

Antimalarial Effects of Aqueous Extract of *Senna singueana* Leaf against *Plasmodium falciparum*

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ABSTRACT

Malaria remains a significant public health concern, exacerbated by the emergence of drug-resistant *Plasmodium falciparum* strains. This study evaluated the anti-malarial effect of *Senna singueana* against clinical strains of *P. falciparum* using an ex vivo assay. The percentage viability and inhibition data revealed a clear dose-dependent reduction in parasite viability and growth inhibitory effects against the parasite. The % viability reduces with increase in extract concentration while the % inhibitory effects of *Senna singueana* extract increases with increase in extract concentration. The % inhibitory concentration of the extract against *P. falciparum* growth was significantly ($p < 0.05$) higher than the negative control at 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$. While no significant difference was observed with other concentrations. *Senna singueana* extract exhibited anti-malarial activity, with an IC_{50} value of 4.311 $\mu\text{g/mL}$. Quinine phosphate, used as a positive control, demonstrated an IC_{50} value of 1.988 $\mu\text{g/mL}$. This study highlights the potential of aqueous extract of *Senna singueana* leaf as an anti-malarial agent and underscores the importance of ex vivo assays in the discovery and development of novel anti-malarial therapies

Keyword: Malaria; Resistant; Viability; Activity.

INTRODUCTION

Malaria is a life-threatening disease caused by *Plasmodium* parasites, transmitted through the bite of infected *Anopheles* mosquitoes (WHO, 2020). The disease is a major public health concern globally, particularly in tropical and subtropical regions. Malaria has been a human affliction for thousands of years, with evidence of its existence dating back to ancient civilizations in Egypt, Greece, and Rome (Bruce-Chwatt, 2016). The disease was a major obstacle to human development and progress, particularly during the colonial era (Packard, 2017). Malaria is a significant global health problem, with 241 million cases reported in 2020, resulting in 627,000 deaths (WHO, 2020). The disease is endemic in 109 countries, with the majority of cases and deaths occurring in Africa. Malaria has a profound impact on individuals, communities, and economies. The disease causes significant morbidity and mortality particularly among children under five and pregnant women (WHO, 2024). Furthermore Malaria can have a devastating impact on economic development, particularly in endemic countries. According to the world health organization (WHO, 2019). Malaria can increase poverty by reducing productivity, increasing healthcare costs, and decreasing economic opportunities (WHO, 2019). In

addition a study by the World Bank estimated that malaria cost Africa approximately \$12 billion annually in lost productivity and healthcare expenses (World Bank, 2019). Despite the availability of effective treatments, malaria control efforts face significant challenges such as widespread resistance to conventional anti-malarial drugs, such as chloroquine and sulfadoxine-pyrimethamine, has compromised their effectiveness (WHO, 2019). The Emergence of multi-drug resistant *Plasmodium falciparum* strains underscores the need for new treatments (Ménard *et al.*, 2018). Additionally Limited access to effective treatments in many endemic areas, due to factors such as cost, availability, and healthcare infrastructure, exacerbate the problem (WHO, 2019). Investigating new treatments for malaria is crucial for addressing these challenges.

The life cycle of *Plasmodium falciparum* is complex and involves multiple stages, Sporozoite Stage: The life cycle of *P. malariae* begins when an infected female Anopheles mosquito bites a human host and injects sporozoites into the bloodstream (Collins *et al.*, 2019). Exo-Erythrocytic Stage: Once in the bloodstream, the sporozoites travel to the liver, where they infect hepatocytes and undergo exo-erythrocytic schizogony (Battle *et al.*, 2019). This stage is longer in *P. malariae* than in other Plasmodium species, lasting around 10-14 days.

Erythrocytic Stage: The merozoites released from the liver infect red blood cells (RBCs), where they undergo erythrocytic schizogony (van Schaijk *et al.*, 2019). *P. malariae* infects mature RBC and can cause mild anemia.

