Original Research Article

Comparative Repellency of Selected Plants to Adult *Anopheles gambiea* complex (Gilles) in the Laboratory

ABSTRACT

Aims: The study evaluated the repellency effects of some tropical plants and shrubs found in semi- rural communities of Badagry Area of Lagos state; which are acclaimed to have the potentials of repelling mosquitoes from human dwellings. The repellency effects of *Moringa oleifera, Moringa indica, Magnifera indica* and *Phyllanthus muellrianus* to *adult Anopheles gambiea* was evaluated in the Laboratory.
Study Design: the study was carried out at Central Research Laboratory of Lagos State University, Ojo, Lagos, Nigeria AND Central Research Laboratory of University of Lagos, Akoka, Lagos, Nigeria.
Methodology: Plants were prepared into powder forms and rubbed on the fore arms volunteers and they were exposed to 0-2 two day old adult mosquitoes in a glass chamber and repellency was observed for a period of 180 minutes, with knockdown counts taken every 30 minutes. The plants were subjected to qualitative and quantitative phytochemical analysis.
Results: Results showed that all test plants were able to repel *Anopheles* mosquitoes in the study, repellency was shown in descending order *Moringa oleifera* with 88%, *Magnifera indica* 83%, *Phyllantus muellerians* 80% and *Morinda lucida*72%. The result of phytochemical screening of the test plants showed that only M.indica is the only plant that indicated presence of saponing (36.99). while M.oleifera has highest phenol content (45.63), Alkaloid (38.68), steroid (24.89) and Tannin (33.19). Flavinoid and reducing sugar quantity was highest in M. indica (39.39) and (55.18) respectively.
Conclusion: The plants were able to show repellency to *Anopheles gambiae* a nuisance malaria vector of serious medical importance. These plants are available in all tropical areas of Africa, they can therefore be used to prevent nuisance and painful mosquito bites which could be a sustainable way to prevent mosquito vectored diseases.

Keywords: Anopheles gambiae, Moringa oleifera, Moringa indica, Magnifera indica, Phyllanthus muellrianus Lethal Dossage.

1. INTRODUCTION

Mosquitoes are the most important single group of insects in terms of public health importance because they are vectors of serious human diseases such as malaria, filariasis, Japanese encephalitis, dengue fever, and yellow fever. Mosquito-transmitted diseases are a major cause of illness and death in the world, particularly in tropical and subtropical Africa [1]. They are considered the deadliest insects in the world and are major human and animal

health problem as they transmit diseases to more than 700 million people annually [2,3]. World Health Organization [4] estimated that more than a million people die annually from malaria and other mosquito borne diseases with 300 to 500 million people being affected. It is also estimated that 100 children under one year of age and 200 children under five years out of 1000 respectively die annually of malaria in Nigeria [5].

Mosquito control in Africa and its environs is becoming a great challenge because oftentimes synthetic chemical insecticides use for it control are harmful to man and animals, non-degradable and non-selective and are considered to be environmental pollutants and bioaccumulates both in the body and build up in the food chain has discouraged their use and hence some of them have been banned in developed Countries [6,7].

The problems associated with the use of chemical insecticides has challenged researchers to explore traditional non chemical control methods that were practiced before the advent of chemical insecticides. Such as mosquito avoidance practices like burning of plant parts, rubbing of ash and oils to ward of mosquitoes and consequently avoid bites. Plant botanicals have been used from time immemorial because they are cheap, safe and effective means of mosquito vectors control [8].

