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Original Research Article

Ovipositional Deterrence, Ovicidal and Developmental Effects of *Pithecellobium dulce* (Roxb.) Benth. (Fabaceae) Aqueous Leaf Extract against Dengue Vector Mosquito *Aedes aegypti* (Diptera: Culicidae).

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A b s t r a c t

Mosquito-borne diseases have an economic impact, including loss in commercial and labor outputs, however, no part of the world is free from vector-borne diseases. One of the most effective alternative approaches under the environmental sustainable control program is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple method of mosquito control. Plant secondary metabolites are considered to be a potential alternative approach against different species of mosquitoes and their various immature stages due to their richness in bioactive compounds, easy availability, environmental safety etc. The current study was undertaken to investigate the ovicidal activity, ovipositional deterrence activity and developmental effects of aqueous leaf extract *P. dulce* against of *Ae. aegypti*. The hatchability of *Ae. aegypti* eggs was decreased when placed in media of aqueous leaf extract. The reduction in percent hatch was inversely proportional to the concentration of aqueous leaf extract used. The aqueous leaf extract of *P. dulce* were found to deter mosquitoes from oviposition. Oviposition Active Index and Oviposition Deterrent Index indicated a low preference of *Ae. aegypti* to leaf extract treated medium for egg laying. Extension in larval development time was in correlation to concentrations of aqueous leaf extract used. The results indicated interference of bio – active compounds with the normal hormonal activity of the larvae. These results could be useful in the search for novel, more selective, and biodegradable compounds for the control of the dengue vector *Ae. aegypti*.

K e y w o r d s

Aedes aegypti

Aqueous leaf extract

Ovicidal activity

Ovipositional deterrence

Pithecellobium dulce

Introduction

The Mosquitoes, commonly called "flying syringes", as they are sanguivorous vectors, cause more sufferings to the human than any other organism. It is not time to forget the recent rampage of Chikungunya and Dengue fever all over the state, pushing the people into death or at least to permanent ill health. Due to inadequate management of land and water resources, and failure to solve problems of waste management, more productive habitats for mosquito continue to grow and, cause diseases and intolerable annoyance. "The world most dangerous animal is the mosquito," according to a BBC World Service health program on 9th September, 2009, 12:45pm.

Aedes aegypti is a medium- sized blackish mosquito easily recognized by a silvery- white Iyre- shaped pattern of scales on its scutum. The colouration of both males and females is similar. It is breeds in many types of household containers, such as water storage jars, drums, tanks and plant or flower containers (Harrington et al., 2005**)**. Compared to any other species of *Aedes, Ae. aegypti* shows more dependency on human blood (Scott et al., 1993**)**. *Ae. aegypti* breeds throughout the year. The eggs laid singly on the side of containers at or above the water line and also on the water surface. Hatching can take place in 2 or 3 days. These mosquitoes go through distinct stages of development: egg, larva, pupa and adult. The life cycle can be completed in about 10 days. The adult life-span of a mosquito is 50-55 days or approximately two months (Park and Park, 1987).

Today, the geographic distribution of dengue epidemics includes 124 countries worldwide whereby 3.61 billion people are at risk of being infected and every year 500 million of them contract infection effectively (Beatty et al., 2010).The increase in dengue cases is considered to be a reflection of the rampant development towards massive infrastructure as well as urbanization, which is a favorable factor for breeding of *Ae. aegypti* (Azami et al., 2011 and Gubler, 2002).

Dengue or 'break bone' fever had been known in our country for every long time. It is estimated that 34% of the global cases are from India (Bhatt et al., 2013) and the country is known to be endemic , with all four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) circulating throughout the year in different parts (Gupta et al., 2012). Dengue fever can manifest as the classic form of the diseases, which debilitates the patient for a week or more, or as the haemorrhagic form which, in many cases leads to death (Neves-Filho et al., 2009). Epidemic outbreaks of dengue fever have also been reported in India. For instance, in 1980 a total of 4,601 cases were recorded (Park and Park, 1987). In October 2001, an outbreak of dengue resulting in 16 deaths was reported in Chennai (Tamil Nadu) India (The Hindu, 2001). In October, 2006, a total of 5,710 cases were recorded in India. Delhi had the highest (1,637) patients. Tamilnadu, India had 307 patients; 103 deaths were also reported (The Hindu, 2006). In 2010, there were a total of 28, 292 cases and 110 deaths (NVBDCP, 2011). In 2012 a total of 9,000 cases and 50 deaths were reported in Madurai, Tirunelveli and Kanyakumari districts (Tamil Nadu) (The Hindu, 2012). According to the Central Health Ministry of India in 2013, 75,454 people affected by this disease, in Tamilnadu alone 4,000 affected by dengue which led to 167 deaths (The Dinamani, 2013**).** According to Health Minister of Tamilnadu in Sivagangai district, nearly 1,400 people were affected by dengue fever (The Dinamalar, 2014a). In Coimbatore Corporation alone during 2014, 172 people were affected by dengue fever (The Dinamalar, 2014b). Recently in 2015 February, 17 people were affected by dengue fever in Coimbatore Corporation (The Dinakaran, 2015)

