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RESEARCH ARTICLE

Ovicidal and Larvicidal Activities of Ethanolic Leaf Extracts of Three Botanicals Against the Malaria Vector - *Anopheles Gambiae*

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ABSTRACT

Malaria, transmitted by *Anopheles gambiae*, has been a major public health concern in Africa. Chemicals used in the control of *A. gambiae* have caused a lot of havoc in the environment and to non-target organisms. More so, a high rate of resistance by these mosquitoes has been recorded. This study evaluated the ovicidal and larvicidal activities of ethanolic leaf extracts of *Duranta erecta*, *Tridax procumbens* and *Pennisetum purpureum* against *A. gambiae*. Phytochemical analysis of these plants revealed the presence of tannins, saponins, alkanoids, flavonoids, glycosides and anthroquinone. Ground dry leaves of each plant material were concentrated in 7 litres of 95% ethanol for 72 hours followed by filtration and evaporation. *D. erecta, T. procumbens* and *P. purpureum* yielded 617.2g, 598.3g and 552g of extracts respectively. The WHO standard for mosquito bioassay was adopted and concentrations 40, 100, 140 and 200 parts per million (PPM) were tested against 20 eggs and 25 larvae using emersion method. The hatching rate and % larval mortality of the extracts were recorded in which a concentration dependent increase was observed. High ovicidal activity (low egg hatchability) was recorded in *D. erecta* (LC_{50} -10.037 PPM) followed by *P. purpureum* and *T. procumbens* with LC_{50} values of 17.380 and 39.198 respectively. The highest larvicidal activity was observed in *D. erecta* (LC_{50} -76.943 PPM) compared to *P. purpureum* and *T. procumbens* (LC_{50} - 213.410 PPM and 214.217 PPM). Evidently, *D. erecta* ethanolic leaf extracts showed the best efficacy in the control of *A. gambiae* in this study. *D. erecta* is an environmentally friendly alternative in reducing the use of chemicals for mosquito control.

Keywords: Anopheles gambiae, Duranta erecta, Tridax procumbens, Pennisetum purpureum, Mosquito, Larvicidal, Ovicidal, Mosquito, Malaria

1 Introduction

Malaria is one of the serious scourges inflicted upon humanity causing high level of mortality along with great financial losses. Malaria and filariasis rank among the world most prevalent tropical infectious diseases, [1] with malaria being the most important vector borne disease in the world [2]. Globally, malaria infects more than 500 million humans each year [3] with one or two million deaths reported annually [3][4]. In 2015, 3.3 billion people in 97 countries were reported by World Health Organization [5] to be at risk of malaria with an estimated number of 200 million cases and 600, 000 deaths and in 2016, there was an increase of over 5 million cases.

In 2015, 90% of the malaria cases were reported in sub-Saharan Africa and 92% of the malaria deaths were reported worldwide. Two countries in sub-Sahara Africa, Nigeria and Republic of Congo have been reported to have contributed to high malaria burden, as 36% of the malaria cases worldwide occurred in the two countries. More so, the prevalence was reduced by half and the incidence of clinical disease fell by 40% between 2000 and 2015[6] owing to the use of mosquito treated nets. However, malaria still remains a

major health problem in sub-Saharan Africa countries [1].

In Nigeria, the entire population has been reported to be at risk of malaria. In 2009 the World Health Organization reported 4,295,686 confirmed cases, 658,732 were inpatient malaria cases and 7,522 malaria attributed deaths [2]. Furthermore, in 2016 malaria killed more than 100, 000 people in Nigeria [7].

Anopheles gambiae is the major vector of *Plasmodium falciparium* – the causal agent of malaria – in Africa, thus, it is commonly called the African malaria mosquito. Its blood meal come almost exclusively from human, its larvae develop in temporary bodies of water produced by human activities through agricultural irrigation, flooded human or domestic animal footprints among others while adults rest primarily in human dwellings. *A. gambiae* complex comprises six named species which include: *A. arabiensis*, *A. bwanbae*, *A. melas*, *A. merus*, *A. quadriannulatus* and *Anopheles gambiae sensu stricto*; one unnamed species and several 'incipient' species [8].

