



## **Potential of the Ethanol Extract of Aerial Parts of *Diplazium esculentum* (Retz) SW. AS Larvicide against *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors UIA and UPME designed the study, performed the statistical analysis, wrote the protocol as well as the first draft of the manuscript. Authors UEJ, BDN and JIV were part of the field and laboratory studies. Author JIV managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

Plant products have been tested as insecticides against mosquitoes as they are promising candidates to replace conventional insecticides. This study was carried out to evaluate the larvicidal potential of ethanol extract of the aerial parts of *Diplazium esculentum* against *Anopheles gambiae* and *Culex quinquefasciatus*. Ethanol extract of the aerial parts of *D. esculentum* was screened for its phytochemical constituents and used for larvicidal assay. A stock solution of the extract (5 g in

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100 ml of water) was prepared. From the stock solution, 0.45, 0.60, 0.75, 0.90 and 1.05% w/v concentrations of the extract were obtained for the study. Each concentration of the extract had 3 replicates. The control was also replicated. Twenty (20) third instar larvae each of *Anopheles gambiae* and *Culex quinquefasciatus* were separately exposed to each extract concentration for a duration of 48 hours. Larval nutrient was added to each experimental set up. Observations were made after 24 and 48 hours exposure period. Phytochemical screening revealed the presence of some plant metabolites. Mortality of larvae exposed to the extract increased with increased concentration and exposure time. This study revealed a differential susceptibility of larvae of the two mosquito species to the extract as evident by the 24 h LC<sub>50</sub> values obtained which were 0.355 and 2.468% w/v for *An. gambiae* and *Cx. quinquefasciatus* respectively. Exposure of *An. gambiae* larvae to the extract resulted in 100% mortality even with the least concentration of 0.45% w/v after 48 hours exposure period while the highest concentration of extract (1.05% w/v) resulted in 53.33% mortality of *Cx. quinquefasciatus* larvae, after an exposure period of 48 hours. Results obtained from this study suggest that the aerial parts of *D. esculentum* if further explored would be useful in the control of *An. gambiae* and *Cx. quinquefasciatus*.

**Keywords:** *Anopheles gambiae*; *Culex quinquefasciatus*; *Diplazium esculentum*; Potency.

## 1. INTRODUCTION

Mosquitoes are fragile, insects belonging to the family Culicidae. They are among the best known groups of insects because of their medical importance to man as vectors of some of the most deadly human diseases. Diseases such as malaria, lymphatic filariasis, viral encephalitis, dengue, yellow fever and zika are all vectored by different species of mosquitoes [1].

*Anopheles* mosquitoes are important vectors of malaria and lymphatic filariasis (LF), which are major public health diseases in Africa. *Anopheles gambiae* species complex has been implicated as an efficient vector of these diseases in the Afrotropical region. *Culex* species are reported to be the most widespread mosquito species across the world [2]. They are known to be highly opportunistic, feeding on both humans and animals, a behaviour which increases their potential to transmit zoonotic diseases and makes them important threat to public health [3]. *Culex quinquefasciatus* species are known to cause nuisance, they also transmit diseases such as lymphatic filariasis, Japanese and Saint Louis encephalitis, Rift valley fever and West Nile Virus [1].

Malaria and lymphatic filariasis have been leading causes of death and long term disability in Nigeria. Annually, Nigeria contributes about 81,640 malaria deaths which accounts for 19% of global deaths and 45% of deaths in West Africa [4]. This devastating disease affects the country's economic productivity, resulting in an estimated monetary loss of approximately 132 billion naira in treatment cost, prevention and other indirect

costs [5]. Also, Nigeria has a significant burden of lymphatic filariasis caused by the parasite *Wuchereria bancrofti*. Nigeria with an estimated population of 186 million people is Africa's most endemic country with approximately 80 to 120 million people at risk of lymphatic filariasis [6]. The disease is prevalent and widespread in the six geo-political zones of the country [7]. The socio-economic and psychological burden of the disease is enormous, these include direct cost of treatment and losses resulting from incapacitation and loss of labour [7].