Chikungunya, a febrile disease is caused by Chikungunya virus which is transmitted by *Ae. aegypti*. Chikungunya virus, a member of alpha virus genus is of considerable public health concern in Southeast Asian and African countries (Pastorino et al., 2005). There was an outbreak of this disease in Calcutta in 1963-1964 and another in Madras (Chennai) in 1965 which gave rise to 3,00,000 cases in Madras city alone (Park and Park, 1987). According to Central Health Secretary of India, in 2006, 13 lakh people affected by this disease. In Tamil Nadu alone 63,000 persons were affected by this disease (The Hindu, 2006**)**. In 2013, a total of 500 cases were reported in Thirunelvali district (Uthakulam village) Tamilnadu, India (The Dinamani, 2013). These diseases devastate Indian economy every year (Jaswanth et al., 2002).

At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of this disease is mosquito control (Sarita

et al., 2012). The control methods should aim at the weakest link of the life cycle of the mosquito, which is the larval stage. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. During the immature stage, mosquitoes are relatively immobile; remaining more concentrated than they are in the adult stage (Rutledge et al., 2003).

Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides such as malathion, DDT and deltamethrin have an adverse impact on the environment and disturb ecological balance (Yang et al., 2005). Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides may enter into the food chain. They even result in mutation of genes and these changes become prominent only after a few generations (Ghose, 1991). These problems have warranted the need for developing alternative strategies using eco-friendly products.

The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. The phytochemicals derived from plant resources can act as larvicidal, ovicidal, oviposition deterrence, growth and reproduction inhibitors, repellents, growth regulation, fecundity suppression, male sterility (Elimam et al., 2009a, b). Some of the plant leaf extract tested for their diverse insecticidal properties on the medically important mosquitoes are: methanolic extracts of *Derris elliptica* leaves (Prempree and Sukhapanth,1990); aqueous extract of *Senna didymobotrya* leaves (Ojewole et al., 2000); aqueous extract of *Solanum nigrum* leaves (Singh et al., 2001); acetone extract of *Solanum trilobatum* leaves (Rajkumar and Jebanesan, 2004); aqueous extracts of *Gymnema sylvestre* and *Eclipta prostrate* leaves (Khanna and Kannabiran, 2007); methanol, benzene and acetone leaf extracts of *Cassia fistula* (Govindarajan, 2009); petroleum ether extract of *Azadirachta indica, Ocimum gratissimum* and *Hyptis suaveolens* leaves (Okigbo et al., 2010); aqueous and chloroform extracts of *Leucas aspera* leaves (Ramanibai et al., 2011); ethanolic extract of *Datura stramonium* leaves (Swathi et al., 2012); aqueous extract of *Spathodea campanulata* leaves (Saranya et al., 2013 a, b,c); methanolic extract of *Spathodea campanulata* leaves (Kathika Devi et al., 2013);acetone extracts of *Spathodea campanulata* leaves (Pravin et al., 2014); acetone, benzene, petroleum ether, chloroform and aqueous extracts of *Nerium oleander* leaves (Fakoorziba et al., 2015); methanol and water extract of *Indigofera arrecta* leaves (Neema et al., 2015); water, ethanol and petroleum ether extracts of *Citrullus colocynthis* leaves(Satti and Edriss, 2015); petroleum ether extract of *Zizyphus jujube* leaves (El-Husseiny and El-Kholy, 2015); methanol and petroleum ether extract of *Mallotus repandus* leaves (Hasan et al., 2015); petroleum ether, ethyl acetate, chloroform and methanol extracts of *Rhinacanthus nasutus* leaves (Jayapriya and Shoba, 2015); methanol , ethyl acetate and hexane extracts of *Ageratum houstonianum* leaves (Samuel et al., 2015); aqueous , ethanol, methanol, acetone and chloroform extracts of *Lantana camara aculeate* leaves (Hemalatha et al., 2015); hexane, ethyl acetate, benzene, chloroform and methanol extracts of *Erythrina indica* leaves (Govindarajan and Sivakumar, 2015); acetone extract of *Cipadessa baccifera* leaves (Ramkumar et al., 2015); aqueous extract of *Argemone mexicana* leaves (Zeinab , 2015); aqueous extract of *Cleistanthus collinus* leaves (Arivoli et al ., 2015) ethanol extract of *Ixora coccinea* and *Allamanda violacea* leaves (Rahul et al ., 2015).

