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Bioefficacy of *Duranta erecta* leaf extract on yellow fever and dengue vector, *Aedes aegypti* Linn. in Nigeria

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Mosquitoes are considered as a major problem in public health. Control measures mainly by chemical insecticides, have led to several environmental and health problems in addition to development of resistance. The bioefficacy of aqueous extract of leaves of *Duranta erecta* against the yellow fever and dengue vector, *Aedes aegypti* was evaluated as a step towards developing a safe and eco-friendly agent to combat the problems of mosquitoes and mosquito-borne diseases. Pulverized leaves of *D. erecta* were macerated in 2 L of distilled water for 24 h, filtered and concentrated. The aqueous extract of *D. erecta* leaves at different dilutions such as 50, 200, 500, 700, 900 and 1000 mg/L were tested against 525 eggs and 1050 fourth instar larvae of *A. aegypti* using emersion method. The percentage ovicidal, larvicidal activities, pupal and adult emergence, adult immergence and fecundity inhibition were studied. A concentration dependent increase in ovicidal, larvicidal, inhibition of adult emergence and fecundity were recorded. High ovicidal activity (low egg hatchability) was recorded with LC₅₀ (95% CL) values of 340.085 mg/L (250.85-430.17mg/L). High larvicidal, emergence and fecundity inhibition were recorded with LC₅₀ value of 420.59 mg/L (33.34-520.06 mg/L). Preliminary phytochemical analysis showed the presence of tannins, saponins, alkanoids, flavonoids, glycosides and anthroquinone. The results indicated that these botanicals may play an important role in reducing the use of chemicals for mosquito control. In-depth investigations to elucidate the active ingredients of the extract responsible for mosquitocidal activity in *Aedes aegypti* should be conducted and small scale field trials are needed for utilization.

Key words: *Duranta erecta*, aqueous extract, *Aedes aegypti*, mosquitocidal activity.

INTRODUCTION

Mosquitoes in general and *Aedes aegypti* in particular constitute a major problem in public health and lead to

serious human diseases such as malaria, encephalitis, yellow fever, dengue, hemorrhagic fever, filariasis and

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other arboviruses. Nearly 700 million people get a mosquito-borne illness each year resulting in more than one million deaths (Caraballo and King, 2014). *A. aegypti* is a known vector of several viruses including dengue and yellow fever viruses (WHO, 2015). Yellow fever and dengue viruses occur in tropical areas of Africa and South America (Tomori, 2004; Dawurung et al., 2010), and are regarded as two of the most important arbovirus-causing diseases in Nigeria (Monath et al., 1974; DeCock et al., 1988). Over 100 million Nigerians are reportedly at risk of YF while DENV has been identified as an emerging cause of fever alongside the commonly known malaria (Oyero and Ayukekbong, 2014). Dengue and other *A. aegypti* related diseases are increasing global public health concerns due to their rapid geographical spread and increasing disease burden due to an ongoing range expansion fuelled by increased global trade and travel (Kraemer et al., 2015; Stanaway et al., 2016). In addition to horizontal transmission of the disease, vertical transmission (from mother to child) during pregnancy or at birth has been reported in man, while transovarial transmission has been reported in mosquitoes (Carod-Artal et al., 2013), further facilitating the spread of the diseases and increasing the difficulties involved in combating them.

Aedes aegypti exhibits ecological plasticity. It is currently distributed in Africa, the surrounding tropics and subtropics, south eastern USA, the Middle east, South east Asia, Pacific and Indian island and Northern Australia (WHO, 2015). *A. aegypti* are container-inhabiting mosquitoes. They thrive in urbanized areas, in close contact with people making them an exceptionally successful vector. *A. aegypti* are extremely common in areas lacking piped water systems, and depend greatly on stored water for breeding sites (Nwangangi et al., 2007; Medronho et al., 2009).

