

Research Article

Comparative Activities of Stem Bark Extracts of *Anthocleista vogelii*, *Bligha sapida*, *Voacanga africana* and *Momordica charantia* Leaf against *Plasmodium berghei-berghei* in Mice

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Abstract

Background: The search for antimalarial molecules from plants necessitates comparative studies of ethnomedicinal antimalarial plants to quickly identify those that may be used in further search. Therefore, the median lethal dose, LD₅₀, and the antiplasmodial activities of the methanol extracts of the stem barks of *Anthocleista vogelii*, *Bligha sapida*, *Voacanga africana*, and the leaf of *Momordica charantia* were evaluated against *Plasmodium berghei berghei*-infected mice using prophylactic, chemosuppressive, and curative models to compare their activities and identify the most active for further evaluation.

Methods: The plant materials were collected, authenticated, and voucher specimens were deposited at the Faculty of Pharmacy Herbarium, OAU, Ile-Ife. They were separately macerated in methanol, and the median lethal dose, LD₅₀ determined using Lorke's method. The percentage parasitaemia, percentage reduction, chemosuppression and clearance, survival time, and percentage survivor of each, in the three models of antiplasmodial test against *Plasmodium berghei berghei* infected mice were assessed. Pyrimethamine and Chloroquine were positive controls, while normal saline was a negative control. One-way analysis of variance (ANOVA) followed by Student Newman-Keuls *post hoc* test ($p < 0.05$) was used for the analysis of data.

Results and Conclusion: The lowest prophylactic ED₅₀ and ED₉₀ values 304 and 624 mg/kg of AV, comparable chemosuppressive ED₅₀ values of all extracts and the significantly ($p < 0.05$) lower values of ED₅₀ and ED₉₀ of MC and VA in the curative assay can guide the selection of the plant extract(s) for further antimalarial evaluation.

Introduction

Even though sustained efforts and interest in screening plants for secondary metabolites with potent pharmacological activities have increased globally, only about 17% of the 250,000 plants worldwide have been investigated thoroughly for medicinal potential [1], thus implicating the plant kingdom as a potential reservoir of potent drug molecules and medicinal recipes for which more discovery should be focused. The plant kingdom, therefore, needed to be explored for valuable drugs. Also, since *Cinchona succirubra*'s stem

bark provided quinine, the first antimalarial medication, numerous plant stem barks and leaves have been studied for their potential to inhibit malaria, and numerous others still need to be studied. Also, there have been reports of the use of *Chrysophyllum albidum* leaf and stem-bark, *Citrus aurantifolia* leaf and fruit, *Sorghum bicolor* leaf, *Mangifera indica* stem-bark, foliage, or leaf, and *Anacardium occidentale* stem-bark as antimalarial remedies in Ogun and Osun States of Nigeria [2,3] and the antimalarial properties of a decoction made from the leaves of *Mangifera indica*, *Alstonia boonei*, *Morinda lucida*, and *Azadirachta indica* in a ratio of 1:1:1:1 and other

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Keywords: Comparative; Antimalarial models; *Anthocleista vogelii*; *Bligha sapida*; *Voacanga Africana*; *Momordica charantia*



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ratios in mice have also been documented [4,5]. The decoction of the roots of *Sphenocentrum jollyanum*, *Zingiber officinale*, stem bark of *Khaya grandifoliola*, root and stem bark of *Senna spectabilis*, root of *Zanthoxylum xanthoxyloides*, and leaves of *Ocimum basilicum* were also reported to be active against fever [6-8]. Despite this avalanche of investigation on plants, more plants still need to be investigated, and an emphasis on comparative antimalarial studies on plants is desirable as a means of identifying potential candidates not only for the formulation of herbal remedies but also for the discovery of putative compounds for antimalarial drug discovery. The resistance posed by resistant forms of the parasite to drugs currently used in treating malaria, including Artemisinin Combination Therapy [9,10], may also compel comparative antimalarial studies that could eventually identify new and more effective antimalarial chemicals from plants.

Anthocleista vogelii is an evergreen tree native to Nigeria. The decoction of the leaf and stem bark is used traditionally in Nigeria and Ghana for the prevention and treatment of malaria, and also to alleviate symptoms such as fever and Decussatin, isolated from this plant, has demonstrated very weak antiplasmodial activity [11,12]. *B. sapida* is used in Sub-Saharan Africa traditionally to manage fever in young children [13-15]. Some of its extracts and isolated compounds have shown some antiplasmodial activities [16]. Though the extracts of *Voacanga africana* are used by the Africans for various ethno-medical practices such as treatment for leprosy, diarrhea, generalized edema, convulsions, curing of orchitis, ectopic testes, as well as gonorrhea, and madness figures [17], the *in vivo* and *in vitro* anti-malarial activity of voacamine (an ibogavobasine type alkaloid) isolated from *V. africana* was significant [18,19]. The fruit juice and leaf tea extracted from *M. charantia* have been used for the treatment of malaria and fevers [20]. The antiplasmodial properties of three bark drugs (*Anthocleista vogelii*, *Bligha sapida*, *Voacanga africana*) and one leaf antimalarial ethno medicinal drug (*Momordica charantia*) are being compared in this study using prophylactic, chemosuppressive, and curative models of antiplasmodial test in order to identify their antimalarial potential and so be able to select candidate plants for antimalarial drug molecule isolation or preparation of herbal remedies.

Materials and methods

Plant material

Collection and authentication: The stem bark of *Anthocleista vogelii* (Loganiaceae) was collected at the back of the Faculty of Pharmacy, Obafemi Awolowo University, Ife; the stem barks of *Bligha sapida* (Sapindaceae) and *Voacanga africana* (Apocynaceae) were collected at the back of the Faculty of Sciences, while the leaf of *Momordica charantia* (Cucurbitaceae) was collected in front of Oduduwa Hall of the same University. The plants were identified and authenticated at the Faculty of Pharmacy Herbarium, Ife, by Mr. I. I.