Over the years, the use of a number of synthetic insecticides in mosquito control programme has been limited due to enormous challenges such as increasing insecticide resistance, toxic effect on human health and other non-target organisms, high cost of synthetic insecticides, environmental persistence, higher rate of biological accumulation etc. These challenges have resulted in the urge to search for environmentally sustainable, biodegradable, affordable and target specific insecticides against mosquito species. Consequently, the application of environment friendly alternatives has become the main focus of the control programme to replace synthetic insecticides [8,9]. One of the most effective alternative approaches under the environment friendly control programme is to utilize botanicals as insecticides as they are safer, simple to use, affordable and sustainable.

*Diplazium esculentum* (Retz) Sw, is an edible fern found throughout Asia and Oceania and has been introduced in other continents like Africa. The plant also occurs widely and commonly throughout India, China, Cambodia, Laos,

Thailand and Vietnam. [10]. *D. esculentum* commonly called vegetable fern belongs to the Athyriaceae family. It is a terrestrial fern with a short and erect rhizome that is occasionally trunk-like or can be creeping. The Ibibios of Akwa-Ibom State call it *Nyama idim*, because of its abundance near rivers, streams, and swamp areas. It is known as 'paco' in English and in Philippines 'pacupaku', in Malaysia, 'linguda', in Northern India 'phakktu' [11]. The vegetable of different species of *Diplazium* including *D. esculentum* are known to have laxative properties and is often used to treat colitis and constipation [12]. *D. esculentum* is among several species of pteridophytes with medicinal properties. The fronds are eaten and believed to have antimalarial effects. They are believed to have the potentials for treating jaundice, constipation, ear ache, measles, dermatitis and fever [12]. Different parts of *D. esculentum* especially the leaves have traditionally been used for curing headache, pain, cough, fever, wounds, dysentery, glandular swellings, diarrhea and skin infections by various ethnic groups and folklore throughout the world [12]. In Akwa Ibom State, besides being used as vegetable for soup, the immature fronds of *D. esculentum* are effective in the treatment of various stomach ailments, these stomach ailments are treated by boiling the young fronds and consuming them.

Despite its many ethnomedicinal and nutritional uses, there is a paucity of information on the larvicidal efficacy of the extract of *D. esculentum* against *An. gambiae* and *Cx. quinquefasciatus*. Thus, the need to evaluate the larvicidal efficacy of the ethanol extract of the aerial parts of *D. esculentum* for possible activity against *An. gambiae* and *Cx. quinquefasciatus*.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Plant Material

The experimental plant (*D. esculentum*) was collected from the botanical garden of the Department of Botany and Ecological Studies, University of Uyo, located at the main campus of the University along Nwaniba road, Uyo, Akwa Ibom State, Nigeria. The plant was authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo. Herbarium specimen with voucher number (James UUH4057) was prepared and deposited in the same department for future referencing.

### 2.2 Processing of Plant Material

The aerial parts of the plant were thoroughly washed and shade dried on laboratory tables for 21 days. They were thereafter pulverized with manual grinder and weighed.

### 2.3 Extraction

The extraction process was carried out in the laboratory of the Department of Pharmacognosy and Natural medicine, University of Uyo, Nigeria. The pulverized plant material (350 g) was extracted by maceration (cold extraction) at room temperature ( $27 \pm 2^\circ\text{C}$ ) using 70% ethanol for 72 hours with intermittent stirring using a glass rod. This was followed by filtration using muslin cloth, filter funnel, Whatman No 1 filter paper and non-absorbent cotton wool as described by Ubulom et al. [13]. This method was used in order to completely get rid of any marc in the filtrate. The filtrate was concentrated *in vacuo* at  $40^\circ\text{C}$  using a rotary evaporator. The dried extract obtained was weighed and percentage yield determined. Dried extract was stored in a refrigerator at  $4^\circ\text{C}$  prior to further studies.

### 2.4 Phytochemical Screening

The qualitative screening was carried out on the ethanol extract of *Diplazium esculentum* aerial part according to standard methods of Sofowara [14] and Evans [15]. This was done to identify the classes of bioactive compounds present. Phytochemical constituents screened were Alkaloids, Anthraquinones (free and combined), Cardiac glycosides, Flavonoids, Saponins and Tannins.

