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Full Length Research Article

INHIBITORY ACTIVITY OF *ANOGEISSUS LEIOCARPUS* AQUEOUS LEAF EXTRACT AGAINST *PLASMODIUM FALCIPARUM* SCHIZONTS

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ABSTRACT
Malaria is an infectious disease caused by protozoan parasites of the genus *Plasmodium*. The widespread emergence of drug-resistant strains of *P. falciparum* underscores the urgent need for novel antimalarial agents. This study investigated the schizont growth inhibition effect of *Anogeissus leiocarpus* (Marke), a medicinal plant widely used in African traditional medicine. Using an in vitro schizont maturation inhibition assay, the aqueous leaf extract of *A. leiocarpus* was tested against *P. falciparum*. The half-maximal inhibitory concentration (IC_{50}) was determined over a concentration range of 100–1.56 µg/mL. Results showed a dose-dependent inhibition of schizont maturation, with an IC_{50} of 1.828 µg/mL. Morphologically, the highest schizont growth inhibition of 86.32 % occurred at 100 µg/mL. A significant reduction in plasmodial viability compared with the negative control was observed. This study highlights the bioactivity of *A. leiocarpus* as an affordable, accessible, and effective alternative to synthetic antimalarial drugs. Future research should focus on isolating active compounds, elucidating their mechanisms of action, and evaluating their efficacy and safety in vivo.

Keywords: Antimalarial activity; schizont inhibition; *Plasmodium falciparum; Anogeissus leiocarpus;* Leaf extract.

INTRODUCTION

Malaria has been recognized as a serious health problem since the earliest historical times. This disease is caused by protozoan parasites belonging to the genus *Plasmodium* (World Health Organization, 2023). The strong negative pressure of the disease has likely forced the evolution of human populations in malaria-endemic regions and the selection of some unique genetic variants. Malaria continues to be a major global health challenge, impacting millions annually and disproportionately affecting low-income countries. According to the World Health Organization (WHO), malaria is endemic in 87 countries, with an estimated 247 million cases reported globally in 2022. The disease remains particularly severe in sub-Saharan Africa, where 95% of global cases and 96% of malaria deaths occur. Among the most affected nations are Nigeria, the Democratic Republic of Congo, and Uganda, collectively contributing to over 50% of global malaria-related mortality. The burden of malaria is a significant barrier to achieving global health goals, including the Sustainable Development Goals (SDGs). Specifically, SDG 3 (Good Health and Well-being) aims to end the epidemic of malaria and other communicable diseases by 2030. While progress has been made in reducing malaria mortality rates over the past two decades, the emergence of drug-resistant *Plasmodium falciparum* strains poses a formidable challenge to global elimination efforts. Synthetic antimalarial drugs, though initially effective, face several challenges, including high production costs, limited accessibility in low-income regions, and the rapid development of drug resistance (Mpiana *et al*., 2018). Moreover, adverse side effects associated with some synthetic treatments further complicate their widespread use (Ibrahim *et al*., 2021). These challenges highlight the pressing need for alternative therapeutic options that are affordable, accessible, and effective against resistant strains of *P. falciparum*.

However, despite its ethnomedicinal applications, the specific antiplasmodium potential of *A. leiocarpus* leaf extracts against *P. falciparum* has not been rigorously studied.

The implications of this research extend beyond academic inquiry. Findings from this study may inform the development of plant-based therapeutics that align with the global agenda for malaria eradication. Moreover, identifying effective and affordable alternatives to synthetic drugs could improve treatment outcomes for malaria patients in endemic regions. This research also has the potential to contribute to the conservation and sustainable utilization of medicinal plants by emphasizing their value in modern medicine (Tchokouaha *et al*., 2020).

