J Herbmed Pharmacol. 2020; 9(4): 374-381.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2020.47

Journal of Herbmed Pharmacology

# *In vitro* and *in vivo* antiplasmodial assays of selected Nigerian commercial herbal formulations

**Chinedu Joseph Ikem[1,2], Regina Appiah-opong[3], Angus Nnamdi Oli[2], Malachy Chigozie Ugwu[2], Patrick Amoateng[4], Kojo Agyemang[4], David Chinemerem Nwobodo[2,5], Charles Okechukwu Esimone[2]**

[1]Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, Madonna University, Elele, River State, Nigeria
[2]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria
[3]Department of Clinical Pathology, Noguchi Memorial institute for Medical Research, College of Health Sciences, University of Ghana, Ghana
[4]Department of Pharmacology & Toxicology, School of Pharmacy, College of Health Sciences, University of Ghana, Ghana
[5]Department of Microbiology, Renaissance University, Enugu, Nigeria

**ARTICLE INFO**

***Article Type:***
Original Article

***Article History:***
Received: 12 December 2019
Accepted: 5 February 2020

***Keywords:***
Antiplasmodial activity
Herbal formulations
Malaria
SYBR Green
*Plasmodium falciparum*
*Plasmodium berghei*

**ABSTRACT**

**Introduction:** Malaria remains a life-threatening disease, mainly in tropical and sub-tropical countries of the world. The problem caused by the disease is further compounded by the emergence and spread of multidrug resistant *Plasmodium falciparum*. Coupled with the poor distribution of modern health facilities, there is resurgence in the use of herbal remedies to treat malaria. In this study, we evaluated the antiplasmodial activities of six commercially available herbal formulations using *in vivo* and *in vitro* methods to assess their claimed antimalarial properties.

**Methods:** The antiplasmodial activities of the six herbal formulations were assessed using Chloroquine sensitive *P. falciparum* parasite strain 3D7 using the SYBR Green *in vitro* method and the *in vivo* curative test (established infection) in *Plasmodium berghei* infected *Mus musculus*.

**Results:** The six herbal formulations had values of $IC_{50}$ > 100 µg/mL on 3D7 strain of *P. falciparum* compared to controls which had $IC_{50}$ values of 6.92nM (Chloroquine) and 0.75nM (Artesunate). In the curative evaluation (*in vivo*) the herbal formulations significantly reduced parasitaemia on day 4 (26.3%-77.3 %) and day 7 (45.54%-94.81%) post-treatments ($P<0.05$) when compared to the untreated group, which recorded high mortality rate.

**Conclusion:** Findings made in this study lend support to the claim that these herbal formulations have antiplasmodial activities. Percentage inhibitions of parasitaemia of the formulations were all above 50% except M&T capsule which had lower percentage inhibition of parasitaemia.

*Implication for health policy/practice/research/medical education:*
The tested formulations could serve as good antiplasmodial therapy and the constituents as lead for the discovery of novel antiplasmodial compounds.

*Please cite this paper as:* Ikem CJ, Appiah-opong R, Oli AN, Ugwu MC, Amoateng P, Agyemang K, et al. *In vitro* and *in vivo* antiplasmodial assays of selected Nigerian commercial herbal formulations. J Herbmed Pharmacol. 2020;9(4):374-381. doi: 10.34172/jhp.2020.47.

## Introduction

Malaria remains a major public health problem especially in tropical and sub-tropical countries of the world (1-3). This acute infection is caused by five species (*vivax, falciparum, ovale, malariae* and *knowlesi*) of the protozoan genus *Plasmodium*. However, *P. falciparum* causes the most serious complications and significant number of deaths. The parasite is transmitted to humans through the bite of the female anopheles mosquito which thrives in humid, swampy areas and breeds in stagnant water (4). In 2017, nearly 50% of the world's population were at risk of the disease (3). An estimated 1.2 billion people are at high risk of transmission (≥1 case per 1000 population), half of which live in the African regions; 80% of such cases are concentrated in 13 countries, and over half in Nigeria, Congo, Ethiopia, Tanzania and Kenya (5). So, Africa has the highest incidence rate of malaria disease and the highest death rate in the world. This is attributed to the

***Corresponding author**: Angus Nnamdi Oli,
a.n.oli@live.com

prevalence of *P. falciparum* and the effective mosquito vector, *Anopheles gambiae*, in Africa (6). Mortality has risen in recent years, probably due to increasing resistance to antimalarial medicines (7,8).