Control measures against this vector in the short term are the use of conventional insecticides (Cao et al., 2004). Since “adulticides” may only reduce the adult population temporarily, most mosquito control programmes target the egg and larval stages in their breeding sites with ovicides and larvicides (ElHag et al., 1999, 2001; Mohan et al., 2005). Nevertheless, repeated use of insecticides leads constantly to the risk of contamination of water used for domestic purposes, animals and humans by pesticides residues. It is also important to note the high cost of chemical pesticides and the development of resistances, a major issue from mosquito species vectors (WHO, 2013; Kraemer et al., 2015). Other health and environmental issues include toxicity of non-target organisms as well as an increasing burden on the environment and human health. These issues have necessitated the search for plant-based control methods using bio-insecticides. The biological agents are easily degradable into less or nontoxic compounds and proven to be safely used for mosquito control programs, with minimal or absent undesirable

effect on environment and human health.

This research explored the potential for *D. erecta* (family Verbenaceae), a widely cultivated ornamental plant in tropical and subtropical gardens throughout the world to provide locally available biopesticide against mosquito vectors. *D. erecta* is known to produce many secondary chemicals e.g, phenolics, alkaloids, saponins, glycosides (Lobna et al., 2007; Sharama et al., 2012; Manjunatha et al., 2013) and have been tested against key insect pests including *Culex quinquefasciatus* larvae (Say) (Culicidae) (Mc Connell et al., 2010; Chennaiyan et al., 2016). Recent research by Chennaiyan et al. (2016) has demonstrated high antifeedant, larvicidal, pupicidal and ovicidal activities of ethyl acetate leaf extracts of *D. erecta* against Armyworm *Spodoptera litura* and cotton bollworm *Helicoverpa armygera*. In addition, *D. erecta* has numerous medical uses including antifungal (Sharma et al., 2012) antimicrobial (Ogbuagu et al., 2015), antiplasmodial (Majunatha et al., 2013), antioxidant (Dressler et al., 2014) and treatment of abscesses (Manjunatha et al., 2013). The insecticidal property of *D. erecta* has not been evaluated against *A. aegypti*. The present study was done to evaluate the insecticidal property of crude leaf aqueous extract of *D. erecta* Linn. against different developmental stages of *A. aegypti*.

MATERIALS AND METHODS

Collection of *A. aegypti*

The eggs used for the ovicidal experiment and larval rearing were recruited from the egg colony held at Arthropod Borne Viral Diseases (ABOVIRUS) Center, Ministry of Health, Enugu State, Nigeria, where they were reared in the laboratory.

Larval rearing of *A. aegypti*

The mosquito larvae were hatched from the egg colony using modified methods reported by Elumalai et al. (2012). The eggs were washed with 0.01% formaldehyde solution for 30 to 40 min as a precaution against possible microsporidian infections which might interfere with the normal development of the immature stages of mosquitoes and soaked in water to facilitate hatching. After hatching, first instar larvae were distributed in buckets 40 cm in diameter and 15 cm in depth. The containers were large in size to prevent overcrowding until development to early 4th instar larvae required for the study. The larvae were kept in the plastic buckets half filled with tap water and fed with larval food (powdered white oats and yeast in the ratio of 3:1) once a day initially and twice during the later stages of development. To prevent scum from forming on the water surface, water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. The mouth of the buckets were covered with bridal net held in place with a rubber band to prevent unwanted mosquitoes from laying eggs in the plastic buckets.

Collection of plant materials

Fresh leaves of *D. erecta* were collected from the residential

quarters within the University of Nigeria, Nsukka. They were identified and authenticated by Dr. Nzekwe U. of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Sample specimen of the leaves were deposited in the herbarium with the voucher number IDR No.1276.

Preparation of the aqueous extract of *D. erecta*

The collected leaves of *D. erecta* were washed thoroughly with tap water and shed dried under room temperature for two weeks at the Department of Home Science, Nutrition and Dietetics. The dried leaves were pulverized using electric blender and sieved through kitchen strainer (Medium sieve: mesh size = 1/16inch). 624 g of the ground leaves was soaked in 2 l of distilled water for 24 h, after which it was filtered using Whatman's No. 1 filter paper, concentrated (solid) at room temperature and stored in the refrigerator until needed. The solid extract was weighted and the percentage yield was calculated. 624 g of pulverized leaves yielded 385.11 g of aqueous extract (61.72%). The extract obtained was in a dried, powdery form. The extraction was carried out according to the protocols described by Ekpenyong et al. (2012).

