

Preclinical Antimalarial Evaluation and Quality Assessment of Obiri Mixture: A Polyherbal Product Used For the Management of Uncomplicated Malaria in Ghana

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Abstract

Background and Objectives: Herbal medicines are widely utilized in Ghana for the management of malaria, with polyherbal formulations increasingly integrated into mainstream healthcare. However, most of these products are prescribed without sufficient data on their quality, safety, and efficacy, raising concerns among the scientific community. Obiri Mixture is a registered polyherbal product used for the management of uncomplicated malaria. This study aimed to assess the antimalarial activity of Obiri Mixture.

Materials and Methods: The antimalarial activity of Obiri Mixture was evaluated *in vitro* against chloroquine-sensitive (3D7) and resistant (Dd2) *Plasmodium falciparum* strains using the SYBR Green I assay. Cytotoxicity was assessed on human erythrocytes using the MTT assay. Acute oral toxicity was assessed in rats at 2,000 mg/kg following guidelines of the Organisation for Economic Co-operation and Development. *In vivo* antimalarial efficacy was evaluated using the 4-day suppressive and Rane's curative tests in *Plasmodium berghei*-infected mice. Phytochemical, physicochemical, elemental, and microbial analyses were performed according to standard procedures.

Results: Obiri Mixture exhibited potent *in vitro* activity with a high selectivity index. Acute toxicity was recorded above 2,000 mg/kg. There was significant ($p < 0.05$) suppression of parasitaemia in the curative assay at 300 mg/kg, with prolonged survival of treated mice. Phytochemical screening revealed secondary metabolites. Physicochemical parameters and microbial load were within the recommended limits of the World Health Organization.

Conclusion: Obiri Mixture demonstrates potent antimalarial activity, a safety profile, and acceptable quality standards. This provides scientific support for its use and potential integration into evidence-based malaria management.

Keywords: Efficacy, Obiri Mixture, Polyherbal, Quality, Safety, Uncomplicated malaria



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Introduction

Medicinal plants and products derived from them have long played an important role in primary healthcare across Africa, where a significant proportion of the population relies on traditional remedies to treat various ailments (1). In Ghana, the use of herbal medicine is rooted in culture and continues to expand as these remedies are increasingly developed into standardised products. Such products offer a convenient alternative for individuals, especially those living in urban areas, who may not have direct access to raw medicinal plants but still seek plant-based therapies (2, 3).

Recognizing the widespread use of herbal remedies, member states of the World Health Organization (WHO) were urged to advance and institutionalize traditional medicine into their national healthcare systems (4). Therefore, Ghana took deliberate steps to integrate herbal medicine into the mainstream healthcare system. Dedicated herbal medicine units have been established in selected government hospitals, and several polyherbal formulations have been listed in the national “recommended herbal medicines list” for use in these facilities (5). Despite their inclusion in formal healthcare delivery, most of these products lack accessible, comprehensive data on efficacy, safety, and quality. While the Food and Drugs Authority (FDA) of Ghana oversees their registration, this process does not include pharmacological validation of therapeutic claims for some diseases. This gap raises concerns about the reliability of such products and necessitates the need for scientific evaluation.

Obiri Mixture is one such FDA-registered polyherbal product that is widely used in Ghana for the management of uncomplicated malaria. Notwithstanding, scientific evidence supporting its efficacy, safety, and quality remains scarce. Given that malaria remains a leading cause of morbidity and mortality in Africa, with Ghana among the countries bearing the highest burden (6), the validation of herbal products used for malaria treatment remains important. Conventional antimalarial drugs, including artemisinin-based combination therapies, remain the cornerstone of malaria management (7).

However, cultural preference and accessibility drive many patients to seek herbal alternatives (8), such as Obiri Mixture.

This study aimed to evaluate the antimalarial activity of Obiri Mixture using *in vitro* and *in vivo* models, assess its toxicity, and establish some quality parameters. These findings will provide scientific justification for its continued use and contribute to the evidence-based integration of standardised herbal products into malaria control strategies in Ghana.

Materials and Methods

Sample collection

Ten (330 ml) bottles each from three different batches (OM-003, OM-005, and OM-007) of *Obiri Mixture* with FDA Registration numbers: FDA/HDI. 9-10353 were procured from Abibidurofie at Ghana Industrial and Commercial Estate Limited, Accra, Ghana.