Ogunlowo of the Pharmacognosy Department, OAU, Ile-Ife, where voucher specimens, FPI 2429, FPI 2432, FPI 2431, FPI 2430, were deposited, respectively.

Extraction of the plant materials

Each of the plant materials was separately air-dried and powdered. A quantity (200 g) of the dried powders was separately macerated in 2,500 mL methanol for 72 hours with intermittent shaking. The resultant extracts were filtered, evaporated to dryness *in vacuo*, weighed, and the % yields were obtained.

Animal experiment

Ethical approval: The protocol used for this study was approved by the Health Research Ethics Committee (HREC), Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria, with the HREC Number IPH/OAU/12/2266 and the Board of Postgraduate College, OAU, with the Registration Number PHP19/20/H/1671. Guidelines on the handling and use of laboratory animals [21], as well as extant local and national laws, were strictly followed.

Preparation of the mice

Seven-week old Swiss mice of either sex weighing between 18 to 24 g (male and female, not pregnant) were obtained from the Animal House, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife where they were housed in aluminum cages with wood shavings used as beddings and allowed free access to water and food (Growers' mash) under 12 hours, day/night cycle. They were acclimated for at least seven days before use. The mice were handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals [21]. They were subsequently randomly divided into groups of five mice each for the experiments.

Determination of median lethal dose (LD₅₀)

The median lethal dose (LD₅₀) determination was conducted using Lorke's method, briefly divided into two phases: Phase 1 involved nine mice, which were divided into three groups of three mice each. Each group of mice was administered with 10, 100, and 1000 mg/kg of the extract, respectively, and observed for 24 hours to monitor their mortality (no mortality in Phase 1 would enable the experiment to proceed to Phase 2; otherwise, the LD₅₀ will be decided at Phase 1).

Phase 2 involved the use of three mice, which were grouped into three groups of one mouse each. The mice were administered higher doses (1600, 2900, and 5000 mg/kg) of the extract, respectively, and then observed for 24 hours for behaviour as well as mortality (no mortality at this stage confirmed the extract as being non-toxic [22,23]. Then the LD₅₀ was calculated from the formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$ where D_0 = the Highest dose that gave no mortality, and D_{100} = the Lowest dose that produced mortality.

Rodent parasite

The donor Swiss mouse containing the rodent parasite, *Plasmodium berghei-berghei* NK 65, and with rising parasitaemia was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan. It was maintained by serial passaging in mice and close monitoring of the parasitaemia level.

Preparation of the test extracts and standard drug

Doses of 100, 200, 400, and 800 mg/kg were prepared by dissolving 40, 80, 160, and 320 mg each of the extract of *Anthocleista vogelii* (AV) in 4.0 mL of normal saline for the prophylactic model. Doses of 100, 200, 400, and 800 mg/kg were prepared by dissolving 50, 100, 200, and 400 mg each of the extract of *Anthocleista vogelii* (AV) in 5.0 mL of normal saline for the chemosuppressive model. Doses of 100, 200, 400, and 800 mg/kg were prepared by dissolving 60, 120, 240, and 480 mg each of the extract of *Anthocleista vogelii* (AV) in 6.0 mL of normal saline for the curative model. Other extracts of *Bligha sapida* (BS), *Voacanga africana* (VA), and *Momordica charantia* (MC) were prepared similarly as reported above for all three models. Chloroquine (10 mg/kg) and pyrimethamine (1.2 mg/kg) were prepared by dissolving 8.33 mg and 10.08 mg of their tablets in 5 mL and 4 mL of normal saline, respectively.

In vivo antiplasmodial activity of the extracts

Prophylactic (Repository) test model: Swiss albino mice (30) were grouped into 6 groups of 5 mice each. Mice in Groups I – IV were orally treated with the methanol extract of the stem bark of AV at doses of 100, 200, 400, and 800 mg/kg, dissolved individually in normal saline, respectively. The same doses were repeated daily for two consecutive days (D1-D2), while mice in Groups V and VI were administered with normal saline and pyrimethamine (PYR) at 1.2 mg/kg/day as negative and positive controls, respectively. The mice were then inoculated with *P. berghei*-infected red blood cells on day four (D3) of extract administration, followed by taking the rectal temperature for three days (D0 – D2). Blood smears were then made from each mouse after 72 h post-inoculation to evaluate the parasitaemia levels and to calculate the percentage reduction. Other methanol stem bark extracts of BS, VA, and the leaf extract of MC were treated similarly as reported above.

Four-day chemosuppressive test model: The *in vivo* chemosuppressive antiplasmodial activities for the extracts were assessed using the four-day test. Swiss mice (30) were randomly divided into six groups of five mice each and inoculated with the inoculum. Two hours after inoculation, 100, 200, 400, and 800 mg/kg of the methanol extract of AV, dissolved individually in normal saline, were administered to each of Groups I-IV, respectively. The same doses were repeated daily for three consecutive days (D1-D3), after

measuring their rectal temperatures. Normal saline and chloroquine (10 mg/kg) were administered to Groups V and VI to serve as negative and positive controls, respectively. The levels of parasitaemia were assessed on the fifth day (D4) for each mouse by withdrawing blood from the tail of each of the mice to calculate the percentage parasitaemia and percentage chemosuppression. Other extracts of BS, VA, and MC were treated similarly as reported above.