### 2.5 Larvicidal Assay

Larvicidal assays reported in this study were carried out in the Malaria Vector Research Laboratory and Insectary of the Department of Animal and Environmental Biology, University of Uyo, Uyo, Nigeria. Third instar larvae of *An. gambiae* and *Cx. quinquefasciatus* were obtained from the Insectary of the Malaria Vector Research laboratory, of the Department of Animal and Environmental Biology, University of Uyo. The method used for the bioassay followed the guidelines of WHO, [16]. The stock solution was prepared by adding 100 ml of tap water to 5 g of extract and mixed thoroughly. Heat was applied to the stock solution of the extract over a water bath at a temperature of  $40^\circ\text{C}$  for 2 minutes, after which the solution was removed

and left for 30 minutes to cool before assays were carried out as described by Ubulom et al. [9]. This was to reactivate the phytoconstituents from possible inactivation due to refrigeration. From the stock solution, graded concentrations were prepared to obtain 0.45, 0.60, 0.75, 0.90 and 1.05% w/v of the extract in a final formulation of 100 ml. Twenty (20) third instar larvae each of *Anopheles gambiae* and *Culex quinquefasciatus* were separately transferred to small disposable test cups containing larval nutrient. The larval nutrient was a pinch of pulverized Quaker oat added to each assay cup. The mosquito larvae were exposed to five concentrations (0.45, 0.69, 0.75, 0.90 and 1.05% w/v) of the extract and were assessed for their larvicidal activity. Three replicates were set up for each concentration. There was a control experiment which consisted of 20 third instar larvae each of *Anopheles gambiae* and *Culex quinquefasciatus* separately immersed in 100 ml of tap water to which larval nutrient only was added and mortality was recorded after 24 and 48 hours exposure period. The larvae were considered dead when they failed to move and did not respond to stimulus with a Pasteur pipette.

## 2.6 Data Analysis

Data obtained from this research were statistically analyzed and presented as means and standard error. The median lethal concentration (LC<sub>50</sub>) in each case, determined by Probit analysis, as described by Finney, [17]. Microsoft excel version 2013 was used for analysis of data.

## 3. RESULTS

### 3.1 Percentage Yield and Phytochemical Constituents of the Extract of *D. esculentum*

The Percentage yield of the ethanol extract of the aerial parts of *D. esculentum* was 5.90%. The phytochemical screening of crude ethanol extract of *D. esculentum* aerial parts revealed the presence of alkaloids, anthraquinones (free and combined), cardiac glycosides, flavonoids, saponins and tannins (Table 1).

### 3.2 Larvicidal Effect of Ethanol Extract of *D. esculentum* on *An. gambiae* and *Cx. quinquefasciatus*

Findings from the present study revealed that the crude ethanol extract of the aerial parts of *D.*

*esculentum* tested against third instar larvae of *An. gambiae* and *Cx. quinquefasciatus* had larvicidal effect. Mortality increased in both mosquito species as the concentration of the extract increased. The percentage mortality of the extract of *D. esculentum* aerial parts against *An. gambiae* and *Cx. quinquefasciatus* larvae are presented in Tables 2 and 3 respectively. The extract was more potent against *An. gambiae* larvae. Even the least concentration (0.45%w/v) of the extract resulted in a 100% mortality of *An. gambiae* larvae, after 48 hours exposure period (Table 2). Exposure of larvae of *Cx. quinquefasciatus* to the highest concentration (1.05% w/v) resulted in mortality of 53.33%, after an exposure period of 48 hours (Table 3).

### 3.3 Median Lethal Concentration (LC<sub>50</sub>) of the Extract

The 24 hour LC<sub>50</sub> values of the extract were 0.355 and 2.468 for *An. gambiae* and *Cx. quinquefasciatus* respectively (Table 4). Thus, *An. gambiae* larvae were more susceptible to the crude ethanol extract of the aerial parts of *D. esculentum*, as judged by the 24 h LC<sub>50</sub> value. The 48 h LC<sub>50</sub> value of the extract for *Cx. quinquefasciatus* larvae was 1.269% w/v.

