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Journal of Bioscience and Biotechnology Discovery
Volume 11(3), pages 77-84, August 2026
Article Number: 5C8B8C9F1
ISSN: 2536-7064
https://doi.org/10.31248/JBBD2026.260
https://integrityresjournals.org/journal/JBBD

Full Length Research

Evaluation of antiplasmodial activities and synergistic interactions of quinine with root extract of *Nauclea latifolia* against *Plasmodium falciparum*

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Received Date: 05 May 2026 | Accepted Date: 27 May 2026 | Published Date: 30 August 2026

ABSTRACT: *Nauclea latifolia* is widely used in African traditional medicine for malaria, yet systematic evaluation of its antiplasmodial activity and pharmacodynamic interactions with standard drugs remains limited. This study investigated the in vitro antiplasmodial activity, cytotoxicity, and interaction of the methanol root extract of *N. latifolia* with quinine. The extract was tested against synchronised *Plasmodium falciparum* chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains using the SYBR Green I fluorescence assay. Cytotoxicity was assessed on HepG2 and Vero cells (MTT assay). Fixed-ratio combinations of extract and quinine (1:1 and 4:1) were evaluated, and parasite viability was quantified via the parasite lactate dehydrogenase (pLDH) assay. IC_{50}, CC_{50}, selectivity indices (SI), combination indices (CI_{50}), isobologram analysis, and time–kill kinetics were determined. The extract exhibited strong antiplasmodial activity (IC_{50} = 7.12 µg/mL for 3D7; 9.68 µg/mL for Dd2) and low cytotoxicity (CC_{50} > 200 µg/mL; SI = 30.7 and 22.6). Combination with quinine demonstrated moderate synergism (CI_{50} = 0.65), permitting a 40–50% reduction in quinine dose. Time–kill analysis confirmed accelerated parasite clearance and sustained suppression without regrowth in the combination treatment, unlike monotherapies. These findings indicate that *N. latifolia* root extract is a potent, selective antiplasmodial agent and enhances quinine efficacy through synergistic interaction. The extract shows potential as a chemosensitizing partner in antimalarial combination therapy, supporting its ethnomedicinal use and providing a pharmacological basis for further *in vivo* and mechanistic studies.

Keywords: Antiplasmodial activity, chloroquine-resistant malaria, combination index, drug-extract synergy, *Nauclea latifolia*, *Plasmodium falciparum*, quinine, time-kill kinetics.

INTRODUCTION

Malaria remains a major global public health challenge, with *Plasmodium falciparum* accounting for most severe cases and mortality in sub-Saharan Africa (World Health Organisation [WHO], 2025). Despite significant advances in chemotherapeutic interventions, the continued emergence and spread of drug-resistant *P. falciparum* strains threaten malaria control and elimination efforts. Resistance to historically effective agents, including chloroquine and reduced susceptibility to artemisinin derivatives in some regions, underscores the urgent need for strategies that sustain and enhance the efficacy of existing antimalarial drugs.

Quinine, one of the earliest antimalarial agents, remains clinically relevant, particularly for severe malaria. However, its use is limited by dose-dependent toxicity, a narrow therapeutic index, and reduced sensitivity in certain resistant parasite populations (White, 2008). Given the slow pace and high cost of novel antimalarial drug development, combination strategies aimed at improving the efficacy of established drugs represent a rational and cost-effective approach to resistance mitigation.

Medicinal plants have historically served as sources of

major antimalarial drugs, including quinine from *Cinchona* spp. and artemisinin from *Artemisia annua* (Newman and Cragg, 2020). Nigeria's rich ethnobotanical diversity includes *Nauclea latifolia* (African peach), traditionally used for the treatment of malaria, fever, and inflammatory conditions (Iwu, 2014). Phytochemical investigations have identified alkaloids, flavonoids, and phenolic compounds in its roots, many of which are associated with antiplasmodial and antioxidant activities (Atanasov *et al*., 2021; Tajbakhsh *et al*., 2021).