In vitro antiplasmodial activity

In vitro antiplasmodial activity of Obiri Herbal Mixture was assessed against the chloroquine-sensitive (3D7) and resistant (Dd2) strains of *Plasmodium falciparum* using the SYBR green method (9). Approximately 200 µg/mL of the test drug was prepared in a complete culture medium. Test concentrations were made using a two-fold dilution approach to obtain 8 dose titrations within the range of 100-0.78 µg/ml.

In a 96-well plate, test wells were seeded with a 90 µL suspension of ring-stage parasitized red blood cells (pRBCs) with 2% haematocrit and 1% parasitaemia. The wells were topped up with approximately 10 µL of the various concentrations of test drug prepared earlier. Negative control wells had no drug/inhibitor, while positive control wells contained Artesunate. The tests were conducted in triplicate. Plates were incubated at 37 °C for 48 h. Thereafter, about 100 µL of SYBR Green I in lysis buffer was mixed with the contents of the wells, and the mixture was incubated in a dark space for 30 min at room temperature.

Absorbance was determined using a multi-well plate reader (Guava easycyte HT FACS machine, Millipore, USA) at excitation and emission wavelengths of 485 and 530 nm, respectively.

The percentage inhibition of parasite growth at each concentration was calculated relative to the untreated control wells. A dose–response curve was generated by plotting log-transformed concentrations against percentage inhibition. The half-maximal inhibitory concentration (IC_{50}) was determined using nonlinear regression analysis with a four-parameter logistic (4PL) sigmoidal dose–response model in GraphPad Prism Software (version 5.0, San Diego, CA, USA). The IC_{50} values were expressed as mean $\pm$ SD from two independent experiments performed in duplicate (10).

In vitro cytotoxicity of obiri mixture

Cytotoxicity of the hydroethanolic extracts of Obiri Herbal Mixture was evaluated against human erythrocytes using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (10). A final concentration range of 1.5–100 μ g/mL of the product was used. In a 96-well plate, 90 μ L of packed RBCs (haematocrit of 2.0%) were seeded. In each well, an aliquot of 10 μ L of each concentration was added in triplicate and incubated for 72 h at 37 °C. The negative control received no drug/inhibitor.

Afterwards, 20 μ L of 2.5 mg/mL MTT solution was incorporated and further incubated for 4 h. Each well was then treated with 0.15 mL of acidified isopropanol to dissolve the formed formazan crystals and incubated in the dark at room temperature (25 °C) overnight. Tecan Infinite 200®PRO microplate reader was used to measure absorbance at 540 nm (Grodig, Austria). The percentage cell viability was determined, and consequently, percentage cell viability was calculated relative to untreated control cells. A concentration–response curve was generated by plotting log-transformed concentrations against percentage viability. The half-maximal cytotoxic concentration (CC_{50}) was calculated using nonlinear regression analysis based on a four-parameter logistic model in GraphPad Prism (version 5.0). The CC_{50} values were expressed as mean $\pm$ SD from duplicate experiments (11).

In vivo assays

Ethical Considerations

The *in vivo* experiments were conducted following the standard operating procedures of

the institution. Guidelines of the Declaration of Helsinki for the ethical treatment of experimental animals were strictly followed (12). Animal experimental procedures were conducted after the study received ethical approval from the Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (KNUST/Cology/073).

Experimental animals

Sprague Dawley mice and rats were obtained from the Centre for Plant Medicine Research. Animals were housed at the Department of Pharmacology, KNUST. They were exposed to a 12:12-h light-dark cycle with *ad libitum* access to clean drinking water and pellet diet. Before the experiment, all involved animals were acclimatised to the environment (13).

Acute toxicity

The limit test at a single dose of 2,000 mg/kg body weight was adopted (14) for assessing the acute toxicity of Obiri Herbal mixture. Female rats were starved overnight and given water *ad libitum*. Following the starvation period, rats were weighed and dosed with 2,000 mg/kg body weight of extract using a stomach tube. Feeding was delayed for another 3 h after drug administration, during which the rats were individually monitored at 30-min intervals for the first 1 h and then occasionally for the subsequent 24 h, with particular attention paid to the first 4 h. The animals were observed daily for 14 days for signs of toxicity, including gross physical and behavioural changes. This study was conducted at the animal house of the Department of Pharmacology, KNUST. This section was conducted in line with the Animal Research: Reporting of In Vivo Experiments Guidelines for reporting animal research (15).