Various cultural control measures targeted at reducing and eradicating the breeding sites of mosquitoes have been suggested and implemented over the years. Synthetic insecticides such as the organochlorines, organophosphates, and cabamates proved very effective until their havoc to the environment and non-target species were discovered. There are many negative impacts and short comings of these strategies which include environmental pollution, dangers to non-target organisms and most importantly the development of resistance in mosquitoes. There is still needed to search for more stable and effective technique to end the malaria scourge in Africa.

It has been discovered that plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs [9]. Varieties of plants such as *Citrus paradise* [10], *Clausena anisate* [11], *Zanthoxylum heitzii* [12], *Lippia adoensis* [13], *Chromolaena odorata* [14], *Ageratum conyzoides* [15] with insecticidal activities have been reported.

Duranta erecta (Linn), also known as golden dewdrop, pigeon berry, angel whisper, or sky flower in English, is a shrub usually 1 to 3 m in height. It is a native plant of Southern America, Mexico, Central America, Caribbean, and other areas that spread throughout the tropical and warm subtropical regions and the most common ornamental plant in south western Nigeria [16].

D. erecta has been shown to possess various antimicrobial, antioxidant and insecticidal properties [17]. It contains various primary and secondary metabolites such as glycoside, saponins, sterols, flavonoids, phenols, tannins, alkaloids, carbohydrates and proteins. Saponins in the fruits and foliage cause gastro enteric irritation, drowsiness, fever nausea, vomiting, and convulsions [18]. Few studies have been published on the bio-efficacy of *D. erecta* against some different developmental stages of mosquitoes. The bio-efficacy of its leaf extracts only on the eggs and larvae of the yellow fever and dengue vector- *Aedes aegypti* [19] and its efficacy as a larvicidal agent against *Culex quinquefasciatatus* [20] have been reported.

Pennisetum purpureum commonly known as napier grass or elephant grass, is a native grass species in the tropical grasslands of Africa which is spreading rapidly in many parts of the world. It is a major forage crop in the wet tropics of the world [21]. It is named elephant grass because it is mainly used for feeding elephants in Africa. Its widespread occurrence may be attributed to its aggressive behaviour, very high seed production potential and suppressive effects on neighbouring plants through allelopathic interactions [21].

P. purpureum contains several phytotoxic chemicals which also makes it a potential herbicide [21]. It is rich in tannin, alkaloids, flavonoids and saponins with tannin having the highest concentration [22] which have conferred upon it antimicrobial, anti- inflammatory, antioxidant, anti-allergic, hepatoprotective, anti-thrombic, antiviral and anti-carcinogenic properties. No published work has been done on the bio efficacy of *P. purpureum* extracts as insecticide on any class of insects.

Tridax procumbens Linn. is a common grass, a native of tropical America which naturalized in tropical Africa, Asia, and Australia. It is found in tropical southern part of Nigeria, growing

primarily during raining season and also throughout India [23].

T. procumbens contains different secondary metabolites. Chloroform extract of the leaves shows the presence of Steroid, Saponin, Coumarins, Alkaloids, Amino acids, Diterpenes, Phenol and Flavonoids while the Acetone-Water extract shows the presence of Steroid, Tannin, Saponin, Anthocyanin, Coumarins, Alkaloids, Diterpenes, Phenol and Flavonoids [23]. High flavonoids, alkaloids, hydroxycinnamates, tannins and phytosterols, moderate benzoic acid derivatives and lignans, and low carotenoids contents in *T. procumbens* have been reported [24]. The presence of these phytochemicals portrays the potential of *T. procumbens* as a health-promoting food, food supplements and an insecticide.

Primary and secondary metabolites extracted from plants have the potential to be used as effective and ecofriendly control agents for mosquito vectors [25]. Several groups of these phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities [26]. They are extracted either from the whole body of little herbs or from various parts like fruits, leaves, stems, barks, roots, etc., of larger plants or trees [27]. Naturally-occurring phyto-compounds that are rich sources of bioactive chemicals appear to be the most likely candidates for the environmentally safe and degradable products targeted specifically against mosquitoes [28], especially those that cause Malaria.