One of the major challenges faced in the fight against malaria is the ability of the most virulent causative agent, *P. falciparum*, to develop resistance to antimalarial drugs and how quickly the resistance spread. In some parts of the world, including Nigeria, chloroquine (CQ) and sulphadoxine-pyrimethamine (SP) are no longer considered drug of choice for treating malaria due to their recorded high failure rate (9,10). Although vaccines are anticipated to be the best long-term control option, they are still undergoing clinical trials (11-15).

Interestingly, most malaria endemic countries are found in areas of the world whose populations still rely heavily on traditional medicines of plants' origin for their primary healthcare needs. Due to factors such as: high cost of western medicine, the common belief that medicines of plant origin are cheap, easily available, safe and effective (16,17); several antimalarial formulations of plants origin have flooded the Nigerian market. It seems logical then to assess the therapeutic claims of these formulations. In view of the general acceptability of the selected herbal formulations, this study was set to assess their therapeutic claims using both *in vivo* and *in vitro* methods and compare their effects with standard antimalarial drugs. According to ethno-botanical survey in Nigeria (18), it was reported that some 98 species from different families of plants are used in traditional medicine singly or in combination to treat malaria infection. These plants used for malaria treatment or prevention represent more than half of Nigeria medicinal species (19).

Till date, no data are available for validating the antimalarial use of these herbal formulations (Ruzu bitters, Deep root, African Iba, M&T capsule and Blood purifier) but most of the extracts have been used singly for assessment of antimalarial properties *in vitro* and/or *in vivo* (20,21). *Alstonia bonnie*, *Markhamia tomentosa*, *Moringa oleifera*, *Mangifera indica*, *Cymbopogon citratus*, *Psidium guajava* which have different species widely distributed in Africa, Asia and America have been reported to be effective in treatment of several diseases including Malaria (19,22-24). In this study, the antiplasmodial activities of six commercially available herbal formulations were studied using *in vivo* and *in vitro* methods to assess their claimed antimalarial properties.

*Mus musculus* – the animal model chosen for this study- provides an easy to manage, cheap and excellent animal model for human malarial which cell-based studies do not offer at the moment. Also, cell-based study does not offer the physiological environments which animal model offers. Environmental factors can readily be controlled in *M. musculus* compared to human studies. These obvious factors informed our choice of *M. musculus* as a model animal for the study.

## Materials and Methods

### *Plasmodium* parasites

The *P. falciparum* strain 3D7 used for the *in vitro* study (chloroquine sensitive parasite) was obtained from Immunology Department, Noguchi Memorial Institute for Medical Research, Legon, Accra, Ghana and chloroquine-sensitive strain of *P. berghei*, maintained in mice, from the National Institute of Pharmaceutical Research and Development, Nigeria. The parasite was subsequently inoculated into fresh mice, which served as donor mice in this study.

### Drug extraction/preparation

Six herbal formulations were obtained from commercial drug markets in Anambra State, Nigeria. Twenty millilitres (20 mL) of each herbal formulation was measured and dispensed into Eppendorf tube and stored in the freezer at -20°C. The herbal formulations were removed and placed in a freeze dryer (Labconco FreeZone 6, USA) with reduced pressure of 12 mbar at -40°C to remove all moisture for complete dryness. The dry extracts were weighed using electronic weighing balance, transferred into Eppendorf tubes and stored for further analysis.

### Herbal formulation preparation

Stock concentrations of 50 mg/mL of each extract of the 6 herbal preparations (Ruzu Bitters, Deep Root, Chuka Trin Cleanser, Blood Purifier, M&T Capsule and African Iba), as well as chloroquine and artesunate were prepared in 50% ethanol and filter-sterilized through a 0.22 µm membrane (Millipore) filter. The extracts, artesunate and chloroquine stocks were diluted to 2000 µg/mL, 630nM and 1000nM, respectively in complete parasite medium and further diluted three-fold serially into six different concentrations for the assays with extracts, chloroquine and artesunate (final concentrations, 0.41–100 µg/mL, 0.41–100nM and 0.03–6.3nM, respectively). Both chloroquine and artesunate served as the positive controls.