Gametocyte Stage: Some of the parasites in the RBCs develop into gametocytes, which are the sexual stage of the parasite (Chu *et al.*, 2019). The gametocytes are responsible for transmitting the infection to other humans through the bite of an infected mosquito.

Treatment of malaria involves the use of antimalarial medications to eliminate the parasites from the body. The choice of treatment depends on the severity of the disease, the species of the parasite, and the patient's age, weight, and medical history (WHO, 2020). For uncomplicated malaria, treatment typically involves the use of artemisinin-based combination therapies (ACTs), which are effective against all species of malaria parasites (White, 2019). Examples of ACTs include artemether-lumefantrine, artesunate-mefloquine, and dihydroartemisinin-piperazine (Llanos-Cuentas *et al.*, 2019).

Prevention and control of malaria involve a combination of measures aimed at reducing the transmission of the disease and protecting individuals from infection. One of the most effective ways to prevent malaria is through the use of insecticide-treated bed nets (ITNs), which can reduce the risk of malaria transmission by up to 50% (WHO, 2020). Other measures include indoor residual spraying (IRS) with insecticides, which can reduce the population of malaria-carrying mosquitoes (CDC, 2020). In addition to these measures, individuals can also take steps to protect themselves from malaria. This includes wearing protective clothing, applying insect repellents, and avoiding peak mosquito hours (NIAID, 2020). Chemoprophylaxis, which involves taking antimalarial medications before traveling to malaria-endemic areas, is also an effective way to prevent malaria (CDC, 2020). Integrated vector management (IVM) is a comprehensive approach to controlling malaria vectors, which involves combining multiple methods, such as ITNs, IRS, and larval control (WHO, 2020). IVM can be an effective way to reduce malaria

transmission and prevent outbreaks. Early diagnosis and treatment of malaria are also critical components of prevention and control. Prompt treatment of malaria cases can help prevent the spread of the disease and reduce the risk of complications and death (WHO, 2020).

The *ex vivo* anti-malaria activity assay involves several steps. First, human red blood cells are infected with Plasmodium parasites, usually *Plasmodium falciparum* (Olorunfemi *et al.*, 2016). Next, plant extracts, fractions, or isolated compounds are prepared and dissolved in a suitable solvent. Infected red blood cells are then incubated with different concentrations of plant extracts or fractions. After incubation, the parasitemia (percentage of infected red blood cells) is measured using microscopy or flow cytometry (Onyekwelu *et al.*, 2019). Finally, the IC₅₀ value (concentration of plant extract or fraction required to inhibit 50% of parasite growth) is calculated using dose-response curves.

Ex vivo anti-malaria activity assays offer several advantages, including high throughput, cost-effectiveness, and rapid results (Wu *et al.*, 2018). Multiple plant extracts or fractions can be tested simultaneously, and results can be obtained within a few days. However, these assays also have some limitations. Results may not accurately predict *in vivo* efficacy, and *ex vivo* assays do not provide information on the pharmacokinetics of plant extracts or fractions (Liu *et al.*, 2014). Despite these limitations, *ex vivo* anti-malaria activity assays are widely used in the discovery and development of anti-malarial drugs from plants (Kumar *et al.*, 2017). They provide a rapid and cost-effective way to evaluate the efficacy of plant extracts, fractions, or isolated compounds against Plasmodium parasites.

Senna singueana is a shrub-like plant that belongs to the family Fabaceae (Leguminosae) (Kokwaro, 2019). It is native to tropical Africa, including countries such as Kenya, Tanzania, and Uganda (Beentje, 2021). *Senna Singueana* is a deciduous shrub or small tree that grows up to 3-6 meters in height (Kokwaro, 2019). It has a broad, rounded crown and a straight, smooth bole. The leaves are compound, with 4-6 pairs of leaflets that are lanceolate to ovate in shape (Kokwaro, 2019).