As for as our literature survey that there was no information available on the ovicidal, ovipositional deterrence effects and prolongation of larval duration of the aqueous leaf extract of the *P. dulce.* The present study was therefore carried out to evaluate mosquitocidal properties *P. dulce* aqueous leaf extract against the vector mosquito, *Ae. aegypti*. *Pithecellobium dulce* (Roxb.) Benth. (Fabaceae) is a small, medium sized, evergreen tree, native of tropical America and cultivated throughout the plains of India. It is known as Vilayati babul in Hindi and Kodukkapuli in Tamil. *P. dulce* reaches a height of about 10 to 15 m (33 to 49 ft). Its trunk is spiny and its leaves are bipinnate. Each pinna has a single pair of ovate-oblong leaflets that are about 2 to 4 cm long. The flowers are greenish-white, fragrant, sessile and reach about 12 cm in length, though appear shorter due to coiling. The flowers produce a pod, which turns pink when ripe and opens to expose an edible pulp. The pulp contains black shiny seeds that are circular and flat. The leaves have been reported to possess astringent, emolliment, abortifacient antidiabetic, larvicidal and antimicrobial properties in folk medicines (Banerjee, 2005; Shanmugakumaram et al.,

2005). Quericitin, kaempferol, dulcitol and afezilin have been reported from the leaves (Nigam et al., 1997). It is commonly planted as a street tree in south Tamil Nadu.

The aim of the present study is therefore to find out:

- Ovicidal activity of the aqueous leaf extract of *P. dulce* on mosquito eggs.
- Ovipositional preferences of adult mosquitoes to different concentrations of aqueous leaf extract of *P. dulce.*
- Effect of aqueous leaf extract of *P. dulce* on the larval duration of *Ae. aegypti.*

Materials and methods

Colonization of *Aedes aegypti*

Collection of eggs: The eggs of *Ae. aegypti* were collected from National Institute for Communicable Disease (NICD), Mettupalayam, Coimbatore, Tamil Nadu, India without exposure to any insecticide. The eggs were then brought to the laboratory and transferred to enamel trays containing water and kept for larval hatching. They were hatched and reared and have been still maintained for many generations in the laboratory. The eggs and larvae obtained from this stock were used for different experiments.

Maintenance of larvae: The larvae were reared in plastic cups. They were daily provided with commercial fish food (Lymio et al., 1992) *ad libitum.* Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through foreign mosquitoes.

Maintenance of pupae and adult: The pupae were collected from culture trays and were transferred to glass beakers containing water with help of a sucker. The pupae containing glass beaker were kept in side mosquito cage for adult emergence. The cage was made up of steel frame wrapped with mosquito netting. The cage had a provision (a hole) for handling of materials and animals placed inside. The hole was guarded with a sleeve which was useful to close suddenly after being used.

Blood feeding of adult Ae. aegypti and egg laying: The females were fed by hand every alternate day. Feeding mosquitoes on human arm for experimental purposes was suggested by Judson (1967) and Briegel (1990). Both females and males were provided with 10% glucose solution as described by Villani et al. (1983) on cotton wicks. The cotton was always kept moist with the solution and changed every day. An egg trap (cup) lined with filter paper containing pure water was always placed at a corner of the cage. This arrangement made the collection of eggs easier.