### *In vitro* culture of *Plasmodium falciparum*

The chloroquine-sensitive *P. falciparum* strain 3D7 was cultured and maintained as described in earlier studies with slight modification (17,25). The parasites were cultured in a complete media consisting of RPMI 1640 (Sigma) supplemented with 1% L-glutamine, 25mM HEPES, 0.2% sodium bicarbonate, 0.5% Albumax II, 100µM hypoxanthine, and 1% gentamycin and incubated at 37°C. The parasites were cultured in fresh $O^+$ human erythrocytes at 4% hematocrit. Estimation of parasitaemia was done using Giemsa stain and visualization performed under the normal light microscope (X100) using oil immersion.

The antiplasmodial activity of the six herbal formulations

was assessed against *P. falciparum*: 3D7 (chloroquine-sensitive) strain *in vitro* using SYBR® Green assay. The activity of the extract was measured over the six concentrations prepared. All experiments were performed in triplicates. At least two independent experiments were performed.

The SYBR green I-based fluorescence antiplasmodial assay as described by Leidenberger et al (26) was used for the activity screening of the extracts and drugs. Sorbitol synchronized parasites were incubated with the extracts and drugs (100 µL final volume) under culture condition as described above at 2% hematocrit and 1% parasitaemia. Parasites without drug treatment were used as negative controls while the wells containing chloroquine and artesunate were the positive controls. The plates were covered and shaken slightly to ensure a thorough mixing. The cultures were incubated for 72 hours. After incubation, aliquots of 100 µL of SYBR GREEN lysis buffer containing 20mM Tris-Cl (pH 7.5), 5mM EDTA, 0.008% saponin, 0.08% triton-X 100 and 1X SYBR green I (10 000X in DMSO) were added. The plates were shaken gently and incubated in the dark for 3 hours. The fluorescence in each well was read using a Tecan fluorescence (Tecan Infinite M200, Austria) multi-well plate reader at excitation and emission wavelengths of 485 and 530nm, respectively. The experiments were performed in triplicate and each repeated at least once. The intensities of the fluorescence signals were plotted against the extracts or drug concentrations to obtain a concentration response curve. The curves were analysed to determine 50% inhibitory concentrations ($IC_{50}$) of the drugs.

### *In vivo* Assay

#### *Experimental animals*

Forty albino mice –species: *M. musculus* (20-26 g, 5-6 weeks old) of both sexes, caged separately, were tested separately with extracts and controls. The animals were in-bred by the principal investigator and when fully grown, they were taken to the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Agulu Nnamdi Azikiwe University in Anambra State for the experimental procedures. They were first housed in pathogen-free metal cages (n=16) for 7 days to acclimatize under standard environmental conditions of temperature: 26 ± 2°C, relative humidity: 45 ± 2% and 12 hours natural dark-light cycles before the experimentation. Sixteen cages (same sex per cage) were chosen to prevent mating. These standard environmental conditions were maintained throughout the experimental periods. During the acclimatization period and throughout experimental periods, the animals were handled in accordance with established guidelines (27-29) for care and use of laboratory animals. The study protocols were approved by the Proposal/Ethics Committee for animal studies of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka Nigeria.

#### *Induction of infection*

After 7 days acclimatization period, standard inoculums of $1\times10^5$ *P. berghei* infected *M. musculus* erythrocytes were prepared by diluting the infected *M. musculus* blood with 0.9% normal saline such that 0.2 mL of the blood contained approximately $1\times10^5$ infected erythrocytes. A 0.2 mL of the diluted blood was injected intraperitoneally into each experimental animal on the first day.