The aim of the research is to evaluate the repellency effect of four tropical plants namely: *Moringa oleifera, Magnifera indica, Morinda lucida* and *Phyllanthus muelleranius* against *Anopheles gambiae.*

2. MATERIAL AND METHODS

2.1 COLLECTION AND PREPARATION OF TEST PLANTS

Matured whole plants of *Moringa oleifera, Magifera indica, Phyllanthus mullerianuis,* and *Morinda lucida were collected* from local villages around Badagry Area of Lagos state. The Plants were taken to University of Lagos herbarium for identification and deposit. Respective voucher numbers were given: *Magnifera indica* (8591), *Phyllanthus mullerianuis* (8590), *Morinda lucida* (8593) and *Moringa oleifera* (8589).The collected plants were wash

under running water and transported to the laboratory and dried on the laboratory work tables at $26^{+}.2^{O}C$ for about 10 days until fully dried. The dried leaves were grinded into fine powder with JŘ 200^{R} grinding machine. Each powder form of plant leaves was kept separately in an airtight containers and stored in refrigerator until needed.

The stored leaf powders were admixed with powdered coconut husk as inert material at different concentrations . The repellent study was carried out in the laboratory using 40 by 40 netted aluminium cage with arm entry. Three weeks old blood-starved female *Anopheles gambiae* mosquitoes were introduced into the cages. The volunteers hands were washed with mild soap , rinsed with lots of water and made to place their exposed arm that had been treated with different dosages of powder paste in the cage for a period of five minutes. The powder paste was applied at 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 g separately to the exposed area of the fore arm. Only powder coconut husk served as control. The control and treated arms were introduced simultaneously into the mosquito cage, and gentle tapping of the sides of the experimental cages is to activate the mosquitoes. Each test concentration was repeated three times. The volunteers conducted their test of each concentration by inserting the treated and control arms in to the same cage every 30 minutes for 1800 minutes. The mosquitoes that landed and bites received on the hands were recorded after every 30 minutes and then shaken off making out a 180 minute protection. The percentage of repellency was calculated with the formula: % Repellency =[(Ta – Tb)/Ta] *100, where Ta is the number of mosquitoes in the control group and Tb is the number of mosquitoes in the treated group.

2.2 PREPARATION OF PLANTS FOR PHYTOCHEMICAL ANALYSIS

2.2.1 QualitativeTests

2.2.1.1 Test for Tannins

About 0.5 g of the sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

2.2.1.2 Test for Phlobatannins

0.5g extract of each plant sample was boiled with 2mL of 1% aqueous hydrochloric acid for 10 minutes. Deposition of a red precipitate indicates the presence of phlobatannin.

2.2.1.3 Test for Saponin

About 2 g of the sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, formation of emulsion was observed.

2.2.1.4 Test for Flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated tetra oxosulphate vī acid.

2.2.1.5 Test for Steroids

2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml tetra oxosulphate vī acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

2.2.1.6 Test for Terpenoids (Salkowski test)

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated sulfuric acid (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.2.1.7 Test for Cardiac Glycosides (Keller-Killani test)

Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

2.2.1.8 Test for Alkaloid

A 5 mg sample of the extract dissolved in 3 ml of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer's reagent and 1 ml of Dragendroff's reagent were added to 1 ml of the filtrate and turbidity was observed

2.2.2 Quantitative Analysis Tests

2.2.2.1 Estimation of Tannins

500 mg sample of the concentrate was dissolved in 50 ml of distilled water, and shake for one hour. A 5 ml aliquot of the filtrate was mixed with 2 ml of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 720 nm within 10 minutes.

2.2.2.2 Estimation Of Total Phenolic Compound

0.5g sample of extract was dissolved in 50 ml of water. 0.5 ml was added to 0.1 ml of Folin-Ciocalteu reagent (0.5 N) mix and incubate at room temperature for 15 minutes. After this, add 2.5 ml sodium carbonate solution (7.5% w/v) and further incubated for 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

2.2.2.3 Total Flavonoid Content Estimation

1 ml of sample solution (100µg/ ml) was mixed with 3 ml of methanol, 0.2 ml of 10% Aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol.

