ANTIPLASMODIAL EFFICACY, PHYTOCHEMICAL COMPOSITION, AND SAFETY PROFILE OF TEN MEDICINAL PLANT EXTRACTS AGAINST *PLASMODIUM BERGHEI* IN SWISS ALBINO MICE: IMPLICATIONS FOR HERBAL MALARIA THERAPY

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ABSTRACT
Malaria remains a catastrophic public health burden in sub-Saharan Africa, with Nigeria accounting for approximately 30.9% of global malaria-related deaths (WHO, 2024). The emergence of partial artemisinin resistance across African countries has intensified the search for novel antiplasmodial agents from medicinal plant sources. This study evaluated the phytochemical composition, proximate and mineral content, antiplasmodial activity, acute toxicity, and haematological, biochemical, and histopathological safety profiles of aqueous and ethanolic extracts of ten medicinal plants used in traditional malaria management in Ondo State, Nigeria, against *Plasmodium berghei* (NK 65) in Swiss albino mice. The plants studied were *Anthocleista djalonensis, Azadirachta indica, Cajanus cajan, Crescentia cujete, Lawsonia inermis, Lophira alata, Myrianthus pruessii, Nauclea latifolia, Olax subscorpioidea,* and *Terminalia glaucescens.* Phytochemical screening confirmed alkaloids, saponins, tannins, and cardiac glycosides across all ten plants, with highest alkaloid and saponin content in *T. glaucescens* stem bark. All aqueous extracts significantly suppressed parasitaemia from Day 1–4, with gradual recrudescence observed thereafter. The polyherbal combination demonstrated activity comparable to chloroquine. Histopathological examination revealed no serious lesions at standard doses, however dose-dependent tissue changes were observed at 300 mg/kg and above. These plants, individually and in combination, exhibit significant antiplasmodial activity -underpinned by diverse bioactive constituents. While their safety profile at standard doses supports continued ethnomedicinal use, standardisation, dose optimisation, and mechanistic studies are warranted before clinical translation. These formulations represent a promising strategy amid escalating antimalarial drug resistance.
Keywords: *Plasmodium berghei*; antiplasmodial; medicinal plants; phytochemistry; Swiss albino mice; herbal malaria therapy; Ondo State; Nigeria

Introduction
Malaria remains one of the most devastating infectious diseases of the 21st century, disproportionately afflicting populations in tropical and subtropical regions. According to the World Health Organization's World Malaria Report 2024, an estimated 263 million malaria cases occurred globally in 2023, resulting in approximately 597,000 deaths, of which 95% were concentrated in the WHO African Region (WHO, 2024). Nigeria bears the heaviest national burden, accounting for 25.9% of all global malaria cases and approximately 30.9% of malaria-related deaths (WHO, 2024; Severe Malaria Observatory, 2024). Children under five years of age account for approximately 76% of malaria fatalities across sub-Saharan Africa, underscoring the severe immunological vulnerability of this demographic (WHO, 2024).

Historically, management of malaria has relied on chemotherapy, progressing from quinine and chloroquine to artemisinin-based combination therapies (ACTs). However, this pharmacological trajectory has been increasingly threatened by the emergence and spread of drug resistance. Artemisinin partial resistance (ART-R), mediated principally by mutations in the *P. falciparum* Kelch 13 (K13) protein, was initially confined to Southeast Asia but has now been independently confirmed in Rwanda, Uganda, and multiple countries in the Horn of Africa (Rosenthal *et al*., 2024; Uwimana *et al*., 2021). The Lancet Infectious Diseases and Nature Reviews Microbiology have characterised ART-R as an urgent continental health security threat that demands the accelerated development of alternative antimalarial strategies (Rosenthal *et al*., 2024). Resistance to conventional drugs reinforces the imperative to investigate medicinal plants as sources of novel antiplasmodial agents.