Collection of plant materials

P. dulce leaves were collected from Government Arts college campus, Coimbatore, Southern India. The identification of the plant was authenticated at BSI (Botanical Survey of India), Coimbatore.

Preparation of plant extract

The fresh leaves of the plant *P. dulce* were collected in our college campus area. Then the leaves brought to the laboratory. The plant leaves were observed carefully for any kind of diseases or infection and if found any, those parts were separated and not used for the experiment. The selected leaves washed with distilled water in order to clean dust or any particle stuck to them. Then the leaves kept for drying under shade at room temperature (27± 2^{o}C) for about 2 weeks till they dried completely. The leaves were finely powdered using electric blender. Powdered plant material (100g) was soaked in double distilled water (1000 ml) in airtight wide mouth bottle and kept for 4 days with periodic shaking. After that, the cold extracts were filtered using Whatman No. 1filter paper and kept in Petri dishes for drying at room temperature (Kongkathip, 1994). Dried extracts were used for the preparation of stock solution.

Preparation of stock solution and different concentrations of leaf extract

1 g of the concentrated extract of *P. dulce* leaves of was dissolved in 100ml tap water and kept as stock solution. This stock solution was used to prepare the desired concentrations of the extract for exposure of the mosquito larvae.

Ovicidal assay

Effect of aqueous leaf extract of *P. dulce* on the hatchability of *Ae. aegypti* eggs were determined adopting the following procedure (Judson and Gojrati, 1967). Hatching rate was calculated on the basis of non-hatchability of eggs according to Sahgal and Pillai (1993). To ensure non-hatchability, the eggs from any test container were collected after 96 hours. Unhatched and decapped eggs were separated and counted using dissection microscope. Five replications were conducted at each concentration of test compound. The data were statistically examined using Student's *t*- test.

Oviposition bioassay

Fifteen pairs of mosquitoes were kept in a cage and maintained. They were blood fed every alternate day. The effect of aqueous leaf extract of *P. dulce* on oviposition of *Ae. aegypti* was determined under two set of conditions (choice oviposition and no choice oviposition test) as suggested by Beehler and Mulla (1993), Chen et al. (1996) and Dhanakkodi et al. (1999).

Oviposition Active Index (OAI) was calculated as detailed by Vasuki (1991) using the formula. This would indicate whether the effect of the compound on oviposition is positive or negative. Further, the percentage of oviposition deterrence (oviposition deterrent index of Lundgren, 1975) was determined according to the formula given by Dimetry et al. (1995). The data were statistically examined using Student's *t*- test.

Laboratory assay for larval duration

To determine the effect of aqueous leaf extract of *P. dulce* on the length (duration) of the larval stage (larva-pupation), different concentrations (0.05, 0.10, 0.15 and 0.20) test solutions were prepared. Fifty number of first instar larvae were placed in the treated water and allowed developing further. The medium was watched every 24 h. The total larval duration in days was recorded from first instar to pupation. In parallel, the duration of larval stage was calculated for the larvae reared in untreated water for comparison (Gunasekaran et al., 2009). The data were statistically examined using Student's *t*-test.

Results and discussion

Effect of aqueous leaf extract of *P. dulce* on hatching of *Ae. aegypti* eggs

Freshly laid eggs obtained from the general stock of mosquitoes were tested for their hatching ability in relation to the different concentrations of aqueous leaf extract of *P. dulce*. Percent hatch of eggs placed in control medium was 88 % where as in 0.1, 0.4, 0.8 and 0.12% concentrations it was 68, 50 and 11. The dose of 0.12% completely arrested the hatching eggs (Table 1). The decrease in hatchability was found to be dose dependent.

Table 1. Alternation in hatchability of *Aedes aegypti* eggs exposed to different concentrations of aqueous leaf extract of *Pithecellobium dulce* and control.