Experimental design and procedure

Ovicidal bioassay

The experimental design was the modified version of that reported by Elumalai et al. (2012). Seven groups of concentrations of aqueous extracts (aqE) of *D. erecta* leaf A, B, C, D, E, F and G were used for the bioassay experiment. Groups A, B, C, D, E, F and G were various concentrations of the extract: 50, 200, 500, 700, 900, 1000 mg/L and the control (distilled water only) making a total of 7 treatment levels. Each group was replicated thrice bringing the number to 21 treatments and put in 21 emergence cages. 25 *A. aegypti* eggs were added to each of the 21 plastic cups, making it a total of 525 eggs (75 egg per treatment). The setup was left for four days. Records of the daily number of larvae hatched per cup were taken.

Larvicidal bioassay

Standard methods for testing the susceptibility of mosquito larvae to insecticides as stipulated by WHO (1996) were followed. The bioassay were performed at a room temperature of $28 \pm 2^\circ\text{C}$, relative humidity of 75-85%, photoperiod of 12:12 (light: dark) and pH 7.0 of distilled water. The same procedure done for the egg stage experiment was repeated but instead of eggs, fourth instar larvae were used. 50 *A. aegypti* fourth instar larvae were added to each of the 21 plastic cups. The larvae in all the bowls were fed every twenty four hours on equal amount of larval food which was spread evenly across the water surface. The setup was checked thrice daily for any mortality at 0900, 1200 and 1500 h. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiment was run for 15 days. The following records were taken for each treatment: Total number of dead larvae, larval mortality in minutes, pupal and adult emergence every 24 h.

The results of the bioassay experiments were analyzed as percent mortality and corrected for control mortality with Abbott's formula (Abbott, 1987): $\% M = [(N.D.L.T. - N.D.L.C.) / (Total N.L. - N.D.L.C.)] \times 100$ during treatment of *D. erecta* extracts; N.D.L.C. = number of dead larvae in the control (untreated); Total N.L. = total number of larvae used in each treatment.

Fecundity experiment

Adults from the larval experiment were used to check for the effect of *D. erecta* leaf extract on the fecundity of adult *A. aegypti*. The fecundity experiment lasted for 19 days and was a modified method reported by Appadurai et al. (2015). In place of the plastic bowls with the concentrates, oviposition traps were placed in the cages. The oviposition cups used for this survey were plastic cups with a capacity of 300 ml. The trap was poplin cloth (white) measuring 8 cm long and 5 cm wide. The white poplin cloth when placed into the cups constituted the ovitraps (oviposition traps).

The mosquitoes were fed every two days with blood meal from partially de-feathered chicken strapped to the top of the cage. One ovitrap was placed into each cage to capture the mosquito eggs. One petri dish containing 10% glucose solution soaked in cotton wool was placed in each of the cages and replaced daily. The mosquitoes fed on the 10% glucose as glucose is necessary for flight energy (Cutwa et al., 2007). The calico material was changed daily. Records of onset of oviposition and daily number of eggs laid/mosquito in each treatment were taken.

Fecundity was calculated: eggs laid/adult female mosquitoes. Percentage inhibition of fecundity was calculated as follows: $\text{Number of eggs laid by the control} - \text{number of eggs laid by the treated mosquitoes} \times 100 / \text{number of eggs laid by the control mosquitoes}$.

Phytochemical analysis

Qualitative phytochemical screening

The qualitative phytochemical screening was conducted using the following analytical methods; for the analysis of tannins and alkaloid, the method used was the method of Treas and Evans (1996), while saponins, glycoside, carbohydrates and terpenoid were studied using the method as described by Harbourne (1984). Steroids and acidic compounds were analyzed using the methods described by Akpuaka (2009).

Quantitative phytochemical analysis

The quantitative phytochemical screening were conducted using the analytical methods of Umeh and Ogbuagu (2012) for the analysis of flavonoids, saponins and alkaloids, Makkar et al. (1993) for the analysis of tannins, while for glycosides, methods of Kanoman et al. (2014) and Trease and Evans (1999) were employed.

Data analysis

The mean values and standard deviations were calculated from replication data. One-way analysis of variance (ANOVA) was used to determine the significance of the treatments and means were separated by Tukey's test of multiple comparisons using SPSS software (version 11.5; SPSS Inc., Chicago, IL, USA). Significant differences at p-value less than 0.05 was kept significant.