Ethnobotanical knowledge accumulated over centuries in sub-Saharan Africa represents an underexplored reservoir of potential antimalarial leads. A systematic review of antiplasmodial and antimalarial activities of African medicinal plants identified 502 plant species from 200 studies, predominantly conducted in Nigeria, with *Azadirachta indica* and *Nauclea latifolia* among the most frequently investigated species (Tajbakhs *et al*, 2021). Traditional healers across Ondo State, southwestern Nigeria, have long employed a polyherbal recipe integrating ten plant species for malaria treatment: *Anthocleista djalonensis*, *Azadirachta indica*, *Cajanus cajan*, *Crescentia*

cujete, *Lawsonia inermis*, *Lophira alata*, *Myrianthus pruessii*, *Nauclea latifolia*, *Olax subscorpioidea*, and *Terminalia glaucescens*. Several of these plants have attracted scientific investigation individually, but comprehensive, comparative data on their collective phytochemical profiles, safety parameters, and antiplasmodial efficacy as a synergistic recipe remain limited in the peer-reviewed literature.

The rodent malaria model employing *Plasmodium berghei* (NK 65 strain) in Swiss albino mice is a well-validated preclinical platform for antimalarial drug discovery, directly underpinning the development of several conventional antimalarials including quinine, chloroquine, halofantrine, and mefloquine (Alaribe *et al*., 2020). The four-day suppressive test, developed by Peters (1975) and continuously refined, remains the gold standard in vivo assay for evaluating antiplasmodial efficacy at the erythrocytic stage.

This study was therefore designed to: (i) document the qualitative phytochemical, proximate, and mineral element profiles of the ten plant samples; (ii) evaluate the in vivo antiplasmodial activity of their aqueous and ethanolic extracts, individually and in combination, against *P. berghei* NK 65-infected Swiss albino mice; (iii) assess the acute toxicity, haematological, serum biochemical, and histopathological safety parameters of the extracts; and (iv) generate evidence-based pharmacological data to support or refine the traditional use of these plants as complementary and alternative antimalarial treatments.

Literature Review

Global and Regional Malaria Burden

The WHO African Region continues to shoulder a disproportionate share of the global malaria burden, accounting for 94% of cases and 95% of deaths in 2023 (WHO, 2024). Nigeria, the Democratic Republic of Congo, Niger, and Tanzania collectively account for more than half of all global malaria fatalities, with Nigeria contributing the single largest share at 30.9% (WHO, 2024). These figures underline the extraordinary urgency of malaria control in the Nigerian context. The WHO World Malaria Report 2024 estimates that 2.2 billion cases and 12.7 million deaths have been averted globally since 2000, yet progress has stalled, and case incidence has plateaued over the past decade at approximately 60.4 cases per 1,000 population at risk (WHO, 2024; *The Lancet Microbe*, 2025).

Antimalarial Drug Resistance and the Case for Phytomedicine

The history of malaria pharmacotherapy has been repeatedly punctuated by the emergence of parasite resistance. Resistance to chloroquine, once the cornerstone of malaria treatment, spread from Southeast Asia to Africa with devastating consequences (Rosenthal *et al*., 2024). ACTs subsequently became the global standard of care; however, artemisinin partial resistance mediated by *Plasmodium falciparum* K13 mutations has now been confirmed in Rwanda, Uganda, and nations across the Horn of Africa, prompting WHO to launch a dedicated antimalarial drug resistance strategy for Africa in November 2022 (WHO, 2022; Rosenthal *et al*., 2024a). Rosenthal and colleagues (2024), writing in *The Lancet Infectious Diseases*, identify expanded genomic surveillance, policy adaptation, and accelerated development of new regimens as the most pressing responses to this crisis.

In this context, the pharmacological validation of medicinal plants as antimalarial agents assumes renewed strategic significance. The complex phytochemical matrices of herbal extracts containing multiple bioactive compounds simultaneously are generally considered less susceptible to the single-point resistance mutations that compromise synthetic mono-therapies (Angupale *et al*., 2024). Poly-herbal formulations, in particular, introduce a multiplicity of mechanisms of action that make selective parasite resistance considerably more difficult to evolve.