Parasite cultivation and inoculation

Rats previously infected with the ANKA strain of *Plasmodium berghei* were used as donors. Donor animals were euthanised via cervical dislocation after parasitaemia was determined using thin blood smears, and their blood was collected via cardiac puncture into heparinised vacutainer tubes. The collected blood was diluted with physiological saline (0.9%) to obtain 5×10^7 infected RBCs per millilitre of blood. The diluted

blood was then administered intraperitoneally to each animal at a volume of 0.2 mL (containing 1×10^7 infected RBCs) (16).

Four-day suppressive antimalarial test

The *in vivo* antimalarial activity of *Obiri Mixture* was initially evaluated using Peter's 4-day suppressive assay (17). The mice were randomly divided into five (5) groups of five (5) and inoculated with the standard inoculum prepared earlier. Three hours post-infection, mice in groups II, III, and IV were treated with *Obiri* herbal mixture at doses of 30, 100, and 300 mg/kg, respectively, for four consecutive days (day 0–3). The choice of 30–300 mg/kg dose levels for *in vivo* antimalarial studies of a herbal product is generally based on a combination of preliminary pharmacological screening, safety margins, and standardized protocols for evaluation of natural products. Group V (positive control) received 4 mg/kg/day of artesunate, and group I (negative control) received the vehicle (normal saline). Thin blood smears were prepared from blood obtained from the tail vein and used to determine percentage parasitaemia and suppression (18).

Curative test (Rane's test)

The curative potential of *Obiri Mixture* was evaluated on the ANKA strain of *P. berghei* (19). On the first day (Day 0), 25 male and female mice were inoculated with the standard inoculum intraperitoneally. Seventy-two hours post-infection, the infected mice were randomly divided into five groups, with five mice in each group. Groups I and V were designated vehicle-treated and positive control (artesunate at 4mg/kg/day) groups, respectively. The test groups (II, III, and IV) were dosed with 30, 100, and 300 mg/kg/day of the product, respectively. Thin blood smears were prepared on day 3 post-inoculation and on day 7 after treatment to assess parasitaemia. Body weights were taken before and after treatment on days 3 and 7 post-infection, and the survival of animals was monitored over 30 days starting from the day of inoculation.

Quality assessment of *Obiri Mixture*

Phytochemical Screening

Obiri Mixture was qualitatively screened for secondary metabolites, including tannins, alkaloids, glycosides, triterpenoids, phytosterols,

coumarins, and flavonoids, using standard procedures (20).

Physicochemical studies

pH determination

A digital calibrated pH meter (PL-700 PV, Taiwan) was used to determine the pH of the product.

Total solid residue

Total solid residue (TSR), which refers to the total solid content and indicates the concentration of active medicinal components in liquid formulations, was determined. About 10 mL of *Obiri Mixture* was measured into a ceramic evaporating dish of known weight (W_0), which was kept in a water bath in the hood to be dried. The dish was removed and further dried at 70 °C to a constant weight in an oven. It was removed and allowed to cool to room temperature, after which its weight (W_1) was determined. The Total solid residue was determined using the formula:

$$TSR = \frac{W_1 - W_0}{\text{Volume of sample evaporated}} \times 100\%.$$

Where:

TSR: total solid residue

W_0 : initial weight of ceramic evaporating dish

W_1 : final weight of *Obiri Mixture* +dish after oven drying.

Specific Gravity

A known weight of a dried, empty gravity bottle was first recorded. The bottle was then filled with *Obiri Mixture* and corked with its stopper. It was wiped dry before it was weighed. The contents in the gravity bottle were emptied, and distilled water was used to rinse the bottle. Thereafter, it was completely filled again with distilled water, wiped to dryness, and weighed. Specific Gravity of the sample was determined as the ratio of the weight of the sample to the weight of the water using the following formula:

$$SG = \frac{W_1 - W_0}{W_2 - W_0}$$

Where:

SG: specific gravity

W_0 : Weight of a dried empty gravity bottle

W_1 : Weight of gravity bottle + *Obiri Mixture*

W_2 : Weight of gravity bottle + distilled water

Elemental analysis

Elemental and heavy metal content in Obiri Herbal Mixture was determined using standard procedures (21).

