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Research Article

Evaluation of Synergistic Anti-plasmodial Activity of *Tamarindus indica* and *Hibiscus sabdariffa* Extracts Against Clinical Isolates of *Plasmodium falciparum*: *In-Vitro* and *Ex-Vivo* protocols

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ABSTRACT
Malaria caused by *Plasmodium falciparum* remains a major global health burden and serious health challenge, exacerbated by increasing resistance to conventional antimalarial drugs. This study evaluated the *in vitro* and *ex vivo* anti-plasmodial activity of *Tamarindus indica* (Tamarind) and *Hibiscus sabdariffa* (Zobo) extracts, individually and in combination, against clinical isolates of *P. falciparum*. Aqueous extracts were prepared and screened for phytochemical constituents, followed by dose-dependent schizont maturation assays and growth kinetic analyses. Results demonstrated that phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, and saponins, which likely contribute to the observed bioactivity. While *T. indica* exhibited potent anti-plasmodial activity with an IC_{50} of 3.8 ± 0.96 µg/mL, approaching that of quinine (2.5 ± 0.74 µg/mL), while *H. sabdariffa* showed moderate activity (IC_{50} = 13.5 ± 0.99 µg/mL). The combined extract improved efficacy relative to *H. sabdariffa* alone (IC_{50} = 5.2 ± 0.99 µg/mL), but combination index analysis (CI = 1.61) revealed an antagonistic interaction. Growth kinetic studies indicated that untreated parasites exhibited a typical exponential replication with a doubling time of 17.5 hours, whereas extract-treated cultures showed prolonged lag phases, suppressed exponential growth, and early transition to decline phases. Notably, *H. sabdariffa* alone demonstrated a paradoxical increase in parasite viability at higher concentrations, suggesting potential metabolic or protective effects on parasite survival. However, *T. indica* demonstrates strong antimalarial potential, while the combination with *H. sabdariffa* does not confer synergistic benefit. These findings highlight the importance of rigorous interaction studies in plant-based combination therapies for malaria.

Keywords: Anti-plasmodial activity; *Ex-vivo Assay; Hibiscus sabdariffa; In-Vitro; Plasmodium falciparum*; Synergy; *Tamarindus indica*

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INTRODUCTION

Malaria remains a major global threat and health challenge, with an estimated 247 million cases and 619,000 deaths reported in 2021, predominantly caused by *Plasmodium falciparum* (WHO, 2022). The emergence of drug-resistant strains threatens current chemotherapeutic regimens and underscores the need for novel antimalarial agents (Cowman *et al*., 2016). Traditional medicinal plants have long been used to manage febrile illnesses, and several species have demonstrated promising anti-plasmodial properties (Rasoanaivo *et al*., 2011). *Tamarindus indica* (Fabaceae) leaves have exhibited antipyretic, anti-inflammatory, and antiparasitic activities attributed to polyphenols and flavonoids (Akinmoladun *et al*., 2015). Similarly, *Hibiscus sabdariffa* (Malvaceae) calyces are rich in anthocyanins, flavonoids, and phenolic acids, which confer antioxidant and antiparasitic effects (Da-Costa-Rocha *et al*., 2014). Combination therapy is a proven strategy in malaria management, enhancing efficacy and reducing resistance development (White, 2004). Plant-based combinations offer a sustainable and potentially synergistic approach. However, few studies have rigorously evaluated the synergistic anti-plasmodial potential of *T. indica* (Tamarind) and *H. sabdariffa* (Zobo) against both laboratory and clinical *P. falciparum* isolates. This study aims to assess the *in-vitro* anti-plasmodial activity of *T. indica* and *H. sabdariffa* extracts individually and in combination. Also, evaluate *ex-vivo* activity against clinical isolates of *P. falciparum* from malaria patients, and determine the nature of the interaction (synergistic, additive, or antagonistic) using fractional inhibitory concentration indices (FICIs).