The absence of rigorous studies evaluating the antiplasmodial potential of *A. leiocarpus* with a focus on its schizont growth inhibition activity is a critical gap. There is also a lack of data on the plant's effectiveness against molecularly characterized clinical strains of *P. falciparum*, which are pivotal for understanding drug resistance and efficacy. By systematically investigating the schizont growth inhibition effect of *Anogeissus leiocarpus*, this study seeks to provide a scientific foundation for the plant's potential as a source of novel antimalarial agents and contribute to the fight against malaria.

Natural products have historically been a reliable source of antimalarial compounds, such as quinine and artemisinin, underscoring the potential of plants as reservoirs for new antimalarial agents (Ntie-Kang *et al*., 2019). *Anogeissus leiocarpus* (Marke) is a medicinal plant widely utilized in African traditional medicine for its antiparasitic, antimicrobial, and anti-inflammatory properties (Adeyemi *et al*., 2018). Despite its ethnomedicinal importance, there is limited scientific evidence on its specific activity against *P. falciparum*, particularly during the schizont stage of the parasite's life cycle—a critical stage for replication and survival. Moreover, the potential of *A. leiocarpus* to inhibit growth in molecularly characterized *P. falciparum* clinical strains remains unexplored, leaving a significant gap in the scientific validation of its traditional uses.

The absence of rigorous studies evaluating the antiplasmodial potential of *A. leiocarpus* with a focus on its schizont growth inhibition activity is a critical gap. There is also a lack of data on the plant's effectiveness against molecularly characterized clinical strains of *P. falciparum*, which are pivotal for understanding drug resistance and efficacy. By systematically investigating the schizont growth inhibition effect of *Anogeissus leiocarpus*, the research aim is to evaluate the schizont growth inhibition effect of *Anogeissus leiocarpus* (Marke) against *Plasmodium falciparum* clinical strains, with a view to identifying its potential as a natural source of antimalarial agents and contributing to the development of alternative treatments for malaria, particularly in the face of increasing drug resistance.

MATERIALS AND METHODS

Sample Collection and Identification: Fresh leaves of *Anogeissus leiocarpus* were collected from the Tudun Wada area, Kaduna, Kaduna State, Nigeria. The plant material was taxonomically identified and authenticated at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and deposited under the voucher number ABU-01738.

Preparation of Plant Material

The leaf was oven-dried at 40–50°C and then pulverized into a fine powder using a mortar and pestle.

Preparation of Extract Concentrations

A stock solution of the *Anogeissus leiocarpus* extract was prepared by dissolving a known weight of the dried extract in an appropriate solvent to obtain a concentration of 100 µg/mL. From this stock, a series of two-fold serial dilutions was prepared using the assay medium to obtain final concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.56 µg/mL.
The control group consisted of parasite cultures treated with the solvent or culture medium only, without the plant extract.

Collection of Blood Sample

Blood samples were collected from volunteers in the study group with their consent through a finger prick and analyzed immediately. Microscopic Detection of *Plasmodium falciparum* A malaria screening was conducted to confirm the presence of *Plasmodium falciparum* by preparing a blood smear on a slide, air-drying, and fixing it with methanol. The slides were then stained with a 2% Giemsa solution by immersion and allowed to stain for 12–15 minutes, after which they were removed and washed with distilled water and allowed to dry. The slides were then examined under ×100 oil immersion to confirm the presence of malaria parasites.

Preparation and Deactivation of Serum

About 10 ml of O^+ blood was collected from the veins of a consented volunteer from the study group in a plain sample bottle. The blood was allowed to clot for 30 minutes, after which it was centrifuged at 1,500 rpm for 5 minutes. It was then placed in a water bath at 56°C for 30 minutes to deactivate the serum.