#### *Malarial curative test (schizonticidal activity in established infection)*

The evaluation of the curative potential of the herbal formulations on established malarial infection was evaluated using the method described by Fidock et al (30) and modified by Tarkang et al (31). The animals were first placed into 8 groups of each sex with 5 animals per group and after 72 hours post-infection; the treatment began with oral administration of single daily dose and lasted for 3 days (parasitemia was checked before commencement of treatment). Oral route was chosen to mimic the natural means of administration of the herbal formulation in human. All the animals were allowed free access to feed and clean water throughout the experimental period. Group 1 animals (serving as the negative control) were administered 0.2 mL of Distilled Water (DW); Group 2 (the positive control) was given artemether/lumefantrine (AL) combination (20 mg/120 mg) at 0.04 mg/kg body weight; Group 3 received 0.2 mg/kg body weight Ruzu Herbal Mixture; Group 4 received 0.36 mg/kg body weight of Chuka trin Cleanser; Group 5 received 0.47 mg/kg body weight of African Iba Herbal Mixture; Group 6 received 0.52 mg/kg body weight of Deep root Herbal Mixture; Group 7 received 0.5 mg/kg body weight of Blood purifier Herbal Mixture while group 8 received 0.7 mg/kg body weight of M&T Herbal capsule. Oral route was used for the administration of the drugs (controls and tests). The doses were determined from the dose equivalent for a 70 kg man, using the weight and surface area of the animals (32).

$$Dose\ equivalent = \frac{Weight\ of\ Mus\ musculus\,(g)\times\ Human\ dose}{Human\ weight\,(g)\times 0.081}$$

Animals' allocation into groups was such that the mean weights of the groups were equal (or near equal) and there were approximately equal number of each sex per group. The administration of the herbal drugs and results recording were all done by the investigator. The animals were allowed free access to their feed and water throughout the period of the study. All treatments were carried out in the animal house in the morning periods. On days 4 and 7 post-treatment, blood samples were collected from the

tip of the tail of each lab animal for the preparation of thin smears. The smears were prepared, fixed with methanol, and stained with 10% Giemsa solution at pH 7.2 for 25 minutes and examined under microscope with an oil immersion objective (×100 magnification power).

All the animals used in the study were observed for 30 days for signs of morbidity and for mortality.

### *Parasitaemia monitoring*

Parasitaemia was determined by collecting blood sample from the tail on day 0 (before treatment), day 4 and day 7 post treatment. Parasitaemia was assessed by thin blood films made by collecting blood from the cut tip of the tail, dry blood films were fixed with methanol for 15 seconds and subsequently stained with 10% Giemsa for 25 min. They were then washed with water and allowed to dry. The slides were then microscopically examined using ×100 magnification in oil immersion. Triplicate experiments were performed. The level of parasitaemia was determined by counting the number of parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope.

The percentage parasitaemia was determined using the following equation (33):

$$\%Parasitaemia = \frac{Total\ number\ of\ parasitized\ erythrocytes}{Total\ number\ of\ erythrocytes}$$

For the *in vivo* study, the mean percentage parasitaemia was used for each treatment group in the basal and corresponding days, and the percentage inhibition was also calculated thus (33);

$$\%\ Inhibition\ of\ activity\ = (100 - \frac{a}{b} \times 100)$$

Where 'a' is the mean percentage parasitaemia in the corresponding days (day 4 and 7) of the treatment groups and 'b' is the mean percentage parasitaemia in the basal day (day 0) of the treatment groups.

Fifty percent inhibitory concentrations ($IC_{50}$) for the *in vitro* study were obtained from plots of extract concentration versus percentage inhibition.

### Statistics and data analysis

The results were presented as the mean ± SEM (standard error of mean) for each group of experiments. The test groups were compared with the negative control group using one-way analysis of variance (ANOVA). All data were analysed at a 95% confidence interval using GraphPad Prism 3 Software, San Diego, California, USA. *P* values less than 0.05 were considered statistically significant. The $IC_{50}$ values were obtained from the log-linear regression analysis of log-dose response curves using the GraphPad Prism 3.

## Results

Table 1 shows the Latin binomial nomenclature of the constituents of the herbal formulations that were investigated.

The results of the *in vitro* antiplasmodial assay (Table 2) showed that the six herbal formulations had $IC_{50}$ values of > 100 µg/mL on 3D7 strain of *P. falciparum*. The products were considered less potent and had low percentage inhibition of parasitaemia when compared with the controls, chloroquine and artesunate, with $IC_{50}$ values = 6.92nM and 0.75nM, respectively.

Effect of the herbal medicines on uninfected human red blood cells (RBCs) is also shown on Table 2. None of the extracts could inhibit the viability of untreated RBCs, suggesting that they are not harmful to the cells.

