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PHYTOCHEMICAL, TOXICOLOGICAL, AND PHARMACOLOGICAL INVESTIGATION OF INDIGENOUS MEDICINAL PLANTS FROM ONDO/OGUN STATES FOR ANTI-MALARIA DRUG DISCOVERY

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Abstract

Malaria remains a major public health challenge in sub-Saharan Africa, compounded by increasing resistance of *Plasmodium falciparum* to existing anti-malarial therapies. This study investigated the phytochemical composition, toxicological safety, and pharmacological efficacy of selected indigenous medicinal plants from Ondo and Ogun States, Southwest Nigeria, traditionally used for malaria treatment. An ethnobotanical survey guided plant selection, followed by solvent extraction, phytochemical screening, chromatographic isolation, and spectroscopic characterisation of bioactive compounds. In-vitro anti-plasmodial activity was evaluated against chloroquine-resistant *P. falciparum* strains, while in-vivo efficacy was assessed using *Plasmodium berghei*-infected albino mice. Acute toxicity studies were conducted in accordance with OECD guidelines. Results revealed that several plant extracts, particularly *Morinda lucida*, *Enantia chlorantha*, and *Vernonia amygdalina*, exhibited strong anti-plasmodial activity with IC_{50} values below 12 µg/mL and significant parasite suppression in vivo. Toxicological findings indicated wide safety margins with no severe adverse effects at therapeutic doses. The study validates indigenous anti-malarial knowledge and identifies promising plant-derived candidates for further drug development. These findings highlight the potential of locally sourced medicinal plants as affordable and culturally acceptable leads in the search for new anti-malarial drugs.

Keywords: *Anti-malarial plants; ethnopharmacology; phytochemistry; toxicology; Plasmodium falciparum; Southwest Nigeria*

Introduction

Malaria remains one of the most persistent and deadly infectious diseases in sub-Saharan Africa, accounting for a disproportionate share of global morbidity and mortality. Nigeria alone contributes a substantial percentage of worldwide malaria cases and deaths, placing immense pressure on public health systems and household livelihoods (WHO, 2023). Although artemisinin-based combination therapies (ACTs) have significantly reduced malaria mortality over the past two decades, growing evidence of *Plasmodium falciparum* resistance, sub-optimal treatment outcomes, and limited access to affordable medicines in rural communities underscore the urgent need for new, safe, and effective anti-malarial agents (Adepoju & Ogundipe, 2022).

Historically, natural products have played a central role in anti-malarial drug discovery. Landmark drugs such as quinine and artemisinin originated from medicinal plants, demonstrating the enduring relevance of ethnomedicine as a foundation for modern pharmacotherapy. In recent years, renewed global interest in plant-based drug discovery has emerged, driven by the search for novel bioactive compounds with unique mechanisms of action and improved safety profiles (Ayoola et al., 2022). This renewed focus is particularly relevant for malaria, where drug resistance continues to threaten current treatment regimens.

Southwest Nigeria, particularly Ondo and Ogun States, is endowed with rich floral biodiversity and a long tradition of herbal medicine. Indigenous communities in these states routinely use medicinal plants to manage febrile illnesses commonly diagnosed as malaria. This ethnomedicinal knowledge, transmitted orally across generations, represents a valuable but increasingly fragile repository of therapeutic insights (Fasola et al., 2021). Despite widespread use and anecdotal reports of efficacy, many of these plants have not been subjected to systematic phytochemical, toxicological, or pharmacological evaluation using modern scientific tools.

The absence of rigorous scientific validation poses two major challenges. First, potentially valuable anti-malarial compounds remain undiscovered, limiting innovation in drug development. Second, the safety of commonly used herbal remedies is often uncertain, exposing users to risks associated with unverified toxicity, inappropriate dosing, and herb–drug interactions (Babalola & Olorunfemi, 2021). As global pharmacological research increasingly prioritises evidence-based herbal medicine, there is a clear need to bridge traditional knowledge systems with laboratory-based drug discovery pipelines.

Advances in phytochemistry, pharmacology, and toxicology now provide robust methodologies for identifying, isolating, and characterising bioactive plant compounds. Techniques such as chromatographic separation, spectroscopic analysis, in-vitro anti-plasmodial screening, and in-vivo efficacy and toxicity testing enable a comprehensive assessment of medicinal plant potential. Integrating these tools within an ethnobotanical framework ensures cultural relevance while meeting international standards for drug discovery research (Adepoju & Ogundipe, 2022; WHO, 2023).

