The Efficacy of *Cola millenii* K. Schum. (Malvaceae) against malarial vector *Anopheles stephensi* (Diptera: Culicidae)

ABSTRACT

Cola millenii (Monkey kola) is a wild plant of tropical and sub-tropical countries. The persistent increase in the incidence of antibiotic resistant strains of organisms has led to the search for newer and more potent antimicrobials. Some plant materials have traditionally been used in folk medicine, as well as in the extension of shelf-life of foods, in the case of those with antimicrobial activity. The *C. millenii* is such a plant, that is widely distributed throughout tropical Africa. This research investigated *C. millenii* leaf for its insecticidal properties against mosquitoes. The extracts of *C. millenii* leaf was obtained using ethanol and n-Hexane as the solvents. The insecticidal efficacy of the plant extracts were evaluated in the laboratory against larvae of *Anopheles stephensi*. 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, 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. The killing effect of the coil made from ethanol leaf extract was comparable to the commercially available coil 5 hr after exposure recording 100 % mortality in the mosquitoes. Phytocoil and phytospray were produced from the leaf extracts of the plant which gave promising results in the control of the malaria parasite vector.

Keywords: Mosquitoes, Cola millenii K. Schum, malaria

INTRODUCTION

Mosquitoes are a major threat to human beings since they are vectors of some dangerous disease causing organisms which affects lots of people throughout the world especially, in the developing countries (WHO, 2010), and they have become the most notorious of all the arthropods to the public. Mosquitoes cause more human suffering than any other organism and transmits about ten terrible diseases of man in many countries leading to the death of about two million people every year (Klempner *et al.,* 2007). A number of species of this insect pest are carriers of pathogens of different diseases ranging from fever (malaria, dengue and hemorrhagic) filariasis and encephalitis. Moreover, it has been reported that bites from this insect may lead to intense skin pain by causing allergic reactions to the saliva of the mosquito leading to itching (Abdullah *et al.,* 2003).

In order to control this vector, it is becoming clearer that controlling the developmental stages of mostquitoes in water environment may be the best way to achieve success in this regard. The application of chemically formulated insecticides is the most popular method used all over the world for the control of this important vector. However, its application though effective against the targeted organism do affect non-targeted population as well, this is due to overuse, inappropriate application and release into the environment. This has gradually led to major environmental hazards by buildup of non-biodegradable recalcitrant toxic constituents of the insecticide in the ecosystem, as well as the progressive resistance of mosquitoes to synthetic insecticides, bioaccumulation in food-chain and detrimental effects on people's health (Devine and Furlong, 2007; Bansal *et al.,* 2011). This has provoked a wide and deepe level of research into seeking for different approaches cutting across various options like the adoption of effective mosquito management techniques that centered on educating the public, monitor and observation, source reduction and environmental friendly and less toxic control of the mosquito larva.

Plants and their produts have been exploited traditionally by man from time immemorial as alternative way of controlling insects and these products have been found to be very safe to man, his environment and the ecosystem at large since they are easily degraded by microorganisms (Kalu *et al.,* 2007) phytochemical extracts from different plants have been assessed for their potency against mosquitoes (Pavela, 2008) with varying degree of successes.

Phytoproducts on accounts of lesser hazardous effects on the ecosystem and their high level of availability in nature offer promise in mosquito control programme. Plant products have revolutionized the field of vector control as they possess different bioactive components and can be used as general toxicant against various larvae stages of the mosquito (Sharma *et al.,* 2004; Mohan *et al.,* 2005).

Botanically phytoconstituents with insecticidal potentials are now documented as effective substitute for the chemical insecticides used for mosquito control programmes owing to their great ability to kill mosquitoes and phytochemical secondary metabolites have been anticipated as a potential tool in future mosquito control programmes as they are reported to inhibit the general function, development as well as reproduction in insects (Sukumar *et al.,* 1991). Roark (1949) described over 1,000 different species of plant that possess insecticidal potential, while Sukumar *et al.,* 1991) have streamlined these to about 344 plant species that possess mosquito killing effect.