## 4. DISCUSSION

The yield (5.90%) of the ethanol extract of *D. esculentum* was low compared with the amount of pulverized sample. This was due to the fact that the cold extraction method was used. Maceration has been reported to result in low yield but has the advantage of preserving the thermolabile components of the plant [18]. The present study has shown the larvicidal potential of *D. esculentum* against 3rd instar larvae of *An. gambiae* and *Cx. quinquefasciatus* mosquitoes. Some of the phytochemicals detected in the aerial parts of *D. esculentum* have been reported to have good larvicidal efficacy. The larvicidal potency of alkaloids, saponins, flavonoids and tanins have been reported [19-22]. Thus, the larvicidal effect of *D. esculentum* reported in this study is attributable to the presence of these bioactive compounds. These compounds may have acted jointly or independently to contribute to larval mortality. This was substantiated by the fact that no mortality was recorded in the control experiments as the larvae were still agile and actively wriggling. These findings are in agreement with previous reports of Ubulom et al. [13], Opara et al. [8] and Ubulom et al. [9]. They all reported the efficacy of plants extracts against different mosquito species.

**Table 1. Phytochemical constituents of ethanol extract of *Diplazium esculentum***

| Phytochemical Compound | Test Method                          | Inference |
|------------------------|--------------------------------------|-----------|
| Alkaloids              | (i) Dragendorff's test               | +         |
|                        | (ii) Meyer's test                    | +         |
| Anthraquinones         | (i) Test for combined anthraquinones | +         |
|                        | (ii) Test for free anthraquinones    | +         |
| Cardiac glycosides     | (i) Salkowski's test                 | +         |
|                        | (ii) Keller-Killiani test            | +         |
| Flavonoids             | (i) Magnesium metal test             | +         |
|                        | (ii) Sodium hydroxide test           | +         |
|                        | (iii) Ammonia test                   | +         |
| Saponins               | (i) Frothing test                    | +         |
|                        | (ii) Sodium bicarbonate test         | +         |
| Tannins                | (i) Ferric chloride test             | +         |

Key: + = present; - = absent

**Table 2. Larvicidal activity of ethanol extract of *D. esculentum* on *Anopheles gambiae***

| Conc. (% w/v)             | Number of larvae introduced | Percentage Mortality |                   | Percentage Mortality |                   |
|---------------------------|-----------------------------|----------------------|-------------------|----------------------|-------------------|
|                           |                             | 24h exposure period  | Mean $\pm$ S.E    | 48h exposure period  | Mean $\pm$ S.E    |
| 0.45                      | 60                          | 73.33                | 14.67 $\pm$ 1.333 | 100                  | 20.00 $\pm$ 0.000 |
| 0.60                      | 60                          | 83.33                | 16.67 $\pm$ 3.333 | 100                  | 20.00 $\pm$ 0.000 |
| 0.75                      | 60                          | 90.00                | 18.00 $\pm$ 1.154 | 100                  | 20.00 $\pm$ 0.000 |
| 0.90                      | 60                          | 100                  | 20.00 $\pm$ 0.000 | 100                  | 20.00 $\pm$ 0.000 |
| 1.05                      | 60                          | 100                  | 20.00 $\pm$ 0.000 | 100                  | 20.00 $\pm$ 0.000 |
| Control (100 ml of water) | 60                          | 0.00                 | 0.00 $\pm$ 0.000  | 0.00                 | 0.00 $\pm$ 0.000  |