Established infection (Curative) test model: Swiss albino mice (30) were inoculated with *P. berghei* and randomly divided into 6 groups of 5 mice each. Seventy-two hours after inoculation, mice in Groups I – IV were orally treated with the methanol extract of AV at doses of 100, 200, 400, and 800 mg/kg, dissolved individually in normal saline, respectively. The same doses were repeated daily for four consecutive days (D1-D4), after measuring their rectal temperatures, while mice in Groups V and VI were administered with normal saline and CQ at 10 mg/kg/day as negative and positive controls, respectively. Determination of rectal temperature, preparation of blood smears collected from the tail, and microscopic examination of parasitized cells to assess the parasitaemia levels were carried out daily for 5 days (D0 – D4). The percentage clearance was also determined [24]. The same method stated above was repeated for the extracts of BS, VA, and MC.

Determination of average percentage parasitaemia

Each of the stained blood films prepared was mounted on the microscope stage, and ten fields of view with uniform distribution of red blood cells were viewed using an oil immersion (x100) objective. For each of the fields selected, the numbers of parasitized (Np) as well as unparasitized (Nu) red blood cells were counted.

The percentage parasitaemia for each field of view was then calculated from the formula:

$$\% \text{ Parasitaemia} = \frac{N_p}{N_u + N_p} \times 100$$

Where Np: number of parasitized red blood cells; Np: total number of parasitized and Nu: total number of unparasitized red blood cells. The averages of these percentage parasitaemia for the 10 fields per mouse were calculated, while the average of these results for five mice gave the average percentage parasitaemia per dose with their respective $\pm$ SEM values [25].

Estimation of percentage reduction, percentage chemosuppression, and percentage clearance

From the Average percentage parasitaemia, the percentage reduction, percentage chemosuppression, and percentage clearance for each extract/fraction, depending on the model, were afterwards calculated using this formula:

$$\% \text{ Chemosuppression} / \% \text{ Clearance} / \% \text{ Reduction} = \frac{(PNC - PTD)}{PNC} \times 100$$

Where PNC: Average parasitaemia in the negative control, PTD: Average parasitaemia in the test dose. The values were recorded as percentage reduction in parasitaemia $\pm$ SEM, percentage chemosuppression $\pm$ SEM, and percentage clearance $\pm$ SEM for the prophylactic, chemosuppressive, and curative antiparasmodial test, respectively.

Estimation of mean survival times and percentage survivors of mice

From the first day of the administration of the drug, each treated mouse was observed for mortality for 28 days. This was done to determine the survival times and percentage of survivors elicited by the extracts/fractions in each of the mice. The survival time for each mouse was recorded as days, and the average for each group was determined as days $\pm$ SEM. The percentage survivor for each dose group was estimated from the average survival time for each mouse. The percentage of mice eliciting survival time that falls within the average for the whole group is the Percentage Survivor (PS).

Estimation of the median effective doses ED₅₀ and ED₉₀

A graph of the test doses in mg/kg against reduction in parasitaemia, chemosuppression and clearance in percentage was automatically plotted using Microsoft Excel 2007 from which the median effective doses ED₅₀ and ED₉₀ the doses that would give 50% and 90% percentages reduction in parasitaemia, chemosuppression and clearance for prophylactic, chemosuppressive and curative antiparasmodial test were forecast and recorded as mg/kg $\pm$ SEM.

Statistical analysis

Statistical analysis was performed on the percentage parasitaemia, percentage reduction in parasitaemia, percentage chemosuppression, percentage clearance, effective doses and mean survival times of the extracts. Values were expressed as mean $\pm$ SEM and analyzed statistically using One-way Analysis of Variance (ANOVA) followed by Student Newman-Keuls post-hoc for comparisons to determine the source of significant difference for all values. Values of $p < 0.05$ were considered to be statistical significance.

Results

In the order VA > MC > AV > BS were 20.13, 15.23, 13.12, 2.08% respectively. For the LD₅₀ of extracts, only VA produced mortality at 1600 mg/kg while AV, BS and MC were greater than 5000 mg/kg. No sign of toxicity, behavioral changes, or death was observed in all animal post-administration of the extracts. The LD₅₀ of VA, was calculated to be 1,264.91 mg/kg while those of MC, AV, BS, were greater than 5000 mg/kg.

Percentage parasitaemia

For the prophylactic experiment, each of the extract displayed percentage parasitemia significantly ($p < 0.05$)

lower than the value of $6.48 \pm 0.40\%$ produced by the negative control. All the extracts elicited percentage parasitaemia that were comparable to that of the positive control at 400 and 800 mg/kg except MC at 400 mg/kg. The lowest percentage parasitaemia of $1.61 \pm 0.18\%$ was recorded for AV at 800 mg/kg which was significantly ($p < 0.05$) lower than that of the negative control but comparable to the effects displayed by the positive control (Table 1).

For the chemosuppressive experiment, each of the extract displayed percentage parasitemia which is significantly ($p < 0.05$) lower than the $8.56 \pm 0.68\%$ produced by the negative control. All the extracts elicited activity comparable to that of the positive control at 400 and 800 mg/kg. While there were no significant variations in values given by the different doses of AV and VA. The percentage parasitaemia of $1.38 \pm 0.10\%$ was recorded for MC at 800 mg/kg, which was significantly ($p < 0.05$) lower than the negative control but comparable to the positive control. However, chloroquine (the positive control) displayed the lowest percentage parasitaemia of $1.20 \pm 0.08\%$ (Table 2).

At Day 4, in the curative experiment, each of the extract displayed remarkable percentage parasitemia significantly different ($p < 0.05$) from the $11.07 \pm 0.25\%$ produced by the negative control. All the extracts elicited percentage parasitaemia values significantly higher than that of $1.08 \pm 0.03\%$ elicited by the positive control. The lowest value in % parasitaemia elicited by the extracts is $1.64 \pm 0.07\%$ which was recorded for MC at 800 mg/kg (Table 3).