Several reports have been made on the inhibitory activities of different extracts of plants extracts against mosquito larva. For instance, dried leaf of *Adhatoda vasica, Azadirachta indica* and *Ocicum sanctum* when ignited release aromatic smoke that repels *Armigers subaltatus* and *Culex quinquerfrasciatus* to prevent their bitting activity for 6-8hours (Pandian and Manoharan, 1995). Also, petroleum ether extracts of *Annona squamosa* seeds revealed larvicidal action on *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stehnes* (George and Vincent, 2005).

Reports abound on the traditional medicinal uses of *Cola* species. However, little is known about the insecticidal properties of the plant extracts of *Cola millenii* (Monkey cola) against mosquitoes. It is viewed that the exploration of this medically useful plant could either promote its wider use particularly in controlling mosquitoes.

METHODOLOGY

Collection and processing of plant materials

The monkey cola (*Cola millenii*) was collected fresh from the farm centre at Owo, Ondo State and was authenticated at the Herbarium Section of the Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo where Voucher specimens were deposited before it was transferred to the laboratory for analysis. The authenticated plants were washed and cleaned thoroughly with tap water and then air-dried under shade for six weeks. The dried samples were then ground into coarse powder, stored in clean air- tight containers and kept in a cool, dry place until required for use.

Sterilization of Materials

Glass wares such as Petri dishes, beakers, conical flasks, test tubes and pipettes were washed in soap solution, rinsed with sterile distilled water, wrapped in aluminum foil and oven dried at 160 °C at 15 psi for 1h. Inoculating loops and forceps were flamed to red hot and then dipped in 70% ethanol. Workbenches were swabbed with cotton wool soaked in 70 % ethanol before and after investigations.

Preparation of Plant Extracts.

A 100g of the powdered sample was soaked in 300 ml aqueous, ethanol and n-hexane respectively for 72 hr with intermittent stirring using sterile spatula. The plant extracts were then filtered through Whatman No1 filter paper into Bijou bottles and then concentrated *in vacuo* using rotary evaporator model. Different concentrations of the extracts were prepared by diluting 0.10 g, 0.20 g, 0.30 g, 0.40 g and 0.50 g of the extracts in 100 ml of 0.01% Tween-20 to obtain concentrations of 10 mg/ml, 20 mg/ml, 30 mg/ml, 40mg/ml and 50mg/ml respectively (Opawale *et al.* 2015).

Insecticidal Assays

Mosquito Test organism

The test organism larvae of *Anopheles stephensi* were collected from stagnant water within the student hostel of Rufus Giwa Polytechnic, Owo and were kept in a plastic container until testing for bioassays (Ileke and Ogungbite, 2015).

Test for Larvae Activity

In the larvicidal assays, third and fourth instars larvae of *Anopheles stephensi* were exposed to test concentration 2, 4, 8 and 10 ml of the plant extracts from leaf and pulp in 100 ml of distilled water. A 100 ml volume of distilled water was taken in series of 200 ml plastic beaker. The plant extracts was added to the distilled water in the beakers. A control was also maintained by not

adding any known concentration of the plant extract to the distilled water in the beaker. Ten (10) larvae per concentration were used for all the larvae experiment (Ansari *et al.*, 2005).

Each concentration of the plant extract had 3 replicates each and were arranged in Complete Randomised Design (CRD). The number of dead larvae was recorded at the end of 24 hours respectively. The percentage mortality value was calculated.

$$\text{Percentage mortality} = \frac{\%\text{ mortality in treated} - \%\text{ mortality in control} \times 100}{100 - \%\text{ mortality in control}}$$

Method for Production of Phyto-Insecticides

Production of coils from the plant extract

For the production of the bio-insectide coil, taxopon (150g) was poured into a 2L container and Phenol (300 mL) was added then stirred continuously until all was dissolved; however, menthol was added to aid the dissolution. *Cola. millenii* (200 g) was added to the mixture and stirred until the solution homogenized. Cassava starch (500 g) was cooked in boiling water (100 ml) placed in a water bath to form a paste. The paste was stirred into the prepared mixture until a consistent paste was formed. The paste was then made into different forms and shapes, placed in the drying cabinet with the temperature set at 60 ^{o}C to until the shapes reached a constant texture (Ileke and Ogungbite, 2015).

Test of phytocoils efficacy

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.

Data Analysis

Unless otherwise indicated, results are expressed as means ± SEM of three replicates. Data were subjected to one-way analysis of variance (ANOVA) using SPSS version 25.0. The Duncan's Multiple Range test was used to separate the means at the 5% level of probability.