No published work has been repoorted on the efficacy of *D. erecta, P. purpureum* and *T. procumbens* against the different stages of *A. gambiae*. Therefore, this study focused on evaluating the ovicidal and larvicidal activities of *D. erecta, T. procumbens* and *P. purpureum* extracts against the malaria vector – *A. gambiae.*

2 Materials and Methods

2.1 Collection and Identification of Plant Materials

Fresh leaves of *D. erecta, T. procumbens* and *P. purpureum* were collected from the residential quarters within the University of Nigeria, Nsukka. Identification of the plants was verified at the International Centre for Ethnomedicin and Drug Development, Nsukka. Sample specimen of the leaves of each plant were deposited in the herbarium and allocated a voucher number, InterCEDD/16292, InterCEDD/16291 and InterCEDD/844 for *D. erecta, T. procumbens* and *P. purpureum* respectively.

2.2 Preparation of Plant Extracts

The plant samples were washed with tap water and then surface sterilized in 10% sodium hypochlorite to prevent contamination of any microbes. Thereafter, the plant materials were washed with tap water to remove all the unwanted impurities and finally they were thoroughly rinsed with sterile distilled water and then, the plant materials were dried under room temperature (20^{o}C) followed by oven drying by an electric oven at 60 °C for half an hour. The oven dried plant materials were grind using electric blender and the powdered materials were packed in air-lock plastic containers.

2.3 Preparation of Concentrated Extracts of Plant Materials

1kg of dried and ground leaves of each plant were concentrated in 7liters of 95% ethanol each and separately. *D. erecta, T. procumbens* and *P. purpureum* yielded 617.2g, 598.3g and 552g of extracts respectively. They were allowed to stay for 72 hours before filtration. The plant extracts were filtered using a funnel packed with cotton wool. The filtered extracts were thereafter evaporated to dryness using a rotary evaporator. The concentrated extracts were stored in a refrigerator until use.

2.4 Phytochemical screening

The qualitative phytochemical screening for alkaloids and phenols were conducted using the analytical methods of Khalid *et al* [29]; flavonoids, tannins and glycosides screening done using the methods of Ajuru *et al.* [30]; saponins and phytosteroids, with the methods of Mumtaz and Raza [31] while proteins, carbohydrates and triterpenoids were studied using the methods of Bandiola [32].

2.5 Test Organism

Anopheles gambiae eggs were recruited from the egg colony at the Arthropod Borne Viral Diseases (ARBOVIRUS) Center, Ministry of Health, Enugu State, Nigeria. The eggs were reared to larvae and adults for the larvicidal bioassays and repellent activities, at the Entomology Laboratory of the University of Nigeria Nsukka, Nigeria.

2.6 Larval Rearing of *A. gambiae* Eggs

The mosquito larvae were hatched from the egg colony using modified methods of Agwu *et al* [19]. The eggs were washed with 5 ml of 0.01% formaldehyde solution for 30 minutes in a 500ml distilled water, 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, the first instar larvae stages were distributed in buckets 40 cm in diameter and 15 cm in depth. The containers were large enough in size to prevent overcrowding until development to early 4th instar larvae which is the required larvae stage 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 at the beginning of the experiment and twice during the later stages of development. In order 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 was all covered with bridal net held in place with a rubber band to prevent unwanted mosquitoes from laying eggs in the plastic buckets.

2.7 Preparation of Plant Extracts Solutions for Bio-assay Experiments

The ovicidal and larvicidal potential of the leaf extracts against *A. gambiae* were observed using the standard procedures recommended by the World Health Organization [33]. The stock solution (20 ml of 1%) of each plant extracts was prepared by weighing 200 mg of the extract and adding 20 ml solvent (ethanol). Thereafter, the stock solution was serially diluted in ten-fold ethanol (2 ml solution to 18 ml solvent).

2.8 Determination of Test Concentrations of the Plant Extracts

The mosquito eggs and larvae were initially exposed to a wide range of test concentrations (0.1 – 2.0 ml of extract solution in 200ml of distilled water) and a control to establish the activity range of the plant materials under test. After determining the mortality of larvae in this wide range of concentrations, a narrower range of 4 aliquots (0.4ml, 1.0ml, 1.4ml and 2.0ml) yielding between 10% and 95% mortality in 24 h were used to determine LC_{50} and LC_{90} values. Three replicates and an equal number of controls were set up for each concentration simultaneously with tap water, to which 1 ml of ethanol was added.