Pharmacological Profiles of the Study Plants

Azadirachta indica (Neem)

Among the most extensively studied of the ten plants, *A. indica* (family Meliaceae) has demonstrated consistent antiplasmodial activity in both in vitro and in vivo models. Ofoego *et al*. (2025), in the *Journal of Complementary and Alternative Medical Research*, reported significant hepatoprotective and antiplasmodial activity of its ethanolic leaf extract against *P. berghei*-infected Swiss albino mice. Aqueous and ethanolic leaf extracts produced dose-dependent chemosuppression rates of up to 78.32% against *P. berghei* in BALB/c mice, with an LD50 exceeding 1 g/kg, indicating a favourable safety margin (IJPSR, 2025). The antiplasmodial activity of neem has been attributed principally to limonoids (azadirachtin, nimbin), alkaloids, and flavonoids that appear to exert oxidative stress on the intraerythrocytic parasite (Olanlokun *et al*., 2024).

Nauclea latifolia

The stem bark and roots of *N. latifolia* (family Rubiaceae) represent one of the most validated antimalarial plants in the Nigerian pharmacopoeia. Asanga *et al*. (2024), in *BMC Complementary Medicine and Therapies*, conducted in vivo antiplasmodial assays and molecular docking studies, isolating betulinic acid and ursolic acid from root

fractions, achieving parasite growth inhibition of 83.4% by Day 5. The residual fraction demonstrated therapeutic responses comparable to both amodiaquine (80.5%) and artesunate (85.1%) as reference standards. Alaribe *et al*. (2020) similarly documented 91.32% chemosuppression with *N. latifolia* root fractions, comparable to chloroquine (100%) in the Peters four-day suppressive test.

Terminalia glaucescens

Ethanol and aqueous extracts of *T. glaucescens* (family Combretaceae) stem bark have been demonstrated to inhibit the erythrocytic cycle of *P. falciparum* at the trophozoite-to-schizont transition. IC50 values for the ethanol extract against chloroquine-resistant *P. falciparum* strains ranged from 0.35 - 43.40 µg/mL, with a favourable cytotoxicity/antiplasmodial index exceeding 20 for the ethanol extract (Banzouzi *et al*., 2000). *T. glaucescens* is also notable in the present study for its exceptional phytochemical richness, containing the highest alkaloid (0.75%) and saponin (0.36%) concentrations, the highest carbohydrate content (67.58%), and the highest calcium level (35,142 mg/kg) among all ten samples.

Cajanus cajan (Pigeon Pea)

Bioassay-guided fractionation of *C. cajan* (family Fabaceae) leaf extract has yielded cajachalcone (2',6'-dihydroxy-4-methoxychalcone) with an in vitro IC50 of 2.0 µg/mL against chloroquine-sensitive *P. falciparum* strain 3D7 (Banzouzi *et al*., 2004). Stilbenes; specifically longistylin A and C and betulinic acid have also been isolated from *C. cajan* with moderately high antiplasmodial activity. A comprehensive review published in *Molecules* in 2022 confirmed the phyto-pharmacological versatility of *C. cajan*, including its antimalarial, anti-inflammatory, and hepatoprotective properties (Gargi *et al*., 2022).

Lawsonia inermis (Henna)

The leaf extracts of *L. inermis* (family Lythraceae) have documented activity both in vitro against *P. falciparum* and in vivo against *P. berghei*-infected mice. An ethnobotanical survey and in vivo assessment in Kwara State, Nigeria, confirmed moderate antiplasmodial activity with good repository (prophylactic) properties for *L. inermis* leaf extracts (Afolayan *et al*., 2023). The principal bioactive constituent, fraxetin, suppresses parasitaemia and ameliorates Plasmodium-induced oxidative stress by augmenting glutathione redox defences, as demonstrated in *P. berghei*-infected mice (Singh *et al*., 2017).

Other Study Plants

The remaining five plants *A. djalonensis, C. cujete, L. alata, M. pruessii*, and *O. subscorpioidea* have been documented in Nigerian ethnomedicinal surveys as components of antimalarial recipes used by traditional healers in Akure and Ondo State, with collective evidence suggesting synergistic antiplasmodial effects when combined (Tajbakhs *et al*, 2021; Alaribe *et al*., 2020). Individual pharmacological studies on these species remain relatively limited in the peer-reviewed literature, further justifying the present comprehensive investigation.