Effect of the methanol extract of the selected plants on percentages reduction, chemosuppression and clearance in mice

Each of the extract displayed remarkable percentage reduction significantly different ($p < 0.05$) from that produced by the negative control. Both AV and VA at 400 mg/kg and 800 mg/kg, MC at only 800 mg/kg elicited comparable activity with the positive control. The percentage reduction of 70.46 ± 1.51 and $73.91 \pm 3.98\%$ recorded for AV at 400 and 800 mg/kg, respectively, were significantly ($p < 0.05$) higher than that of the negative control but comparable to the positive control. However, chloroquine (the positive control) displayed the highest effects with value of $74.73 \pm 1.48\%$ (Table 1).

For the chemosuppressive experiment, each of the extract displayed percentage chemosuppression significantly ($p < 0.05$) higher than that of the negative control AV at 100, 200 and 400 mg/kg gave percentage chemosuppression significantly ($p < 0.05$) lower than that of the positive control. While at 800 mg/kg, AV elicited value that was comparable to that of the positive control (Table 2). BS and VA at all the doses (100, 200, 400 and 800 mg/kg) gave percentage chemosuppression which were significantly ($p < 0.05$) lower than that of the positive control (Table 2). MC at 100, 200 and 400 mg/kg gave percentage chemosuppression that were

Table 1: Percentage parasitemia and percentage reduction. in parasitaemia in mice in prophylactic antimalarial test of the methanol extract of the selected plant at different doses

Doses (mg/kg)	% Parasitaemia			
	AV	BS	MC	VA
NC	6.48 ± 0.40 ^c (0.00 ± 0.00 ^a)	6.48 ± 0.40 ^c (0.00 ± 0.00 ^a)	6.48 ± 0.40 ^d (0.00 ± 0.00 ^a)	6.48 ± 0.40 ^c (0.00 ± 0.00 ^a)
100	2.94 ± 0.12 ^b (50.45 ± 5.74 ^b)	3.29 ± 0.22 ^b (45.30 ± 2.48 ^b)	3.69 ± 0.14 ^c (40.23 ± 6.27 ^b)	3.87 ± 0.29 ^b (35.88 ± 1.70 ^b)
200	2.67 ± 0.16 ^b (56.80 ± 4.53 ^b)	3.33 ± 0.04 ^b (43.94 ± 5.25 ^b)	3.01 ± 0.35 ^{b,c} (45.44 ± 5.91 ^b)	2.78 ± 0.39 ^a (52.58 ± 9.59 ^c)
400	1.85 ± 0.09 ^a (70.46 ± 1.51 ^c)	2.53 ± 0.07 ^{a,b} (57.71 ± 3.00 ^c)	2.70 ± 0.33 ^b (36.31 ± 6.32 ^b)	2.41 ± 0.20 ^a (59.83 ± 3.66 ^{c,d})
800	1.61 ± 0.18 ^a (73.91 ± 3.98 ^c)	2.11 ± 0.12 ^a (64.54 ± 3.45 ^c)	2.40 ± 0.09 ^{a,b} (62.23 ± 3.23 ^c)	1.93 ± 0.15 ^a (67.80 ± 2.51 ^d)
PYR	1.515 ± 0.07 ^a (74.73 ± 1.48 ^c)	1.515 ± 0.07 ^a (74.73 ± 1.48 ^d)	1.515 ± 0.07 ^a (74.73 ± 1.48 ^c)	1.515 ± 0.07 ^a (74.73 ± 1.48 ^d)

Keys: AV : *Anthocleista vogelii*; BS: *Bligha sapida*; MC: *Momordica charantia* and VA: *Voacanga africana*. Data show the mean ± SEM, n = 5, NC = Negative Control (Tween 80 in normal saline); PYR = Pyrimethamine (1.2 mg/kg). Only values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls’ post hoc test).

Table 2: Percentage parasitaemia in Mice in Chemosuppressive Antimalarial Test of the Methanol Extract of the Selected Plants at Different Doses.

Doses (mg/kg)	% Parasitaemia			
	AV	BS	MC	VA
NC	8.56 ± 0.68 ^c (0.00 ± 0.00 ^a)	8.56 ± 0.68 ^d (0.00 ± 0.00 ^a)	8.56 ± 0.68 ^c (0.00 ± 0.00 ^a)	8.56 ± 0.68 ^c (0.00 ± 0.00 ^a)
100	2.40 ± 0.10 ^a (71.59 ± 1.52 ^b)	3.32 ± 0.18 ^c (59.89 ± 2.43 ^b)	2.84 ± 0.25 ^b (66.13 ± 3.61 ^b)	2.70 ± 0.19 ^{a,b} (67.61 ± 3.14 ^b)
200	2.36 ± 0.12 ^a (71.30 ± 3.43 ^b)	2.81 ± 0.34 ^{b,c} (65.41 ± 5.88 ^b)	2.34 ± 0.09 ^{a,b} (72.16 ± 1.91 ^{b,c})	2.50 ± 0.27 ^a (69.09 ± 5.21 ^b)
400	2.03 ± 0.08 ^a (75.45 ± 2.58 ^b)	2.16 ± 0.15 ^{a,b,c} (74.34 ± 2.29 ^c)	2.12 ± 0.13 ^{a,b} (74.44 ± 2.99 ^c)	2.01 ± 0.14 ^a (75.10 ± 3.47 ^b)
800	1.48 ± 0.08 ^a (81.79 ± 1.83 ^c)	1.93 ± 0.09 ^{a,b} (76.84 ± 2.39 ^c)	1.38 ± 0.10 ^a (83.36 ± 1.86 ^d)	1.86 ± 0.13 ^a (77.46 ± 2.98 ^b)
CQ	1.20 ± 0.08 ^a (85.75 ± 1.16 ^c)	1.20 ± 0.08 ^a (85.75 ± 1.16 ^d)	1.20 ± 0.08 ^a (85.75 ± 1.16 ^d)	1.20 ± 0.08 ^a (85.75 ± 1.16 ^c)

Keys: Methanol extracts of AV: *Anthocleista vogelii*; BS: *Bligha sapida*; MC: *Momordica charantia* and VA: *Voacanga africana*. Data show the mean ± SEM, n = 5, NC = Negative Control (Tween 80 in normal saline); CQ = Chloroquine (10 mg/kg). Only values with different superscripts (a, b, c or d) within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls’ post hoc test).