Screening of different concentrations of *Senna singueana* is crucial in evaluating the plant's anti-malarial activity. This involves testing various concentrations of the plant extract or fractions against Plasmodium parasitemia (Kumar *et al.*, 2017). The significance of screening different concentrations of *Senna singueana* lies in its potential to identify the most effective and safe concentration for anti-malarial activity.

The aim of this study is to evaluate the *ex vivo* anti-plasmodial activity of *Senna singueana* against *Plasmodium falciparum* clinical strain.

MATERIALS AND METHODS

RPMI-1640 medium (Thermo Fisher Scientific, USA), Absolute ethanol (Sigma-Aldrich, USA, 99.9% purity), Phosphate buffer saline (PBS) (Thermo Fisher Scientific, USA, 1X, pH 7.4) Methanol (Fisher Chemical, USA, 99.9% purity, 2% Giemsa solution (Sigma-Aldrich, USA, staining solution) and Distilled water.

Plant Collection and Preparation

Senna singueana leaf was harvested fresh from a garden at Kabala Costain, Kaduna. The plant was identified in a herbarium section of Pharmacy Department at Kaduna State

University. A voucher specimen number was deposited KASU/PCG/HERB/298. The freshly collected leaf was rinsed with distilled water to remove dirt and debris then oven dried at 40 - 50 degree Celsius for a few days. The dried leaf was subsequently pulverized into fine powder using a Mortar and Pestle.

To prepare the extract, 80 g of powdered leaves were measured and transferred to a 2-liter conical flask. One liter of distilled water was added, and the mixture was agitated for several hours. The flask was then left undisturbed at room temperature (25-30°C) for 48 hours, with occasional agitation to facilitate extraction. The mixture was filtered using Whatman No. 1 filter paper to separate the liquid extract. The filtrate was collected and evaporated using a water bath at 40-60°C to concentrate the extract. The resulting solid residue was weighed, and 5 g of the crude aqueous extract were dissolved in distilled water to prepare a 5 mg/mL working stock. Quinine phosphate a standard anti malaria drug was also prepared as a positive control to validate the anti-malaria activity of the extract.

Serial dilutions of *Senna singueana* extract was prepared in RPMI 1640 medium using a 2-fold dilution series, resulting in 4-7 concentrations. Quinine phosphate a standard anti malaria drug was also prepared in parallel as a positive control. The diluted extracts and quinine phosphate were added to the wells containing the culture mixture. The micro titer plate was placed in the desiccator. A low oxygen environment was created using a lit candle flame. The desiccator was sealed and incubated at 37 degree Celsius for 48 hours.

Collection of Blood Sample

Blood samples were collected from human volunteers within the research group. Each volunteer donated 10 mL of blood, which was collected and stored in a sterile container. A malaria test screen was subsequently conducted to confirm the presence or absence of malaria parasites in the collected blood samples. To confirm the presence of malaria parasites in the collected blood samples, a microscopic examination was performed using the method of Muziazia *et al.*, 2020. Thin blood smears were prepared on glass slides, air-dried, and fixed with methanol. The slides were then stained with 2% Giemsa stain by immersion for 12-15 minutes. After staining, the slides were washed with distilled water and allowed to dry. The stained slides were subsequently examined under a microscope to detect the presence of *Plasmodium falciparum* parasites. This step was crucial in confirming whether the collected blood samples were infected with malaria parasites.

Preparation and Deactivation of Serum

Ten milliliters (10 mL) of whole positive blood was collected from the veins of volunteer participants from the study group into a plain sample bottle. The blood was allowed to clot for 30 minutes at room temperature. The clotted blood was then centrifuged at 1500 RPM for 5 minutes to separate the serum. The serum was subsequently heat-inactivated by gentle placement in a water bath at a 56°C for 30 minutes.

