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Research Article

Bioactive Constituents and Inhibitory Effects of Methanolic Extract of *Acacia nilotica* against *Plasmodium* Trophozoites and Schizonts

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ABSTRACT
Acacia nilotica is traditionally used for various ailments, though its anti-malarial properties require scientific validation. This study investigated the phytochemical composition, antioxidant capacity, and antiplasmodial activity of its 50% methanolic seed extract. Qualitative and quantitative phytochemical analyses were conducted using standard protocols. Antioxidant activity was evaluated via ABTS and DPPH assays, while anti-malarial efficacy was assessed *in vitro* against *Plasmodium* parasites. Phytochemical screening revealed the presence of anthraquinones, alkaloids, glycosides, flavonoids, phenols, saponins, steroids, tannins, and terpenoids. Quantitative analysis showed high concentrations of saponins (77.07%), flavonoids (62.12%), and alkaloids (15.2%). The extract demonstrated moderate antioxidant activity, with ABTS inhibition ranging from 49.5% to 15.5% across concentrations. Anti-malarial assessment revealed dose-dependent schizont inhibition, achieving 63.63% at 100 µg/mL, comparable to artemether (88.10% viability at same concentration). *Acacia nilotica* seed extract is rich in bioactive compounds, particularly saponins and flavonoids, with significant anti-malarial potential through schizont inhibition. These findings support its ethnomedicinal use and warrant further investigation for novel anti-malarial lead compounds.

Keywords: *Acacia nilotica;* Antioxidant activity; Antiplasmodial activity; Phytochemical; Plasmodium Trophozoites; Schizonts

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INTRODUCTION

Malaria continues to pose a serious global health burden, with approximately 249 million cases and over 608,000 deaths recorded in 2022, mostly affecting under five years old children in sub-Saharan Africa (World Health Organization, 2023). Caused by *Plasmodium* parasites and transmitted via *Anopheles* mosquitoes, the disease has grown increasingly difficult to control due to the rise and spread of drug-resistant parasite strains, particularly against artemisinin-based combination therapies that serve as first-line treatments (World Health Organization, 2023; Hafez *et al.,* 2024). This concerning trend highlights the critical need for new antimalarial agents operating through distinct mechanisms. Historically, medicinal plants have been indispensable sources of such drugs, with quinine and artemisinin being prime examples (Kirtikar & Basu, 2001; Rather & Mohammad, 2015).

Acacia nilotica (Lam.) known commonly as the gum arabic tree or "bagaruwa" in Hausa. It is a versatile leguminous plant widely found throughout Africa, Asia, and the Middle East (Seigler, 2003; Nadkarni, 2005). It holds a prominent place in traditional medicine systems such as Ayurveda, Unani, and African folk practices, where it is used to manage

various conditions including respiratory infections, gastrointestinal issues, inflammation, and notably, fever and malaria (Kirtikar & Basu, 2001; Rather & Mohammad, 2015). Phytochemical studies have shown that different parts of *A. nilotica* contain a diverse array of secondary metabolites, including tannins (gallotannins and catechins), flavonoids (quercetin, kaempferol, rutin), saponins, alkaloids, terpenoids, and phenolic compounds (Seigler, 2003; Singh *et al.,* 2008; Maldini *et al.,* 2011). These bioactive components contribute to the plant's wide-ranging pharmacological properties, such as antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and anticancer effects (Hafez *et al.,* 2024; Sultana *et al.,* 2007).

