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

Chinedu Joseph Ikem1,2 ID, Regina Appiah-opong3 ID, Angus Nnamdi Oli4 ID, Malachy Chigozie Ugwu2 ID, Patrick Amoateng5 ID, Kojo Agyemang4 ID, David Chinemerem Nwobodo2,5 ID, Charles Okechukwu Emione2 ID

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

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

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 IC50 > 100 µg/mL on 3D7 strain of P. falciparum compared to controls which had IC50 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 antimalarial 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 antiprotozoal 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
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 antimalarial use of these herbal formulations (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. Both chloroquine and artesunate 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.

### Drug extraction/preparation

Stock concentrations of 50 mg/mL of each extract of the herbal formulations were obtained from commercial drug markets in Anamba State, Nigeria. Twenty milliliters (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 \textit{P. falciparum}: 3D7 (chloroquine-sensitive) strain \textit{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 (10000X 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) multiwell 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.

\textbf{In vivo Assay}

\textbf{Experimental animals}

Forty albino mice – species: \textit{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.

\textbf{Induction of infection}

After 7 days acclimatization period, standard inoculums of 1×10$^6$ \textit{P. berghei} infected \textit{M. musculus} erythrocytes were prepared by diluting the infected \textit{M. musculus} blood with 0.9% normal saline such that 0.2 mL of the blood contained approximately 1×10$^6$ infected erythrocytes. A 0.2 mL of the diluted blood was injected intraperitoneally into each experimental animal on the first day.

\textbf{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).

\begin{equation}
\text{Dose equivalent} = \frac{\text{Weight of Mus musculus} \times \text{Human dose}}{\text{Human weight}} \times 0.081
\end{equation}

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
erthrocytes out of 200 erythrocytes in random fields of
the microscope.
The percentage parasitaemia was determined using the
following equation (33):

\[
\text{% Parasitaemia} = \frac{\text{Total number of parasitized erythrocytes}}{\text{Total number of erythrocytes}}
\]

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):

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

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\textsubscript{50}) for
the in vitro study were obtained from plots of extract
centration 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 us-
ing 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\textsubscript{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\textsubscript{50} values of
> 100 µg/mL on 3D7 strain of \textit{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\textsubscript{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
\textit{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 \textit{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 congesta 35%.                                    |
| African Iba                | Kigelia Africana 17%, Nauclea latifolia-linn 43%, Water.                           |
± 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 *Plasmodium 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 2. The Effect of the herbal medicines on *Plasmodium falciparum* 3D7 strain and on uninfected red blood cells

| Herbal formulations/Drugs | IC$_{50}$ (µg/mL) | CC$_{50}$ (µg/mL) |
|---------------------------|-------------------|------------------|
| Ruzu Bitters              | >100              | >100             |
| Deep Root                 | >100              | >100             |
| Chuka Trin Cleanser       | >100              | >100             |
| Blood Purifier            | >100              | >100             |
| M&T Capsule               | >100              | >100             |
| African Iba               | >100              | >100             |
| Chloroquine               | 6.92              | 0.075            |
| Artesunate                | ND                | 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.
Antiplasmodial herbal formulations

of some other medicinal plant extracts showed varying comparisons between the IC\textsubscript{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 \textit{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 \textit{in vivo} assays showed that they possessed varying antiplasmodial activity against \textit{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\textsubscript{50} (µg/mL) values > 100 are non-toxic to RBC (34,39).

The \textit{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 of 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). \textit{M. indica}, \textit{C. citratus}, \textit{P. guajava}, \textit{Uvaria chamae}, \textit{Morinda lucida}, have all been reported to possess good antimalarial properties \textit{in vivo} (24,35,42,43).

Similarly, a good \textit{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 \textit{Carica papaya}, \textit{Uvaria chamae} and \textit{Morinda lucida} which have all been reported to exhibit good antiplasmodial activity \textit{in vivo} (18,35,43,44). A similar result has been reported by Ihekwere et al (33) that indicated high % parasitaemia reduction by fruit pulp of \textit{Chrysophyllum albidum} (Sapotaceae) against \textit{P. berghei} \textit{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 \textit{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 \textit{M. oleifera} has appreciable antiplasmodial property.

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 \textit{in vitro} assays showed that all herbal formulations had no inhibitory effect on chloroquine sensitive \textit{P. falciparum} strain 3D7. The difference in activity between the \textit{in vivo} and \textit{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 \textit{in vivo} into active antimalarial drugs.

\textbf{Conclusion}

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

\textbf{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 notwithstanding, this study offers scientific information on the usefulness of the formulations in treating human malarial infection.