### Results of *in vivo* curative activity using mice infected with *Plasmodium berghei*

Prior to treatment, the animals were in good health status as evidenced by their agility and good feeding habit and their mean age was 24 ± 0.63 days. Mean parasitaemia of the *M. musculus* was of the range 9.05 % ± 0.37 to 15.90 % ± 1.08 for day 0 (Basal parasitaemia), 1.60 % ± 0.44 to 8.25 % ± 0.77 for day 4 post treatment and 0.55 % ± 0.18 to 6.11 % ± 0.76 for day 7 post treatment (Table 3). The mean parasitaemia in Artemether and Lumefantrine (AL) group (positive controls) were 10.65 %±0.9, 1.00 % ± 0.14 and 0.4 % ± 0.20 for days 0, 4 and 7 post treatment, respectively showing good curative effect of the drug while the mean parasitaemia of untreated (negative) control were 16.45 %

**Table 1.** Botanical constituents of the commercial herbal formulations

| Herbal products | Active ingredients |
|---|---|
| Ruzu bitters | *Curculigo pilosa* (root) 40%, *Uvaria chamae* (stem) 20%, *Citrullus colocynthis* (bark) 40%. |
| Deep root herbal mixture | *Cymbopogon citratus* 13%, *Carica papaya* leaves 12%, *Magnifera indica* bark 11%, *Moringa Oleifera* leaf 11%, *Citrus limon* 9%, *Psidium guajava* 9%, *Zingiber officinale* root 9%, *Allium sativa* 6%, Water. |
| Chuka Trin Cleanser | *Moringa oleifera 100%*, water |
| Blood purifier | *Aloe barbadensis* 15%, *Xylopia aethiopica* 20%, *Gongronema latifolia* 22%, *Dichrostachys cinerea* 17%, Water. |
| M&T malaria cap | *Markhamia tomentosa* 65%, *Alstonia congensis* 35%. |
| African iba | *Kigelia Africana* 17%, *Nauclea latifolia-linn* 43%, Water. |

**Table 2.** The Effect of the herbal medicines on *Plasmodium falciparum* 3D7 strain and on uninfected red blood cells

| Herbal formulations/Drugs | | Ruzu Bitters | Deep Root | Chuka Trin Cleanser | Blood Purifier | M&T Capsule | African Iba | Chloroquine | Artesunate |
|---|---|---|---|---|---|---|---|---|---|
| Inhibitory effects on *P. falciparum* 3D7 strain | $IC_{50}$ (µg/mL) | >100 | >100 | >100 | >100 | >100 | >100 | 6.92 | 0.075 |
| Effects on uninfected red blood cells | $CC_{50}$ (µg/mL) | >100 | >100 | >100 | >100 | >100 | >100 | >100 | ND |

ND: Not determined; $CC_{50}$: Concentration of extracts /drugs which causes 50% cytotoxic effect.

**Table 3.** Parasitaemia after treatment with extracts

| Treatment group | Dose (mg/kg) | Basal parasitaemia | Day 4 post treatment | Day 7 post treatment |
|---|---|---|---|---|
| DW (Negative control) | 0.02 | 10.50 ± 0.63 | 16.45 ± 0.85 | 19.85 ± 1.19 |
| AL (Positive control) | 0.04 | 10.65 ± 0.90 | 1.00 ± 0.14* | 0.40 ± 0.20* |
| Ruzu bitters | 0.20 | 15.90 ± 1.08 | 3.60 ± 0.87* | 1.55 ± 0.56* |
| Chuka Trin cleanser | 0.36 | 10.10 ± 1.23 | 5.00 ± 0.96* | 3.30 ± 0.52* |
| African Iba | 0.47 | 9.40 ± 0.65 | 4.25 ± 0.62* | 4.00 ± 0.32* |
| Deep root | 0.52 | 10.60 ± 0.52 | 1.60 ± 0.44* | 0.55 ± 0.18* |
| Blood purifier | 0.50 | 9.05 ± 0.37 | 5.00 ± 1.09* | 2.45 ± 0.54* |
| M & T capsule | 0.70 | 11.20 ± 0.63 | 8.25 ± 0.77* | 6.11 ± 0.76* |

DW: Distilled water, AL: Artemether 20 mg + Lumefantrine 120 mg.
Data are expressed as mean ± SEM.
* Significant difference in value ($P$<0.05) compared with untreated group.