Preparation of 50% Hematocrit Red Blood Cells

The method described by moody (2002) was used for the preparation of hematocrit red blood cells. Exactly 10 mL of O-positive blood was obtained from a confirmed malaria-positive volunteer and collected into an EDTA tube. The blood sample was gently mixed to distribute the anticoagulant evenly. Centrifugation at 1500 RPM for 5 minutes

followed, resulting in the separation of blood components. The buffy coat, containing white blood cells and platelets, was carefully removed using a pasture pipette. The remaining erythrocytes were washed with an equal volume of phosphate-buffered saline (PBS) solution. This washing process was repeated twice to ensure thorough removal of contaminants. In the final washing step, RPMI 1640 medium was added to the erythrocytes, mixed, and incubated for 30 seconds. After centrifugation at 1500 RPM for 5 minutes, the supernatant was discarded. An equal volume of RPMI 1640 medium was then added to achieve a 50% hematocrit.

Seeding and culture initiation

The culture mixture was prepared by combining 4 mL of heat-inactivated serum, 2 mL of washed RBCs (50% hematocrit), 14 mL of RPMI 1640 medium. Using a multichannel pipette, 100 μ L of the culture mixture was added to each well of the micro titer plate, including the control wells.

Post-Incubation Processing and Slide Preparation

Following 48-hour incubation, the supernatant from each well was carefully aspirated and discarded. The remaining cell pellets were gently resuspended, and a small aliquot from each well was transferred onto glass slides. Thin blood films were prepared, air-dried, and methanol-fixed for 3-5 seconds. The dried films were stained with 2% Giemsa stain for 12-15 minutes, rinsed with distilled water, and air-dried. Slides were then examined under a microscope to evaluate parasite growth. The number of schizonts per 200 asexual parasites were recorded and the percentage inhibition of parasites growth was calculated for each concentration of *Senna singueana* extract.

The slides were mounted on a microscope using 100 \times oil immersion objective lens, different field of the slide were viewed and the number of infected cells were counted, followed by counting the total number of trophozoites and then the schizonts and using the following relationships described by Abdelkodoose *et al.*,(2009).

To calculate the percentage schizont inhibition, it was calculated using:

% Schizont inhibition

$$= \frac{\text{Number of schizont in control} - \text{Number of schizonts in treated}}{\text{Number of schizonts in control}} \times 100$$

To calculate the percentage viability, the following formular was used:

$$\% \text{ viability} = \frac{\text{Number of schizonts in treated}}{\text{Number of schizonts in control}} \times 100$$

Statistical Analysis

Data were presented as \pm standard deviation. Graph pad Prism version 10.4.1 was used for the statistical analysis. Mean values were compared using one way analysis of variance followed by post hoc analysis. Values of $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Malaria remains a significant public health concern globally, particularly in tropical regions where the disease is endemic. The emergence of drug-resistant *Plasmodium falciparum* strains has necessitated the discovery of novel anti-malarial agents. In this context, the present study evaluated the anti-malarial activity of *Senna singueana* against

clinical strains of *P. falciparum* using an ex vivo assay. The percentage viability and inhibition data revealed a clear dose-dependent reduction in parasite viability. At the highest concentration (100 µg/mL), the extract achieved significant inhibition, reaching 86.15% ± 0.62 (Table 1). This finding is in compliance with the results of Munoz *et al.*, 2000 where the level of parasitemia was reduced in a dose-dependent manner, which is an indicator of the antiplasmodial activity of *the extract used*. He stipulated that an extract is considered active if its inhibition is ≥30%.

Clarkson (2004), reported that plant extracts, according to their activities against *P. falciparum*, are categorized as promising (IC₅₀ < 10 µg/ml), moderate (IC₅₀ 10 - 20 µg/ml), good (IC₅₀ 20 - 40 µg/ml), marginally potent (IC₅₀ 40 - 70 µg/ml) and poor (IC₅₀ > 70 µg/ml) . This present study revealed IC₅₀ value of *Senna singueana* to be 4.311 µg/mL which demonstrated the potent anti-plasmodial activity of the extract as promising IC₅₀. As the concentration of the extract increased, parasite viability decreased, accompanied by a corresponding rise in inhibition percentage (Table 1).