Synergistic Poly-herbal Formulations

The rationale for evaluating a poly-herbal recipe, as opposed to individual plant extracts alone, is well-grounded in both ethno-botanical tradition and pharmacological science. The complexity of phytochemical matrices in multi-plant preparations is theorised to reduce the likelihood of parasite resistance through simultaneous multi-target interference (Angupale *et al*., 2024). Polyherbal combinations employing plants with complementary mechanisms such as those producing alkaloids that intercalate parasite DNA alongside saponins that perturb parasite membrane integrity may demonstrate superior efficacy compared to any single extract.

Materials and Methods

Plant Collection, Identification, and Preparation

Ten medicinal plant species were collected from Akure and its environs in Ondo State, Nigeria, following documented ethnomedicinal use by traditional healers in the management of malaria. The plant parts used were as follows: *Anthocleista djalonensis* A. Chev. (stem bark); *Azadirachta indica* A. Juss. (stem bark and leaf); *Cajanus cajan* (L.) Huth. (leaf); *Crescentia cujete* L. (stem bark); *Lawsonia inermis* L. (leaf); *Lophira alata* Banks ex C.F. Gaertn. (stem bark); *Myrianthus pruessii* Engl. (leaf); *Nauclea latifolia* Sm. (stem bark); *Olax subscorpioidea* Oliv. (root); and *Terminalia glaucescens* Planch ex Benth. (stem bark and root). All plant samples were authenticated by a certified plant taxonomist. Plant materials were air-dried under shade for three weeks, ground to fine powder using an electric blender, and stored in sealed containers prior to extraction.

Preparation of Extracts

Aqueous extracts were prepared by boiling 100 g of powdered plant material in 1,000 mL of distilled water for 30 minutes, filtering through Whatman No. 1 filter paper, and freeze-drying to obtain dried aqueous extract. Ethanolic extracts were prepared by maceration of 100 g of powdered material in 500 mL of 96% ethanol for 72 hours with intermittent shaking, followed by filtration and concentration under reduced pressure using a rotary evaporator at 40°C. Combined polyherbal extracts (recipe) were prepared by pooling equal weights of all ten plant powders prior to extraction using the same protocols. All extracts were stored at 4°C until use.

Phytochemical Screening

Qualitative phytochemical screening of all dried plant samples was conducted using the standard methods described by Harborne (1973) and Trease and Evans (2002) for the detection of alkaloids, saponins, tannins, flavonoids, anthraquinones, and cardiac glycosides. Quantitative alkaloid and saponin determination was performed using the gravimetric methods of Obadoni and Ochuko (2002). Tannin content was determined by the Folin–Denis spectrophotometric method.

Proximate and Mineral Analysis

Proximate composition (moisture, crude protein, crude fat, crude fibre, ash, and carbohydrate) was determined using the AOAC (2000) standard procedures. Mineral element concentrations (calcium, phosphorus, magnesium, sodium, and zinc) were determined by atomic absorption spectrophotometry (AAS) after acid digestion of dried plant samples.

Experimental Animals

Swiss albino mice (20–25 g body weight) of both sexes were obtained from a standard Animal House and housed in well-ventilated cages under standard laboratory conditions (12-hour light/dark cycle; room temperature 25 ± 2°C) with free access to standard rodent chow and water. All animal procedures were conducted in strict accordance with the ethical guidelines for the use of laboratory animals (NIH Guide for the Care and Use of Laboratory Animals, 2011) and were approved by the institutional ethical review committee.

In vivo Antiplasmodial Assay

The chloroquine-sensitive *Plasmodium berghei* (NK 65 strain) was maintained by serial intra-peritoneal passaging in donor Swiss albino mice. For the four-day Peters' suppressive test (Peters, 1975), experimental mice were inoculated intra-peritoneally on Day 0 with 1×10^7 parasitised erythrocytes. Mice were then randomly allocated into treatment groups (n = 5 per group). Beginning on Day 0, mice received daily oral doses of: (a) plant extracts at doses of 100, 200, and 300 mg/kg body weight; (b) polyherbal recipe at equivalent doses; (c) chloroquine phosphate at 10 mg/kg as positive control; or (d) 0.2 mL normal saline as negative control. On Day 4, thin blood smears were prepared from tail-vein blood, fixed with methanol, stained with 10% Giemsa, and examined microscopically. Parasitaemia was assessed by counting at least 200 erythrocytes per field.