Results and Discussion

Larvicidal activity of the *C. millenii* against the mosquito larvae

The larvicidal effect of *C. millenii* extracts on mosquito larva is presented in Table 1. 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, 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: Percentage mortality of mosquito larva at different concentrations of *C. millenii* leaf extracts

		Hexane				Ethanol	
Dosage (ppm)	**Initial larvae**	**No. of survivors**	**No. of deaths**	**% mortality**	**No. of survivors**	**No. of deaths**	**% mortality**
Leaf							
1000	20	0	20	100	0	20	100
100	20	6	14	70	3	17	85
10	20	8	12	60	6	14	70
1	20	12	8	40	9	11	45
LC_{50}				**4.18**			**1.71**
Pulp							
1000	20	10	10	50	10	10	50
100	20	14	6	30	13	7	35
10	20	17	3	15	15	5	25
1	20	19	1	5	16	4	20
LC_{50}				**930.91**			**792.34**

Mosquiticidal activity of the *C. millenii* leaf extract

The mosquiticidal effect of phytocoil produced from *C. millenii* leaf extracts on adult mosquito is presented in Tables 2 and 3. The killing effect of the coil made from ethanol leaf extract was comparable to the commercially available coil 5 hr after exposure recording 100 % mortality in the mosquitoes. Also, the phytocoil produced from the n-hexane extract was mildly active against the mosquitoes as a mere 58.35% death were achieved 5 hours after exposure.

Table 2: Percentage mortality of adult mosquito exposed to phytocoil produced from ethanol leaf extract of *Cola millenii* over time (hrs)

Concentration (%)	**Hours After Treatment**				
	1	**2**	**3**	**4**	**5**
1	15.33 ± 0.01^{b}	35.00 ± 0.00^{b}	40.33 ± 0.00^{b}	40.33 ± 0.00^{b}	40.33 ± 0.00^{b}
2	20.67 ± 0.00^{c}	45.33 ± 0.10^{c}	50.67 ± 0.00^{c}	51.67 ± 0.00^{c}	51.67 ± 0.00^{c}
5	42.20 ± 0.00^{d}	71.67 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Unexposed	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Coil	50.00 ± 0.00^{e}	95.67 ± 0.00^{e}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}

Table 3: Percentage mortality of adult mosquito exposed to phytocoil produced from n-hexane leaf extract of *Cola millenii* over time (hrs)

Concentration (%)	Hours After Treatment				
	1	**2**	**3**	**4**	**5**
1	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	20.67 ± 0.00^{b}	30.17 ± 0.00^{b}	35.33 ± 0.00^{b}
2	0.00 ± 0.00^{a}	21.33 ± 0.10^{b}	27.17 ± 0.00^{c}	31.67 ± 0.00^{b}	40.00 ± 0.00^{c}
5	15.20 ± 0.00^{b}	31.67 ± 0.00^{c}	38.12 ± 0.00^{d}	46.67 ± 0.00^{c}	58.35 ± 0.00^{d}
Unexposed	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Coil	50.00 ± 0.00^{c}	95.67 ± 0.00^{d}	100.00 ± 0.00^{e}	100.00 ± 0.00^{d}	100.00 ± 0.00^{e}

Discussion

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. Similarly, the pulp extract of the plant had lower larvicidal activity at all the test concentrations used. A 5% mortality was recorded at 1 ppm against the n-hexane extract of the pulp and an LC_{50} of 930.91 ppm was obtained while mortality of 20% at 1 ppm of the ethanol extract of the pulp with an LC_{50} of 792.34 ppm was obtained.

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 *Alstonia 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 Bhattacharya *et al.* (2014) that botanical oils have a considerable effect on the swimming ability of larvae and pupae of mosquito and reduction in their surviving rate.

Incidentally, the pulp of the *C. millenii* did not have the same effect on the larvae of the mosquito recording a rather high LC50 of 930.91 ppm and 792.34 ppm respectively for the n-hexane and ethanol extracts. The difference observed may be due to the array of phytochemicals present in the two plant parts. The phyochemicals in a plant have been reported to work synergistically to effect actions in a biological entity.