Microbial load evaluation

The guidelines provided by the World Health Organisation were used to assess the microbial quality of the product. The presence or absence of various organisms was determined using standard procedures (22).

Statistical analysis

GraphPad Prism software for Windows (version 5.0, San Diego, CA, USA) was used for all

statistical analyses. All data were expressed as mean $\pm$ SEM and SD (duplicate measurements).

Results

In vitro antiplasmodial and cytotoxicity of Obiri Mixture

Obiri Mixture demonstrated remarkable *in vitro* antiplasmodial activity against both sensitive and resistant strains of *P. falciparum*. However, it was more active against the resistant strain relative to the sensitive strain (Table 1). Obiri Mixture showed cytotoxic (CC₅₀) levels above 100 μ g/mL in human red blood cells (Table 1). This was consequently employed in obtaining the selectivity index of the extract.

Table 1. Half-maximal inhibitory concentration (IC₅₀) values of Obiri Mixture against 3D7, Dd2 and clinical strains of *Plasmodium falciparum*

Drug/Extracts	IC ₅₀ (3D7) (μ g/ml)	IC ₅₀ (Dd2) (μ g/ml)	CC ₅₀ (μ g/ml)	Selectivity Index
Obiri Mixture	$1.196 \pm 4 \times 10^{-3}$	$0.325 \pm 22 \times 10^{-3}$	> 100	> 83.61
Artesunate	$0.001 \pm 3 \times 10^{-5}$	$0.001 \pm 1 \times 10^{-5}$	> 100	> 8333.33

Acute toxicity

Administration of Obiri Mixture at 2,000 mg/kg caused no observable physical and behavioural changes for 24 h. No mortality was recorded for the 2-week observation period. Henceforth, the LD₅₀ was estimated to be more than 2,000 mg/kg.

In vivo antimalarial activity

Peter's 4-day suppressive assay

Obiri Mixture exhibited a significant ($p < 0.05$) dose-dependent suppression, with the highest suppression seen at 300 mg/kg (78.68%). At all tested doses, the survival time of mice in the extract-treated groups was prolonged, with the 300 mg/kg dose producing the greatest survival time of 21.80 days (Table 2).

Table 2. Effect of Obiri Mixture on parasitaemia, suppression, and survival time of the *Plasmodium berghei*-infected mice

Dose (mg/kg)	% Parasitaemia	% Suppression	MST
NC	62.43 ± 1.31	NS	8.80 ± 1.46
30	$36.13 \pm 0.51^*$	42.13 ± 3.46	$11.00 \pm 2.38^*$
100	$16.06 \pm 0.56^*$	74.28 ± 2.05	$20.80 \pm 7.02^*$
300	$13.31 \pm 0.70^*$	78.68 ± 1.46	$21.80 \pm 5.50^*$
PC (4)	$1.87 \pm 0.02^*$	97.00 ± 0.25	$30.00 \pm 0.00^*$

% Parasitaemia presented as Mean $\pm$ SD, N = 5, NC = Negative Control group, NS = No Suppression, PC = Artesunate, MST = Mean Survival Time. *Values are significantly different at $p < 0.05$, compared to the negative control group.

Rane's curative test

Obiri Mixture demonstrated a significant ($p < 0.05$) decrease in parasitaemia levels on day 7 relative to day 3 at all tested doses. The highest suppression of parasitaemia (82.16%) was seen at

300 mg/kg/day. The mice treated with Obiri Mixture had a significantly longer survival time at all doses relative to the vehicle-treated group Table 3.

Table 3. Effect of Obiri Mixture on parasitaemia and survival time of the *P. berghei*-infected mice in the Rane's test

Dose (mg/kg)	% Parasitaemia		% Suppression	MST
	Day 3	Day 7		
NC	60.39 ± 7.09	69.52 ± 4.93 ^a	NS	9.250 ± 0.62
30	65.60 ± 10.03	48.10 ± 2.26 ^{a*}	30.81	17.00 ± 2.53
100	57.79 ± 4.13	21.88 ± 2.00 ^{a*}	68.53	20.50 ± 2.15
300	63.28 ± 9.86	12.40 ± 2.62 ^{a*}	82.16	26.00 ± 3.00
PC (4)	62.23 ± 1.72	2.74 ± 0.21 ^{a*}	96.05	30.00 ± 0.0

% Parasitaemia represented as Mean ± SEM, N = 5, NC = Negative Control group, PC = Artesunate, NS=No Suppression. Values are significantly different at $p < 0.005$, ^a compared to day 3 and ^{*}the negative control group.