Percentage chemosuppression was calculated as:

$$\% \text{ Chemosuppression} = [(\text{Parasitaemia of negative control} - \text{Parasitaemia of treated group}) / \text{Parasitaemia of negative control}] \times 100$$

Acute Toxicity (LD50) Determination

Acute toxicity was assessed using the up-and-down method as described by the OECD Guidelines for Testing of Chemicals (TG 425). Groups of three Swiss albino mice of each sex received single oral doses of plant extracts at 100, 200, 300, 500, and 1,000 mg/kg body weight. Animals were observed for signs of toxicity and mortality over 24 hours post-dosing, and the LD50 was estimated by graphical interpolation of the dose mortality relationship.

Haematological and Biochemical Analyses

At Day 4 post-treatment, blood samples were collected by cardiac puncture under light anaesthesia. EDTA-anticoagulated blood was used for haematological analysis (PCV, RBC, WBC count, haemoglobin, platelets) using an automated haematology analyser. Serum was separated by centrifugation at 3,000 rpm for 10 minutes and used for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total and direct bilirubin, urea, and creatinine using commercial enzyme-linked diagnostic kits.

Histopathological Examination

Liver and kidney tissue samples were harvested at Day 4 post-treatment, fixed in 10% neutral buffered formalin, processed by routine paraffin embedding, sectioned at 5 µm, and stained with haematoxylin and eosin (H&E). Stained sections were examined by light microscopy for cellular architecture, inflammatory infiltrates, necrosis, and vascular changes.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Comparisons between groups were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. Significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., USA).

Results

Qualitative Phytochemical Screening

Qualitative phytochemical analysis (Table 1) confirmed the presence of alkaloids, saponins, tannins, and cardiac glycosides in all ten plant samples. Flavonoids were absent in eight of ten samples, with only a trace quantity (0.01%) detected in *C. cujete* stem bark. Similarly, anthraquinones were detected only as a trace (0.01%) in *O. subscorpioidea* root. Quantitative analysis revealed that alkaloids and saponins were highest in *T. glaucescens* stem bark (0.75% and 0.36%, respectively). Tannin content was generally low across all samples. Cardiac glycoside content was highest in *L. alata* stem bark.

Table 1: Qualitative Phytochemical Profile of Ten Medicinal Plant Samples

Plant Sample	Alkaloids	Saponins	Tannins	Flavonoids	Anthraquinones	Cardiac Glycosides	
A. djalonensis (SB)	+	+	+	–	–	+	
A. indica (SB)	+	+	+	–	–	+	
A. indica (L)	+	+	+	–	–	+	
C. cajan (L)	+	+	+	–	–	+	
C. cujete (SB)	+	+	+	Tr	–	+	
L. inermis (L)	+	+	+	–	–	+	
L. alata (SB)	+	+	+	–	–	++	
M. pruessii (L)	+	+	+	–	–	+	
N. latifolia (SB)	+	+	+	–	–	+	
O. subscorpioidea (R)	+	+	+	–	Tr	+	
T. glaucescens (SB)	++	++	+	–	–	+	
T. glaucescens (R)	+	+	+	–	–	+	

+ = Present; ++ = Present in high concentration; – = Absent; tr = Trace; SB = Stem bark; L = Leaf; R = Root

Proximate and Mineral Composition

T. glaucescens stem bark contained the highest carbohydrate content at 67.58%, while *C. cajan* leaf recorded the highest crude protein (17.76%), ash (11.69%), and phosphorus (2,279 mg/kg) concentrations. *M. pruessii* leaf recorded the highest magnesium content at 5,837 mg/kg. Calcium was highest in *T. glaucescens* stem bark (35,142 mg/kg), and zinc was highest in *A. indica* stem bark (402.18 mg/kg). Fat content was generally low across all samples, while crude fibre values were notably high, particularly in *L. inermis* and *A. djalonensis*. Sodium concentrations were low in all samples. These nutritional characteristics are consistent with recognised bioactivity-supporting mineral profiles in medicinal plants.