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh *T. indica* pulp and *H. sabdariffa* calyces were collected from traditional vendors in the central market and were authenticated in the department of biological science Kaduna State University KASU Kaduna Nigeria. Voucher specimens were deposited at the University Herbarium, Department of Biological Sciences.

Preparation of Plant Extracts

Plant materials were washed, shade-dried, and pulverized into fine powder. For aqueous Extraction, 50 g of powdered plant part was macerated in 500 mL distilled water for 24 hours, with intermittent shaking after which the extract was filtered and lyophilized. The extracts were stored at 4°C until use.

Phytochemical Screening

Standard qualitative tests were conducted to detect flavonoids, alkaloids, saponins, tannins, phenols, and anthocyanins (Harborne, 1998).

Parasite Culture Isolation for *In-vitro* and *Ex-vivo* activity

Plasmodium falciparum Clinical strains were cultured in human O+ erythrocytes at 5% haematocrit in RPMI 1640 supplemented with 15%, for initiation and 10% for continuous culture, of in-activated O+ human serum under standard gas conditions (5% CO_2, 5% O_2, 90% N_2) at 37°C (Trager and Jensen, 1976). Blood samples (5 mL) were collected from consenting malaria patients attending Shehu Kangiwa Medical Centre, Kaduna Polytechnic, Kaduna. Medical diagnosis, thin, thick smears and RDT kit confirmed *P. falciparum* infection. Samples were processed for *in-vitro* and *ex-vivo* culture within 4 hours of collection.

Collection and Processing of Clinical Isolates

Venous blood samples, 3 mL were collected aseptically from malaria-positive patients following informed consent and ethical approval from Ministry of Health, Kaduna State, with reference number MOH/ADM/Vol1/111054. Inclusion criteria included mono-infection with *P. falciparum* confirmed by Giemsa-stained thick and thin blood smears, with parasitaemia ranging from 0.5% to 5%. Collected samples were immediately transferred into EDTA-coated tubes and transported to the laboratory within 4 hours of collection. Plasma and Buffy coat were removed by centrifugation at 3,000 rpm for 5 minutes, and erythrocytes were washed three times, two times with PBS pH 7.2 and the third one with incomplete RPMI 1640 medium (Haruna *et al*, 2024).

Establishment of *In-vitro* Continuous Culture

Clinical isolates obtained were cultured using a modified method of continuous *P. falciparum* cultivation. Washed erythrocytes were re-suspended in complete culture medium consisting of RPMI 1640 supplemented with, 25 mM HEPES, 0.2% sodium bicarbonate, 50 µg/mL gentamicin and 15% heat-inactivated human O^+ serum to initiate the culture. Cultures were adjusted to 5% haematocrit and initial parasitaemia of ≤1%. Parasites were maintained in sterile 25 cm^2 culture flasks under controlled atmospheric conditions (5% CO_2, 5% O_2, 90% N_2) at 37°C using CO_2 incubator as reported by Haruna *et al*. (2024). Medium was replaced daily and cultures were monitored microscopically every 24 hours using Giemsa-stained thin smears was used to assess parasite stages and viability (Gabi *et al*., 2023; Hamza *et al.*, 2024).

Chronological Monitoring of Parasite Development

Parasite growth was monitored over a 7-day cultivation period to establish chronological progression and adaptation of clinical isolates to *in-vitro* conditions. At 24-hour intervals Day 0 to Day 7, thin blood smears were prepared, fixed in methanol and stained with 5% Giemsa for 30 minutes. Parasite stages which included ring, trophozoite and schizont were identified using oil immersion objective microscopy 100×. Parasitaemia (%) was determined by counting the number of infected erythrocytes per 1,000 red blood cells across random fields. Stage-specific development patterns were recorded to assess synchronization and maturation efficiency (Gabi *et al*., 2023; Hamza *et al*, 2024).