Quinine phosphate was used as a positive control to benchmark the anti-plasmodial activity of *Senna singueana*. At the highest concentration used in this work (100 µg/mL), quinine phosphate achieved a significantly higher inhibition, reaching 93.79% ± 0.55, with an IC₅₀ value of 1.988 µg/mL. The dose-response model for quinine phosphate highlights the potency of quinine phosphate as a standard anti-malarial compound. (Table.2). This is in agreement with the report of Oaikhena, 2021 where quinine displayed significant potency against plasmodial activity.

Table 1: Percentage Viability and Percentage Inhibition at Different Concentrations of *Senna singueana* against *P. falciparum*

Concentration µg/ml	Sch CNT	% Viability	% Inhibition
0	162.5± 5.1 ^a	100 ^a	0
1.56	61 .0± 1.9 ^b	37.8±2.2 ^b	62.5±2.3 ^a
3.12	54.5 ± 2.0 ^{bc}	34.4±0.9 ^b	66.5±0.9 ^a
6.25	47.5 ± 2.4 ^c	32.2±0.8 ^b	70.8±0.8 ^a
12.5	45.5 ± 1.7 ^c	28.2±0.9 ^{bc}	71.9±0.8 ^a
25	34.0 ± 0.9 ^d	23.2±4.6 ^c	79.1±4.6 ^{bc}
50	29.5 ± 0.75 ^{de}	18.3±3.29 ^c	81.8±3.3 ^c
100	22.5 ± 0.45 ^e	13.3±0.6 ^c	86.2±0.6 ^c

Values having different superscripts down the column are significantly different(p ≤ 0.05).

IC₅₀ 4.311 ± 0.6

Table 2: Schizont Count, Percentage Viability and Inhibition at Different Concentrations of Quinine Phosphate (positive control)

Concentration µg/ml	Schizont Count	% Viability ± SD	%Inhibition ± SD
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0	274± 6.3 ^a	100	0
1.56	146.5± 4.2 ^b	53.47 ± 1.1 ^a	46.53± 1.2 ^a
3.12	116.5± 4.3 ^c	42.52 ± 0.9 ^b	57.48± 0.9 ^b
6.25	91.5± 3.2 ^d	33.39 ± 0.9 ^c	66.60± 1.0 ^c
12.5	51.5± 2.4 ^e	18.79 ± 0.7 ^d	81.21± 0.7 ^d
25	41.5± 0.5 ^e	15.14 ± 0.9 ^{de}	84.85± 1.3 ^d
50	27± 1.5 ^f	9.85 ± 0.5 ^{ef}	90.15± 1.4 ^{de}
100	17± 0.7 ^g	6.21 ± 0.6 ^f	93.79± 1.7 ^e

Values having different superscripts down the column are significantly different ($p \leq 0.05$).

IC₅₀ = 1.988

The findings of this study have implications for malaria treatment and control. The discovery of novel anti-malarial agents is essential for combating the emergence of drug-resistant *P. falciparum* strains. *Senna singueana* may offer a potential solution for the treatment of malaria, particularly in resource-poor settings where access to conventional anti-malarial drugs is limited. Further research is necessary to fully explore the potential of *Senna singueana* as an anti-malarial agent. Investigations into the plant's mechanism of action, toxicity, and pharmacokinetics are essential for determining its therapeutic potential. Additionally, studies on the cultivation and formulation of *Senna singueana* extracts could provide valuable insights into its potential for large-scale production and commercialization.

CONCLUSION

In conclusion, *Senna singueana* extract exhibits moderate anti-malarial activity against clinical strains of *P. falciparum*, with an IC₅₀ value of 4.311µg/mL. These findings support the potential of *Senna singueana* as a natural anti-malarial agent, warranting further investigation.

CONFLICT OF INTEREST: There is no any conflict of interest declared by the authors.

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