No. of larvae per replicate = 20, No. of replicates = 3, S.E = Standard Error

**Table 3. Larvicidal activity of ethanol extract of *D. esculentum* on *Culex quinquefasciatus***

| Conc. (%w/v)              | Number of larvae introduced | Percentage mortality |                  | Percentage mortality |                   |
|---------------------------|-----------------------------|----------------------|------------------|----------------------|-------------------|
|                           |                             | 24h exposure period  | Mean $\pm$ S.E   | 48h exposure period  | Mean $\pm$ S.E    |
| 0.45                      | 60                          | 3.33                 | 0.67 $\pm$ 0.333 | 13.35                | 2.67 $\pm$ 0.000  |
| 0.60                      | 60                          | 13.33                | 2.67 $\pm$ 0.333 | 33.33                | 6.67 $\pm$ 0.000  |
| 0.75                      | 60                          | 16.67                | 3.33 $\pm$ 0.333 | 50.00                | 10.00 $\pm$ 0.000 |
| 0.90                      | 60                          | 16.67                | 3.33 $\pm$ 0.333 | 53.33                | 10.67 $\pm$ 0.333 |
| 1.05                      | 60                          | 20.00                | 4.00 $\pm$ 0.000 | 53.33                | 10.67 $\pm$ 0.333 |
| Control (100 ml of water) | 60                          | 0.00                 | 0.00 $\pm$ 0.000 | 0.00                 | 0.00 $\pm$ 0.000  |

No. of larvae per replicate = 20, No. of replicates = 3, S.E = Standard Error

**Table 4. The 24h LC<sub>50</sub> value of ethanol extract of the aerial Parts of *D. esculentum***

| Mosquito species            | LC <sub>50</sub> Value (%w/v) | 95% Confidence interval (%w/v) |
|-----------------------------|-------------------------------|--------------------------------|
| <i>An. gambiae</i>          | 0.355                         | 0.064 – 0.0466                 |
| <i>Cu. quinquefasciatus</i> | 2.468                         | 0.392 – 0.171                  |

Interestingly, *An. gambiae* larvae were more susceptible to ethanol extracts of *D. esculentum* aerial parts than *Cx. quinquefasciatus*. The

difference in susceptibility could be attributed to the difference in the physiological characteristics between the two species of mosquito [23]. The

higher susceptibility of *An. gambiae* to the ethanol extract of *D. esculentum* aerial parts was as judged by the 24hLC<sub>50</sub> value of 0.355% w/v while for *Cx. quinquefasciatus* it was 2.468% w/v. The 48hLC<sub>50</sub> value for *Cx. quinquefasciatus* was 1.269%w/v demonstrating a higher tolerance to the extract than *An. gambiae*. Several studies have reported *Anopheles* mosquito larvae to be more susceptible to plant extracts than mosquitoes from other genera. For example, Govindarajan et al. [24], found out that *Anopheles stephensi* was more susceptible (LC<sub>50</sub> 61.65 µg/mL) to *Origanum scabrum* essential oil than *Ae. aegypti* (LC<sub>50</sub> 67.13 µg/mL), *Cx. quinquefasciatus* (LC<sub>50</sub> 72.45 µg/mL), and *Culex tritaeniorhynchus* (LC<sub>50</sub> 78.87 µg/mL). Similarly, Veni et al. [25], reported *Anopheles stephensi* to be more susceptible to *Terminalia chebula* extract than *Ae. aegypti* and *Cx. quinquefasciatus*, with LC<sub>50</sub> values of 87.13, 93.24, and 111.98 ppm, respectively.

Syed et al. [26] reported that the efficacy of plant extracts against mosquitoes may vary depending on plant species, plant parts, age of plant, solvent used in extraction and mosquito species tested. This may have also accounted for the variation in the susceptibility of the mosquito species to the extract. The plant extract exhibited a concentration dependent activity against the two mosquito species tested. Mortality was observed to increase with increased concentration and period of exposure. This corroborates the reports of Ubulom et al. [13], Opara et al. [8], Iqbal et al. [27] and Ubulom et al. [9] who all reported a concentration dependent activity of various plant extracts against different mosquito species.

## 5. CONCLUSION

Over the years, there has been increased interest in the control of insect vectors using natural products such as plant derived products. The findings from this study have emphasized the need to explore the possibility of using plant-based larvicides as supplementary measures to mosquito control in order to reduce the chemical burden on the environment and human health. *D. esculentum* is a plant well known for its nutritive and medicinal values. Results from the present study reveals that the ethanol extract of the aerial parts of *D. esculentum* have larvicidal effect on *An. gambiae* and *Cx. quinquefasciatus*, with *An. gambiae* being more susceptible to the plant extract than *Cx. quinquefasciatus*. Consequently, the ethanol extract of the aerial parts of *D.*

*esculentum* should be further investigated for the isolation and characterization of its active principle for industrial applicability.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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