Table 3: Percentage Clearance in an *in vivo* Antimalarial Activities (Clearance) Test of the Methanol Extract of the Selected Plant at Different Doses at Day 4.

Doses (mg/kg)	% Parasitaemia			
	AV	BS	MC	VA
NC	11.07 ± 0.25 (0.00 ± 0.00 ^a)	11.07 ± 0.25 (0.00 ± 0.00 ^a)	11.07 ± 0.25 (0.00 ± 0.00 ^a)	11.07 ± 0.25 (0.00 ± 0.00 ^a)
100	4.69 ± 0.14 (57.59 ± 0.87 ^b)	4.63 ± 0.19 (58.19 ± 1.62 ^b)	3.40 ± 0.22 (69.18 ± 2.30 ^b)	3.93 ± 0.19 (64.54 ± 1.27 ^b)
200	3.98 ± 0.12 (63.86 ± 1.85 ^c)	2.99 ± 0.04 (72.94 ± 0.51 ^c)	2.37 ± 0.08 (78.63 ± 0.55 ^d)	2.91 ± 0.17 (73.58 ± 1.92 ^c)
400	2.74 ± 0.11 (75.21 ± 0.94 ^d)	2.25 ± 0.07 (79.62 ± 0.43 ^d)	1.80 ± 0.06 (83.75 ± 0.56 ^e)	1.92 ± 0.12 (82.61 ± 1.33 ^c)
800	1.82 ± 0.03 (83.52 ± 0.68 ^e)	1.87 ± 0.10 (83.05 ± 0.99 ^e)	1.64 ± 0.07 (85.16 ± 0.65 ^e)	1.69 ± 0.08 (84.66 ± 0.86 ^e)
CQ	1.08 ± 0.03 (90.27 ± 0.17 ^f)	1.08 ± 0.03 (90.27 ± 0.17 ^f)	1.08 ± 0.03 (90.27 ± 0.17 ^f)	1.08 ± 0.03 (90.27 ± 0.17 ^f)

Keys: AV: *Anthocleista vogelii*; BS: *Bligha sapida*; MC: *Momordica charantia* and VA: *Voacanga africana*. Data show the mean ± SEM, n = 5, NC = Negative Control (Tween 80 in normal saline); CQ = Chloroquine (10 mg/kg). Only values with different superscripts (a, b, c, d, e, f or g) within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls’ post hoc test).

significantly ($p < 0.05$) lower than the positive control. The percentage chemosuppression of $83.36 \pm 1.86\%$ recorded for MC at 800 mg/ kg was comparable to the positive control (Table 2). However, chloroquine displayed the highest effects with value of $85.75 \pm 1.16\%$ (Table 2).

At Day 0, in the curative test, the extracts at all doses elicited percentage clearance that were comparable to both the negative and positive controls (Table 3). At Day 1, the extracts at all doses elicited percentage clearance that were

significantly ($p < 0.05$) higher than the negative control. The extracts at all doses gave percentage clearance that were significantly ($p < 0.05$) lower than the positive control, except BS and MC at 800 mg/kg which gave comparable activities with the positive control (Table 4). At Days 2 and 3, the extract across all doses displayed percentage clearance significantly ($p < 0.05$) higher than that produced by the negative control but significantly ($p < 0.05$) lower to that of the positive control (Table 3). At Day 4, each of the extract displayed remarkable

percentage clearance significantly ($p < 0.05$) higher than that produced by the negative control but significantly ($p < 0.05$) lower to that of the positive control. The highest percentage clearance of the extract ($85.16 \pm 0.65\%$) was recorded for *M. charantia* at 800 mg/kg (Table 3)

Survival times and percentage survivor

The average survival time for all the mice treated with the plant extracts and the standard drug in the prophylactic experiment showed that all the extracts at the different doses with the standard drug showed a comparable values to that of the negative control (Table 4).

In the curative test, the survival time for AV at 100 and 800 mg/kg showed significantly ($p < 0.05$) lower survival time value than that of the positive control but comparable ($p > 0.05$) to the negative control. While at 200 and 400 mg/kg, the survival times were significantly ($p < 0.05$) higher than the negative control and comparable to the positive control (Table 5). All the mice treated with BS extracts gave significantly ($p < 0.05$) higher values of survival times than the negative control but the values were comparable with that of the positive control (Table 5). The survival time elicited by MC at 100 mg/kg were comparable to both the positive and negative

controls, while the survival times at 200, 400 and 800 mg/kg were significantly ($p < 0.05$) higher than the negative control and comparable to the positive control (Table 5). The extract VA gave survival times at 100 mg/kg that was significantly of lower ($p < 0.05$) value than that of the positive control but comparable to that of the negative control, while at 200, 400 and 800 mg/kg, the survival times were comparable to that of the positive control but significantly ($p < 0.05$) higher than that of the negative control (Table 5).

Median Effective Doses (ED_{50} and ED_{90})

For the prophylactic antimalarial assay, BS and VA gave ED_{50} and ED_{90} values which were comparable to that of AV and MC. The ED_{50} and ED_{90} values of AV were significantly ($p < 0.05$) lower than the values of MC (Table 6).

For the chemosuppressive antimalarial assay, the ED_{50} values of all the extras were comparable. While ED_{90} values of AV and VA were comparable to that of BS and MC. The ED_{90} values of MC was significantly ($p < 0.05$) lower than the values of BS (Table 6).

For the curative antimalarial assay, the ED_{50} and ED_{90} values of MC and VA were significantly ($p < 0.05$) lower than the values of AV and BS (Table 6).