Against this backdrop, this study undertakes a systematic investigation of selected indigenous medicinal plants from Ondo and Ogun States, combining ethnobotanical documentation with phytochemical profiling, toxicological evaluation, and pharmacological testing. By advancing beyond descriptive surveys to experimental validation, the research seeks to identify safe and effective anti-malarial candidates that are locally available, affordable, and scientifically credible. The study contributes to natural-product-based drug discovery, supports the preservation of indigenous knowledge, and aligns with global and national efforts to strengthen locally sourced healthcare solutions in malaria-endemic regions.

Literature Review

Malaria continues to pose a significant public health challenge, particularly in sub-Saharan Africa, where climatic suitability, vector abundance, and socio-economic constraints sustain transmission. Despite substantial progress following the introduction of artemisinin-based combination therapies

(ACTs), emerging resistance of *Plasmodium falciparum* to artemisinin derivatives and partner drugs has been reported in several endemic regions, raising concerns about the long-term effectiveness of current treatment options (WHO, 2023). Studies indicate that drug resistance, coupled with limited access to affordable medicines in rural communities, necessitates the continuous search for novel anti-malarial compounds with alternative mechanisms of action (Adepoju & Ogundipe, 2022).

Natural products have historically provided a rich source of therapeutic agents, with over half of modern anti-malarial drugs derived directly or indirectly from plants. Quinine from *Cinchona* species and artemisinin from *Artemisia annua* exemplify the success of ethnobotany-guided drug discovery. Contemporary pharmacognosy research continues to demonstrate that medicinal plants contain diverse secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds with potent anti-plasmodial activity (Ayoola et al., 2022). These compounds often exhibit multi-target effects, reducing the likelihood of rapid resistance development.

Southwest Nigeria is recognised for its high plant biodiversity and extensive reliance on traditional medicine for primary healthcare. Ethnobotanical surveys in Ondo and Ogun States have documented numerous plant species used locally to treat malaria-like symptoms, including febrile conditions, headaches, and chills (Fasola et al., 2021). However, most documented studies remain descriptive, focusing on plant identification and traditional uses without advancing to laboratory-based validation. This gap limits the translation of indigenous knowledge into evidence-based therapeutic interventions.

Phytochemical screening serves as a critical first step in identifying classes of bioactive compounds responsible for therapeutic effects. Nigerian studies on plants such as *Vernonia amygdalina* and *Morinda lucida* have confirmed the presence of alkaloids and flavonoids with antioxidant and anti-plasmodial properties, yet comprehensive compound isolation and pharmacological profiling remain limited (Babalola & Olorunfemi, 2021). In other African contexts, integrated approaches combining chromatographic separation, spectroscopic characterisation, and in-vitro assays have yielded promising leads against chloroquine-resistant *P. falciparum* strains (Ayoola et al., 2022).

Safety assessment is a critical component of herbal drug discovery, as natural origin does not inherently guarantee safety. Acute and sub-chronic toxicity studies are necessary to determine safe dosage ranges and identify potential organ toxicity. Adepoju and Ogundipe (2022) emphasised that many traditional anti-malarial remedies lack toxicological validation, posing risks of hepatotoxicity and adverse herb–drug interactions. Integrating toxicological evaluation alongside efficacy testing is therefore essential for responsible drug development.

Although existing studies highlight the anti-malarial potential of medicinal plants, few Nigerian investigations adopt a full drug discovery pipeline encompassing ethnobotanical documentation, phytochemical characterisation, anti-plasmodial screening, and toxicity evaluation. This study addresses this gap by systematically investigating indigenous medicinal plants from Ondo and Ogun States using modern pharmacological tools. By combining traditional knowledge with rigorous laboratory validation, the research contributes novel evidence toward the discovery of safe, effective, and locally sourced anti-malarial drug candidates.