Table 2: Selected Proximate and Mineral Values of Key Plant Samples (Highlights)

Plant Sample	Crude Protein (%)	Carbohydrate (%)	Calcium (mg/kg)	Phosphorus (mg/kg)	Zinc (mg/kg)
A. indica (Leaf)	16.43	–	–	–	–
A. indica (SB)	–	–	–	–	402.18
C. cajan (Leaf)	17.76	–	–	2279	–
L. inermis (Leaf)	15.55	–	–	–	–
M. pruessii (Leaf)	16.35	–	–	–	–
C. cujete (SB)	11.59	–	–	–	–
T. glaucescens (SB)	–	67.58	35142	–	–

SB = Stem bark; – = not the highest value for that analyte in the sample set

Antiplasmodial activity in animal model

All aqueous plant extracts and the polyherbal recipe produced statistically significant ($p < 0.05$) suppression of parasitaemia in *P. berghei*-infected mice compared to the negative control from Day 1 through Day 4 of treatment (Table 3). Optimum antiplasmodial activity was recorded on Day 4, with all individual plant extracts reducing parasitaemia to their lowest recorded levels at this time point. The polyherbal recipe demonstrated the highest overall chemosuppression among the experimental groups, achieving activity statistically comparable to chloroquine at 10 mg/kg (positive control). Beyond Day 4, a gradual, statistically significant increase in mean percentage parasitaemia was observed across all plant extract treatment groups, consistent with partial recrudescence a pattern suggesting that while the extracts reduce parasite load substantially, they do not achieve complete parasite clearance comparable to chloroquine during this treatment window.

Table 3: Mean Percentage Parasitaemia (%) in Treated and Control Groups on Days 1–4 and Day 7 (Representative Data)

Treatment Group	Day 1	Day 2	Day 3	Day 4	Day 7
Negative control (saline)	5.1 ± 0.4	9.8 ± 0.7	16.3 ± 1.1	22.7 ± 1.6	34.5 ± 2.1
Chloroquine 10 mg/kg	3.2 ± 0.2	1.1 ± 0.1	0.3 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
A. indica 200 mg/kg	4.0 ± 0.3	3.2 ± 0.2	2.8 ± 0.3	2.4 ± 0.2	4.8 ± 0.5
N. latifolia 200 mg/kg	3.9 ± 0.3	2.9 ± 0.2	2.1 ± 0.2	1.8 ± 0.2	4.2 ± 0.4
T. glaucescens 200 mg/kg	3.8 ± 0.3	2.7 ± 0.2	2.0 ± 0.2	1.7 ± 0.2	4.5 ± 0.4
Polyherbal recipe 200 mg/kg	3.3 ± 0.2	2.0 ± 0.2	1.1 ± 0.1	0.8 ± 0.1	2.4 ± 0.3

Values represent mean ± SEM (n = 5). Day 4 = optimum activity; Day 7 = recrudescence period. Selected representative plants shown.

Acute Toxicity

No mortality was recorded at doses up to 300 mg/kg for any of the ten plant extracts. At doses of 500 mg/kg and above, signs of mild sedation, piloerection, and reduced locomotor activity were observed in some groups. At doses of 1,000 mg/kg, mortality was recorded in mice treated with *L. alata* and *A. djalonensis* extracts. Estimated LD50 values ranged from > 1,000 mg/kg for *A. indica* and *C. cajan* (indicating wide safety margins) to approximately 640–780 mg/kg for *L. alata* and *A. djalonensis*. These findings indicate that all ten plants are relatively safe at the therapeutic doses employed in antiplasmodial testing (100–200 mg/kg), but that caution is warranted at doses of 300 mg/kg and above for certain species.

Haematological and Biochemical Safety Parameters

Plasmodium berghei infection in untreated mice produced significant anaemia (reduced PCV, RBC, and haemoglobin) and elevated WBC count, consistent with the haemolytic pathophysiology of murine malaria. Treatment with plant extracts and the polyherbal recipe significantly ameliorated these haematological derangements at doses of 100 and 200 mg/kg ($p < 0.05$), with values approaching those of the chloroquine-treated group at 200 mg/kg. At doses of 300 mg/kg, some extracts, particularly *L. alata* and *T. glaucescens* produced mildly elevated ALT and AST activities, suggesting subclinical hepatocellular stress at higher doses that warrants further investigation.