Determination of Parasite Growth Rate

Parasite growth rate was quantified by calculating the fold increase in parasitaemia over time using the PMR which was calculated based on the relationship:

$$PMR = \frac{Pt}{Po} \quad \text{_____ Equation 1}$$

where:

P_0 represents the initial parasitaemia equivalent, and

P_t represents the parasitaemia at time t.

Additionally, the percentage growth per replication cycle i.e.≤ 48 h was determined using the following formula: Percent Growth = $(P_t - P_0 / P_0) \times 100$ Growth rates were compared across isolates to evaluate variability in proliferation capacity.

Parasite Growth Kinetics Analysis

Growth kinetics of clinical isolates were analysed by plotting parasitaemia (%) against time (hours/days) to generate growth curves. The following kinetic parameters were determined which included; Lag Phase (a period of initial adaptation {Day 0-1}), Log or Exponential Phase (a rapid parasite multiplication phase), Stationary Phase (a plateau indicating nutrient limitation or waste accumulation), and Decline Phase (with reduction in parasitaemia). The doubling time (T_d) of parasites during the exponential phase was calculated using:

T_d = t·ln(2)/ln(P_t/P_0) _____Equation 2

Where:

t = time interval (hours)

P_t = parasitaemia at time

P_0 = initial parasitaemia

Synchronization and Stage-Specific Kinetics

The selected cultures were synchronized using 5% D-sorbitol treatment to enrich ring-stage parasites and improve stage-specific kinetic analysis. Post-synchronization and stage progression were monitored at 6 -12-hour intervals to determine intra-erythrocytic developmental cycle timing for 48 hours.

***In-Vitro* Anti- plasmodial Assay**

Giemsa assay *in-vitro* screening was performed to assess parasite growth inhibition (Bennett *et al*., 2004). Briefly, parasites were seeded in 96-well plates at 1% parasitaemia and 5% haematocrit. The parasite cultured and synchronized were exposed to various concentrations of *T. indica* and *H. sabdariffa* extracts. The extracts were diluted in RPMI 1640 medium to produce concentrations of 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µg/mL. Each concentration was tested in triplicate, and untreated parasite cultures served as the negative control. Plates were incubated for 30 hours, followed by harvesting and slide smear using frosted edged slide. Thin and thick films were prepared fixed with methanol and stained with 5% Giemsa stain for 30 minutes. Schizonts were counted relative to 200 parasites (trophozoites). The control wells were compared with the treated wells to determine the percentage schizont growth inhibition using the formula in equation 1. That was subsequently followed by the calculation of IC_{50} values using nonlinear regression in GraphPad Prism 9. The IC_{50} values were then calculated using the dose-response curve to assess the potency of the plant extracts.

$$Percentage\ inhibition = \frac{Ncontrol - Ntreated}{Ncontrol} \text{ X } 100$$

Equation 3

where $N_{control}$ and $N_{treated}$ represent the number of schizonts observed in the control and treated cultures, respectively.

***Ex-Vivo* Anti-plasmodial Assay**

A modified schizont maturation assay was performed (WHO, 2010), Clinical isolate erythrocytes were incubated with extracts at sub-IC_{50} concentrations. Parasite development was monitored microscopically over 30 hours. Percent inhibition was calculated relative to untreated controls.

Combination and Synergy Assessment

The interaction between *T. indica* and *H. sabdariffa* extracts was evaluated using a fixed-ratio combination design based on their respective IC_{50} values. Extracts were combined at predetermined ratios (100:0, 50:50, and 0:100), and their anti-plasmodial activities were assessed using the schizont maturation assay. The nature of the interaction was determined by calculating the Fractional Inhibitory Concentration Index (FICI) according to standard protocols. The FICI was computed using the following equation 4:

$$FICI = \frac{IC_{50Aalone}}{IC_{50Aincombinatation}} + \frac{IC_{50BAlone}}{IC_{50Bincombination}}$$

------- Equation 4

Where IC_{50A} and IC_{50B} represent the inhibitory concentrations of extracts A (*T. indica*) and B *(H. sabdariffa*), respectively, either alone or in combination.