± 0.84 and 19.85 % ± 1.19 showing significant increase in mean parasitaemia in this group. Within the 30 days of observation, all untreated animals died while there was no death in the treatment group animals.

On days 4 and 7 post treatment, the % change in parasitaemia was higher in Deep root than all the other herbal formulations (Table 4). This change was comparable to the positive control. On the other hand, M&T capsule recorded the lowest change in parasitaemia on both days. Generally, the percentage inhibition was observed to increase with increase in days of administration of the herbal formulation. It was observed that the animals administered M&T capsule and those given African Iba developed sluggish movement on the 29th and 30th day respectively.

## Discussion

This study utilised both *in vitro* and *in vivo* methods to assess the effectiveness of six herbal products in controlling malarial infection. A combination of *in vitro* and *in vivo* studies may help show if the herbal products and/or their metabolites are responsible for the observed antimalarial actions. Also, instead of taking the blood samples of the animals immediately, the study refined the method by first anaesthetising the animals with isoflurane cotton balls to minimize pain and distress.

The six herbal formulations did not exhibit inhibitory activity against chloroquine sensitive *P. falciparum* 3D7 strain ($IC_{50}$ > 100 µg/mL), according to the criteria described by Kamaraj *et al.* (34). Results of earlier *in vitro* and *in vivo* investigations into anti-plasmodial activities

**Table 4.** Percentage change in post treatment parasitaemia inhibition

| Treatment drug | Compositions | Dose (mg/kg) | Day 4 (%) | Day 7 (%) |
|---|---|---|---|---|
| DW | Distilled Water (Negative Control) | 0.2 mL | 0 | 0 |
| AL | Artemether 20 mg + Lumefantrine 120 mg - (Positive Control) | 0.04 | 90.61 | 95.87 |
| Ruzu bitters | *Curculigo pilosa, Uvaria chamae, Citrullus colocynthis* | 0.20 | 77.30 | 90.25 |
| Chuka Trin cleanser | *Moringa oleifera*, water | 0.36 | 50.49 | 67.33 |
| African Iba | *Kigella africana, Nauclea latifolia* Linn, water | 0.47 | 54.79 | 57.54 |
| Deep Root Herbal Mixture | *Cymbopogon, citratus, Carica papaya, Mangifera indica, Moringa oleifera, Citrus limonia, Psidium guajava, Allium sativa, Zingiber officinale*, water | 0.52 | 84.91 | 94.81 |
| Blood purifier | *Aloe barbadenis, Xylopia aethiopica, Gongroneria latifolium, Dichrostschys cinerea*, Water | 0.50 | 44.75 | 72.93 |
| M&T capsule | *Markhamia tomentosa, Alstonia congensis* | 0.70 | 26.34 | 45.54 |

of some other medicinal plant extracts showed varying comparisons between the $IC_{50}$ values for chloroquine -sensitive 3D7 strain of *P. falciparum* (23,35,36). Some of the constituents of the test herbal formulations have been reported to show *in vitro* antimalarial activity individually (37,38). The undetected inhibitory activity in this study could be due to the extracts acting as pro-drugs or the constituent of the mixtures of the medicines diminishing the effects of other active compounds. It is therefore advisable to investigate the individual components and the interactions between two herbal extracts before combining them into a co-formulation. Further studies should also be performed to determine possible pro-drug action of the medicines since *in vivo* assays showed that they possessed varying antiplasmodial activity against *P. berghei*. None of the extracts inhibited viability of untreated RBCs. This suggests that they are not cytotoxic to the cells. It has been documented that herbal medicines with $CC_{50}$ (µg/mL) values > 100 are non-toxic to RBC (34,39).