Preparation of 50% Hematocrit Red Blood Cells (RBC)

Exactly 50% Hematocrit Red Blood Cells (RBC) were prepared in accordance with the method reported by Zahra *et al.* (2013). Ten milliliters (10 ml) of O+ blood was collected from a confirmed positive volunteer into an EDTA bottle. The sample was gently mixed to incorporate the anticoagulant. The blood was then centrifuged at 1,500 rpm for 5 minutes, after which the buffy coat, WBC, platelets, and plasma were removed using a Pasteur pipette. An equal volume of washing solution PBS was then added and swirled for 30 seconds, and then centrifuged. The supernatant was removed, and the process was repeated twice. For the third time, RPMI 1640 was added, mixed well, and further centrifuged for 5 minutes. The supernatant was removed, and an equal volume of RPMI 1640 was added to make 50% hematocrit.

Seeding and Culture Initiation

The culture mixture was prepared using the method described by Inga *et al*. (2008). It involves combining 4 mL of heat-inactivated serum, 2 mL of washed RBCs (50% hematocrit), and 14 mL of RPMI 1640 medium in a single solution. Using a multichannel pipette, 100 µL of the culture mixture was added to each well of the microtiter plate, including the control wells. The plate was placed inside a desiccator, and a lit candle was used to create a low-oxygen environment. The desiccator was sealed and incubated at 37°C for 48 hours.

Post-incubation and Slide Preparation

After 48 hours, the top layer of each well was carefully removed and discarded. The bottom layer was gently mixed, and a small drop from each well was transferred to separate slides. Thin blood smears were prepared, air-dried, and fixed in methanol for 3–5 seconds. The thin smears were stained with a 2% Giemsa solution for 12–15 minutes, washed with distilled water, and allowed to dry. The slides were examined under a microscope to assess parasitemia. The number of schizonts per 200 asexual parasites was recorded to determine the percentage inhibition of parasite growth.

In vitro micro test (Mark III)] test

Plant extracts were assessed for antiplasmodial activity *in vitro* using the modified WHO (2023) method micro test (Mark III), based on assessing the inhibition of schizont maturation. The assay was performed in duplicate in a 96-well microtiter plate. RPMI 1640 (Sigma Company, USA) was the culture medium used for the cultivation of *P. falciparum*. The concentrations prepared by dilution were (100, 50, 25, 12.5, 6.25, 3.125, and 1.56µg/ml). Negative control treated by solvent and positive control (Quinine) were added to each set of experiments. Two hundred microliters from the blood mixture media were added to each well in a 96-well microtiter plate and incubated in CO_2 conditions at 37.5°C for 24–30 h. After incubation, contents of the wells were harvested and stained on corresponding slides for 30 min in a 2% Giemsa solution, pH 7.2. After that, the developed schizonts were counted against the total asexual parasite count of 200. The count process was done in duplicate, and the data were analyzed by using a computerized mathematical log-concentration-response probit analysis model for result interpretation.

Percentage (%) Parasitemia and IC_{50} Determination

The stained slides were mounted on a microscope using a 100 × oil immersion objective lens. Prepared Giemsa-stained thin blood smears were examined under a light microscope using a 100× oil immersion objective lens. Slides were systematically scanned in multiple fields, and the number of *Plasmodium falciparum*–infected red blood cells (RBCs) was counted alongside the total number of RBCs, with counting continued until at least 1,000 erythrocytes had

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been assessed per slide to ensure statistical reliability. Percentage parasitemia was calculated using the formula:
Percentage Parasitemia (%) =
Total Number of RBCs Counted / Number of Parasitized RBCs× 100
The half-maximal inhibitory concentration (IC_{50}) was determined as the extract concentration that inhibited 50% of parasite growth. Quinine or chloroquine was included as a standard reference drug for comparison.
The percentage parasitemia can be calculated using:

% Paracetemia = No. of parasitized RBCs / Total no of RBCs ×100

IC_{50} represents the concentration of the extract that reduced parasitemia by 50%.

Statistical Analysis
The results were expressed as mean ± standard deviation (SD). The data were analyzed using a one-sample t-test with the Graphpad Prism version 10 with a post hoc-test. The mean percentage inhibition of schizonts or parasitemia in the treated group was compared to the hypothesized value (e.g., 0% inhibition for no effect). A p-value of less than 0.05 was considered statistically significant (P ≤ 0.05).