Table 4: Average Survival Time and Percentage Survivor of Mice in the Prophylactic Antimalarial Test of the Methanol Extract of Selected Plants

Doses (mg/kg)	Average Survival Time (days) $\pm$ SEM (% survivor)			
	AV	BS	MC	VA
NC	11.6 ± 4.68^a (40)	11.6 ± 4.68^a (40)	11.6 ± 4.68^a (40)	11.6 ± 4.68^a (40)
100	16.80 ± 6.86^a (60)	13.40 ± 5.52^a (60)	$15.40 \pm 5.41^{a,b}$ (40)	17.00 ± 6.74^a (60)
200	18.60 ± 5.93^a (60)	15.80 ± 6.34^a (60)	9.60 ± 5.16^a (40)	16.80 ± 6.86^a (60)
400	18.40 ± 4.79^a (80)	15.20 ± 6.22^a (60)	27.6 ± 0.40^b (80)	16.80 ± 6.86^a (60)
800	18.40 ± 4.79^a (80)	13.40 ± 5.31^a (60)	28.00 ± 0.00^b (100)	16.80 ± 6.86^a (60)
PYR	17.20 ± 6.62^a (60)	17.20 ± 6.62^a (60)	17.20 ± 6.62^a (60)	17.20 ± 6.62^a (60)

Keys: AV: *Anthocleista vogelii*; BS: *Blighia sapida*; MC: *Momordica charantia* and VA: *Voacanga africana*. Data show the mean $\pm$ SEM, $n = 5$, NC = Negative Control (Tween 80 in normal saline); PYR = Pyrimethamine (1.2 mg/kg). Only values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keuls' post hoc test).

Table 5: Average Survival Times and Percentage Survivor of Mice in the Curative Antimalarial Test of the Methanol Extract of the Selected Plants

Doses (mg/kg)	Average Survival Time (days) $\pm$ SEM (% survivor)			
	AV	BS	MC	VA
NC	9.50 ± 0.87^a (40)	9.50 ± 0.87^a (40)	9.50 ± 0.87^a (40)	9.50 ± 0.87^a (40)
100	13.80 ± 1.77^a (60)	21.60 ± 3.20^b (60)	$16.20 \pm 3.89^{a,b}$ (40)	11.80 ± 3.40^a (20)
200	19.20 ± 3.63^b (40)	20.25 ± 5.42^b (40)	22.60 ± 3.60^b (60)	21.80 ± 3.80^b (60)
400	20.40 ± 4.70^b (80)	25.00 ± 1.48^b (60)	19.80 ± 5.31^b (60)	22.40 ± 2.69^b (60)
800	11.20 ± 5.23^a (40)	26.20 ± 1.20^b (60)	26.60 ± 1.40^b (80)	24.50 ± 5.43^b (60)
CQ	25.20 ± 2.80^b (80)	25.20 ± 2.80^b (80)	25.20 ± 2.80^b (80)	25.20 ± 2.80^b (80)

Keys: AV: *Anthocleista vogelii*; BS: *Blighia sapida*; MC: *Momordica charantia* and VA: *Voacanga africana*. Data show the mean $\pm$ SEM, $n = 5$, NC = Negative Control (Tween 80 in normal saline); CQ = Chloroquine (10 mg/kg). Only values with different superscripts within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student-Newman-Keuls' post hoc test).

Table 6: Comparative ED₅₀ and ED₉₀ of the Selected Plants' Extracts under the Three Different Antimalarial Test Models.

EXTRACTS	Effective Doses (mg/kg) per Model Type					
	ED ₅₀			ED ₉₀		
	PRO	CHEM	CUR	PRO	CHEM	CUR
<i>Anthocleista vogelii</i> (Stem bark)	304.00 ± 13.65 ^a	240.45 ± 8.08 ^a	256.58 ± 3.88 ^c	624.26 ± 24.51 ^a	471.30 ± 16.40 ^{ab}	549.97 ± 7.95 ^c
<i>Bligha sapida</i> (Stem bark)	373.91 ± 15.10 ^{ab}	264.26 ± 10.94 ^a	243.05 ± 3.15 ^b	758.86 ± 26.93 ^{ab}	529.74 ± 19.68 ^b	503.77 ± 5.79 ^b
<i>Momordica charantia</i> (Leaf)	395.68 ± 26.20 ^b	243.78 ± 8.70 ^a	224.98 ± 1.57 ^a	782.89 ± 49.29 ^b	496.05 ± 19.48 ^a	450.32 ± 4.85 ^a
<i>Voacanga africana</i> (Stem bark)	365.52 ± 20.56 ^{ab}	253.34 ± 10.64 ^a	232.15 ± 2.67 ^a	738.91 ± 43.70 ^{ab}	491.97 ± 19.10 ^{ab}	477.71 ± 5.70 ^a

Keys: PRO: Prophylactic; CHEM: Chemosuppressive; CUR: Curative. Data show the mean ± SEM, $n = 5$. Only values with different superscripts (a, b or c) within columns are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' post hoc test).