The *in vivo* studies, however, revealed that the herbal formulations were active as % parasitaemia was reduced at least by over 50% in most of the products on days 4 and 7. Deep root formulation exhibited the best reduction in parasitaemia, i.e. 84.91% and 94.81% on days 4 and 7, respectively. This activity is comparable with that the positive control, artemether and lumefantrine, which exhibited 90.61 % and 95.87 % reduction on days 4 and 7, respectively. This could be attributed to the combination of the extracts from plants with known antiplasmodial activity. Although, there has been no previous study on the antimalarial activities of these herbal formulations, many of the medicinal plants that constitute the herbal formulations have been individually reported to exhibit diverse pharmacological actions (35,40,41). *M. indica*, *C. citratus*, *P. guajava*, have all been reported to possess good antimalarial properties *in vivo* (24,35,42,43).

Similarly, a good *in vivo* antiplasmodial activity was observed with Ruzu bitters formulation, with percentage parasitaemia reduction of 77.36% and 90.25% on day 4 and 7, respectively. This could be linked to the presence of *Carica papaya*, *Uvaria chamae* and *Morinda lucida* which have all been reported to exhibit good antiplasmodial activity *in vivo* (18,35,43,44). A similar result has been reported by Ihekwereme et al (33) that indicated high % parasitaemia reduction by fruit pulp of *Chrysophyllum albidum (Sapotaceae)* against *P. berghei in vivo* using mouse model. The results obtained are indicative of the curative potential of these herbal formulations. The lowest antiplasmodial activity was observed in M&T formulation with % parasitaemia reduction of 26.34%, and 45.54% on days 4 and 7 post treatment, respectively. These values are below the 50%, thus regarded as demonstration of poor parasitaemia reduction. This could be as a result of the fewer antiplasmodial constituents of the herbal formulation. Chuka Trin Cleanser, with *M. oleifera* and water as the sole constituents and having % parasitaemia reduction of 50.5%, and 67.3% on days 4 and 7 post treatment, may show that *M. oleifera* has appreciable antiplasmodial property. Other researchers (19,22,45) have also proved the antimalarial property of the plant. This investigation has demonstrated that the Deep root and Ruzu bitters herbal formulations have very strong inhibitory effect against *P. berghei* in *M. musculus*.

Our results lend support to the claims of the traditional medicine practitioners that these herbal formulations, which are used in traditional medicine practice against malaria, possess significant anti-malarial potential and justify their use in traditional medicine. The *in vitro* assays showed that all herbal formulations had no inhibitory effect on chloroquine sensitive *P. falciparum* strain 3D7. The difference in activity between the *in vivo* and *in vitro* assays may suggest that these herbal formulations act as pro-drugs. In this case, these precursors of the active compounds have to be metabolized *in vivo* into active antimalarial drugs.

## Conclusion

The results of this study confirmed that the six herbal formulations possess antimalarial properties with good curative activity against *P. berghei* (*in vivo* assay), which is the animal model of the human *P. falciparum*. Percentage inhibitions of parasitaemia of the formulations were all above 50% except M&T capsule which had low percentage inhibition of parasitaemia.

## Limitations

First, our study did not include the identification of the active principles in these herbal formulations. Secondly, neither mechanistic nor molecular bases of the observed antiplasmodial effects were investigated. Also, comprehensive safety profile in an animal model at the therapeutic dosage was not carried out. These limitations not-withstanding, this study offers scientific information on the usefulness of the formulations in treating human malarial infection.

## Acknowledgements

Authors wish to acknowledge West African research Association (WARA), which graciously provided the travel grant used for the *in vitro* aspects of the work

## Authors' contributions

COE and RA conceptualized and designed the study. CJI, PA and KA did the laboratory investigations (experiments), CJI and ANO drafted the manuscript and did data analysis and interpretation; RA, DCN and MCU revised the manuscript for important intellectual content and assisted in literature search. All authors read and approved the final manuscript.

## Conflict of interests

There is no competing interest to declare. The funders were not involved in the writing, editing, approval or decision to publish this manuscript.

## Ethics considerations

The study protocols were approved by the Proposal/ Ethics Committee for animal studies of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka Nigeria. Approval Number: FPhS/AEC/Vol.1.002

## Funding/Support

The *in vitro* part of this work was made possible due to the Travel Grant graciously given to CJI by West African research Association (WARC Travel Grant Spring 2016). The *in vivo* study did not receive sponsorship from external agent but rather was self-sponsored. Funder has no influence whatsoever in the design and execution of the study, analysis and interpretation of data, in the writing of the manuscript nor in the decision to publish the work.

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