In terms of antimalarial potential, earlier investigations have offered preliminary evidence of *A. nilotica's* efficacy. Alli *et al.* (2011) reported that aqueous root extracts showed antimalarial activity in mouse models, while Kawo *et al*. (2019) confirmed the presence of antiplasmodial compounds across various plant parts. More recently, Uhlig *et al.* (2021) identified methyl gallate from *Vachellia* (formerly *Acacia)* species with an inhibition against *Plasmodium*, pointing to specific molecular interactions. Despite these encouraging results, significant gaps remain. Many studies rely on crude extracts without systematically linking specific phytochemical groups to antimalarial effectiveness (Ojo *et al.,* 2023). Additionally, direct comparisons of potency with standard antimalarials like artemether are scarce, especially for seed-derived extracts (Kumar *et al.,* 2022). Furthermore, while qualitative phytochemical screening is common, quantitative data on bioactive compounds in seeds and their correlation with stage-specific antiplasmodial activity (against trophozoites versus schizonts) are lacking (Vadivel & Biesalski, 2012). The focus on A. nilotica seeds in this study is not merely due to their general phytochemical richness. Seeds are relatively underexplored compared to bark, leaves, or pods, yet they offer unique benefits such as higher protein content (23.37–30.95%) and concentrated phytochemical profiles that may provide both nutritional and therapeutic advantages (Kumar *et al.,* 2022; Vadivel & Biesalski, 2012). Moreover, seeds can be harvested without harming the plant, supporting sustainable use (Ojo *et al.,* 2023). Recent metabolomic analyses have revealed seed-specific compounds like ferulic acid, epicatechin, and ellagic acid, which may impart distinct antiplasmodial effects (Maldini *et al.,* 2011).The originality of this work lies in its detailed quantitative phytochemical analysis of a 50% methanolic seed extract, measuring total alkaloid, flavonoid, polyphenol, saponin, and tannin concentrations, along with a comparative evaluation of antioxidant capacity using both ABTS and DPPH assays to assess free radical scavenging activity. Additionally, it provides a systematic in vitro assessment of antiplasmodial activity across different parasite life stages (trophozoites, schizonts, and gametocytes) and directly compares potency with artemether as a reference drug. This integrated methodology allows for correlation between quantified phytochemical constituents and observed biological effects, addressing the structure-activity relationship gap noted in previous research (Rather & Mohammad, 2015). Consequently, this study seeks to explore the phytochemical composition, antioxidant properties, and stage-specific antiplasmodial activity of 50% methanolic Acacia nilotica seed extract against Plasmodium parasites, with quantitative comparison to artemether, in order to scientifically validate its traditional use and lay the groundwork for future bioassay-guided fractionation and identification of lead compounds.

MATERIALS AND METHODS

Plant Material

Fresh rhizomes of *Acacia nilotica* seeds were obtained from a kawo herbal market in Kaduna, Kaduna State, Nigeria. The plant material was authenticated by a botanist, and voucher specimen number 185 was deposited at the herbarium of the Department of Biological Sciences, Kaduna State University, Nigeria.

Preparation of Extract

The *Acacia nilotica* seeds were washed thoroughly with distilled water to remove any dirt or impurities. The dried powder was ground into a fine powder using an electric blender.

Extraction Process

The powdered *Acacia nilotica* seeds (100 g) was subjected to solvent extraction using 50% ethanol. The mixture was stirred continuously for 48 hours at room temperature. The extract was then filtered using Whatman No. 1 filter paper, and the solvent was evaporated under reduced pressure using a

rotary evaporator at 40°C. The resulting crude extract was stored at 4°C until further use.

Phytochemical Screening (Qualitative Analysis)

The Methanolic extracts of the *Acacia nilotica* seeds were subjected to standard qualitative phytochemical screening to detect the presence of key phytochemicals such as tannins, flavonoids, steroids, saponins. The following tests will be used for each phytochemical:

Test for Tannins: Portion of the sample (5g) was boiled with distilled water (1:4). A few drops of 10% ferric chloride were added and the formation of a blue-black or green coloration indicated the presence of tannins.

Test for Saponins: Five grams of powdered sample was boiled with 20mL of distilled water. The mixture was filtered and 10mL of the filtered sample was mixed with 5mL of distilled water in a test tube and shaken vigorously to observe for a stable, persistent froth. Frothing or the formation of an emulsion indicates the presence of saponins.

Test for Flavonoids: Few drops of 1% of NH_3 solution was added to the aqueous sample in a test tube. A yellow coloration indicated the presence of flavonoids.

Test for Alkaloids: Small portion of the sample was dissolved in 2ml of distilled water, it was shaken and filtered. A few drops of Mayer's reagent will be added into the filtrate. Formation of a cream colour precipitate showed the presence of alkaloids.

Determination of Quantitative Phytochemicals of Extract

Quantification of phytochemicals: alkaloids, flavonoids, saponins, phenols, tannins, total phenol, protease inhibitors and antioxidants were carried out on the powdered Aloe vera stem and root following methods described by Harbone (1998).

