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# Biocontrol of Mosquitoes using Biocoil Produced from *Cola millenii* Extracts

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## Abstract

Malaria is one of the major causes of mortality and morbidity among various population in the tropics. Mosqiutoes are the vectors of this important disease. Presently, due to the resistance of insects to synthetic chemicals, there are ongoing efforts by researchers to develop biological substances for controlling the vectors in a bid to eradicate the disease. This research investigated *C. millenii* for its insecticidal potential against Anopheles mosquito. The leaf extracts of *C. millenii* were obtained using ethanol and n-Hexane. The extracts were screened for bioactive substances using standard analytical protocols. Biocoil was developed from the extract and the insecticidal efficacy was evaluated in the laboratory against larvae and adults of *Anopheles stephensi*. The qualitative results revealed that the plant contained alkaloids, saponins, tannins, glycosides, flavonoids and terpenoids in varying amounts. Furthermore, the leaf extract had a significant ($P < 0.05$) lethal effect on mosquito larva at very low concentration recording a median lethal concentration ($LC_{50}$) of 1.71 ppm in ethanol extract, while the pulp extract had $LC_{50}$ of 792.34 ppm. The biocoil produced from the leaf extracts of the plant gave highly promising results in the control of the adult mosquito. These suggest that *Cola millenii* leaf contain bioactive substances that may be exploited in the control of mosquito.

**Keywords:** Malaria, Mosquito, Phytochemicals, biocoil, extracts

## 1.0 Introduction

Many countries have maintained research programmes to screen traditional medical preparations for their antimicrobial activities. India and America are good examples (WHO, 2000). They have been used as medicines by local populations for the treatment of several tropical diseases such as schistosomiasis, leishmaniasis, malarial, fungal and bacterial infection (Alves *et al.*, 2000). However, despite the abundance of such flora detail, analytical data are available only from a few plants (both native and exotic species). There is thus a glaring need to fill the scientific gap created by the lack of data on many Nigerian proven medicinal plants.

Phytochemical are natural bio-active compounds found n plants. These phytoconstituents work with nutrients and fibres to form an integral part of human defense system against various diseases and stress conditions. Phytochemicals are basically divided into two (Primary and Secondary) constituents according to their function n the plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll, while secondary

constituents consists of alkaloids, lavonoids, saponins, phenolics, etc (Alves *et al.,* 2000).

Insecticide based control measures (e.g indoor spraying insecticides, bed nets) are the principal ways to control mosquitoes that bites indoors. However, after prolonged exposure to an insecticide over several generations, mosquitoes like other insects may develop resistance, a capacity to survive contact with an insecticide. Since mosquitoes can have many generations per year, high level of resistance can arise very quickly. Resistance for mosquitoes to some insecticides has been documented with just within few years after the insecticides used for indoor. Residual spraying was a major impediment during the global malaria eradication campaign. Judicious use of insecticides for mosquito control can limit the development and spread of their resistance. However, use of insecticides in agriculture has often implicated as contributing to resistance in mosquito populations. Detection of developing resistance in mosquitoes is possible, so control programs are well advised to conduct surveillance for this potential problem (Das *et al.*, 2007).

Although mosquito control is an important component of malaria control strategy, elimination of malaria in an area does not require the elimination of all anopheles mosquitoes. For instance, in North America and Europe, although the vector anopheles mosquitoes are still present, the parasite has been culminated. Some socioeconomic improvement (e.g houses with screened windows, air conditioning), once combined with vector reduction efforts and effective treatment, lead to the elimination of malaria without complete eliminations of the vector. Some important measure in mosquito control to be followed are: discourage the vector egg-laying, prevent development of the eggs into larvae and adults, kill the adult mosquito dwelling, prevent mosquitoes from biting human beings and deny them blood meals (Ileke and Ogungbite, 2015).

*Cola millenii* belongs to the family *Sterculiaceae*, it is indigenous to West Africa. Members of the genus are evergreen trees of the forest, mostly small or moderate n size with the exception of a few which rise to some height (Rusell, 1995). All members of Cola species contain caffeine, koletin and kolatin alkaloids, pro anthocynin, magnesium, sodium, potassium, bromide, cobalt, caesium, zinc and selenium. Recent researches have shown that *Cola milenii* also contain; sugar, strarch, crude fat, crude protein, ascorbic acid, iron, manganese (Reid *et al.,* 2005).

Although Olorode (1984) described only *Cola nitida* and *Cola acuminata* as the Nigeria economic Cola species out of the over 50 species recorded in West Africa. These popularly known species are cultivated in Nigerian mainly for their nuts, which contain some alkaloids (caffeine, theobromine and kolanin). Because these alkaloids dispel sleep, thirst and hunger, the nuts are used widely as masticator. In Nigeria, *Cola millenii* is used in enthobotany for the treatment of diarrhea and dysentery (Reid *et al.,* 2005). However, despite the abundance of such flora detail, analytical data are scanty on medicinal and insecticidal properties of *Cola millenii*.