Effect of Obiri herbal mixture on body weight

Treatment with Obiri Mixture prevented the decline in percentage change observed in the vehicle-treated groups (NC) in both suppressive (Figure 1A) and curative (Figure 1B) assays.

Similarly, there was no weight decline in the artesunate-treated group (n=5). Nonetheless, the product did not cause a significant change in percentage body weight relative to the negative control groups (n=5) in both assays.

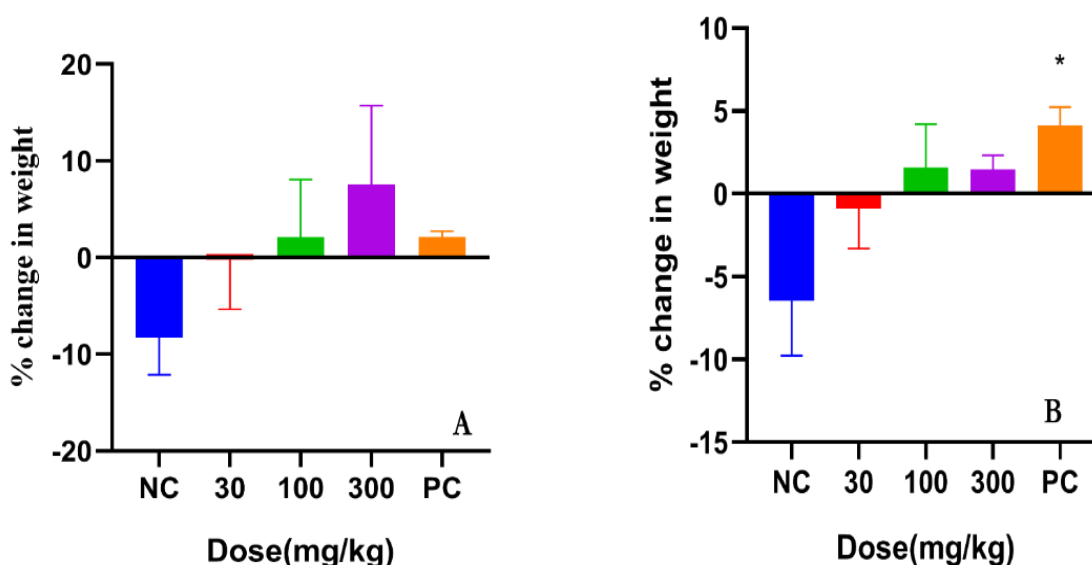


Figure 1. Percentage change in weight of mice infected with *Plasmodium berghei* on day 0 and day 4 in the suppressive assay (A) and on day 3 and 7 in the curative test (B). *Values are significantly different at $p < 0.05$, compared to the vehicle-treated groups.

Quality parameters of Obiri Mixture

Organoleptic and physicochemical properties of Obiri Mixture

All purchased samples had the manufacturer's details, a list of active ingredients, the total volume (330 ml), no physical evidence of

deterioration, and the manufacturing and expiration dates. Samples also had Ghana's FDA registration numbers. Obiri mixture is an aqueous decoction, yellowish-brown in color and bitter in taste. The product had an aromatic odor. Physicochemical evaluation of Obiri Mixture

revealed a slightly acidic pH of 5.21 ± 0.15 . It has a specific gravity of $1.01 \pm 1 \times 10^4$ and a total dissolved solids content of $5.48 \pm 0.29\%$ w/v (Table 5).

Phytochemical screening

Qualitative phytochemical screening showed the presence of all tested secondary metabolites, including tannins, alkaloids, saponins, coumarins, flavonoids, triterpenoids, phytosterols, and reducing sugars in *Obiri Mixture*.

Elemental analysis

Iron, an essential element, was detected in the product. Lead, cadmium, mercury, and arsenic were below the detection limits. Levels of all

identified elements were within the permissible range (23). Results of the elemental analysis of three different batches of the product are summarized in Table 4.