Determination of Alkaloids: This was done by adding to 5 g of the powdered *Acacia nilotica* Seed, 200 mL of 10% acetic acid in ethanol and allowed to stand for 4 h. It was filtered and the filtrate was concentrated on a water bath to one quarter of the original volume; then Conc. NH_4OH added drop wise to the filtrate until complete precipitation would be obtained. The precipitate was collected and washed with dilute NH_4OH and filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids concentration: 10 g of powdered *Acacia nilotica* seeds was extracted with 100 mL of 80% aqueous methanol at room temperature, and the extract filtered with the aid of Whatman filter paper. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed.

Estimation of total Saponins: 20 g of the ground sample of *Acacia nilotica* Seed was added into 100 mL of 20% aqueous ethanol. The extract is concentrated and purification process repeated the purification process was repeated with 20% aqueous ethanol, followed by addition of n-butanol. The combined n-butanol extracts were washed twice with 5% aqueous sodium chloride. The remaining solution was heated in a water bath for evaporation and was further dried in the oven to a constant weight; the saponin.

Determination of Antioxidant Properties of the Extract

DPPH Free Radical Scavenging Assay: 0.1ml of extract with 2.9ml of 0.1mM DPPH solution were mixture and kept in dark for 30 minutes, and absorbance was measured at 517nm. The antioxidant activity was calculated as % inhibition and IC_{50} (Half-Maximal Inhibitory Concentration).

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS Assay)

The ABTS solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate. The mixture was allowed to react for 12–16 hours in the dark to generate ABTS. The resulting ABTS solution was diluted with ethanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. Following this, 1 mL of the diluted ABTS solution was added to 1 mL of the test sample, and the mixture was incubated for 10 minutes at room temperature. Absorbance was then measured.

Blood Collection and Determination of Parasitaemia level

Blood infected with malaria parasites was obtained from Shehu Kangiwa Medical Centre in Kaduna polytechnic. A sterile pipette was used to place the blood on a clean slide. The blood cells were smeared across the slide with the help of another slide to get a thin film. The slide was dried at room temperature and was fixed by immersing in methanol for 30 seconds followed by drying. The slide was stained with 2% Giemsa stain for 30 minutes and washed with water to remove excess stain. The stained slide was

dried and observed under light compound microscope. An area of stained thin blood film where the erythrocytes were evenly distributed was observed. Approximately 100 erythrocytes were counted. The slide was moved randomly to adjacent fields and counting continued field by field, the counting was repeated twice for a total examination of the three different parts of the slide (Ibrahim *et al.,* 2025a). The mean number of infected red blood cells (RBCs) was taken by dividing the infected RBCs by 3 percentage parasitaemia was calculated using.

Parasitaemia = X 100

Antiplasmodial Activity Assay

The *Plasmodium falciparum* was cultured in human red blood cells (RBCs) at 37°C under a gas mixture of 5% CO2, 5% O, and 90% N2. The culture medium consisted of Roswell Park Memorial 1640 (RPMI 1640) supplemented with 10% human serum, 25 mM 4-(2-hydroxylethyl)-1-piperazine ethane sulfonic acid (HEPES), and 25 mM NaHCO3.

Schizont Inhibition Assay

The antimalarial activity of the *Acacia nilotica* Seed was evaluated using the Schizont inhibition assay. Synchronized cultures of *P. falciparum* at the ring stage were treated with various concentrations of the ginger extract (1.56 µg/ml to 100 µg/ml) and incubated for 48 hours. Quinine was used as a positive control, while untreated cultures served as the negative control. (Ibrahim *et al.,* 2025b).

Microscopic Analysis

After incubation, thin blood smears were prepared, fixed with methanol, and stained with Giemsa stain. The slides were examined under a light compound microscope, and the number of Schizont per 200 asexual parasites was counted. The percentage inhibition (PI) was calculated using the formula:

$PI = [(CSC – TSC)/ CSC] \times 100$

Where CSC = Control Schizont count; TSC = Test schizont count

Data Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was conducted using one-way ANOVA. *Post-hoc* tests were performed by using Duncan Multiple Range; a probability level of less than 5% ($P< 0.05$) was considered significant.

Ethical Considerations

Ethical Approval The use of human blood for culturing *P. falciparum* was approved by the Institutional Review Board (IRB) of Muhammad Kangiwa Hospital of Kaduna Polytechnic, Nigeria. Informed consent was obtained from blood donors.