2.2.2.4 Total Antioxidant Capacity Determination

solution of the samples extract (1ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 950 C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm. The total antioxidant capacity was expressed as equivalent of ascorbic

2.2.2.5 DPPH Radical Scavenging Activity Assay

An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/ ml) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature.After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression

% inhibition = [A0-A1] x 100/A0 . Where A0 is the absorption of the blank sample and A1 is the absorption of the extract

2.2.2.6 In vitro Lipid Peroxidation Assay

The reaction mixture containing liver homogenate(0.2ml), Tris−HCl buffer (20 mM pH 7.0,0.1ml), FeCl2 (2 mM,0.1ml), ascorbic acid (10 mM,0.1 ml), and 0.5 ml plant extract (25−100 µg/ml) in a final volume of 1 ml. The reaction mixture was incubated at 37 °C for 1 hour. Lipid peroxidation was measured as malondialdehyde (MDA) using trichloro acetic acid (TCA), thiobar bituric acid (TBA) and HCl (TBA-TCA reagent: 0.375 % w/v TBA; 15 % w/v TCA and 0.25 N HCl). The incubated reaction mixture was mixed with 2 mL of TBA-TCA reagent and heated in a boiling water bath for 15 minutes. After cooling, the flocculent precipitate was removed by centrifugation at 10,000 g for 5 minutes. Finally, malondialdehyde concentration in the supernatant fraction was determined spectro photometrically at 535 nm. Ascorbic acid was used as standard.

2.2.2.7 Nitric Oxide Scavenging Activity Assay

4 ml sample of plant extract or standard solution of different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 mMin phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30 °C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthyle thylenediaminedihydrochloride in 2% H3PO4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyle thylenediamine was measured at 550 nm [9]. Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from t.he following equation:

[(A0 – A1)/A0] x 100.

Where, A0 is the absorbance of the Control and A1 is the absorbance of the extract or standard

2.2.2.8 Reducing power assay

Various concentrations of the extracts (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50cfor 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1to 16µg/ml) was used as standard.

3. RESULTS

Table 1. Quantitative Phytochemical Screening Of Test Plants

	Saponin	*Phenol*	*Flavonoid*	*Alkaloid*	*Steroid*	*Reducing Sugar*	*Tannin*
M. oleifera		45.63	31.02	38.63	24.89	43.09	33.19
M. indica	36.99	39.39	22.53	30.05	14.89	55.18	28.65
M. lucida		27.01	33.47	24.51	19.98	39.99	19.65
P.muellerianus		29.66	57.31	26.28	23.77	45.27	21.58

Table 2. Qualitative Phytochemical Screening Of Test Plants

Sample	*Saponin*	*Phenol*	*Flavonoid*	*Tannin*	*Alkaloid*	*Reducing Sugar*	*Phlobatannin*	*Steroid*	*Terpenoid*
M. oleifera	-	+	+	+	+	+	-	+	+
M.indica	+	+	+	+	+	+	-	+	+
M. lucida	-	+	+	+	+	+	-	+	+
P.muellerianus	-	+	+	+	+	+	-	+	+