Parameters	Control	Concentrations (%)			
		0.1	0.4	0.8	0.12
Number of eggs introduced/trials	20	20	20	20	20
Mean number of eggs hatched #	17.66	13.66	10.00	2.333	0
S.D.	±0.210	±0.101	±0.102	±0.244	±0.211
Percent hatchability	88	68*	50*	11*	0*
Percent reduction over control		20	38	77	100

#, Mean (± SD) of 5 replicates; *, Significantly different from control , $p<0.001$

Choice oviposition test

Mosquitoes showed more preference towards control ovitrap for oviposition, though; media of different concentrations of aqueous leaf extract of *P. dulce* were available along with control (Choice oviposition test). The total number of eggs laid in ovitraps containing any concentration of the aqueous leaf extract of *P. dulce* was always less than that in the control. Among the total number of eggs laid, 55.03% was present in control medium when placed along with ovitraps with 0.10, 0.15, 0.20 and 0.25 % aqueous leaf extract of *P. dulce* in which appeared 24.81, 14.55, 5.597 and 0.9328 % of eggs respectively. This was

also indicated by ODI values (37.850, 58.176, 81.538 and 96.666). Rate of oviposition in ovitraps with any concentration of test compounds was significantly (p<0.001) less than in control (Table 2).

No-choice oviposition test

The ovipositional deterrence of aqueous leaf extract of *P. dulce* against *Ae. aegypti* was also confirmed by the results of 'no – choice test' where ovitrap with any one of the concentrations accompanied the control. Percent oviposition in 0.10, 0.15, 0.20 and 0.25% of aqueous leaf extract of *P. dulce* was 25.00, 21.91, 11.11 and 1.960% which were significantly (p<0.001) less compared to their control counterparts 75.00, 78.08, 88.88 and 98.03 %, respectively. The data of no–choice oviposition test clearly exhibited interference of aqueous leaf extract of *P. dulce* on the oviposition preference of mosquitoes (Table 3).

Table 2. Changes in the indices of oviposition deterrence at different concentrations of aqueous leaf extract of *Pithecellobium dulce* against *Aedes aegypti* under choice oviposition test.

Parameters	Control	Concentrations (%)			
		0.10	0.15	0.20	0.25
Total number of eggs laid*	590	266	156	60	10
Percent oviposition	55.03	24.81#	14.55#	5.597#	0.9328#
Percent reduction in oviposition over control		45.08	26.44	10.16	1.6949
Reduction in number of eggs compared to control		324	434	530	580
Oviposition active index (OAI)		-0.378	-0.581	-0.815	-0.966
Oviposition deterrent index (ODI)		37.850	58.176	81.538	96.666

* - Total number of eggs in 10 replicates; # - Significantly different from control; p<0.001.

Table3. Changes in the indices of oviposition deterrence at different concentrations of aqueous leaf extract of *Pithecellobium dulce* against *Aedes aegypti* under no-choice oviposition test.

Parameters	Concentrations (%)							
	C	0.10	C	0.15	C	0.20	C	0.25
Total number of eggs laid*	300	100	285	80	400	50	500	10
Percent oviposition	75.00	25.00#	78.08	21.91#	88.88	11.11#	98.03	1.960#
Percent reduction over control		33.33		28.07		12.5		2.000
Reduction in number of eggs compared to control		200		205		350		490
Oviposition active index (OAI)		-0.500		-0.561		-0.777		-0.98
Oviposition deterrent index (ODI)		50.00		56.10		77.70		98.00

* - Total number of eggs in 3 replicates; # - Significantly different from control; p<0.001; C- Control.

Effect of aqueous leaf extract of *P. dulce* on total larval duration of *Ae. aegypti*

The aqueous leaf extract of *P. dulce* at 0.05, 0.10, 0.15 and 0.20% tested against the *Ae. aegypti* was found to prolong larval and pupal period. In the control it took 8 days for all the larvae to become pupae, whereas the aqueous extract at 0.05, 0.10 and 0.15% took 12, 16 and 20 days respectively. In 0.20% the larvae required 26 days to become pupae (Table 4).

Table 4. Prolongation of larval duration of *Aedes aegypti* with effect of aqueous leaf extract of *Pithecellobium dulce.*

Concentrations (%)	Larval duration (Days)				
	I-II	II-III	III-IV	IV-Pupa	Total larval development (Days)
Control	2	2	2	2	8 ± 0.001
0.05	2	2	4	4	12*± 0.012
0.10	2	4	4	6	16 *± 0.001
0.15	2	3	5	10	20*± 0.011
0.20	2	4	7	12	26* ± 0.010

Mean (± SD) of 3 replicates; * - Significantly different from control, p<0.001.