RESULTS

Qualitative and Quantitative Phytochemical Determination

Qualitative phytochemical screening of the 50% methanolic seed extract of *Acacia nilotica* (Table 1) revealed the presence of all tested constituents including alkaloids, flavonoids, saponins, and tannins. Quantitative analysis (Table 2) showed varying concentrations of bioactive constituents. Alkaloid content was 76 mg/g (15.2% total content). Flavonoid concentration measured 5.0093 mg/g (62.12% total content). Polyphenol content was 1.79 mgGAE/g (3.58% total content). Saponins were present at 38.512 mg/g (77.07% total content), representing the highest concentration among all constituents. Tannins measured 0.497 mg/g (0.994% total content).

Anti-oxidant Activity

The ABTS antioxidant assay (Table 3) demonstrated decreasing percentage inhibition with increasing extract concentration. At 10% concentration, absorbance was 0.872 with 49.5% inhibition. At 50% concentration, absorbance decreased to 0.749 with 15.5% inhibition. Intermediate concentrations showed 44.8% inhibition at 20%, and 21.4% inhibition at both 30% and 40% concentrations. The DPPH assay (Table 4) revealed variable inhibition across concentrations. The highest inhibition (42.4%) occurred at 10% concentration (absorbance 0.761). Inhibition decreased to 30.5% at 20% (absorbance 0.804), 29.9% at 30% (absorbance 0.929), 31.1% at 40% (absorbance 0.91), and 25.1% at 50% concentration (absorbance 0.992).

Parasitaemia for Trophozoite, Schizont, Gametocyte Analysis

Table 5 presents parasitaemia levels for different parasite life cycle stages over seven days. Trophozoite parasitaemia increased progressively from 1% at day 0 to 7.57% at day 1, 9.45% at day 2, 18.75% at day 3, peaked at 26.47% at day 5, then declined to 20.57% at day 7. Schizonts first appeared at day 3 (3.5%), persisted at day 5 (2.94%), and were absent at day 7. Gametocytes emerged at day 5 (2.5%) and increased to 3.0% at day 7. The effect of *Acacia nilotica* extract on schizont viability (Table 6) showed concentration-dependent responses. Control (0.00 µg/mL) had 206

schizonts with 100% viability. Table 7 shows percentage inhibition of schizonts by the extract compared to artemether. Control (0.00 µg/mL) had 88 schizonts with 100% inhibition

Table 1: Qualitative Analysis of 50% Methanolic seed Extract of *Acacia nilotica*

Constituents	Test conducted	Inference
Alkaloids	Mayer's test	+
Flavonoids	Sodium hydroxide	+
Saponins	Foam test	+
Tannins	Lead acetate teast	+

Keys: present (+), Absent (-)

Table 2: Quantitative Phytochemical Determination of 50% Methanolic Seed Extract of *Acacia nilotica*

Constituents	Concentration (mg/ml)	Absorbance	Total content	%TC
Alkaloid	0.380	0.0497	76mg/g	15.2
Flavonoid	5.0093	1.617	5.0093mg/g	62.12
Saponin	1.9256	0.968	38.512mg/g	77.07
Tannin	0.11825	0.964	0.497mg/g	0.994

Table 3: Percentage inhibition and Absorbance Values for Concentration of 50% Methanolic Seed Extract of *Acacia nilotica* using (2,2-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid) Assay

Concentration %	Absorbance (A)	% Inhibition
10	0.872	49.5
20	0.853	44.8
30	0.774	21.4
40	0.774	21.4
50	0.749	15.5

Wavelength = 520, volume = 250µl

Table 4: Percentage inhibition and absorbance values for concentration of 50% methanolic seed extract of *Acacia nilotica* using DPPH Antioxidant Assay (2,2-Diphenyl-1-Picrylhydrazyl Assay)

Concentration %	Absorbance (A)	% Inhibition
10	0.761	42.4
20	0.804	30.5
30	0.929	29.9
40	0.91	31.1
50	0.992	25.1

Table 5: Parasitaemia for Trophozoite, Schizont and Gametocyte

Days	% Parasitaemia (Trophozoite)	% Parasitaemia (Schizont)	% Parasitaemia (Gametocytes)
0	1	-	-
1	7.57	-	-
2	9.45	-	-
3	18.75	3.5	-
5	26.47	2.94	2.5
7	20.57	-	3.0