Methodology

This study adopted a sequential mixed-methods, laboratory-based experimental design integrating ethnobotanical documentation, phytochemical analysis, toxicological assessment, and pharmacological evaluation to investigate the anti-malarial potential of indigenous medicinal plants from Ondo and Ogun States, Southwest Nigeria. In the first phase, ethnobotanical surveys were conducted using semi-structured interviews with 20–25 experienced traditional healers across three purposively selected Local Government Areas in each state, based on malaria prevalence and reliance on herbal medicine; informed consent was obtained, and plant-use consensus indices were computed to prioritise species. Identified plants were collected, authenticated by a certified taxonomist, assigned voucher numbers, air-dried, pulverised, and stored under controlled conditions. In the second phase, powdered plant materials were subjected to solvent extraction using methanol and aqueous solvents, followed by preliminary phytochemical screening for alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides using standard protocols. Bioactive fractions were separated through Thin Layer Chromatography (TLC) and Column Chromatography (CC), while structural characterisation of isolated compounds was performed using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography–Mass Spectrometry (GC–MS). In the third phase, in-vitro anti-plasmodial activity was evaluated against chloroquine-resistant *Plasmodium falciparum* strains using standard culture assays, with extracts exhibiting $IC_{50} \leq 20$ μg/mL advanced to in-vivo efficacy testing in *Plasmodium berghei*-infected albino mice following established suppressive and curative models. Acute toxicity (LD_{50}) was assessed in rodents in accordance with OECD guidelines, complemented by liver enzyme and histopathological analyses to evaluate safety profiles. Quantitative data were analysed using ANOVA and probit regression at $p < 0.05$, and all procedures involving human participants and animals received ethical approval from relevant institutional review boards.

Results and Discussion

Ethnobotanical Survey and Plant Selection

The ethnobotanical survey identified 14 medicinal plant species commonly used for treating malaria-like symptoms across the surveyed communities. Based on frequency of citation and healer consensus, six plant species were prioritised for laboratory investigation.

Table 1

Ethnomedicinal Plants Identified and Use Consensus

Plant Species	Family	Plant Part Used	Frequency of Citation (%)	Use Consensus Index
Morinda lucida	Rubiaceae	Leaf	82.0	High
Vernonia amygdalina	Asteraceae	Leaf	76.5	High
Alstonia boonei	Apocynaceae	Bark	69.3	Moderate
Azadirachta indica	Meliaceae	Leaf	64.1	Moderate
Enantia chlorantha	Annonaceae	Bark	61.4	Moderate

Nauclea latifolia	Rubiaceae	Root	57.8	Moderate

The high frequency of citation and use consensus observed for species such as *Morinda lucida*, *Vernonia amygdalina*, and *Enantia chlorantha* underscores their entrenched role in traditional malaria management within Southwest Nigeria. This aligns with earlier ethnobotanical surveys that identified these species as core components of indigenous anti-malarial therapy (Fasola et al., 2021). The strong agreement among traditional healers suggests long-standing experiential validation, which modern pharmacognosy recognises as a critical starting point for natural product drug discovery. The prioritisation of these plants based on consensus indices strengthens the scientific credibility of indigenous knowledge systems.

Phytochemical Screening Results

Preliminary phytochemical screening revealed the presence of multiple secondary metabolites associated with anti-plasmodial activity.

Table 2

Phytochemical Constituents of Selected Plant Extracts

Plant Species	Alkaloids	Flavonoids	Tannins	Saponins	Terpenoids
M. lucida	+++	++	++	+	++
V. amygdalina	++	+++	++	++	+
A. boonei	+++	+	++	+	++
A. indica	++	++	+	++	+++
E. chlorantha	+++	+	++	+	++
N. latifolia	++	++	+	+	+

+++ = high presence; ++ = moderate; + = trace

The phytochemical screening revealed abundant alkaloids, flavonoids, tannins, and terpenoids across the selected plant extracts. These classes of secondary metabolites are widely associated with anti-plasmodial activity through mechanisms such as inhibition of heme detoxification, disruption of parasite mitochondrial function, and modulation of oxidative stress (Ayoola et al., 2022). The particularly high alkaloid content observed in *M. lucida*, *A. boonei*, and *E. chlorantha* is consistent with earlier reports linking alkaloids to potent anti-malarial effects, as exemplified by quinine-derived compounds. This phytochemical richness provides a biochemical basis for the observed pharmacological activity.

In-Vitro Anti-Plasmodial Activity

Methanolic extracts showed stronger anti-plasmodial activity than aqueous extracts. Three species demonstrated high activity against chloroquine-resistant *Plasmodium falciparum*.

Table 3

In-Vitro Anti-Plasmodial Activity of Plant Extracts

Plant Species	Extract Type	IC_{50} (µg/mL)	Activity Level
M. lucida	Methanol	8.6	High
V. amygdalina	Methanol	11.2	High
A. boonei	Methanol	14.5	Moderate
A. indica	Methanol	18.7	Moderate
E. chlorantha	Methanol	9.4	High
N. latifolia	Methanol	21.3	Low

$IC_{50} \leq 10$ µg/mL = high activity

The in-vitro assays demonstrated that methanolic extracts exhibited stronger anti-plasmodial activity than aqueous extracts, a pattern commonly reported in natural product research due to the enhanced solubility of lipophilic bioactive compounds in organic solvents (Babalola & Olorunfemi, 2021). The low IC_{50} values recorded for *M. lucida* (8.6 µg/mL), *E. chlorantha* (9.4 µg/mL), and *V. amygdalina* (11.2 µg/mL) against chloroquine-resistant *Plasmodium falciparum* strains are particularly noteworthy. These results compare favourably with similar African studies and suggest potential effectiveness against drug-resistant malaria, a critical global health challenge (Adepoju & Ogundipe, 2022).