Histopathological Findings

Photomicrographs of haematoxylin-and-eosin-stained kidney and liver sections from mice treated at therapeutic doses (100–200 mg/kg) revealed no serious pathological changes. Hepatic architecture was well preserved, with intact hepatocyte plates, central veins, and portal triads. Glomerular and tubular structures of the kidney appeared normal in these groups. At doses of 300 mg/kg and above, mild periportal inflammatory infiltration and occasional hepatocyte swelling were observed in liver sections of mice receiving *A. djalonensis* and *L. alata* extracts. These findings, while not constituting severe histopathological lesions, underscore the importance of dose calibration and support the recommendation that use of these specific plants at high doses requires caution.

Discussion

This study provides a comprehensive pharmacological characterisation of ten medicinal plants used in a traditional polyherbal antimalarial recipe in Ondo State, Nigeria. The findings demonstrate that all ten plants possess significant antiplasmodial activity against *P. berghei* NK 65, exhibit bioactive phytochemical profiles consistent with their ethnomedicinal applications, and maintain an acceptable safety profile at standard therapeutic doses.

The universal presence of alkaloids, saponins, tannins, and cardiac glycosides across all ten plants provides a mechanistic framework for interpreting the observed antiplasmodial effects. Alkaloids — the most studied class of plant-derived antimalarials, encompassing quinine, berberine, and their synthetic derivatives — are known to interfere with parasite haemoglobin digestion, disrupting haem detoxification and inducing oxidative stress within the intraerythrocytic parasite (Tajbakhs *et al*, 2021). Saponins are believed to destabilise the parasite's erythrocytic membrane, facilitating premature release of the parasite from host red blood cells. Tannins have been shown to form complexes with the parasite surface proteins essential for erythrocyte invasion, while cardiac glycosides may interfere with the ion pump mechanisms of the parasite.

The exceptional phytochemical richness of *T. glaucescens* reflected in its high alkaloid (0.75%), saponin (0.36%), and carbohydrate (67.58%) content is consistent with its documented antiplasmodial efficacy at the trophozoite-to-schizont stage transition (Banzouzi *et al*., 2000). Similarly, the elevated cardiac glycoside content in *L. alata* is consistent with the pharmacological principle that cardiac glycosides can disrupt the Na+/K+-ATPase analogues present on the parasite plasma membrane, interfering with osmoregulation and parasite viability.
The Day 4 parasitaemia peak of antiplasmodial activity followed by recrudescence beyond Day 4 is a pattern that has been previously documented with plant-derived extracts and is mechanistically distinct from the complete parasite clearance achieved by chloroquine (Afolabi *et al*., 2021). This pattern may reflect: (i) the relatively short half-life of plant-derived bioactives in the murine system; (ii) plasmodiastatic rather than plasmodicidal activity of the predominant phytoconstituents at the doses tested; or (iii) induction of early resistance adaptations in the parasite. The observation parallels findings by Afolabi *et al*. (2021) for *A. indica* ethanolic extract, which showed parasite relapse on Day 3 post-optimum activity. The superior performance of the polyherbal recipe compared to individual plant extracts on Day 4 and the extended suppression period supports the synergistic multi-target hypothesis of polyherbal formulations.

The antiplasmodial activity of *N. latifolia* observed in this study is strongly corroborated by recent findings. Asanga *et al*. (2024) achieved 83.4% parasite growth inhibition with *N. latifolia* root fractions and identified betulinic acid and ursolic acid as the principal bioactive triterpenoids through GCMS and molecular docking analyses targeting PfEMP-1 and PfPKG proteins, both critical to the pathogenesis and intraerythrocytic survival of *P. falciparum*. Such mechanistic elucidation positions *N. latifolia* as one of the most pharmacologically promising members of the present recipe.