RESULTS
Microscopic evaluation of Giemsa-stained blood smears corroborated the results of the schizont growth inhibition assay. Parasites treated with higher concentrations of *Anogeissus leiocarpus* extract displayed significant disruptions in schizont development, with fewer mature forms observed. The morphological abnormalities included incomplete schizont formation and reduced parasitemia rates (plate 1). In this study, *Anogeissus leiocarpus* exhibited antiplasmodial activity against *Plasmodium falciparum*, as evidenced by a low IC_{50} value (1.828 ± 0.43 µg/mL). This finding suggests strong potency and significant schizont growth inhibition. The % viability and schizont count were significantly reduced by the *Anogeissus leiocarpus extract* from 100% viability and 164.5± 0.71 schizont count to 22.5% viability and 13.68±0.49 schizont count, respectively (Table 1). The schizont growth inhibition assay demonstrated a dose-dependent reduction in the maturation of *Plasmodium falciparum* schizonts upon treatment with *Anogeissus leiocarpus* aqueous extracts. At a concentration of 100 µg/mL, the extract achieved an inhibition rate of 86.32%, with an IC_{50} value of 1.828 µg/mL, indicating substantial potency. Quinine (Standard drug) used reduced the viability count of *Plasmodium falciparum* from 274 ±1.41 schizont count to 17 ±1.41, having 93.79% inhibition of schizont growth (Table 2).

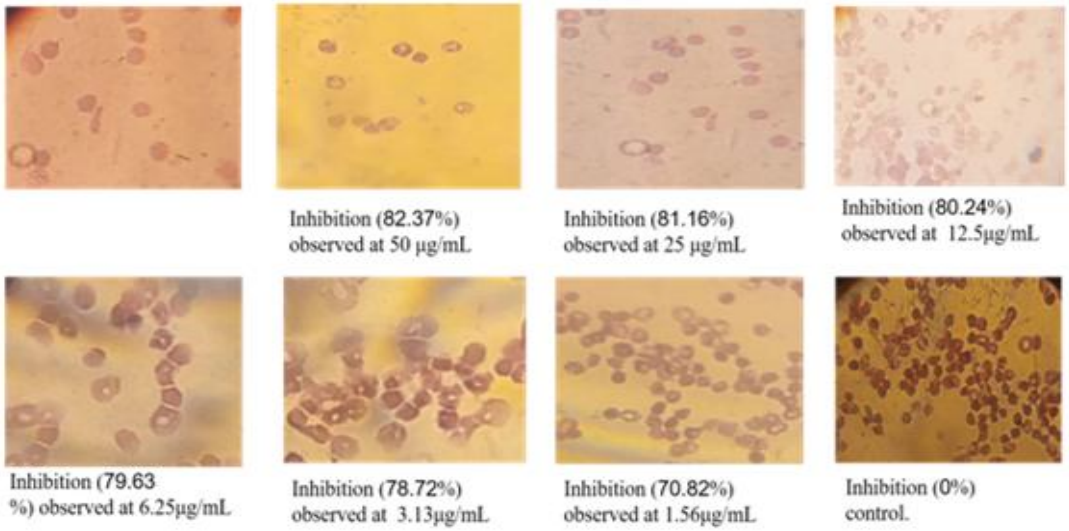

Plate 1: Giemsa-Stained Blood Smears Showing the Effect of *Anogeissus leiocarpus* Extract Concentration on *Plasmodium falciparum* Schizont Maturation

Table 1: Inhibition of *Plasmodium falciparum* Growth by Aqueous Extract of *A. leiocarpus*