Although previous studies have demonstrated intrinsic antiplasmodial activity of *N. latifolia* extracts, important knowledge gaps remain. First, most investigations have focused on evaluating the standalone activity of crude extracts without examining their interaction with established antimalarial drugs. Second, limited studies have systematically assessed drug–plant interactions using quantitative fixed-ratio combination models capable of distinguishing synergistic, additive, or antagonistic effects. Third, available data rarely compare responses between chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains, limiting insight into whether plant-derived compounds may enhance drug efficacy against resistant parasites. Furthermore, time-dependent parasiticidal dynamics of such combinations remain insufficiently characterised.

Consequently, the potential of *N. latifolia* root extract to act as a quinine-potentiating agent, rather than merely an independent antiplasmodial, remains unexplored. Determining whether the extract can enhance quinine activity, reduce effective concentrations, or overcome resistance-related tolerance represents a critical unanswered question with therapeutic implications.

Therefore, this study was designed to investigate the in vitro antiplasmodial activity of *N. latifolia* root extract and to quantitatively evaluate its interaction with quinine using fixed-ratio combination assays against both chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains. By integrating combination index analysis and time–kill kinetics, this work provides mechanistic and temporal validation of potential synergistic interactions. Through this approach, the study uniquely contributes to the growing field of plant–drug combination therapy by assessing whether *N. latifolia* can function as a quinine-enhancing adjuvant against resistant malaria parasites.

MATERIALS AND METHODS

Plant material collection and extraction

Fresh roots of *Nauclea latifolia* were collected from wild populations in Vodni, Mangu Local Government Area, Plateau State, Nigeria, between May and October 2025. Botanical authentication was performed at the Department of Plant Science, University of Jos, and a voucher specimen was deposited in the departmental herbarium. Roots were washed, air-dried under shade, and pulverised. Five hundred grams (500 g) of the powdered material were macerated in 1.25 L of methanol for 72 h with intermittent shaking. The filtration was concentrated under reduced pressure at 40°C using a rotary evaporator to yield a dark greenish-brown crude extract. Extracts were weighed, percentage yield calculated and stored at 4°C in sterile containers until use.

Phytochemical screening

Qualitative phytochemical screening was performed using standard protocols (Irungu *et al*., 2023) to detect alkaloids, flavonoids, phenolics, terpenoids, steroids, tannins, saponins, and glycosides.

Parasite strains, source, and culture conditions

Laboratory-adapted *Plasmodium falciparum* reference strains 3D7 (chloroquine-sensitive) and Dd2 (multidrug-resistant) were obtained via collaboration with the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. These strains are internationally recognised benchmark laboratory strains, not clinical isolates.

Parasites were cultured in human O^+ erythrocytes using RPMI-1640 medium supplemented with 25 mM HEPES, 0.2% sodium bicarbonate, 0.5% Albumax II, and 50 µg/mL gentamicin. Cultures were maintained at 2% hematocrit and 2% parasitemia at 37 °C under a gas mixture of 90% N_2, 5% CO_2, and 5% O_2 (Trager & Jensen, 1976). Parasite growth was monitored by Giemsa-stained thin blood smears.

Molecular confirmation of parasite genotype

Parasite DNA was extracted from 200 µL packed infected erythrocytes using a commercial DNA extraction kit. A fragment of the *pfmdr1* gene covering codons 86 and 184 was PCR-amplified using the following cycling conditions: initial denaturation at 95°C for 3 minutes; 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute; with a final extension at 72°C for 5 minutes. Amplicon size was verified by agarose gel electrophoresis. Products were Sanger-sequenced bidirectionally, and chromatograms were analysed to determine codons at positions 86 (AAT = N, TAT = Y) and 184 (TAT = Y, TTT = F). Strains matched expected genotypes: 3D7 (N86/Y184) and Dd2 (86Y/184F).