RESULTS AND DISCUSSION

Qualitative and quantitative phytochemical analysis

A qualitative test indicated small quantities (+) of alkaloids, flavonoids, tannins, anthroquinone and

Table 1. Qualitative phytochemical screening of *D. erecta* (mg/100 g).

Types of phytochemical constituents	Water
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	++
Steroids	-
Anthroquinone	+
Cardiac glycosides	+

-,Absence of the phytochemical compound;+present in small quantity; ++, moderately present.

Table 2. Quantitative ($\mu\text{g/ml}$) estimate of chosen secondary metabolites of *D. erecta* ($\mu\text{g/ml}$).

Types of phytochemical constituents	Water
Alkaloids	112
Flavonoids	189
Tannins	89
Saponins	429

glycosides in the extract, while saponins, were moderately present (++) , steroids were conspicuously absent (Table 1). The present preliminary phytochemical analysis is similar to that reported earlier by Chennaiyan et al. (2016) and Ogbuagu et al. (2015). The quantitative estimates of four active metabolites shown in Table 2, revealed saponins as the most abundant (429 $\mu\text{g/ml}$), while tannins were the least in quantity.

Earlier studies report saponins, flavonoids and tannins as active insecticidal compounds. Saponins are freely soluble in water and mostly used in the manufacture of insecticides, vaccines and synthesis of steroidal hormones (Gee et al., 1989). Saponins work by interacting with the cuticle membrane of the larvae, changing the microstructure of the cell membranes (Ahmed et al., 2010) and finally disarranging the membrane, which is one of the likely reasons for larval death (Ahmed et al., 2010; Amer and Mehlhorn, 2006). Flavonoids inhibit membrane bound enzymes and this property may explain their antioxidant and anti-inflammatory properties (Susanta et al., 2004). These properties outside inducing toxicity in insects have been gainfully employed in the treatment and management of cancer (Trease and Evans, 1999; Susanta et al., 2004) and malaria from chloroquine sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum* (Ijaz et al., 2010). Acaricidal activities of methanolic leaf extracts of *Jathropa curcas* against the cattle bont tick *Rhipicephalus annulatus* was attributed to tannins (Sanis et al., 2012). Tannin-rich plant extracts from *Acacia pennatula*, *Piscidia piscipula*, *Leucaena*

leucocephala and *Lysiloma latisiliquum* have exhibited acaricidal properties against *Rhipicephalus microplus* (Fernandez-Salas et al., 2011). Generally, flavonoids and tannins are toxic phenolics that disrupt cellular structure, while alkaloids interferes with insect's nerve impulses (Simmonds and Stevenson, 2001; Koul, 2008; Tennyson et al., 2012a).

Ovicidal effect

The extracts at all concentrations tested (50-1000mg/L) blocked hatchability when compared with the control (Figure 1). This indicates high potentials of *D. erecta* as a mosquito ovicide. Number of eggs hatched reduced with increased concentration and ranged between 81-22% against 92% for control. Reductions were significantly different ($P < 0.05$), at 500mg/L and above. None of the eggs hatched at concentrations of 700 mg/L and above. Hatching was also delayed (4 days) in 50, 200 and 500 mg/L concentrations, while it was completed in two days in the control (data not presented).

Previous studies on ovicidal activity of plant extracts against mosquito eggs had also been reported. Methanolic extract of *Cocculus hirsutus* caused 86 and 100% ovicidal activity at 500 and 1000 ppm, respectively against *A. subpictus* (Elango and Rahuman, 2011; Rahuman et al., 2008). In another study, hexane extract of *Limonia acidissima* at 500 ppm caused 79.2 and 60% ovicidal activity on *A. aegypti* and *C. quinquefasciatus*, respectively (Appadurai et al., 2015). In this study, the