2.9 Experimental Design and Procedure for Bioassay Experiment

The bioassay was performed at room temperature of 26 ± 2°C and relative humidity of 80 ± 2% and a photoperiod of 12 h light followed by 12 h dark (12L:12D) for 48 hours.

2.9.1 Ovicidal Bioassay

Twenty five *A. gambiae* eggs were introduced into the plastic container containing 200 ml distilled water. The setup was left for 48 hours. The larvae hatched were visually observed and recorded. The rate of hatching for each treatment level was calculated using the formulae below:

$$Hatching\ Rate = \frac{Number\ of\ hatched\ eggs}{Total\ number\ of\ eggs\ used\ in\ each\ treatment}\ X\ 100$$

2.9.2 Larvicidal bioassay

Twenty five *A. gambiae* 4th instar larvae were introduced into the plastic container which contains 200 ml of distilled water by means of a dropper. Small, unhealthy or damaged larvae were all removed and replaced. After 24 h exposure, larval mortality was recorded. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapables of rising to the surface or not showing

the characteristic diving reaction when the water is disturbed. The results were recorded on the form recommended by the World Health Organization. Percentage of mortality of the larvae were calculated using the formula below:

$$\%\ Mortality = \frac{Number\ of\ Larvae\ Dead}{No.\ of\ larvae}\ X\ 100$$

2.10 Statistical analysis

The hatching rate and percentage of mortality data were subjected to a One-way analysis of variance (ANOVA) to compare the means. A post hoc test - Duncan test of multiple comparisons - was used to determine the significant differences between the treatments. Probit analysis was used to determine lethal dosages causing 50% (LC_{50}) and 90% (LC_{90}) mortality. All statistical analyses were done using the SPSS (Statistical Package of Social Sciences) software version 22. Results with P< 0.05 were considered to be statistically significant.

3 Results

3.1 Phytochemical screening

The phytochemical screening of the ethanol extracts of *D. erecta, T. procumbens* and *P. purpureum* extracts revealed the presence of alkaloids, flavonoids, tannins, saponins and carbohydrates. Phenols were only found to be present in the extracts of *D. erecta*; Glycosides and triterpenoids were found in both *D. erecta* and *P. purpureum* but absent in *T. procumbens*; while phytosteroids and proteins were discovered in *T. procumbens* and *P. purpureum* but found to be absent in *D. erecta* (Table 3.1).

3.2 Ovicidal Bioassay

The three plant extracts tested (*D. erecta, T. procumbens* and *P. purpureum*) partially inhibited the hatchability of the Anopheles eggs exposed to them. However, there were recorded variations in the rate of hatching in the three plants tested as shown in Table 3.2. This indicated that *D. erecta, T. procumbens* and *P. purpureum* are potential ovicides. Only 12.92% of the eggs exposed to *D. erecta* extracts hatched while 21.25% and 30.83% hatched in treatments containing *P. purpureum* and *T. procumbens* leaf extracts (Table 3.3).

Table 3.1: *Phytochemical compounds present in ethanol extracts of the leaves of D. erecta*

Phytochemical constituents	*D. erecta*	*T. procumbens*	*P. purpureum*
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	++
Saponins	++	+	+
Steroids	-	+	++
Phenols	++	-	-
Glycosides	+	-	+
Triterpenoids	++	-	+
Proteins	-	++	+
Carbohydrates	+	+	+

Keys: (-) absence; (+) small quantity; (++) moderately

Table 3.2: *Mean hatching rate of A. gambiae eggs exposed to extracts*

Concentration (PPM)	Mean Hatching Rate ± SD		
	D. erecta	*T. procumbens*	*P. purpureum*
40	20.00 ± 5.00^{b}	46.67 ± 17.56^{ab}	31.67 ± 5.77^{b}
100	18.33 ± 2.89^{b}	36.67 ± 17.56^{ab}	25.00 ± 5.00^{b}
140	13.33 ± 2.89^{b}	25.00 ± 13.23^{ab}	21.67 ± 5.77^{b}
200	0.00 ± 0.00^{a}	15.00 ± 8.66^{a}	6.67 ± 2.89^{a}
Control	58.33 ± 10.21^{c}	55.00 ± 27.83^{b}	50.00 ± 10.00^{c}