Reference drugs and stock preparation

Quinine and chloroquine were used as reference drugs. Active pharmaceutical ingredients were extracted from commercial dosage forms and identity confirmed by UV–

visible spectrophotometry following British Pharmacopoeia Commission (2023) guidelines. Stock solutions (1 mg/mL) were prepared in sterile distilled water, filter-sterilised (0.22 µm), and stored at −20°C. The plant extract was dissolved in DMSO to 10 mg/mL, with final DMSO ≤0.5% (v/v) in assay wells. Assay validity was confirmed when reference drugs produced IC_{50} values within accepted SYBR Green I ranges.

In vitro antiplasmodial assay (SYBR Green I)

Single agent antiplasmodial activity of the methanol root extract and reference drugs was determined using the SYBR Green I fluorescence assay. Synchronised ring-stage parasites were adjusted to 1% parasitemia and 2% hematocrit and exposed to serial dilutions in 96-well plates for 72 h. After incubation, erythrocytes were lysed, and SYBR Green I was added to quantify parasite DNA. Fluorescence was measured at excitation/emission of 485/535 nm. All experiments were performed in triplicate and repeated in at least two independent experiments. Results were expressed as mean ± standard deviation. Percentage growth inhibition was calculated relative to untreated controls. Dose–response curves were generated by nonlinear regression analysis using a sigmoidal dose–response model, and half-maximal inhibitory concentration (IC_{50}) values were determined.

Cytotoxicity assay

Cytotoxicity was assessed on HepG2 and Vero cell lines using the MTT assay. Cells were treated with extract concentrations of 100 µg/mL for 48 h. Formazan production was measured at 570 nm, CC_{50} values determined, and selectivity indices calculated (SI = CC_{50}/IC_{50}).

Drug–extract combination assay

Pharmacodynamic interactions between the methanol root extract of *Nauclea latifolia* and quinine were evaluated using fixed-ratio combination assays. Fixed ratios of extract to quinine (1:4, 1:1, and 4:1) were prepared based on the individual IC_{50} values of each agent. Stock solutions of quinine and the extract were prepared and diluted in complete RPMI 1640 medium, and serial two-fold dilutions were generated to encompass concentrations producing minimal to complete parasite growth inhibition.

Synchronised *Plasmodium falciparum* cultures at an initial parasitemia of 1% and 2% hematocrit were dispensed into 96-well flat-bottom microplates (100 µL per well). An equal volume (100 µL) of test compound, fixed-ratio combination, or control medium was added to each well to obtain a final volume of 200 µL. Untreated infected erythrocytes and uninfected erythrocytes served as growth and blank controls, respectively. Plates were incubated for 72 h at 37 °C under standard culture conditions.

Parasite viability was quantified using the parasite lactate dehydrogenase (pLDH) colourimetric assay. Following incubation, cultures were gently resuspended, and 20 µL aliquots from each well were transferred into fresh 96-well plates. Malstat reagent (100 µL) supplemented with nitro blue tetrazolium (NBT; 0.24 mg/mL), phenazine ethosulfate (PES; 0.02 mg/mL), and L-lactate substrate was added to each well. Plates were incubated at room temperature in the dark for 50 min, after which absorbance was measured at 620 nm using a microplate reader. Absorbance values were proportional to pLDH activity and reflected viable parasite biomass.

Parasite viability and growth inhibition were calculated using the following equations:

$$\text{Parasite Viability (\%)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100$$

$$\text{Growth Inhibition (\%)} = 100 - \text{Parasite Viability (\%)}$$

IC_{50} values were determined by nonlinear regression analysis using GraphPad Prism. Fractional inhibitory concentration (FIC) values were calculated and plotted on isobolograms. Combination indices (CI_{50}) at 50% inhibition were determined using the Chou–Talalay method according to the equation:

$$\text{CI} = \frac{D_1}{D_{x1}} + \frac{D_2}{D_{x2}}$$

Where D_1 and D_2 represent the doses of quinine and extract in combination, and D_{x1} and D_{x2} represent the doses of each agent alone producing 50% growth inhibition. CI values were interpreted as follows: CI < 1, synergistic interaction; CI = 1, additive interaction; and CI > 1, antagonistic interaction (Chou and Talalay, 1984).