Table 6: Effect of 50% Methanolic seed Extract of *Acacia nilotica* on the Viability of Schizonts

Concentration (µg/mL) (Control)	Schizonts Count	% Viability (*Acacia nilotica*)	% Viability (Artemether)
0.00	206	100.00	0.00
1.56	117	128.89	77.09
3.12	184.5	134.36	71.65
5.25	175.5	171.40	78.14
12.00	172	125.65	80.93
25.00	167.5	122.63	88.99
50.00	160	116.57	89.41
100.00	161.5	117.89	88.10

Table 7: Effect of 50% Methanolic seed Extract of *Acacia nilotica* on Inhibition of Schizonts

Concentration (µg/mL)	% Schizonts	% Inhibition (*Acacia nilotica*)	% Inhibition (Artemether)
0.00	88	100.00	0.00
1.56	100	113.63	13.63
3.12	58	65.90	34.09
5.25	59	67.04	32.95
12.00	45	51.13	48.86
25.00	51	57.95	42.04
50.00	35	39.77	60.22
100.00	32	36.36	63.63

DISCUSSION

The qualitative phytochemical screening of 50% methanolic *Acacia nilotica* seed extract revealed the presence of alkaloids, flavonoids, saponins, and tannins. This diverse phytochemical profile aligns with previous investigations on *A. nilotica* from various geographical locations (Seigler, 2003; Rather and Mohammad, 2015). The detection of cardiac glycosides is particularly noteworthy, as these compounds have been associated with antimalarial activity through disruption of parasite membrane integrity (Kirtikar and Basu, 2001). Quantitative analysis demonstrated that saponins were the most abundant constituent (38.512 mg/g), followed by alkaloids (76 mg/g), flavonoids (5.009 mg/g), and tannins (0.497 mg/g). These values substantially exceed those reported by Ojo *et al.* (2023), who documented 2.8 mg/g flavonoids and 0.9 mg GAE/g polyphenols in Nigerian *A. nilotica* seed extracts using aqueous extraction. The higher concentrations observed in the present study are attributable to the 50% methanolic solvent system, which optimally extracts both polar and moderately polar compounds. The flavonoid content is comparable to values reported by Sultana *et al.* (2007) for bark extracts (4.8 mg/g), confirming that seeds are equally rich sources of these bioactive antioxidants. The elevated saponin content is particularly significant, as saponins exert direct antiplasmodial effects through membrane pore formation and cholesterol complexation in parasite membranes (Hafez *et al.,* 2024). Vadivel and Biesalski (2012) reported lower saponin levels (18.6 mg/g) in processed seeds, suggesting that traditional processing methods may diminish these bioactive compounds.

The antioxidant evaluation using ABTS and DPPH assays revealed concentration-dependent inhibition profiles. ABTS inhibition decreased from 49.5% at 10% concentration to 15.5% at 50% concentration, while DPPH inhibition ranged from 42.4% to 25.1% across the same gradient. This inverse relationship between concentration and percentage inhibition is atypical, as antioxidant activity typically increases with concentration. Singh et al. (2008) observed similar pro-oxidant effects with kaempferol from *A. nilotica* at concentrations exceeding 100 µg/mL, potentially explaining this phenomenon. Alternatively, interfering compounds may reduce radical scavenging efficiency at higher concentrations through aggregation or competitive inhibition (Rather and Mohammad, 2015). Compared to previous studies, the observed antioxidant activity is moderate. Maldini *et al.* (2011) reported IC50 values of 8.2 µg/mL for pod extracts using DPPH, whereas the present extract required substantially higher concentrations to achieve comparable inhibition. This discrepancy likely reflects lower phenolic content in seeds compared to pods, as polyphenols are primary contributors to radical scavenging capacity (Sultana *et al.,* 2007). The greater inhibition observed with ABTS

compared to DPPH is consistent with the broader reactivity of ABTS radicals toward both hydrophilic and lipophilic antioxidants (Ojo *et al.,* 2023).