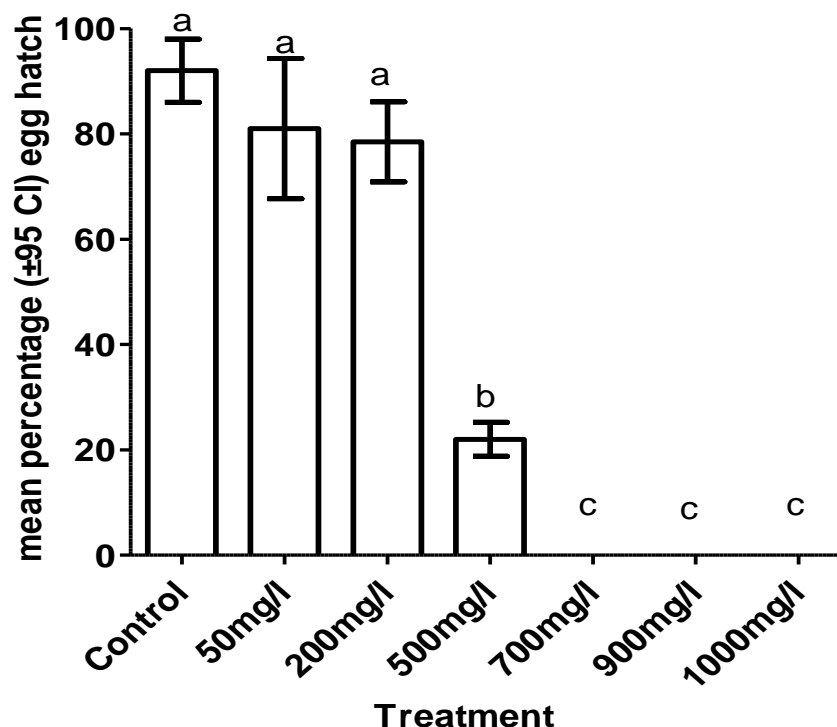


Figure 1. Ovicidal activity (\pm 95% CI) of 6 concentration levels of *D. erecta* on *A. aegypti* eggs. Different lower case letters represent statistically significant differences between treatments.

concentration that effectively achieved ovicidal activities were lower than those reported above earlier studies. This may be an indication of the high efficacy of *D. erecta* leaf aqueous extract against *A. aegypti* eggs.

Larvicidal activity

The extracts at all concentrations tested were toxic to fourth instar larvae of *A. aegypti* (Table 3). Larval mortality increased significantly ($P < 0.05$) with increased concentration of the extract and ranged between 17 – 97% for 50-1000 mg/L, respectively. The extracts considerably blocked pupal and adult emergence with number of emerged pupae and adults reducing significantly ($P < 0.05$) with increased concentration when compared with the control (Table 3). Number of emerged pupae were extremely low for 700,900 and 1000 mg/L concentrations (13, 3.33 and 2.67%, respectively), while only 1.67% of adults emerged from the pupae from 700 mg/L concentration and no adult emerged from the pupae emerged from the larvae subjected to 900-1000 mg/L concentrations. Inhibition of adult emergence also increased with concentration and ranged between 25.52 and 95.9% for 50-700 mg/L. Similar studies on the larvicidal activities of plants belonging to different families have been reported by various authors. According to

Elumalai et al. (2012), methanolic leaf extract of *Gymnema sylvestre* (LC_{50} value of 28.577ppm) was effective against *C. tritaeniorhynchus* larvae, *A. millefolium* methanolic stem extract (LC_{50} value of 120.0ppm) against *C. quinquefasciatus* (Parvela, 2008), 1% Suneem a derivative of neem oil (LC_{50} value of 2mg/L) against larvae of *A. aegypti* (Ndione et al., 2007), aqueous extract of *D. erecta* at 3.0% concentration of leaf extract achieved 100% mortality of fourth instar *C. quinquefasciatus* larvae (MaConnell et al., 2010) and ethyl acetate extract of *Sphaeranthu indicus* whole plant was found to be effective with an LC_{50} value of 201.11 ppm against the larvae of *A. aegypti* followed by hexane leaf extract of *Abailon indicum* (LC_{50} value of 261.31 ppm) (Tennyson et al., 2012b).