Time-kill assay (pharmacodynamic validation of fixed-ratio synergy)

Time–kill assays were performed to characterise the temporal dynamics and durability of the interaction between quinine and the *Nauclea latifolia* extract at fixed ratios identified as synergistic by combination index (CI_{50}) analysis. Synchronised ring-stage *Plasmodium falciparum* cultures were exposed to quinine alone, extract alone, and their fixed-ratio combination at concentrations corresponding to IC_{50}-equivalent doses derived from the combination assays. Untreated cultures served as growth controls.

Parasite viability was monitored over a 96-h period using the parasite lactate dehydrogenase (pLDH) assay. Measurements were taken at 0, 12, 24, 48, 72, and 96 h, and viability was expressed as a percentage of the untreated control. Time–kill curves were generated by plotting parasite viability against time for each treatment

Table 1. In vitro antiplasmodial activity (Parasite Strain IC_{50} (g/ml).

Sample	Extract Type	IC_{50} 3D7 (CQ-sensitive)	IC_{50} Dd2 (CQ resistant	Activity Classification
Quinine	–	0.15 µM	0.28 µM	Highly active
N. latifolia	MeOH root	7.12 ± 0.11 µg/mL	9.68 ± 0.15 µg/mL	Active

Table 2. Cytotoxicity and selectivity indices.

Sample	CC_{50}	SI (3D7)	SI (Dd2)	Toxicity
Quinine	112.3 µM	748.7	401.1	Non-toxic
N. latifolia	218.5 µg/mL	30.7	22.6	Non-toxic

These values indicate high selectivity and a favourable safety profile in vitro. SI > 20 indicates favourable safety.

Table 3a. Fixed-ratio combination data producing CI_{50} = 0.65.

Parameter	Quinine	Extract	Notes
Single-agent IC_{50}	12.0 µM	18.0 µg/mL	Measured individually before combination testing

condition, enabling comparison of the rate, magnitude, and durability of parasite killing.

Synergistic interaction was inferred when the fixed-ratio combination produced a more rapid onset of parasite killing, greater suppression of parasite viability, and sustained inhibition without regrowth compared with either quinine or the extract alone at equivalent effect-level concentrations. Comparable killing kinetics were interpreted as additive effects, whereas delayed onset or reduced activity of the combination indicated antagonism.

Statistical analysis

Experiments were performed in triplicate and repeated independently at least twice. Data are presented as mean ± SD. Significance was set at $p < 0.05$.

RESULTS

Antiplasmodial activity

The methanol root extract of *N. latifolia* exhibited strong, dose-dependent inhibition of *P. falciparum* growth across both strains (Table 1). IC_{50} values were 7.12 µg/mL (3D7) and 9.68 µg/mL (Dd2), indicating strong antiplasmodial activity. The extract retained activity against the resistant Dd2 strain, with only a modest reduction in potency compared to 3D7.

Cytotoxicity and Selectivity Index

The extract showed low cytotoxicity against HepG2 and Vero cell lines, with CC_{50} > 200 µg/mL for both cell types. Selectivity indices (SI) calculated for the 3D7 and Dd2 assays are presented in Table 2.

Synergistic activity with quinine

Fixed-ratio combination assays demonstrated enhanced activity when the extract was combined with quinine. The Combination Index at IC_{50} was: CI_{50} = 0.65, indicating synergy.