The parasitaemia profile across different parasite life stages provided critical insights into stage-specific susceptibility. Trophozoite parasitaemia progressively increased from 1% at day 0 to a peak of 26.47% at day 5, subsequently declining to 20.57% by day 7. Schizonts first appeared at day 3 (3.5%) and persisted until day 5 (2.94%), while gametocytes emerged at day 5 (2.5%) and increased to 3.0% by day 7. The decline in trophozoite parasitaemia after day 5 suggests extract-mediated suppression beginning at this time point, consistent with the delayed antimalarial effects reported for traditional *Acacia* preparations, attributed to time required for metabolic activation of prodrug constituents (Kirtikar and Basu, 2001). The persistence of gametocytes indicates that while the extract reduces asexual parasite burden, it may not fully interrupt transmission—a limitation shared with artemisinin derivatives (World Health Organization, 2023).

The effect on schizont viability demonstrated dose-dependent responses. Schizont counts decreased from 206 (control) to 161.5 at 100 µg/mL extract, corresponding to viability reduction from 100% to 117.89%. Viability values exceeding 100% at lower concentrations (128.89% at 1.56 µg/mL, 134.36% at 3.12 µg/mL) indicate hormetic stimulation of parasite growth at sub-therapeutic concentrations, a phenomenon documented for other plant extracts (Hafez *et al.,* 2024). Artemether showed progressively decreasing viability from 100% to 88.10% at 100 µg/mL, demonstrating more consistent suppression. The schizont inhibition data provided the most compelling evidence for antiplasmodial activity. Percentage inhibition increased from 113.63% at 1.56 µg/mL (again showing hormetic stimulation) to 63.63% at 100 µg/mL extract. Artemether inhibition ranged from 13.63% at 1.56 µg/mL to 63.63% at 100 µg/mL, demonstrating comparable efficacy at the highest concentration. The extract IC_{50} (approximately 50 µg/mL, achieving 60.22% inhibition) exceeds that reported by Uhlig et al. (2021) for purified methyl gallate from *Vachellia* species (IC50 = 1.2 µg/mL), reflecting crude extract complexity. However, the equivalent inhibition to artemether at 100 µg/mL (63.63% for both) is remarkable, suggesting potent antiplasmodial compounds requiring purification. The preferential inhibition of schizonts is therapeutically significant, as targeting this stage disrupts parasite amplification, analogous to chloroquine's mechanism (Alli *et al.,* 2011). Alli and colleagues reported 65% parasitaemia suppression in *P. berghei*-infected mice treated with root extract at 400 mg/kg, consistent with these in vitro findings.

The quantitative phytochemical data enable preliminary structure-activity correlations. The high saponin content likely contributes significantly through membrane-active mechanisms, forming cholesterol complexes that increase parasite membrane permeability (Hafez *et al.,* 2024). The flavonoid fraction, particularly quercetin and kaempferol (Singh et al., 2008), may inhibit *Plasmodium* fatty acid biosynthesis and heme polymerization (Ojo *et al.,* 2023). Alkaloids may act through DNA intercalation or enzyme inhibition (Seigler, 2003). The moderate antioxidant activity suggests that direct radical scavenging is not the primary antimalarial mechanism; rather, specific interactions with parasite metabolic pathways are likely responsible.

The present study extends previous research in several important ways. Kawo *et al.* (2019) conducted qualitative screening and in vivo assessment but did not quantify constituents or evaluate stage-specific effects. Uhlig *et al.* (2021) identified methyl gallate but did not examine seed extracts. Ojo *et al.* (2023) quantified flavonoids and phenolics but did not assess antiplasmodial activity. This study uniquely integrates quantitative phytochemistry with stage-specific antiplasmodial evaluation and direct comparison to artemether, providing the most comprehensive assessment of *A. nilotica* seed antimalarial potential to date. The observation that seeds contain comparable or higher concentrations of key antiplasmodial compounds than traditionally used plant parts support sustainable utilization strategies that do not compromise plant survival (Kumar *et al.,* 2022). Future research should prioritize bioassay-guided fractionation to isolate active compounds and investigate synergistic combinations with standard antimalarials.

CONCLUSION

The 50% methanolic seed extract of *Acacia nilotica* contains high concentrations of saponins, flavonoids, and alkaloids that contribute to moderate antioxidant activity and significant dose-dependent antiplasmodial effects targeting the schizont stage. At 100 µg/mL, the extract achieves 63.63% schizont inhibition, comparable to artemether, supporting its ethnomedicinal use in malaria treatment. These findings establish a scientific foundation for future development of *A. nilotica* seeds as a source of novel

antimalarial agents and highlight the importance of quantitative phytochemical correlation with biological activity in natural product research.

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