S/N	Conc(µg/ml)	Schizont Count	%Viability	%Inhibition
A	Control	164.5± 0.71^{a}	100±0.00^{a}	0.00
B	1.56	48±1.41^{b}	29.18±0.73^{b}	70.82±0.73^{a}
C	3.125	35±1.41^{c}	21.28±0.95^{bc}	78.72±0.9^{5a}
D	6.25	33.5±2.12^{c}	20.37±1.38^{c}	79.63±1.38^{a}
E	12.5	32.5±0.71^{c}	19.76±0.51^{c}	80.24±0.51^{a}
F	25	31±1.41^{c}	18.84±0.78^{c}	81.16±0.78^{b}
G	50	29±0.00^{cd}	17.63±0.08^{c}	82.37±0.08^{ab}
H	100	22.5±0.71^{d}	13.68±0.49^{d}	86.32±0.49^{ab}

Values having different superscripts down the column are significantly different (P ≤ 0.05)
IC_{50} **=** 1.828 ± 0.43 (µg/mL)

Table 2: Inhibition of *Plasmodium falciparum* Growth by Quinine

S/N	Conc(µg/mL)	Schizont Count	% viability	% Inhibition
A	00	274 ±1.41 [a]	100.00±0.00[a]	0.00
B	1.56	146.5 ±2.12 [b]	53.47±1.05[b]	46.53±1.05[a]
C	3.13	116.5 ±2.12 [c]	42.52±0.99[b]	57.48±0.99[a]
D	6.25	91.5 ±2.12 [c]	33.40±0.95[c]	66.60±0.95[b]
E	12.50	51.5 ±2.12 [d]	18.79±0.68[d]	81.21±0.68[c]
F	25.00	41.5 ±2.12 [d]	15.15±0.85[d]	84.85±0.85[cd]
G	50.00	27 ±1.41 [e]	9.85±0.47[e]	90.15±0.47[cd]
H	100.00	17 ±1.41 [f]	6.21±0.55[e]	93.79±0.55[d]

Values having different superscripts down the column are significantly different (P≤0.05)
IC_{50} (µg/mL) = 3.138± 0.74

DISCUSSION

In this study, *Anogeissus leiocarpus* demonstrated significant schizont growth inhibition activity, with an IC_{50} value of 1.828 ± 0.43 µg/mL, highlighting its high potency. *A. leiocarpus* exhibited significant antiplasmodial activity, further validating its traditional use in malaria treatment. This result underscores the therapeutic potential of *A. leiocarpus* and aligns with previous studies by Inger *et al*. (2008) on antiplasmodial activities of *A. leiocarpus* against *P. falciparum* using the fluorescence-activated cell sorting (FACS) method.

This finding complies with the report of Olusegun *et al*. (2012), where *Anogeissus leiocarpus* extracts showed antiplasmodial activity. For comparison with quinine, the standard reference drug, which exhibited an IC_{50} value of 3.138 µg/m in this research findings. The inclusion of quinine as a control established a benchmark for antiplasmodial activity, allowing for the accurate assessment of the extract's efficacy. The comparative analysis underscores the therapeutic potential of *A. leiocarpus* and its applicability in managing drug-resistant strains of malaria. This finding complies with the report of Ohashi *et al.* (2018), where *Anogeissus leiocarpus extract* was shown to have a higher antiplasmodial activity than quinine. These results not only validate the traditional use of *A. leiocarpus* as an antimalarial remedy but also emphasize its superior inhibitory effect on schizont maturation compared to the standard (quinine).

The potential synergistic effects of combining *A. leiocarpus* extracts with conventional antimalarial drugs warrant further exploration. This strategy could reduce drug dosages, thereby mitigating side effects and combating drug resistance. However, critical challenges must be addressed, including variability in plant extract composition, scalability of extraction processes, and standardization for consistent therapeutic outcomes.

Conclusion

Anogeissus leiocarpus aqueous extract exhibited potent, dose-dependent antiplasmodial activity against *Plasmodium falciparum*, achieving an IC_{50} of 1.828 ± 0.43 µg/m. This finding supports the traditional use of *Anogeissus leiocarpus* aqueous leaf extract as an antimalarial drug and its development for complementary or alternative therapy for malaria.

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