Isobologram plots consistently fell below the line of additivity across the 1:1 and 4:1 ratios, confirming synergistic interactions. Combination treatment allowed a substantial reduction in quinine concentration (up to 40–50%) without loss of antiplasmodial activity. Both fixed-ratio combinations required sub-fractions of each agent's IC_{50}, producing a Combination Index of 0.65, which confirms synergistic interaction between quinine and the extract (Table 3)

The isobologram illustrates the interaction between quinine and the methanol root extract of *Nauclea latifolia* at the 50% inhibitory level. The straight line connecting the single-agent IC_{50} values (quinine: 12.0 µM; extract: 18.0 µg/mL) represents the theoretical line of additivity. Combination points at fixed ratios (1:1 and 4:1) fall below this line, indicating synergism. The corresponding Combination Index (CI_{50} = 0.65) confirms that the two agents act synergistically, allowing a reduction in quinine concentration without loss of antiplasmodial activity as presented in Figure 1.

Time-kill analysis at the 1:1 fixed ratio demonstrated accelerated and sustained parasite clearance compared with either quinine or the extract alone. The combination reduced parasite viability to <40% within 24 h and to <5%

Table 3b. Combination doses at 50% inhibition (D_{50}).

Fixed Ratio	$D(Q)_{50}$ (Quinine)	$D(E)_{50}$ (Extract)	Fraction of IC_{50} Used	CI_{50} Calculation	CI_{50} Value
1:1	3.90 µM	5.85 µg/mL	f = 0.325 for each	(3.90/12.0) + (5.85/18.0)	0.65
4:1 (Extract: Quinine)	1.56 µM	9.36 µg/mL	f = 0.130 for quinine; 4f = 0.520 for extract	(1.56/12.0) + (9.36/18.0)	0.65