In-Vivo Anti-Malarial Efficacy

Extracts with $IC_{50} \leq 20$ µg/mL were advanced to in-vivo testing. Significant parasite suppression was observed.

Table 4

In-Vivo Parasite Suppression in Plasmodium berghei-Infected Mice

Plant Species	Dose (mg/kg)	Parasitaemia Suppression (%)	Significance ($p < 0.05$)
M. lucida	400	72.8	Yes
V. amygdalina	400	68.4	Yes
E. chlorantha	400	74.1	Yes
A. boonei	400	61.2	Yes
Chloroquine (control)	10	89.5	Yes

The significant parasite suppression observed in *Plasmodium berghei*-infected mice further confirms the translational relevance of the in-vitro findings. Suppression rates exceeding 70% for *M. lucida* and *E. chlorantha* indicate strong in-vivo efficacy, approaching the performance of standard anti-malarial controls. This reinforces the argument that ethnomedicinal plants can yield pharmacologically active compounds capable of systemic activity, not merely in-vitro inhibition. Similar suppression levels have been reported in East African studies employing integrated phytochemical–pharmacological pipelines (Ayoola et al., 2022).

Acute Toxicity Assessment

All tested extracts exhibited relatively high safety margins, with no mortality recorded at doses up to 2,000 mg/kg.

Table 5

Acute Toxicity (LD_{50}) and Safety Indicators

Plant Species	LD_{50} (mg/kg)	Observed Toxic Signs	Safety Classification
M. lucida	>2000	None	Safe
V. amygdalina	>2000	Mild sedation	Safe
E. chlorantha	>2000	None	Safe
A. boonei	>1500	Mild lethargy	Relatively Safe

The acute toxicity assessments revealed favourable safety profiles, with LD_{50} values exceeding 1,500–2,000 mg/kg for all tested extracts. These findings are consistent with previous toxicological studies on *V. amygdalina* and *M. lucida*, which reported wide therapeutic windows and minimal hepatotoxic effects at pharmacologically relevant doses (Adepoju & Ogundipe, 2022). The absence of severe toxic signs supports the continued traditional use of these plants and strengthens their suitability for further drug development. Importantly, integrating toxicity evaluation alongside efficacy testing addresses a major limitation of many earlier Nigerian studies that focused solely on bioactivity.

Conclusion

This study provides comprehensive scientific evidence supporting the anti-malarial potential of selected indigenous medicinal plants from Ondo and Ogun States, Southwest Nigeria. By integrating ethnobotanical documentation with phytochemical profiling, toxicological assessment, and pharmacological evaluation, the research advances beyond anecdotal use to experimentally validated drug discovery pathways. The high use-consensus recorded among traditional healers confirms the cultural relevance and historical efficacy of plants such as *Morinda lucida*, *Vernonia amygdalina*, and *Enantia chlorantha*. Phytochemical analyses revealed rich profiles of alkaloids, flavonoids, tannins, and terpenoids, which are known to play critical roles in anti-plasmodial activity. The in-vitro assays demonstrated strong inhibitory effects against chloroquine-resistant *Plasmodium falciparum*, while in-vivo studies confirmed significant parasite suppression in *Plasmodium berghei*-infected animal models. Importantly, the favourable acute toxicity profiles observed across all tested extracts indicate wide safety margins, supporting their continued traditional use and potential for pharmaceutical development. Collectively, these findings validate indigenous knowledge systems and highlight locally available medicinal plants as promising sources of novel, affordable, and culturally acceptable anti-malarial drug candidates.

Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. Sub-chronic and chronic toxicity studies, including genotoxicity and reproductive toxicity assessments, are recommended to fully establish safety profiles prior to clinical consideration.

2. Promising plant extracts and isolated compounds should be advanced into preclinical optimisation and, where feasible, early-phase clinical trials in collaboration with pharmaceutical and regulatory bodies.
3. Conservation strategies should be implemented to protect high-value medicinal plant species from overharvesting, including cultivation programmes and community-based resource management.
4. Government and funding agencies should strengthen support for ethnopharmacological research and promote the integration of validated herbal medicines into national malaria control strategies, in line with WHO recommendations.

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