Discussion

The endemic nature of malaria in Africa, coupled with the development of resistance to available orthodox antimalarial drugs has necessitated the continuous search for new molecules to replace or support the existing drugs. Medicinal plants that are used in the treatment of a variety of diseases including malaria abound all over the world [26]. Since the first antimalarial drug, quinine, was isolated from the stem-bark of *Cinchona succirubra* Pavon. a plant belonging to the family Rubiaceae [27], several *in vivo* and *in vitro* investigations of the antiplasmodial activities of medicinal plants, used in various cultures as antimalarial remedies, have been reported with adequate justification of their ethnomedical uses. Investigations on a considerable few of them like *Mangifera indica*, *Bauhinia manca*, *B. monandra*, *Detarium senegalense*, *Polygonatum verticillatum*, *Eucalyptus camaldulensis*, *Erythrina sigmoidea* etc. [28-35] had led to the isolation of their antimalarial constituents. Also, some morphological parts of medicinal plants such as the leaves, roots and stem-barks of some medicinal plants, containing active antimalarial principles have been reported to have antimalarial activities [36,37]. It is therefore not misplaced to search for antimalarial agents from stem-barks and other morphological parts of some medicinal plants. Thus, the stem-barks of *Anthocleista vogelii*, *Bligha sapida*, *Voacanga africana* and the leaf of *Momordica charantia*, ethnomedicinally used as antimalarial remedies, were selected with the aim of ascertaining and comparing their antimalarial potencies, and probably identify their most active and isolate or identify its chemical constituents. The median lethal doses (LD₅₀) of these herbs were assessed in order to obtain the appropriate working doses for their evaluation and their level of safety [38]. Also, a percentage yield data from the extraction would help future workers on the quantity of material for further work and so help in managing the flora effectively and also aid the conservation of medicinal plants that are gradually going extinct. A relative yield of 13.12, 20.08, 20.13 and 15.23% elicited by *Anthocleista vogelii*, *Bligha sapida*, *Voacanga africana* and the leaf of *Momordica charantia* respectively gives an idea of the quantities that may be collected by future workers.

The three models of antiplasmodial activities studies interrogated viz: prophylactic, chemosuppressive and

curative gave an idea of their respective relative potencies as antimalarial agents for appropriate model(s) as determined by their various parameters of percentage reduction in parasitaemia, chemosuppression and clearance respectively including effective doses, particularly the median effective doses, survival times and percentage survivor. The identification of the most effective plant out of the conglomerate was to push the work further intensively on the identified plant.

Acute toxicity study

Using Lorke's method to determine the safety, all the extracts, except *V. africana*, produced neither death, skin changes, aggressiveness, diarrhoea, restiveness, seizures, dizziness, weakness nor withdrawal from food or water at doses administered up to 5000 mg/kg implying their safety for the management of malaria and so freely used in ethnomedicine [22,39-43] while on the other hand, *V. africana* with LD₅₀ of 1264.91 mg/kg cannot be freely used as others but only within the limit of its safety. The different LD₅₀ elicited by each of these plant extracts compel toxicity studies on any of the tested plants especially those that are freely used in ethnomedicine. Non-toxic extracts may be tested at a minimum dose of 20 times lower than the LD₅₀ value [44] putting *V. africana*, at 63 and others at 250 mg/kg minimum testable doses. All were subsequently tested at 100, 200, 400 and 800 mg/kg. The LD₅₀ value of *V. africana*, also confirm the use of its bark as an arrow poison in ethnomedicine and also its fruit been considered to be poisonous [45].

In vivo antimalarial activities of the extracts

The three models of test to which the plant extracts were subjected, are classical methods for the preliminary *in vivo* screening of drugs with potential antimalarial activity [46]. *In vivo* antiplasmodial activities determinations of prophylactic, chemosuppressive and curative have been variously used to ascertain antimalarial potencies of medicinal plants in their various plant parts [47]. An extract that is able to reduce, suppress the multiplication of or clear malarial parasites in mice can be suspected to possess antimalarial activity [27]. Such identified activities of the tested plants against *Plasmodium* parasite vividly confirmed the various claims of their uses in ethnomedicine as antimalarial agents.

The tested plants exhibited various activities with the different models. This may be confirmed by their significantly different percentage parasitaemia from the negative control.

Prophylactic activities of the extracts

For the prophylactic model, the comparable ($p > 0.05$) percentage parasitaemia elicited by all the extracts to that of the positive control at 400 and 800 mg/kg except *M. charantia* which was comparable only at 800 mg/kg implied that the extract were probably active at relatively higher doses. It also indicates that plant extracts can be as active as the standard drugs but at higher doses. This is because the yet to be isolated constituents may be acting synergistically to effect the antiplasmodial actions in the extracts [48]. Also, all the extracts, displayed lower activities than positive control at lower doses of 100 mg/kg and 200 mg/kg except *V. africana* which had similar activity with positive control at 200 mg/kg (Table 4). Plants with relatively high activities at lower doses can be sources of promising antimalarial agents [49]. Voacamine, an ibogavobasine type alkaloid, isolated from *V. africana* showed significant *in vivo* and *in vitro* anti-malarial activities [16,17]. The relatively high percentage reduction in parasitaemia elicited by AV at all doses compared to the other plant extracts seem to confirm its higher prophylactic antimalarial activity. Methanol extract of AV gave 50% activity at the lowest dose and 74% at the highest dose compared to 36% and 68%, elicited by VA respectively and correspondingly. Though lower values of % reduction in parasitaemia were elicited by BS, it still gave comparable activities at lower and higher doses, whereas MC elicited its highest 62%, VA at 68% at the highest dose of 800 mg (Table 4). Of all the plant extracts, AV elicited significantly lower prophylactic ED_{50} and ED_{90} to become the most active plant extract among the four (Table 4). The order of prophylactic activity seems to be $AV > VA = BS > MC$. Leaf of *Momordica charantia* had the lowest prophylactic activity of the four plant extracts. The design of this study was similar to the earlier research reported by [50] on the *in vivo* antiplasmodial potentials of the combinations of four Nigerian antimalarial plants, where the extract *Nauclea latifolia* root gave the best prophylactic antimalarial activity.