Time taken by the different concentrations of the extracts to achieve larval mortality, pupal and adult emergence on *A. aegypti* also varied with the concentration (Table 3). Larval mortality was hastened at high concentrations with time decreasing significantly ($P < 0.05$) with increased concentration for 700-1000 mg/L when compared with the control and ranged between 3600 - 160 min for control – 1000 mg/L respectively. Conversely, pupal and adult emergence in the treated mosquitoes were delayed, with emergence time increasing with concentration and ranged from 96 to 230.88 h for control – 1000 mg/L, respectively for pupae

and 9 to 18 days for control -700 mg/L for adults. When compared with the control, the delay was significant ($P < 0.05$) for 50, 500, 700, 900 and 1000 mg/L for pupae and 50-700 mg/L for adults. Inhibition of adult emergence is a juvenile effect than toxic. This is in harmony with the report by Mahyoub et al. (2014), who indicated that alcoholic extract of *Meliza azedarach* leaf extract at low concentrations (LC_{50} value of 32.6223) significantly inhibited adult emergence of *A. aegypti* and was projected as a possible juvenile hormone analogue. Crude leaf aqueous extract of *D. erecta* may also be considered as a possible juvenile hormone analogue and requires further investigation. No adult emerged from pupae in 900 and 1000 mg/L media (Table 3). High concentration of toxic substances in the mosquito breeding habits has been reported to reduce the surface tension of the water and newly emerged adults have been reported to drown while attempting to rest on the water surface (Clements, 1993). This may be responsible for the absence of adults in the higher concentrations in this study as dead adults were observed floating on the water surface.

In addition, body tissue laceration and deformity which may have contributed to death, were observed only in larvae exposed to *D. erecta* leaf aqueous extract, when compared with the control group (data not presented). Similarly, Ndione et al. (2007) reported that death in fourth instar larvae of *A. aegypti* subjected to neem products was due to gastric caeca and midgut epithelial columnar cells vacuolization and microvilli damages, busting of epithelial cells in the posterior part of the gut, cell degeneration and perturbation of alimentary flow in the alimentary canal in the anterior part of the gut. It is important to investigate the histopathological effects of *D. erecta* on the fourth instar larvae of *A. aegypti* to delineate the cause of death.

Number of eggs laid by adult emerged from the treated larvae were also concentration dependent and significantly ($P < 0.05$) decreased with increased concentration, ranging between 234 - 141 against 512 for control, while fecundity inhibition significantly ($P < 0.05$) increased with concentration ranging between 22.22 - 69.87% for 50-700 mg, respectively (Table 3). Similarly, Mong'are et al. (2012), reported that leaf crude extracts of *Acalypha fruticosa*, *Tagetes minuta* and *Tarhonanthus camphoratus* reduced the fecundity of *Phlebotomus duboscqi* by 73, 53 and 26%, respectively. The number of eggs produced by mosquito females is reported to be dependent on the size of the blood meal and subsequently linked to the quantity of proteins obtained from a blood meal (Maruniak, 2014). Further investigations are needed to understand the role of aqueous extract of leaf of *D. erecta* on mosquito fecundity.

Oviposition time was also concentration dependent, reducing with increased concentration. Oviposition commenced on the third day (data not presented) and

ranged between 5 and 2 days for control, 700 mg/L (Table 3).

Reduction in oviposition duration was not significant ($P < 0.05$) when compared with the control. Curiously, 200 mg/L appeared to have less toxic effects than 50 mg/L on the fourth instar larvae. For instance, higher number of eggs (382.68) was laid by adult females that emerged from larvae treated with 20mg/L, than those from 50 mg/L (234). Inhibition of fecundity was also lower (13.35%) for adults from 200 mg/L than adults from 500 mg/L (22.22%) (Table 3).

The LC_{50} values for *A. aegypti* varied depending on the tested stage. The egg stage had the lower LC_{50} value (340.085 mg/L) and appeared to be more sensitive to this extract than the larvae and also had the widest fiducial limits for the extract (Table 4). The larval LC_{50} value (420.59 mg/L), would be lethal to both egg and larvae of *A. aegypti* and would effectively control *A. aegypti*.

Conclusions

Vector control is facing threats due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides and side effects such as environmental pollution and toxic hazards to humans and other non-target organisms (Don-Pedro, 1976; Don-Pedro and Adegbite, 1985; Rodriguez et al., 2007). The aforementioned problems created awareness of the need for new insecticides for mosquito control which are eco-friendly and target specific (Sukurmar et al., 1991; Shaalan et al., 2005; Sharma and Srivastava, 2006). The screening of local medicinal plants for mosquito larvicidal activity may lead to their use in natural product-based mosquito abatement practices. The current study revealed that leaf aqueous extract of *D. erecta* is a promising potential biocontrol agent against *A. aegypti*, particularly in its markedly ovicidal, larvicidal, adult and fecundity inhibition effects. The extract or isolated bioactive phytochemical could be used for the control of mosquitoes acting as vectors for many communicable diseases. However, further studies on the identification of the active compounds, their modes of action and field trials, are required.