\textbf{Acknowledgements}

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

\textbf{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.

http://www.herbmedpharmacol.com
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.

References
1. Doolan DL, Dobano C, Baird JK. Acquired immunity to malaria. Clin Microbiol Rev. 2009;22(1):13-36, Table of Contents. doi: 10.1128/cmr.00025-08.
2. File T, Dinka H, Golassa L. A retrospective analysis on the transmission of Plasmodium falciparum and Plasmodium vivax: the case of Adama City, East Shoa Zone, Oromia, Ethiopia. Malar J. 2019;18(1):193. doi: 10.1186/s12936-019-2827-6.
3. World Health Organisation (WHO). World Malaria Fact Sheet 2019. https://www.who.int/news-room/fact-sheets/detail/malaria. Accessed 20 June 2019.
4. Greenwood BM, Fidock DA, Kyle DE, Kappe SH, Alonso PL, Collins FH, et al. Malaria: progress, perils, and prospects for eradication. J Clin Invest. 2008;118(4):1266-76. doi: 10.1172/jci33996.
5. World Health Organisation (WHO). World Malaria Report 2018. Geneva: WHO; 2018. https://www.who.int/malaria/publications/world-malaria-report-2018/report/en/. Accessed 20 June 2019.
6. Barabadi H, Alizadeh Z, Rahimi MT, Barac A, Maraho AE, Robertson LJ, et al. Nanobiotechnology as an emerging approach to combat malaria: a systematic review. Nanomedicine. 2019;18:221-33. doi: 10.1016/j.nano.2019.02.017.
7. Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: linking Plasmodium falciparum parasite biology to the clinic. Nat Med. 2017;23(8):917-28. doi: 10.1038/nm.4381.
8. Menard D, Dondorp A. Antimalarial drug resistance: a threat to malaria elimination. Cold Spring Harb Perspect Med. 2017;7(7). doi: 10.1101/cshperspect.a025619.
9. Federal Ministry of Health. National Guidelines for Diagnosis and Treatment of Malaria. National Malaria and Vector Control Division Abuja-Nigeria; May 2015. %20
10. Mullick S, Das S, Guha SK, Bera DK, Sengupta S, Roy D, et al. Efficacy of chloroquine and sulphadoxine-pyrimethamine either alone or in combination before introduction of ACT as first-line therapy in uncomplicated Plasmodium falciparum malaria in Jalpaiguri District, West Bengal, India. Trop Med Int Health. 2011;16(8):929-35. doi: 10.1111/j.1365-3156.2011.02799.x.
11. Bejon P, Lusingu J, Olotu A, Leach A, Lievens M, Vekemenes J, et al. Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. N Engl J Med. 2008;359(24):2521-32. doi: 10.1056/NEJMoa0807381.
12. Guinovart C, Aponte JJ, Sacarlal J, Aide P, Leach A, Bassat Q, et al. Insights into long-lasting protection induced by RTS,S/AS02A malaria vaccine: further results from a phase IIb trial in Mozambican children. PLoS One. 2009;4(4):e5165. doi: 10.1371/journal.pone.0005165.
13. Abdulla S, Salim N, Machera F, Kamata R, Juma O, Shomari M, et al. Randomized, controlled trial of the long term safety, immunogenicity and efficacy of RTS,S/AS02A (D) malaria vaccine in infants living in a malaria-endemic region. Malar J. 2013;12:11. doi: 10.1186/1475-2875-12-11.
14. Moreno A, Joyner C. Malaria vaccine clinical trials: what’s on the horizon. Curr Opin Immunol. 2015;35:98-106. doi: 10.1016/j.coi.2015.06.008.
15. Butler D, Stricker L. Promising malaria vaccine to be tested in first large field trial. Nature News; 2019. doi: 10.1038/d41586-019-01232-4.
16. Oreagba IJ, Oshikoya KA, Amachree M. Herbal medicine use among urban residents in Lagos, Nigeria. BMC Complement Altern Med. 2011;11:117. doi: 10.1186/1472-6882-11-117.
17. Jonville MC, Kodja H, Humeau L, Fournel J, De Mol P, Cao M, et al. Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. J Ethnopharmacol. 2008;120(3):382-6. doi: 10.1016/j.jep.2008.09.005.
18. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: a review. J Ethnopharmacol. 2011;133(2):289-302. doi: 10.1016/j.jep.2010.11.024.
19. Bankole AE, Adefunke AA, Sowemimo AA, Umbece CE, Abiodun O, Gbotosho GO. Phytochemical screening and in vivo antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria. Parasitol Res. 2016;115(1):299-305. doi: 10.1007/s00436-015-4747-x.
20. Ogundapo SS, Ezeanyaki LUS, Uzoegwu PN, Soniran OT, Okoro DO, Okoronkwo I, et al. Evaluation of Moringa oleifera as anti-plasmodial agents in the control of malaria. Niger J Parasitol. 2015;36(1):22-7.
21. Somsak V, Borkaew P, Klusiri C, Donceed K, Bootprom P, Saiphet B. Antimalarial properties of aqueous crude extracts of Gynostemma pentaphyllum and Moringa oleifera leaves in combination with artesunate in Plasmodium berghei-infected mice. J Trop Med. 2016;2016:8031392. doi: 10.1155/2016/8031392.
22. Iyiola OA, Tijani AY, Lateef KM. Antimalarial activity of ethanolic stem bark extract of Alstonia boonei in mice. Asian J Biol Sci. 2011;4(3):235-43. doi: 10.3923/ajbs.2011.235.243.
23. Arrey Tarkang P, Franzoi KD, Lee S, Lee E, Vivarelli D, Freitas-Junior L, et al. In vitro antiplasmodial activities and synergistic combinations of differential solvent extracts of the polyherbal product, Nefang. Biomed Res Int. 2014;2014:835013. doi: 10.1155/2014/835013.
24. Ashley EA, Phylo AP. Drugs in Development for Malaria. Drugs. 2018;78(9):861-79. doi: 10.1007/s40265-018-0911-
35. Ojo OO, Anibijuowo II, Ojo OO. Studies on extracts of three medicinal plants of south-western Nigeria: *Hoslundia opposita*, *Lantana camara* and *Cymbopogon citratus*. Adv Nat Appl Sci. 2010;4(1):93-8.