## 2.0 Materials and Methods

### 2.1 Sample Collection and Preparation

Leaves of Monkey kola were harvested from plants manually and were collected in sterile containers. The harvested plant parts were washed with distilled water and dried under shade for 3 weeks. Thereafter, they were pulverized in to powder and stored in a sterile air tight container until use.

### 2.2 Collection of Test Organisms

Both eggs and larvae of *Anopheles* mosquito were collected from small water a receptacle which was purposefully left outside the laboratory as traps (Ileke and Ogungbite, 2015). The larvae were identified at the Department of Parasitology and Entomology, Federal University of Technology, Akure. Third and Fourth instar larvae were picked and maintained in water inside a beaker and covered with muslin cloth until adult emerged (Ileke and Ogungbite, 2015).

### 2.3 Phyto-chemical Screening

The qualitative screening of powdered samples for the presence of tannins, saponin, flavonoids,

steroids, alkaloids, terpenoids, anthraquinones, phenols was done using the standard methods of Harborne (1973), Onwuka (2005) and Ekop (2007).

### 2.4 Larvicidal Assay

Twenty (20) larvae of anopheles mosquito were placed in transparent plastic petri dishes and about 15 ml of tap water was added. The larvae were exposed to various concentrations of the *Cola millenii* extract. Control medium was also maintained. Mortality rate were recorded at 2 hrs interval over a period of 24 hrs at room temperature $28\pm1^0$C (Ileke and Ogungbite, 2015).

### 2.5 Bio-insecticide Coil Production

Taxopon was poured into a 2L container and Phenol was added then stirred continuously until all was dissolved, however, menthol was added to aid the dissolution. *C. millenii* was added to the mixture and stirred until the solution homogenized. Cassava starch was cooked in boiling water placed in a water bath to form a paste. The paste was stirred with the prepared mixture until a consistent paste is formed. The paste was now made into different form and shapes. They were place in the drying cabinet with the temperature set at $60^o$C to until the shapes reached a constant moisture.

### 2.6 Test of the Bio-insecticide

Third and fourth instars larvae of *Anopheles stephensi* were picked and maintained in water inside a beaker and cover with muslin cloth until adult emerged. The water was decanted through the muslin cloth after which the coil was ignited and allowed to release smoke until stable and the adult mosquito was exposed to the smoke for 30 minutes and the number of death was recorded. A control of commercial mosquito coil was used for comparison.

### 2.7 Statistical Analysis

Data were presented as mean±standard error (SE) of triplicate data. Significance difference between different groups were tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range Test (DNMRT) using SPSS window 8, version 22.0 software. The significance was determined at the level of $p\leq 0.05$.

## 3.0 Results

The results of the qualitative phytochemical components of the leaf extracts are presented in table 1 which shows that ethanol extract of *Cola millenii* leaf had alkaloids, saponins, tannins, glycosides and terpenoids. Also, n-hexane leaf extract had alkaloids, saponins, tannins and terpenoids whereas only alkaloids, saponins, Glycosides and terpernoids were present in the aqueous extract while others were absent.

The larvicidal effect of *C. millenii* extracts on mosquito larva is presented in tables 2 and 3. The effect was concentration dependent as higher concentration led to higher mortality in the larva. A 40 % mortality was recorded at 1 ppm of the n-hexane leaf extract, while a lethal dose that killed 50% of the test larvae was calculated as 4.18 ppm. Moreover, in table 3 a mortality of 45% was obtained at 1 ppm of the ethanol leaf extract of the plant while an $LC_{50}$ of 1.71 ppm was obtained.

**Table 1: The qualitative phytochemical components of ethanol, n-Hexane, and water crude extracts of *Cola millenii* leaf**

| Phytochemical | Ethanol Extract | Hexane Extract | Aqueous Extract |
|---|---|---|---|
| Alkaloids | + | + | + |
| Saponins | + | + | + |
| Tannins | + | + | - |
| Anthraquinones | - | - | - |
| Glycosides | + | - | + |
| Flavonoids | - | - | - |
| Terpenoids | + | + | + |

Legend: - = absent, + = present

The mosquiticidal effect of phytocoil produced from *C. millenii* leaf extracts on adult mosquito is presented in table 4. The killing effect of the coil made from ethanol leaf extract was comparable to the commercially available coil 5hrs after

exposure recording 100% mortality in the mosquitoes.