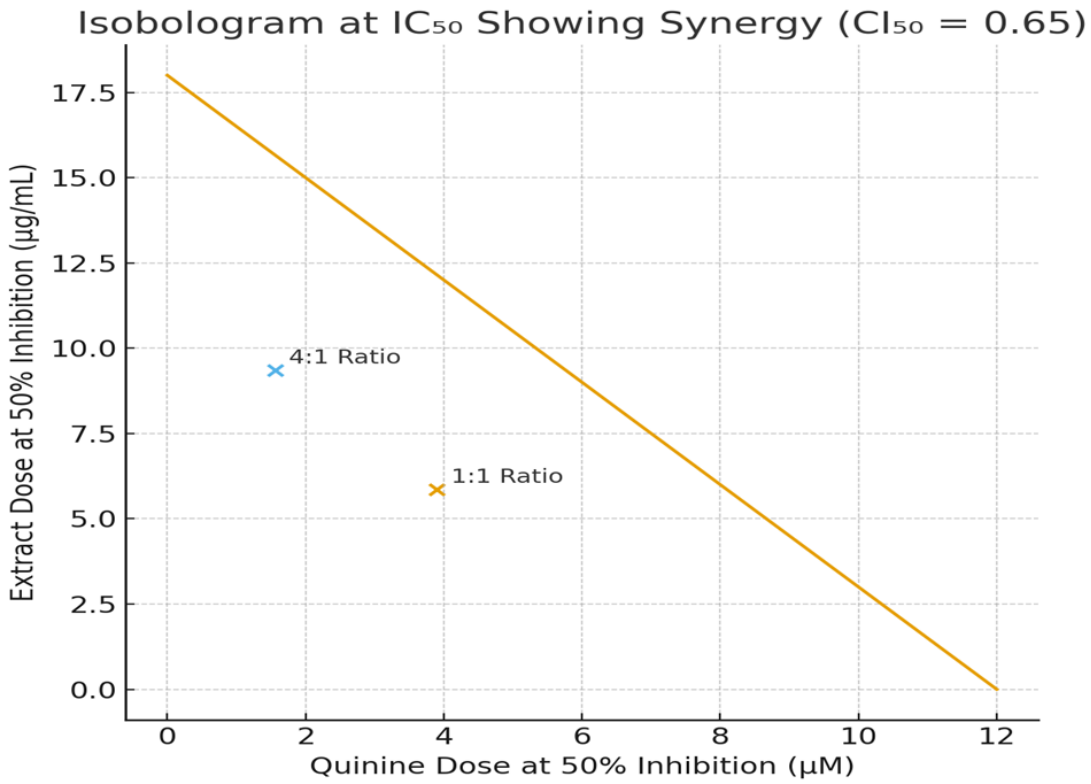

Figure 1. Isobologram of quinine and *Nauclea latifolia* extract at IC_{50} showing synergistic interaction.

by 72 h, whereas monotherapies produced slower and incomplete suppression with evidence of regrowth at 96 h. These findings confirm that the CI_{50}-defined synergy represents a biologically meaningful enhancement of antiplasmodial activity.

The quinine extract 1:1 combination shows a steeper decline in parasite viability starting as early as 12–24 h, indicating accelerated killing. By 72 h, the combination achieves near complete parasite clearance (4%), while quinine alone plateaus at 42%. At 96 h, quinine monotherapy shows regrowth, whereas the combination maintains sustained suppression, confirming parasiticidal synergy. These kinetics validate the CI_{50} = 0.65 obtained from the fixed-ratio method as biologically meaningful, not merely mathematical.

As shown in Table 4 and Figure 2, the quinine–extract combination at the synergistic 1:1 fixed ratio produced faster and deeper parasite killing than quinine alone, with sustained suppression and no regrowth, confirming pharmacodynamic synergy consistent with the CI_{50} value of 0.65.

DISCUSSION

Antiplasmodial activity of *Nauclea latifolia* root extract

The methanol root extract of *Nauclea latifolia* demonstrated strong and reproducible in vitro antiplasmodial activity against both chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strains, with IC_{50} values of 7.12 µg/mL and 9.68 µg/mL, respectively. According to established classification criteria for crude extracts (IC_{50} < 10 µg/mL considered active), the extract qualifies as strongly active (Rasoanaivo *et al*., 2004; Pink *et al*., 2005). These IC_{50} values compare favourably with earlier reports on *N. latifolia*, where root and stem bark extracts typically demonstrated moderate-to-good activity

Table 4. Parasite viability over time (% of untreated control).

Time (h)	Control	Quinine alone	Extract alone	Quinine + Extract (1:1)
0	100	100	100	100
12	118	92	95	72
24	145	78	82	38
48	190	55	60	12
72	235	42	47	4
96*	260	65 (regrowth)	70 (regrowth)	6 (no regrowth)

*96 h measured after continued incubation without drug replenishment.