Results with same letters in the column are not significantly different (P < 0.05)

Table 3.3: *Total Mean Hatching Rate of all Extracts*

Plant	Total Mean Hatching Rate± SD
D. erecta	12.92 ± 8.65^{a}
T. procumbens	30.83 ± 17.68^{b}
P. purpureum	21.25 ± 10.47^{ab}

Results with same letters in the column are not significantly different (P < 0.05)

The most active extract was recorded to be *D. erecta* having a lethal concentration capable of inhibiting 50% of the eggs from hatching (LC_{50}) of 10.037 ppm, followed by *P. purpureum* and *T. procumbens* with LC_{50} values of 17.380 and 39.198 respectively (Table 3.4).

Table 3.4: *Estimates of LC_{50} and LC_{90} values of Plant Extracts against A. gambiae Eggs*

Plants	LC_{50} (PPM) and 95% confidence interval	LC_{90} (ppm)and 95% confidence interval	X^2	df
D. erecta	10.037 (0.020-26.808)	124.657 (79.043-467.187)	9.475	10
T. procumbens	39.198 (1.065-68.125)	412.087 (196.370-120495.460)	18.357	10
P. purpureum	17.380 (0.815-35.874)	271.812 (160.940-2038.029)	6.530	10

Table 3.5: *Mean % mortality of A. gambiae larvae exposed to leaf extracts.*

Conc. (PPM)	*D. erecta*		*T. procumbens*		*P. purpureum*	
	Mean % Mortality ± SD	Mean % survival ± SD	Mean % Mortality ± SD	Mean % survival ± SD	Mean % Mortality ± SD	Mean % survival ± SD
40	29.33 ± 8.33^{b}	70.67 ± 8.33^{d}	13.33 ± 2.31^{b}	86.67 ± 2.31^{c}	10.67 ± 2.31^{a}	89.33 ± 2.31^{d}
100	53.33 ± 6.11^{c}	46.67 ± 6.11^{c}	14.67 ± 2.31^{b}	85.33 ± 2.31^{c}	21.33 ± 2.31^{b}	78.67 ± 2.31^{c}
140	73.33 ± 2.31^{d}	26.67 ± 2.31^{b}	33.33 ± 2.31^{c}	66.67 ± 2.31^{b}	33.33 ± 8.33^{c}	66.67 ± 8.33^{b}
200	82.67 ± 2.31^{e}	17.33 ± 2.31^{a}	57.33 ± 6.11^{d}	42.67 ± 6.11^{a}	53.33 ± 6.11^{d}	46.67 ± 6.11^{a}
Control	5.33 ± 2.31^{a}	94.67 ± 2.31^{e}	2.67 ± 2.31^{a}	97.33 ± 2.31^{d}	2.67 ± 2.31^{a}	97.33 ± 2.31^{d}

Results with same letters in the column are not significantly different ($P < 0.05$)

Table 3.6: *Total Mean Percentage Mortality of Larvae in all Extracts*

Plant	Total Mean Mortality± SD
D. erecta	59.67 ± 21.87^{b}
T. procumbens	29.67 ± 18.87^{a}
P. purpureum	29.67 ± 17.17^{a}

Results with same letters in the column are not significantly different ($P < 0.05$)

Table 3.7: *Estimates of LC_{50} and LC_{90} values of Plant Extracts against A. gambiae Larvae*

Plants	LC_{50} (PPM) and 95% confidence interval	LC_{90} (PPM) and 95% confidence interval	X^2	df
D. erecta	76.943 (62.300-90.862)	307.764 (231.107-496.524)	4.513	10
T. procumbens	214.217 (162.031-381.640)	1024.001 (509.981-6014.077)	12.192	10
P. purpureum	213.410 (169.796-318.820)	1002.144 (558.002-3363.794)	5.527	10