The methanol extract of sea weed leaf exerted 100% egg mortality (zero hatchability) at 240, 300 and 360 ppm for *Ae. aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Rajalakshmi et al., 2013); about 100% mortality was observed of the egg rafts of *Cx. quinquefasciatus* at 500 mg/l for leaf methanol extract of *P. dulce* (Govindarajan and Rajeshwary, 2014a); it has been noticed that higher concentrations of *Pongamia pinnata* leaves extracts possesses strong ovicidal activity 100% at 400 ppm concentration against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. In the same way, methanol extracts showed maximum ovicidal activity at 240, 320, and 400 ppm followed by hexane, dichloromethane and ethyl acetate against vector mosquitoes (Anitha et al., 2014); at 250 mg/L of *Achras sapota* and *Cassia auriculata* leaves hexane extract , 89.2% and 75.2% ovicidal activity was recorded against the eggs of *An. stephensi* respectively (Krishnappa and Elumalai, 2014); maximum percentage of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* eggs was killed when they were exposed to 500ppm concentration of *Clausena excavata* leaves of the methanol extract (Thangarasu et al., 2014); the diethyl ether extract of *Coleus aromaticus* leaves exerted 100% ovicidal activity against *Ae. aegypti* at 200ppm (Baranitharan and Dhanasekaran, 2014); at 100ppm concentration of *Dodonaea viscose* and *Lantana camara* leaves had the most severe effect on *Ae. aegypti* egg hatching rate which were reduced by about 35% and 33.5% compared with 26% for *Ruta chalepensis*. At their highest concentrations (500 ppm), the tree plant extract reduced egg hatchability percentages by about 90%,87% and 69% for *Dodonaea viscose* and *Lantana camara* and *Ruta chalepensis* respectively(Madkour et al., 2014) ; the higher concentrations of *Polygala arvensis* leaf methanol extracts possesses strong ovicidal activity at 200 ppm concentration against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, no egg hatchability was recorded (Deepa et al., 2014); one hundred per cent egg mortality was obtained only in ethyl acetate extract of *Ageratum houstonianum* leaves at 20.0 mg/L against *Ae. aegypti* (Samuel et al., 2015); the methanol and petroleum ether extracts of *Justicia adhatoda* leaves exerted 100% mortality at 240 ppm against *Cx. quinquefasciatus* and *Ae. aegypti* respectively (Jayapriya and Shoba , 2014); the methanol extract of *Ageratum conyzoides* leaves exerted 100% egg mortality at 150, 200 and 250 ppm against *An. stephensi* (Janakan and Ramakrishnan, 2014). In the case of ovicidal activity, exposure of freshly laid eggs was more effective than that of the older eggs (Miura et al., 1976).

The aqueous extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion (Rajkumar et al., 2011). Eggs and egg shells treated with plant extracts become damaged probably due to endosmosis. After the initial phase of swelling, eggs become desiccated, followed by shrinkage and death of larvae trapped within (Arivoli and Samuel, 2011d). The treated eggs contained developed embryos the eclosion of the egg was incomplete (Miura et al., 1976). The findings of the present investigation were comparable with other ovicidal studies and revealed that the aqueous *P. dulce* leaf extract possesses ovicidal activity against the eggs of *Ae. aegypti*.

The ethanolic leaf extract of *Solanum trilobatum* was tested under laboratory conditions for oviposition deterrent activities against the adult *An. stephensi* and the concentrations of 0.01, 0.025, 0.05, 0.075 and 0.1% reduced egg laying by females from 18 to 99% (Rajkumar and Jebanesan, 2005 a); 100% oviposition deterrency was obtained with *Melia azedarach* leaf extract at lg/L against *Ae. aegypti* (Coria et al., 2008); the oviposition active index (OAI) value of acetone, ethyl acetate, and methanol extracts of *Aegle marmelos*, *Andrographis lineata* and *Cocculus hirsutus* leaf at 500 ppm against *An. subpictus* were -0.86, -0.87, -0.90, -0.78 and -0.87, -0.86, -0.91, -0.94 and -0.86 respectively and the OAI values revealed that the solvent plant extracts have deterrent effect, and they caused a remarkable negative response resulting an oviposition of very few eggs (Elango et al., 2009); ethanolic leaf extract of *Andrographis paniculata* observed against *An. stephensi* and OAI values for the species were -0.28, -0.45, -0.49 and -0.59 for extract concentrations of 29, 35, 41 and 46 ppm respectively (Kuppusamy and Murugan, 2008).