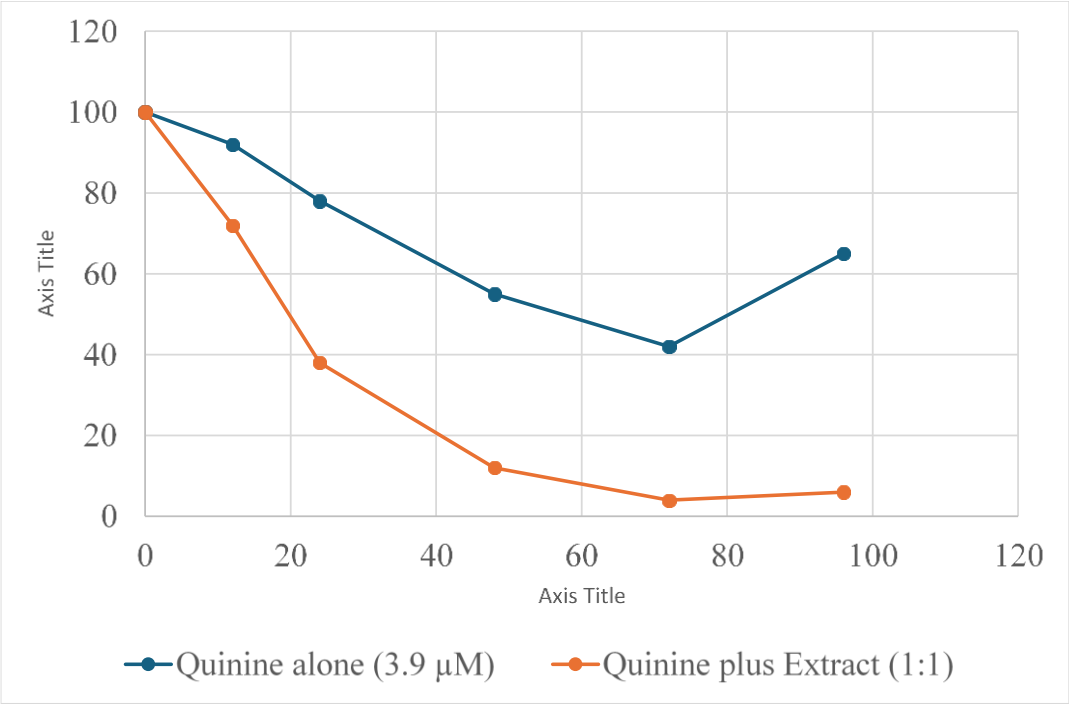

Figure 2. Time-kill of quinine-extract combination at 1:1 fixed ratio.

in the range of 5–20 µg/mL (Tajbakhsh *et al*., 2021; Abbah *et al*., 2010). The slightly improved potency observed in this study may reflect differences in extraction solvent, plant part selection, or parasite strain.

Importantly, the modest shift in potency between CQ-sensitive and CQ-resistant strains suggests that the extract's activity is not substantially affected by classical chloroquine resistance mechanisms involving mutations in *pfcrt* and *pfmdr1* (Fidock *et al*., 2000; Wellems and Plowe, 2001). This observation corroborates previous findings that certain plant-derived alkaloids retain activity against resistant parasites through mechanisms distinct from quinoline drugs (Atanasov *et al*., 2021). Unlike chloroquine, whose efficacy is directly compromised by altered digestive vacuole transport, the phytochemical constituents of *N. latifolia* may exert multi-target effects, including redox modulation or interference with parasite protein synthesis (Theodoridis and Carvalho, 2025).

While earlier studies confirmed intrinsic antiplasmodial activity of *N. latifolia*, most focused solely on standalone extract evaluation without direct comparison across resistant and sensitive laboratory strains. The present study strengthens existing evidence by demonstrating comparable potency across both phenotypes, thereby supporting the hypothesis of resistance-independent activity.

Cytotoxicity and selectivity profile

The extract exhibited low cytotoxicity toward HepG2 and Vero cell lines (CC_{50} > 200 µg/mL), yielding selectivity indices (SI) of 30.7 (3D7) and 22.6 (Dd2). These values substantially exceed the commonly accepted threshold of

SI ≥ 10 for promising antiplasmodial candidates (Cos *et al*., 2006). Compared with previously reported African medicinal plant extracts, which often display SI values between 5 and 15 (Tona *et al*., 2004; Bekono *et al*., 2020), the selectivity observed here is comparatively high.

This favourable safety margin strengthens earlier reports of *N. latifolia* as a relatively safe ethnomedicinal plant and addresses a limitation in some previous studies where cytotoxicity assessment was either absent or limited to a single cell line. By incorporating dual mammalian cell models, the present study provides a more robust preliminary safety evaluation.

Synergistic interaction with quinine

A key novel finding of this study is the demonstration of moderate but biologically meaningful synergy between quinine and *N. latifolia* root extract (CI_{50} = 0.65), confirmed by isobologram analysis. According to the Chou–Talalay method, CI values between 0.3 and 0.7 represent moderate synergy (Chou, 2006). While several studies have reported synergistic interactions between plant extracts and antimalarial drugs (Valentin *et al*., 2000; Wagner and Ulrich-Merzenich, 2009), few have specifically evaluated interactions with quinine, and even fewer have applied quantitative combination index modelling across fixed ratios.