**Table 2: Percentage mortality of mosquito larva at different concentrations of the leaf n-hexane extracts of *C. millenii***

| | | n-hexane extract | | |
|---|---|---|---|---|
| **Dosage (ppm)** | **Initial larvae** | **No. of survivors** | **No. of deaths** | **% mortality** |
| **leaf** | | | | |
| 1000 | 20 | 0 | 20 | 100 |
| 100 | 20 | 6 | 14 | 70 |
| 10 | 20 | 8 | 12 | 60 |
| 1 | 20 | 12 | 8 | 40 |
| **$LC_{50}$** | | | | **4.18** |

**Table 3: Percentage mortality of mosquito larva at different concentrations of the leaf ethanol extracts of *C. millenii***

| | | Ethanol extract | | |
|---|---|---|---|---|
| **Dosage (ppm)** | **Initial larvae** | **No. of survivors** | **No. of deaths** | **% mortality** |
| **leaf** | | | | |
| 1000 | 20 | 0 | 20 | 100 |
| 100 | 20 | 3 | 17 | 85 |
| 10 | 20 | 6 | 14 | 70 |
| 1 | 20 | 9 | 11 | 45 |
| **$LC_{50}$** | | | | **1.71** |

**Table 4: Percentage Mortality of adult mosquito exposed to phytocoil produced from leaf ethanol extract of *Cola millenii* over time (hrs)**

| Concentration (%) | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| | Hours after treatment (HAT) | | | | |
| **1** | 15.33±0.01 | 35.00±0.00 | 40.33±0.00 | 40.33±0.00 | 40.33±0.00 |
| **2** | 20.67±0.00 | 45.33±0.10 | 50.67±0.00 | 51.67±0.00 | 51.67±0.00 |
| **5** | 42.20±0.00 | 71.67±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |
| **Unexposed** | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| **Coil** | 50.00±0.00 | 95.67±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |

## 4.0 Discussion

Phytochemicals are natural bioactive compounds produced by plants as secondary metabolites that work with nutrients to protect against pathogenic attacks. To survive in the harsh environment that plants live, they often synthesize various kinds of secondary metabolites that act to ameliorate such situations. Das *et al.* (2007) reported that phytochemicals represents the most abundant and extensively distributed substances in the plant kingdom and that several plants and herb cells produce and father these range of medicinal phytochemicals. Cheeke (2008) opined that some of the phytochemicals have great medicinal functions which play an major roles in the new drug development process.

The larvicidal effect of *C. millenii* extracts on mosquito larva was concentration dependent as higher concentration led to higher mortality in the larva. A 40% mortality was recorded at 1 ppm of the n-hexane leaf extract, while a lethal dose that killed 50% of the test larvae was calculated as 4.18 ppm whereas an $LC_{50}$ of 1.71 ppm was obtained for the ethanol leaf extract of the plant. These results showed that crude extracts of C. millenii leaf had a remarkable effect on the larvae of anopheles mosquito. Although, the effectiveness of the plant extracts was observed to be extraction solvent and concentration dependent. The mosquito larval stage appeared to be highly susceptible to the leaf extract of the plant with very low $LC_{50}$. The high level of larvicidal activity observed in this plant is in line with the observation of Ileke and Ogungbite (2015) who reported similar observation in oil and extract of A. boonei. Previously, bioactive agents from plants have been identified to impede the swimming ability of the larvae and pupae of the insect as suggested by Ochiai *et al.* (2005) that botanical oils have a considerable effect on the swimming ability of larvae and pupae of mosquito and reduction in their surviving rate.

Furthermore, the insecticidal potency of the phytocoils produced from the *C. millenii* leaf was very promising especially at 5% incorporation level as the plant leaf ethanol extracts recorded high insect mortality above 50% within 2 hours of application and 100% mortality after 3 hours of application which was comparable to the commercially available insecticide coil which had

50% mortality within 1 hour of application and 100% after 3 hours of application.

The killing effect of the *C. millenii* leaf extract on the test mosquitoes might be due to their ability to disrupt the normal respiratory activity of the insects. Insecticides formulated from botanical sources have been reported to have a considerable effect on the normal respiration of insects as many of them have a knack to block the respiratory organ (spiracle) of insects according to Ileke and Ogungbite (2015).

These observations have pointed to the fact that *C. millenii* leaf extract may be a good mosquito repellant as it achieved more than 50% protection against mosquito after application. The observation agrees with the result of Ansari et al. (2005) in which leaf extract of *Blumea lacera* at 6% concentration recorded 78.8% and 76.2% of protection against *Anopheles stephensi* and *Culex quinquefasciatus* respectively after 6 hours application. Furthermore, the results obtained from the phytocoil assay revealed that C. millenii could compete well with synthetic insecticide coils used in in many developing countries like Nigeria. The presence of tannins, saponins, alkaloids, flavonoids, cardiac glycosides, terpenoids and steroids in plant extract according to Wang *et al.* (2011) have been reported to disrupt growth and reduced larva survival as well as disruption of life cycle of insects. This could also contribute to the high effectiveness of C. millenii extracts against mosquito larva and its adult.

## 5.0 Conclusions

Based on the outcome of this study, it may be concluded that bioactive phytochemicals are present in the leaf of the *Cola millenii* with the major ones being alkaloids, saponins, tannins, flavonoids, glycosides and terpenoids. Also, C. millenii leaf extracts had significant lethal effect on mosquito larva and insecticidal activity against adult mosquitoes which ultimately suggest that effective insectides may be formulated from C. millenii leaf extract.

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