Chemosuppressive activities of the extracts

For the chemosuppressive antimalarial test, each of the extracts displayed remarkable % parasitaemia significantly different ($p < 0.05$) from that produced by the negative control with AV and VA giving comparable values to that of the positive control at doses of 200, 400 and 800 mg/kg. BS and *M. charantia* gave significantly higher parasitaemia values at all doses tested with only MC at 800 mg/kg giving comparable values to the positive control. It seems that AV, VA and MC clearly showed better ability to suppress parasites than BS (Table 2). The percentage chemosuppression profile showed that only MC gave comparable activity to the positive control at the highest dose of 800 mg/kg. The very high chemosuppression of up to 60% displayed by the other

extracts at lowest dose of 100 mg/kg and up to 70% at higher doses indicate their very high chemosuppressive activities. Eventually, the effective doses indicate that all the extracts have similar chemosuppressive activities (Table 6). This may infer that all these extracts are strong chemosuppressive agents that may be used as antimalarial drugs barring toxicity that might be displayed especially by VA.

Curative activities of the extracts

The percentage parasitaemia displayed by each of the extracts on Day 4 for the curative test is as shown in Table 3. The positive control gave the lowest parasitaemia consistent with an orthodox drug used in treating malaria. Of all the extracts tested, AV and MC gave dose dependent reduction of parasitaemia while BS and VA was, up to 400 mg/kg which is an indication of a consistent clearance of the parasite in mice. MC gave the lowest value at the highest dose. The percentage clearance of the extract on the parasite on the 4th day revealed that even at the lowest dose of 100 mg/kg, the extracts showed significant clearance of the parasite from the blood of the mice up to 60% and the highest dose about 80% while chloroquine gave 90.3% clearance. Also, a progressive daily increase in the percentage clearance is noticeable in all the extracts, this is an attestation of the curative effectiveness of the plant extracts. Extracts with promising chemosuppressive and curative activities at the same time tend to be more effective in treating malaria than one with either activity exclusively [51].

Comparative median effective doses of the selected plants extracts

The effective doses (ED_{50} and ED_{90}) values have been used to describe and rank the relative antiplasmodial activities of crude extracts, partitioned fractions and isolated chemical constituents of medicinal plants [4]. ED_{50} is the dose of a drug that is pharmacologically effective in 50% of the population exposed to the drug or the dose that gives a 50% response in a biological system.

It can be expressed as the of a substance to a specific positive effect in half of the animal comprising a [52]. Most often, these values are estimated from a graph of dose against % reduction in parasitaemia, % chemosuppression or % clearance in the prophylactic, chemosuppressive and curative antiplasmodial experimental models, respectively. For the purpose of this work, they are the respective doses that will reduce the parasitaemia levels of the untreated mice by 50 and 90%, respectively under standard experimental conditions.

Extract AV which elicited a comparatively lower ED_{50} in the prophylactic test of the three and also which gave comparable ED_{90} with the other three (Table 6) is the most active for that model. For the chemosuppressive model, the ED_{50} and ED_{90} values were all comparable for the four extracts (Table 6) implying that they all have similar chemosuppressive activity. For the curative test MC and VA were most active having



elicited comparable values but BS which is next in activity was significantly the least active. In summary, AV is the most active prophylactic drug, MC and VA are the most active curative drugs while any of the four could easily go for the most active chemosuppressive drug.

Survival time and percentage survivors of mice

The survival time, elicited by an extract, partitioned fraction or isolated chemical constituents of a medicinal plant in mice, is the interval of time between drug administration and death of the mice. It is usually recorded in days $\pm$ SEM and in form of average in a group. The percentage survivor in the same group is the percentage of mice in the group whose survival time falls within the average for the group. Both parameters could be used to determine whether the extract, partitioned fraction or isolated chemical compounds administered to mice in a group can prolong its life beyond that of the negative control drug. They may also be used as an index of relative potency of an extract; partitioned fractions or isolated compounds being investigated for antimalarial activities [53].

Recrudescence which is the reappearance or resurgence of a disease after a supposed period of remission could be expressed by the level of percentage survivors in mice (Popovici, et al., 2019). Recrudescence is capable of reducing the survival of mice in the antiplasmodial experiment and could take its toll on the effectiveness of the tested extract, fraction or isolate. The percentage parasitaemia reduction has often been correlated with percentage survivor rather than with survival time in many previous studies [54-56], probably because the mice was presumed to die or survive on the basis of the residual parasite after being cleared, reduced or suppressed by the drug which has better correlation rather than just reckoning on the number of days in which they survived [57].

For the prophylactic test, though each the four plant extracts (AV, BS, MC and VA) and the standard drug elicited the survival time that elicit comparable ($p > 0.05$) values to that of the negative control, only MC gave relatively higher percentage survivor at the higher doses (400 and 800 mg/kg) tested which slightly corresponded with reduction in parasitaemia (Table 4). The ability of MC to reduce parasite significantly must have enhanced its percentage survivor to 100 at the highest dose. The extracts of BS and VA elicited a value of 60 at all the doses tested, though AV elicited similar at 100 and 200 mg/kg only with relatively higher values of percentage reduction in parasitaemia.

For the curative test, the extracts of BS and MC gave significantly higher values of survival times at all doses than the negative control which were at the same time similar to that of the positive control. Particularly, MC elicited survival time value of 26.60 ± 1.40 at 800 mg/kg that was higher than that of the positive control (25.20 ± 2.80) and a percentage survivor of 80 at 800 mg/kg with AV at 400 mg/kg which happened to be the maximum in the test and similar to that of the positive control (Table 4).

Summary and conclusion

In this comparative study, AV is the most active prophylactic drug, MC and VA are the most active curative drug while any of the four could easily go for the most active chemosuppressive drug. The combined prophylactic and chemosuppressive activities of AV, just as combinable curative and chemosuppressive activities of MC or VA and the preponderance of chemosuppressive activities of BS not only showcases these plants as potential candidate for combination in a herbal remedy but also potential for isolation of active antimalarial compounds. However, AV was chosen as the most active because its lower nominal chemosuppressive ED_{50} value was within the values for the curative activities of the other extracts in addition to its high and better chemosuppressive activities.

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