3.3 Larvicidal Bioassay

Different concentrations of plant extracts showed various percentages of mortality. The highest mean mortality rate was recorded in concentration 200 PPM in all extracts, showing a dose dependent increase in mortality. *D. erecta* increased from 29.33% – 82.67% while *T. prucumbens* increased from 13.33% to 57.33% and *P. purpureum* from 10.67% to 53.33% (Table 3.5). More so, all concentrations were significantly different at $p<0.05$. *D.erecta* displayed the highest larvidical activity with the total mean mortality of 59.67% while *T. procumbens* and *P. purpureum* had same total mean mortality of 29.67% (Table 3.6). The three plant extracts tested against larvae of *A. gambiae* were active. However, the most active extract was recorded to be *D. erecta* with an LC_{50} of 76.943ppm followed by *P. purpureum* and *T. procumbens* having and LC_{50} values of 213.410ppm and 214.217 ppm respectively (Table 3.7).

4 Discussions

Different plant species have been identified to contain various phytochemical constituents which are in form of secondary metabolites majorly for the protection of the plants. Various constituents such as saponins, phenols, alkaloids, flavonoids, terpenoids among others have been extracted from plants.

Flavonoids, tannins, saponins were conspicuously present in all the plant extracts tested. Earlier studies have reported that these phytochemicals are present in *D. erecta* [19] [9] [34] [35], *T. procumbens,* [23][24][36][37][38] [39] and *P. purpureum* [22][40] [41].

The presence of these chemicals attributes to their ovicidal, and larvicidal effect against *Anopheles* mosquitoes in this study. Earlier studies had reported saponins, flavonoids and tannins as active biological compounds. Saponins have been identified to possess a range of biological activities such as antimicrobial [42], membrane-permeabilising, immunostimulant, hypocholesterolaemic, anticarcinogenic, analgesic, anti-nociceptive, antioxidant, antifungal and antiviral properties[43].

Many flavonoids are shown to have antioxidative activity [44] [45], hepatoprotective, anti-inflammatory properties [46][47] developmental

regulators [48] antiviral [49] and antifungal activities [50]. They have been used in the areas of infectious diseases including resistant bacterial infections, tuberculosis, opportunistic infections, viral infections, parasitic infections [51]. Tannins are also known for their antiseptic and antimicrobial properties [52][53] and are being tested against various pathogenic organisms [54] and nematodes [55]. Furthermore, there is ample proof of their anti-inflammatory, cicatrizant and anti-HIV functions [56].

Over the years, plant-based products extracted using different solvents such as hexane, chloroform, benzene, petroleum ether, benzene, ethyl acetate, methanol, and water from the leaves have been tested for their ovicidal activities against Anopheles mosquito species.

From the results obtained *D. erecta* leaf extracts showed high level of ovicidal activity at all concentrations, 40,100,140 and 200 ppm with an LC_{50} value of 10.037 PPM. This indicated a very high potential of this plant as a mosquito ovicide. The effectiveness of this plant could be attributed to the presence of saponin, a phytochemical component known to be insecticidal.

A complete inhibition of egg hatching was observed at 200 PPM in *D. erecta* extracts. This have been reported in *Celosia argentea, Anthocephalus cadamba, Gnetum ula, Solena amplexicaulis* and *Spermacoce hispida* extract against *An. stephensi, Ae.aegypti* and *Cx. Tritaeniorhynchus* [57] and leaf extracts of *Acalypha indica* [58] at 200PPM.

In contrast, zero egg hatchability in the methanol leaf extract of *Coccinia indica* and *Andrographis paniculata* was recorded at 150 PPM for *C. quinquefasciatus* [59], methanol extract of *Cassia occidentalis* and *Euphorbia hirta* [59] 100% mortality was attained against *C. quinquefasciatus* and methanol extract of *Delonix elata* was recorded at 300PPM against *A. stephensi* and *A. aegypti* [60].

The complete ovicidal activity might be as a result of the plant extract being able to block the micropyle region of the egg, thereby preventing the exchange of gases, which eventually killed the embryo in the egg. The disturbance with egg cytoplasm was reflected in the form of dead eggs with black spot stage due to the arrest of further development of embryo inside the egg [19].