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. Nevertheless, the n-hexane extract of the leaf had lower effect on the adult insect as only about 50% were killed after 5 hours exposure at 5% incorporation while lower concentrations were unable to achieve 50% mortality in the mosquitoes (Ileke and Ogungbite, 2015).

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 Singh and Mittal (2014) 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 Yang *et al*. (2006) 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.

Conclusion

Based on the evidence obtained in this study, it can be safely concluded that *Cola millenii* leaf extracts had significant lethal effect on mosquito larva and insecticidal activity against adult mosquitoes. Effective bio-insecticides may be formulated from *C. millenii* leaf extract.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

REFERENCES

Abdullahi K, Muhammad S, Manga SB, and Tunau IM (2003): Chloroquine resistance *Plasmodium falciparu,m* in Sokoto North West Nigeria. *African Journal of Biotechnology*, 2(8): 244-245

Ansari, M.A., Mittal, P.K., Razdan, R.K. and Sreehari, U. (2005). Larvicidal and mosquito repellent activities of Pine (*Pinus longifolia*, family: Pinaceae) oil. *Journal of Vector Borne Diseases*, **42**: 95-99.

Bansal SK, Singh KV, Sharma S, and Shherwani (2011): comparative larvicidal potential of different plant parts of Withania somnifera against vector mosquitoes in the semi-arid region of Rajasthan. *Journal of Environmental Biology*; 32(1): 71-75.

Bhattacharya, S., Iyer, E.P., Iyer, S.C. and Cox, D.N. (2014). Cell-type specific transcriptomic profiling to dissect mechanisms of differential dendritogenesis. *Genomic Data* **2**: 378--381.

Devine GJ, and Furlong MJ (2007): Insecticide use: Contexts and ecological successions. Agriculture and Human values; 24: 281-306

George S and Vincent S (2005): Comparative efficacy of *Annona squamosa Linn* and *Pongamia glabra* Vent to *Azadirachta indica.* a juss against mosquitoes. *Journal of Vector Borne Disease* 42:159-163

Ileke, K.D. and Ogungbite, O.C. (2015). *Alstonia boonei* De Wild oil extract in the management of mosquito (*Anopheles gambiae*), a vector of malaria disease. *Journal of Coastal Life Medicine* **3**(7): 557-563

Kalu IJ, Ofoegbu U, Eroegbusi J, Nwachukwu CU, and Ibeh B (2010): Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus. Journal of Medicinal Plant Research 2010*; 4(6): 496-498

Klempner MS, Unnash TR, and Hu LT (2007): taking a bite out of vector transmitted infectious diseases. *New English Journal of Medicine*-; 356:2567-2569

Mohan LP, Sharma and Srivastava CN (2005): Evaluation of *Solanum xanthocarpum* extracts as mosquito larvicides. *Journal of Environmental Biology*, 26(2): 399-401. 12

Opawale, B.O., Oyetayo, A.M. and Agbaje, R.B. (2015). Phytochemical Screening, Antifungal and Cytotoxic Activities of *Trichilia heudelotii* Planc (Harm). *International Journal of Sciences: Basic and Applied Research* **24**(6): 267-276.

Pandian RS, Manoharan AC, (1995): Herbal smokes a potential repellent and adulticide for mosquitoes. *Insect Environmental* 1:14-15

Pavela R (2008): Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larve (Diptera: Culicidae). *Parasitology Research*, 105:887-892

Roark RC (1949): Some promising insecticidal plants. *Economic Botany* 1: 437-45

Sharma PL, Mohan and Srivastava CN (2004): Larval susceptibility of *Ajuga remota* against anopheline and culicine mosquitoes. Southeast Asian *Journal of Tropical Medicine Public Health*, 35 (3): 608-610

Singh, R.R. and Mittal, S. (2014). Improvement of local subgrade soil for road constuction by the use of coconut coir fiber. *International Journal of Research in Engineering and Technology* **3**: 5.

Sukumar KMJ, Perich and L.R. Boobar (1991): Botanical derivatives in mosquito control: *Journal of the American Mosquito Control Association*, 7(2): 210-237

WHO, (2010): Malaria fact sheets No. 94. WHO Report potential.

Yang, Q., Inoki, K., Ikenoue, T., Guan, K.L. (2006). Identification of Sin1 as an essential TORC2 component required for complex formation and kinase activity. *Genes Development.* **20**(20): 2820--2832.