In oviposition deterrent activity, the highest concentration of (0.1%) ethanolic leaf extract of *Vitex negundo* produced the oviposition deterrent activity of 94.2% in *Ae. aegypti*, 96.4% in *An. stephensi* and 99.8% in *Cx. quinquefasciatus* (Vijaya kumar et al., 2011a); the highest concentration of (0.1%) acetone, chloroform, hexane, petroleum ether and ethanol extracts of *Annona squamosa* leaves produce

oviposition deterrent activity 99.6% against *An. stephensi*, 92.4% against *Cx. quinquefasciatus* and 92.4% against *Ae. aegypti* respectively (Vijaya kumar et al., 2011b); acetone extract of *Prosopis juliflora* leaf found to be strong oviposition deterrent which inhibited egg laying (OAI-0.466) at 100 ppm (Yadav et al., 2014) ; the maximum oviposition deterrent activity was recorded from the highest concentration of methanol, hexane ,dichloromethane and ethyl acetate extracts 400 ppm and the least oviposition deterrent activity was recorded from the 80 ppm concentration of *Pongamia pinnata* leaves extracts against mosquitoes (Anitha et al., 2014).

The oviposition is one of the most important events in the life cycle of mosquitoes (Xue and Barnard, 2001). If oviposition is prevented the mosquito life cycle is disrupted and population growth is reduced. The present oviposition study shows that the aqueous leaf extract of *P. dulce* act as oviposition deterrent, this indicates that *Ae. aegypti* mosquitoes were acutely sensitive to phytochemical stimuli and respond to the odour of the leaf extract. The strong odour produced by higher concentration of aqueous leaf extract produce maximum effective repellence against oviposition. The mosquitoes are known to select or reject their specific oviposition sites by sensing chemical signals that are detected by sensory receptors on the antenna (Davis and Bowen, 1994).

Quershi et al. (1986) reported the prevention of pupation up to day 7 at a dosage of 1000 ppm in the immature of *Ae. aegypti* in ethanolic extract of *Cassia holosericea; Leucas aspera* leaf (500, 1000 ppm) showed prolonged larval and pupal periods among *Ae. aegypti, Cx. quinquefasciatus* and *An. stephensi*. It took 8 days for all the extracts to become pupae; whereas in the aqueous extract it took 11 days for *Ae. aegypti* and 9 days in the case of *An. stephensi* and *Cx. quinquefasciatus* (Arivoli and Samuel, 2011 a) ;the hexane, dimethyl ether, dichloromethane and ethyl acetate extract of *Abutilon indicum* leaf against *Ae. aegypti*, *Cx. quinquefasciatus, An. stephensi* showed that larval and pupal periods were prolonged with an overall increases in the developmental period (Arivoli and Samuel , 2011c); there was a delay in the development of *Cx. pipiens* larvae to pupal stage (21days) when the second instar larvae were exposed to concentrations 40 and 20 mg/ml of methanolic leaf extract of *Azadirachta excela* (Mustafa and AL-Khazraji, 2008); exposure of *An. stephensi* larvae to sub–lethal doses of neem leaves in the laboratory prolonged larval development (Murugan et al .,1996)
In the present study, lengthening of larval and pupal periods indicates the interference of the bio–active compounds of aqueous leaf extract of *P. dulce* with the normal hormonal activity coordination of the metabolic process of the developing stages. Prolongation of development period of mosquito larvae treated with plant extracts were generally attributed to interference of the active ingredients of the extracts with the endocrine system (Zebitz et al., 1986).

Conclusion

The results obtained in the study demonstrated the importance of ovicidal, ovipositional deterrence and prolonged larval duration influence of the extracted plant material *P. dulce* on *Ae. aegypti* mosquitoes. It is anticipated that such effects may be observed when these materials are applied in natural larval breeding habitats in rural as well as urban localities. Moreover, application of these materials is not likely to leave harmful residues in the environment they are naturally occurring among the local flora can be used as an alternative to synthetic insecticides. Furthermore, small scale and field trials should be done with a view to evaluating the efficacy of the plant. We hope that the plants will be acceptable to replace the use of conventional insecticide in mosquito control program. These results could be useful in the search for novel, more selective, and biodegradable compounds for the control of the dengue vector *Ae. aegypti*.

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