Conflict of interests

The authors have not declared any conflict of interest.

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Table 3. Effect of aqueous leaf crude extract of *D. erecta* on developmental stages of *A. Aegypti*.

Concentration (mg/L)	Mean larval mortality	Larval mortality (%)	Larval mortality (min)	Mean pupal emergence (%)	Mean pupal emergence (%)	Mean pupal emergence (h)	Mean adult emergence (%)
Control	4.00±1.24 ^a	1.50±2.0.71 ^{d3*}	3600±12.64 ^a	48.33±4.43 ^a	96.67±8.86 ^a	96±0.00 ^a	41.00±6.39 ^a
50	8.67±1.87 ^a	17.33±2.76 ^a ^{d4}	3600±32.76 ^a	41.00±4.23 ^a	77.33±8.44 ^a	135.12±0.33 ^b	20.67±4.72 ^b
200	4.67±1.57 ^a	9.33±3.164 ^{d3}	3600±28.87 ^a	45.33±2.67 ^a	90.67±5.23 ^a	96.34±0.00 ^a	29.00±3.85 ^c
500	8.33±2.32 ^a	33.66±4.68 ^{d4}	360±4.21 ^a	41.33±4.46 ^a	82.67±8.96 ^a	112.12±0.33 ^c	20.65±4.32 ^{ab}
700	35.67±7.68 ^b	82.66±14.46 ^{d4}	420±18.84 ^b	6.67±1.87 ^b	13.33±3.7 ^c	169.00±0.00 ^{ab}	1.67±0.33 ^b ^c
900	48.32±2.93 ^c	94.77±7.804 ^{d4}	280±22.56 ^c	2.00±0.67 ^{ab}	3.33±2.001 ^d	216±3.00 ^b ^c	0.00±0.00 ^e
1000	48.66±1.74 ^d	97.54±4.799 ^{d4}	160±8.78 ^d	1.33±1.33 ^c	2.67±2.67 ^e	230.88±3.00 ^d	0.00±0.00 ^e
Concentration (mg/L)	Adult mean emergence (%)	Mean adult emergence (days)	*Inhibition of adult emergence (%)	Mean number of eggs laid	Mean number of eggs laid in days	Mean fecundity	Inhibition of fecundity (%)
Control	81.00±12.59 ^a	9±0.33 ^a	17.35	512.00±58.84 ^a	5±0.00 ^a	22.97 ^a	-
50	41.33±6.21 ^b	11.2±0.33 ^b	25.52	234.00±37.12 ^{a2}	5±0.00 ^a	19.07 ^a	22.22
200	57.00±7.71 ^c	12±0.00 ^c	29.63	382.68±38.75 ^{a3}	5±0.00 ^a	20.14 ^a	13.345
500	37.32±4.22 ^d	15±0.00 ^{ab}	53.93	141.33±5.21 ^{a4}	5±0.00 ^a	13.117 ^b	46.54
700	3.33±0.67 ^{ab}	18±0.33 ^{bc}	95.9	30.00±8.54 ^{a5}	2±1.00 ^a	6.92 ^c	69.87
900	0.00±0.00 ^e	0.00±0.00 ^e	-	0.00±0.00 ^{a6}	0±0.00 ^a	0 ^d	-
1000	0.00±0.00 ^e	0.00±0.00 ^e	-	0.00±0.00 ^{a6}	0±0.00 ^a	0 ^d	-

*Corrected using Abbot's equation (Abbot, 1987). Results with same letters in the column are not significantly different (P < 0.05).

Table 4. Dose-mortality responses of eggs and larval stages of *A. aegypti* treated with *D. erecta* leaf aqueous extract.

Tested stage	LC ₅₀ (mg/L)	LC ₉₀ (mg/L)	Slope	X ²
Egg	340.085 (250.85-430.17)	630.07 (230.37-810.01)	0.04421±0.0316	14.744
Larvae	420.59 (330.34-520.06)	780.59 (670.02-960.77)	0.0355±0.017	28.705

the *Aedes aegypti* eggs for the study and some technical assistance.

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