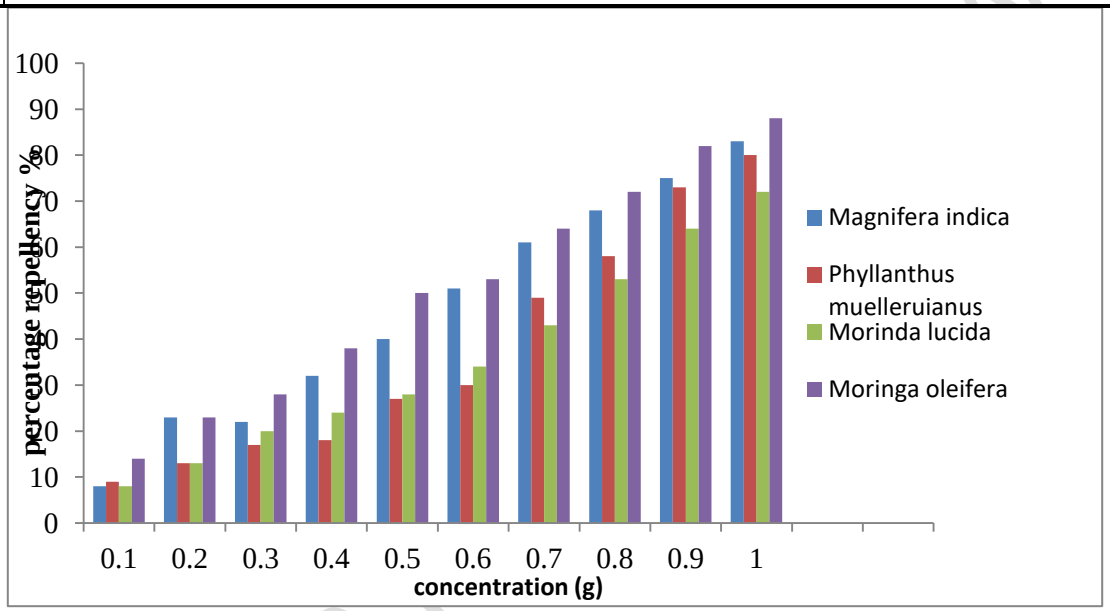

Fig. 1. Chart of Plants showing comparative percentage repellency against concentration.

4. DISCUSSION AND CONCLUSION

The present research evaluated repellency effect of *Moringa oleifera*, *Magnifera indica*, *Morinda lucida* and *Phyllanthus muellerianus* to adult *Anopheles gambiae* in the Laboratory. The result of the research showed that all plants are potent and were able to repel mosquitoes at concentration 0.6 to 1.0g where over 50% population of exposed insects were repelled. *Moringa oleifera* showed highest repellency to *Anopheles gambiae* with repellency of 88% at 1.0g. *Magnifera indica* conferring 83% repellency, *Phyllanthus muellerianus*

conferring 80% and *Morinda lucida* conferring 72% repellency on volunteer after 60 minutes of exposure.

The result equally showed that leaf powder of *Moringa oleifera* is highly repellent to *Anopheles gambiae* adult and it shows a very strong indication of its potential as a repellent. The result from the study is in agreement with Prabhu *et al* [11], that evaluated the repellent potential of *M. oleifera* against adult mosquito; the result showed that repellency is dose dependent, 90.41% repellency was achieved at 100% concentration; while 23,28% was recorded at 20% concentration. The use of plants to ward off nuisance insects is an age long practice. The phytochemical analysis equally showed that *M. oleifera* exhibited highest presence of phenol, alkaloid, steroid and tannin. These phytochemical may be the compounds that is making it show higher repellency. However, there is need to authenticate this observation.

The result justifies studies on botanicals against insects pests as shown by several authors; and not just adherence to traditional beliefs of hanging plants on door and bed post. The insecticidal properties of 10 mosquito repellants plants were well documented; as shown by Egunyomi *et al*. [10]. The methanol and hexane extracts of the plants were investigated for phytochemical compounds with repellent activities against *A.stephensi*. The test plants contained phenols and streroids. The active extracts are promising ethnobotanical repellent at 2mg/ml against *A. stephensi* and could be sources of new natural repellent compounds.

The plants produce certain compounds that repel and ward off insects pests from attacking them and these has provide a basis of use of the plants as repellents. Most branded repellents are made from harmful chemicals; and there has been reports of undesireable side effects to their use; also some of them are non- effective but a way to make consumers part with their hard earned money.

This result agree with work of Denloye et al. [8], who reported that some plants such as Citrus species, whose peels were dried and burnt to repel mosquitoes in Nigeria and have being found to be toxic to some other insects. These plants therefore can serve as very

potent replacement for the chemicals insecticides used in mosquito coils and other repellents, thereby reducing the burden of respiratory disorders.

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