Microbial load analysis of Obiri Mixture

Microbial load assessment of three different batches of the product revealed an average total viable aerobic bacteria count of $2.9 \times 10^3 \pm 7.02$ and a yeast count of $1.57 \times 10^3 \pm 2.52$ CFU/mL. The total viable yeast and aerobic bacteria counts were below the recommended permissible limits of detection established by the WHO guidelines for assessing the quality of herbal medicines (21, 23). *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella Typhi* were not detected (Table 5).

Table 4. Elemental contents of Obiri Mixture

Element	Mean $\pm$ SD (mg/L)	Permissible limits (mg/L)
As	0.016 $\pm$ 0.001	5.0
Cd	0.037 $\pm$ 0.004	0.3
Fe	8.817 $\pm$ 0.206	20.0
Hg	0.012 $\pm$ 0.001	0.5
Pb	0.179 $\pm$ 0.134	10.0

Values are expressed as mean $\pm$ SD. As: arsenic; Cd: cadmium; Fe: iron; Hg: mercury; Pb: lead.

Table 5. Microbial load of Obiri Mixture in different growth media.

Table	Batch 1	Batch 2	Batch 3	Average	Permissible limits
Total aerobic viable count (NA)	$2.6 \times 10^3 \pm 0.04$	$2.4 \times 10^3 \pm 0.15$	$3.7 \times 10^3 \pm 0.16$	$2.9 \times 10^3 \pm 7.02$	$<1.0 \times 10^5$
Yeast and moulds (SA)	$1.8 \times 10^3 \pm 0.19$	$1.6 \times 10^3 \pm 1.44$	$1.3 \times 10^3 \pm 1.79$	$1.57 \times 10^3 \pm 2.52$	$<1.0 \times 10^7$
<i>Escherichia coli</i> (MAC)	0	0	0	0	$<1.0 \times 10^2$
<i>Staphylococcus aureus</i> (MSA)	0	0	0	0	$<1.0 \times 10^2$
<i>Pseudomonas aeruginosa</i> (CET)	0	0	0	0	0
<i>Salmonella Typhi</i> (BS)	0	0	0	0	Absent

Values are expressed as mean $\pm$ SD. NA: nutrient agar (total aerobic viable count); SA: Sabouraud agar (yeast and mould count); MAC: MacConkey agar (*E. coli* detection); MSA: Mannitol salt agar (*S. aureus* detection); CET: Cetrimide agar (*P. aeruginosa* detection); BS: Bismuth sulphite agar (*S. typhi* detection)

Discussion

This study evaluated the preclinical antimalarial potential, safety, and some quality parameters of Obiri Mixture. The aim was to support its use in the management of uncomplicated malaria in Ghana as well as to justify its continual use.

Obiri Mixture inhibited both chloroquine-sensitive (3D7) and resistant (Dd2) strains of *P. falciparum*, with superior potency against the resistant strain (Table 1). This pattern suggests that the mixture contains active phytoconstituents that act through mechanisms independent of chloroquine, thus offering potential advantages in resistance-prone areas, such as the coastal regions of Ghana (24). This result is in line with those of a previous study which found that some herbal antimalarials exhibited antiplasmodial activity (25). However, *in vitro* activity does not necessarily translate directly into clinical effectiveness, and further pharmacodynamic and clinical studies are required.

Obiri Mixture had a CC₅₀ greater than 100 µg/mL in erythrocytes, indicating low cytotoxicity. This observation is noteworthy, given earlier reports of safety concerns with some of the individual plants. For example, *Cochlospermum sanguinolentum* has been associated with DNA damage and genotoxicity at high concentrations (26). However, when combined with *Nauclea latifolia* and *Phyllanthus fraternus*, which are generally regarded as safe, potential toxicity may be mitigated through dose reduction. Additionally, antagonistic interactions at toxicological targets may further reduce the risk of toxicity. This highlights an advantage of polyherbal formulations, where therapeutic synergy may be obtained with reduced toxicity. The acute toxicity study showed no mortality or observable adverse effects in rats at a dose of 2,000 mg/kg, suggesting that the LD₅₀ exceeds this dose. Absence of toxicity at 2,000 mg/kg, coupled with a high *in vitro* selectivity index, suggests the product may have a favorable preclinical safety profile. Nevertheless, sub-

chronic or chronic toxicity, genotoxicity, pharmacokinetic, as well as drug-interaction studies are required to establish its safety.

