetone leaf extract of *Tephrosia purpurea* displayed highest pupicidal activity against *A. aegypti* (Ramesh Venkadachalam *et al*., 2017). The methanol extract of *Artemisia nilagirica* confirmed the pupicidal activity against *A. stephensi* and *A. aegypti* (Panneerselvam *et al*., 2012). This study suggest that this plant *P. hexapetalum* plays a major role in producing toxic effect against the different life cycle stages of the dengue vector *A. aegypti*.

5. Conclusion:

The results obtained from this study indicates that the phyto products extracted from the plant are safer, ecofriendly approach to control the dengue vector. Among the solvents used, ethanol solvent showed more promising and highest activity when compared to other extracts – hexane and dichloromethane extracts. Therefore, *P. hexapetalum* could be used as a best bio-control agent in controlling the vector population efficiently at the different stages. This practice will not only give an holistic approach to control *Aedes aegypti* but also an best way to utilize our naturally available bioresources which are easily biodegradable.

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