T. procumbens and *P. purpureum* in this study, also displayed a potential of being used an ovicide in mosquito control. An LC_{50} value of 39.198 ppm and 17.380 ppm were recorded in both plants respectively. More so, a record of a concentration dependent increase was established in all extracts. Similar studies on the ovicidal activities of plants belonging to different families against *Anopheles* mosquitoes have been reported by various authors with a concentration dependent increase. In the test of the ovicidal potential of *D. erecta* aqueous leaf extracts against the Dengue vector, *Aedes aegypti* a concentration dependent increase was recorded with an LC_{50} value of 340.085 PPM [19]. Similar findings have been documented in *Boswellia dalzielii* leaf extracts [61], ethanolic extract of *Artemisia annua* [62], leaf acetone, ethyl acetate, and methanolic extracts of Aegle *marmelos, Andrographis lineata* and *Cocculus hirsutus* [63], methanolic leaf extract of *Cassia fistula* [64], leaf extract of *Acalypha indica* [65] and those of crude hexane, benzene, chloroform, ethyl acetate, and methanol solvent extracts of *Delonix elata* [60] against Anopheles species.

The increase in the phytochemical constituents present in high concentrations of the plant extract played a remarkable role. Consequently, as the concentration increases, the ovicidal potential of the extract also increases, resulting in an inverse proportionality between the percent hatchability of the eggs and the concentration of extract.

The Larvicidal efficacy of ethanol extracts of *D. erecta*, *T. procumbens* and *P. purpureum* against *A. gambiae* fourth instar larvae revealed that all the tested plant extracts were active against the test organism, as pupation was inhibited in a considerable number of larvae.

Similar studies on the larvicidal activities of plants belonging to different families have been reported by various authors against the *A. gambiae* larvae including *Gnetum ula*, *Spermacoce hispida* [57], *Artemisia annua* [62], *Plumbago zeylanica, Plumbago dawei and Plumbago stenophylla* [66], *Persea Americana* [67], *Citrus reticulata, Citrus limon, Citrus aurantifolia, Citrus sinensis, Citrus paradise* [10], *Clausena anisate* [11], *Zanthoxylum heitzii* [12], *Lippia adoensis* [13],

Chromolaena odorata [14], *Ageratum conyzoides* [15], *Ocimum gratissimum* [68] and *Chenopodium ambrosioides* [13] among other plant based larvicides against *A. gambiae*.

The plants were able to inhibit the larvae growth due to the fact that they contain the three main phytochemicals known to possess insecticidal properties in conspicuous amount, including saponins, tannins and alkaloids. Larval mortality might be traced to these phytochemicals. The changes in the activity of the extracts could be attributed to different concentration of phytochemicals in the extracts. The bioactivities demonstrated by the different extracts may also be attributed to uneven distribution of chemical constituents within these extracts. The phytochemicals could have either exhibited synergistic or additive effects when used in their crude form. Secondary compounds of plants may jointly or independently have activity against mosquito targets from their ovicidal and pupicidal, activity against the adult and inhibition of growth activity [19].

D. erecta showed the highest larvae mortality in all the tested plant which predisposed it as potential larvicide for mosquito control. Other studies have confirmed its larvicidal potential against *A. aegypti* [19] and *Culex quinquefascitatus* [35]. This study, to the best of our knowledge, is the first report of *D. erecta* against *A. gambiae*.

The reason behind the high potential of *D. erecta* leaf extracts as mosquito larvicides was due to high composition of saponins present in the extract compared to other plants tested in this study (*T. procumbens* and *P. purpureum*). Saponins are freely soluble in water and mostly used in the manufacture of insecticides, vaccines and synthesis of steroidal hormones. Saponins work by interacting with the cuticle membrane of the larvae, changing the microstructure of the cell membranes and finally disrupting the membrane, which is one of the likely reasons for larval death.

5 Conclusions

The leaves of *D. erecta, T. procumbens* and *P. purpureum* contain active insecticidal compounds including alkaloids, flavonoids, tannins and saponins which qualify them as potential ovicides and larvicides in the control of mosquitoes. These plants can be used for the development of easily biodegradable ovicides and larvicides which will serve as best alternatives to the expensive, environmentally hazardous and the existing mosquito resistant insecticides. However, further studies on the identification of the major active compounds present in these plants, their modes of action and extensive field trials, are required to further test their efficacy.

6 Declarations

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6.3 Competing Interests

The authors declared that no conflict of interest exist.

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