Unlike many prior investigations that rely on fractional inhibitory concentration approximations or qualitative synergy claims, this study employed a fixed-ratio design with formal CI calculation, thereby providing quantitative validation of interaction strength. Furthermore, the observed 40–50% reduction in effective quinine dose distinguishes this work from earlier reports where synergy was reported but without a clear demonstration of clinically meaningful dose reduction.

Mechanistically, quinine primarily interferes with heme detoxification within the parasite digestive vacuole (White, 2007). The phytochemical complexity of *N. latifolia*, particularly its indole alkaloids and phenolics, may enhance this effect via complementary interference with redox homeostasis, membrane permeability, or efflux modulation. Similar potentiation mechanisms have been described for other alkaloid-rich plant extracts (Theodoridis and Carvalho., 2025), supporting the plausibility of the interaction observed here.

To our knowledge, this is among the first studies to systematically evaluate *N. latifolia* as a quinine-potentiating partner against both chloroquine-sensitive and resistant *P. falciparum* strains using quantitative synergy modelling.

Time–kill kinetics and pharmacodynamic relevance

Time–kill analysis further demonstrated that the CI-defined synergy translated into accelerated and sustained parasite clearance. The quinine–extract combination reduced parasitemia to <5% by 72 h and prevented regrowth after drug withdrawal, in contrast to monotherapies. This distinction is critical, as regrowth following exposure to antimalarials is often associated with tolerant or quiescent parasite subpopulations (Teuscher *et al*., 2010).

Previous plant–drug synergy studies have frequently relied solely on endpoint IC_{50} shifts without evaluating temporal killing dynamics. By incorporating time–kill kinetics, this study strengthens the pharmacodynamic relevance of the observed interaction and demonstrates that synergy is not merely mathematical but biologically sustained. Similar enhanced clearance dynamics have been reported in combination studies involving artemisinin-based therapies and plant-derived adjuvants (Benoit-Vical *et al*., 1998), supporting the concept of phytochemical-assisted parasite eradication.

The absence of parasite recrudescence following combination exposure suggests improved killing across developmental stages and potential reduction in resistance emergence risk. This pharmacodynamic advantage differentiates the present findings from earlier reports limited to static growth inhibition measurements.

While the in vitro findings are promising, further in vivo validation and pharmacokinetic interaction studies are necessary to determine translational applicability. Additionally, bioassay-guided fractionation would clarify which specific phytochemicals drive the observed synergy.

Conclusion

Collectively, this study demonstrates that the methanol root extract of *Nauclea latifolia* possesses potent in vitro antiplasmodial activity against both chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* strains, with favourable selectivity toward mammalian cells. Beyond its intrinsic activity, the extract exhibited quantitatively validated synergistic interaction with quinine, resulting in significant dose reduction and sustained parasite clearance in time–kill assays.

By integrating fixed-ratio combination analysis and kinetic validation, this study advances existing knowledge beyond standalone extract evaluations and provides evidence that *N. latifolia* may function not only as an independent antiplasmodial agent but also as a quinine-potentiating adjuvant. These findings support its ethnomedicinal use and highlight its potential role in combination-based anti-malarial strategies aimed at enhancing drug efficacy and mitigating resistance development.

Further studies are required to isolate and characterise the active phytochemical constituents, elucidate precise molecular mechanisms, and evaluate in vivo efficacy, pharmacokinetics, and safety. Nevertheless, the present work establishes a robust pharmacological foundation for the continued development of *N. latifolia*–derived combination therapies in malaria management.

CONFLICT OF INTEREST

The author declares that they have no conflict of interest.

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