36. Toma A, Deyno S, Fikru A, Eyado A, Beale A. In vivo antimalarial and toxicological effect of crude ethanol extract of *Echinops kebericho* traditionally used in treatment of malaria in Ethiopia. Malar J. 2015;14:196. doi: 10.1186/s12936-015-0716-1.

37. Okpako LC, Ajaiyeoba EO. In vitro and in vivo antimalarial studies of *Striga hermonthica* and *Tabanipusseus silvestris* extracts. Afr J Med Sci. 2004;33(1):73-5.

38. Ayoola GA, Coker HAB, Adesogun SA, Adepoju-Bello AA, Obaweya K, Ezenina EC, et al. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res. 2008;7(3):1019-24. doi: 10.4314/tjpr.v7i3.14686.

39. Kwansa-Bentum B, Agyeman K, Larbi-Akor J, Anyigba C, Appiah-Opong R. In vitro assessment of antimalarial activity and cytotoxicity of *Polyalthia longifolia* leaf extracts on *Plasmodium falciparum* strain NF54. Malar Res Treat. 2019;2019:6976298. doi: 10.1155/2019/6976298.

40. Devi RC, Sim SM, Ismail R. Spasmolytic effect of citral and extracts of *Cymbopogon citratus* on isolated rabbit ileum. J Smooth Muscle Res. 2011;47(5):143-56. doi: 10.1540/jsmr.v7i3.14686.

41. Coppi A, Cabinian M, Mirelman D, Sinnis P. Antimalarial activity of allicin, a biologically active compound from garlic cloves. Antimicrob Agents Chemother. 2006;50(5):1731-7. doi: 10.1128/aac.50.5.1731-1737.2006.

42. Bidla G, Titanji VPK, Joko B, El-Ghazali G, Bolad A, Berzins K. Antiplasmodial activity of seven plants used in African folk medicine. Indian J Pharmacol. 2004;36(4):244-250.

43. Osonwa UE, Mbonu OD, Eluu SC, Oli AN. Antiplasmodial and biochemical effects of combination of ethanolic leave-extracts of *Azadirachta indica* and *Ocinum gratissimum* on *Plasmodium berghei*-infected mice. AJPSP. 2017;5(1):15-29.

44. Memvanga PB, Tona GL, Mesia GK, Lusakibanza MM, Cimanga RK. Antimalarial activity of medicinal plants from the Democratic Republic of Congo: A review. J Ethnopharmacol. 2015;169:76-98. doi: 10.1016/j.jep.2015.03.075.

45. Donkor AM, Oduro-Mensah D, Ani E, Ankamah E,Nsiah S, Ekow Mensah D, et al. In vitro anti-plasmodial activity of aqueous and ethanolic extracts of *Moringa oleifera* and *Phyllanthus amarus*. Int J Biol Chem. 2015;9(4):198-206. doi: 10.3923/ijbc.2015.198.206.

http://www.herbmedpharmacol.com