The mineral element data particularly the exceptionally high calcium content of *T. glaucescens* (35,142 mg/kg), the high magnesium in *M. pruessii* (5,837 mg/kg), and the notable zinc in *A. indica* stem bark (402.18 mg/kg) are relevant beyond nutritive supplementation. Calcium ions are implicated in signal transduction pathways that regulate *Plasmodium* gametocytogenesis and erythrocyte invasion; magnesium is required for parasite DNA replication; and zinc deficiency in the host is associated with impaired innate immune responses to malaria. The mineral richness of these plants may therefore contribute to their antiplasmodial effects via indirect immune-modulatory mechanisms.

The safety data from this study carry important public health implications. The absence of serious histopathological lesions in liver and kidney at therapeutic doses (100–200 mg/kg) provides reassurance regarding the traditional use of these plants within normal decoction quantities. However, the dose-dependent tissue changes observed at 300 mg/kg and above particularly periportal inflammation and hepatocyte swelling with *A. djalonensis* and *L. alata* are consistent with the broader literature cautioning against excessive doses of tannin-rich and cardiac glycoside-rich plant materials. These findings support the traditional recommendation to use herbal remedies in moderation and reinforce the call for standardised dosage guidelines.

From the perspective of the contemporary antimalarial crisis, these findings are particularly timely. The emergence of artemisinin partial resistance across eastern Africa confirmed in Rwanda, Uganda, Eritrea, and Ethiopia (Rosenthal *et al*., 2024a; Rosenthal *et al*., 2024b [Nature Reviews Microbiology]) demands that alternative pharmacological options are urgently validated and advanced toward clinical evaluation. Poly-herbal formulations incorporating bio-actively rich, multi-mechanistic plant combinations such as the recipe described herein represent one credible pathway toward providing complementary and affordable antimalarial options, especially in resource-constrained Nigerian communities where access to ACTs remains limited.

Conclusion and Recommendations

This study confirms that all ten medicinal plants *Anthocleista djalonensis, Azadirachta indica, Cajanus cajan, Crescentia cujete, Lawsonia inermis, Lophira alata, Myrianthus pruessii, Nauclea latifolia, Olax subscorpioidea*, and *Terminalia glaucescens* possess evident antiplasmodial activity against *Plasmodium berghei* NK 65 in Swiss albino mice, strenghtened by various and bioactively relevant phytochemical profiles. This investigation substantiates the antiplasmodial potential of all ten evaluated medicinal plants and affirms the pharmacological

basis for their combined use in traditional ethnomedicinal practice. The superior efficacy of the polyherbal formulation over individual extracts highlights the significance of synergistic phytochemical interactions. The safety profile at standard therapeutic doses (100–200 mg/kg) is acceptable, however, caution is required at doses of 300 mg/kg and above particularly for species with high cardiac glycoside and tannin content.
The following recommendations are proposed:

1. **Bioassay-guided fractionation:** Active compounds should be isolated from the most efficacious individual extracts — particularly *N. latifolia, T. glaucescens*, and *A. indica* and characterised using GC-MS, HPLC, and NMR spectroscopy to identify lead antiplasmodial compounds for drug development.
2. **Mechanistic studies:** In vitro assays against *P. falciparum* 3D7 (chloroquine-sensitive) and Dd2 (chloroquine-resistant) strains, combined with molecular docking studies targeting validated *Plasmodium* proteins (PfEMP-1, PfPKG, PfDHFR), should be conducted to elucidate mechanisms of action.
3. **Extended safety evaluation:** Sub-chronic and chronic toxicity studies across wider dose range and treatment duration are required before any component of this recipe advances to clinical testing.
4. **Standardisation and formulation:** A standardised, quality-controlled phytopharmaceutical preparation of the polyherbal recipe should be developed, with defined specifications for phytochemical content and dosage, consistent with WHO guidelines on herbal medicine standardisation.
5. **Community pharmacovigilance:** Surveillance systems should be established to monitor adverse events associated with traditional use of these plants in Ondo State, informing evidence-based harm reduction messaging for traditional medicine practitioners.

Herbal medicine, properly standardised and scientifically validated, represents a legitimate and complementary component of antimalarial therapeutic strategy particularly in contexts where drug resistance threatens the effectiveness of conventional pharmacotherapy.

Declarations

Ethics Approval: All animal experimental procedures were conducted in accordance with the ethical principles of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (2011).

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