The study sample produced a dose-dependent reduction in parasitaemia and prolonged survival in *P. berghei*-infected mice in both suppressive and curative models. The highest dose (300 mg/kg) achieved > 78% suppression in the 4-day test and > 82% in the curative model. Superior activity of the product in the curative test relative to the suppressive test demonstrates that it may have superior efficacy in treating established infections, supporting its use as an antimalarial. These findings are consistent with those of previous reports on the individual plants comprising the formulation.

Cryptolepis sanguinolenta root extract is known for its potent antiplasmodial activity, largely attributed to the indoloquinoline alkaloid cryptolepine, which intercalates parasite DNA and inhibits topoisomerase II. Several studies have shown IC₅₀ values in the sub-micromolar range against *P. falciparum* (27, 28). *Nauclea latifolia* contains indole alkaloids, such as strictosamide and nauclefidine, which exhibit antiplasmodial and immunomodulatory effects and have demonstrated *in vivo* curative rates of 50–70% (29). *Phyllanthus fraternus*, though less directly potent, contains flavonoids and lignans with hepatoprotective and antioxidant properties that may enhance host defence and mitigate oxidative stress during infection (30).

Activity of Obiri Mixture suggests potential additive or synergistic interactions among its constituents, compared with those of most individual plants. The product additionally improved clinical outcomes, such as body weight and survival. Treatment with the product averted the degree of weight loss observed in the negative control group. However, the differences were not statistically significant. Nevertheless, these findings suggest that a reduction in parasite burden may alleviate factors that lead to weight loss during the infection (31). Moreover, a

significant increase in survival time was observed in the treated animals. Prolonged survival is strongly correlated with reduced parasitaemia, as lower parasite loads alleviate complications, such as anaemia and organ dysfunction (32).

Phytochemical analysis showed the presence of tannins, alkaloids, flavonoids, coumarins, triterpenoids, phytosterols, and reducing sugars, consistent with the secondary metabolite profile of its constituent plants (33, 34, 35). These groups of compounds are known to contribute to antimalarial, antioxidant, and immunomodulatory effects. Physicochemical parameters, such as pH (5.21 ± 0.15), total solid residue ($5.48 \pm 0.29\%$), and specific gravity ($1.01 \pm 1.0 \times 10^{-4}$), were within acceptable ranges, supporting product stability and standardisation. The pH of the test sample was within the normal stomach pH range of 4-6.5. Stability and absorption of products are dependent on a pH within a specified acceptable range.

Elemental analysis showed that essential elements, such as iron, were present in safe concentrations, while toxic heavy metals, such as arsenic, lead, cadmium, and mercury, were within WHO-permissible limits (23). This is critical for consumer safety, as heavy metal contamination is a major concern with herbal preparations (36). Microbial load assessment revealed low levels of aerobic bacteria and fungi, well below WHO-permissible limits, and the absence of pathogenic bacteria, such as *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. typhi*. These results indicate good manufacturing practice and compliance with international quality standards for herbal medicines.

Study Limitations

The present study was limited to the preclinical and quality parameters assessment, the small number of animals ($n=5$), absence of sub-chronic toxicity analysis, and lack of real clinical data.

Conclusion

This study validated and provided preliminary preclinical evidence supporting the antiparasitodal potential, acceptable acute safety profile, and quality compliance of Obiri Mixture under experimental conditions. The findings indicated that the formulation possesses measurable *in vitro* and *in vivo* antimalarial activity in laboratory models and meets selected physicochemical, microbial, and elemental quality standards. Therefore, Obiri Mixture may be considered a promising candidate for further scientific investigation rather than a clinically validated antimalarial therapy at this stage.

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Author Contributions

B. K. T., E. O. P., and A. Y. M. contributed to conceptualization. B. K. T., E. O. P., and R. I. N. contributed to methodology. B. K. T., E. O. P., D. N., and R. I. N. contributed to formal analysis. B. K. T., E. O. P., A. Y. M., and M. L. K. contributed resources. E. O. P., B. K. T., and M. L. K. contributed to writing—original draft preparation. E. O. P., B. K. T., D.N., and M. L. K. contributed to writing—review and editing. B. K. T. and A. Y. M. supervised the study. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics Statement

Ethical approval for animal studies was sought from the appropriate institutional body.

Data Availability Statement

The datasets used during the study are available from the corresponding author on reasonable request.

Artificial Intelligence Statement

No artificial intelligence tools were used in the design, experimentation, analysis, or writing of this article.

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