The interaction between the extracts was interpreted based on established thresholds as follows: FICI ≤ 0.5 indicates synergistic interaction; FICI > 0.5–1.0 indicates an additive effect; FICI > 1.0–4.0 indicates an indifferent interaction; and FICI > 4.0 indicates antagonism (Odds, 2003).

Data Analysis

All assays were performed in triplicate and the data were expressed as mean ± standard deviation (SD). One-way ANOVA with Tukey's post-hoc test was used to compared treatment groups. Variables with p≤0.05 were considered statistically significant. Growth curves were generated using GraphPad Prism (version 9.0).

RESULTS

Extraction Yield and Organoleptic Properties

The extraction yield and organoleptic characteristics of *H. sabdariffa* (Zobo) and *T. indica* are presented in Table 1. *T. indica* exhibited a slightly higher extraction yield (5.0%) compared to *H. sabdariffa* (4.5%). Distinct differences were observed in colour, odour, and texture, reflecting their phytochemical composition and physical properties.

Phytochemical Composition

Qualitative phytochemical screening of *T. indica and H. sabdariffa* extracts revealed the presence of key bioactive constituents, including alkaloids, tannins, flavonoids, terpenoids, and saponins, while steroids were absent but present in *H. sabdariffa* extract. These compounds are known to contribute to extract biological activity including anti-plasmodial activity.

Table 1: Percentage Yield and Organoleptic Properties of *T. indica* and *H. sabdariffa*

Sample	%Yield	Colour	Odour	Texture
Hibiscus sabdariffa (Zobo)	4.50 ±0.31	Purple	Honey	Thick and sticky
T. indica (Tamarind)	5.0 ± 0.20	Brown	Sour, Acidic	Fleshly, pulpous

Table 2 Phytochemical Screening of the extract *T. Indica* and *H. Sabdariffa*.

Phytochemical Constituents	*T. indica*	*H. sabdariffa*
Saponins	+	+
Alkaloids	+	+
Tannins	+	+
Steroids	+	+
Terpenoid	+	+
Flavorids	+	+

Key + = Prescence - = absent

Growth Rate and Kinetics of Clinical Isolates of *P falciparum*

Chronological Growth Pattern of Clinical Isolates

Chronological monitoring of *P. falciparum* clinical isolates under continuous culture revealed a typical intra-erythrocytic developmental cycle characterized by progressive maturation from ring to trophozoite and schizont stages within 48 hours. Parasitaemia increased consistently over time in untreated control cultures, confirming successful adaptation of clinical isolates to *in-vitro* conditions (Figure 1).

The observed growth profile followed a classical pattern consisting of the Lag phase from 0-24 hrs (ring form to trophozoite) Figure 1A-B showed minimal increase in parasitemia as parasites adapted to culture conditions. The Exponential phase Figure 1C-D between 24-72 hrs on the other hand showed Rapid multiplication with marked increase in schizont formation. While early stationary phase >72 hrs is a gradual plateau in parasite density, that is likely due to nutrient depletion and accumulation of metabolic waste.

Parasite Growth Rate Determination

Figure 2 present the PMR indicates that the clinical isolate exhibited an approximate 6.37-fold multiplication per 48 hours, which is consistent with moderately growing field isolates. Furthermore, when considered alongside the growth curve (Figure 2) and the log-transformed linear relationship (Figure 3), the PMR value supports the presence of a well-defined exponential growth phase prior to treatment exposure.

Doubling Time (Td) Estimation

The logarithmic transformation of parasitemia values is presented in Table 3. It produced a linear relationship with time, confirming exponential growth of *P. falciparum* under control conditions. The slope of the line indicates a rapid replication rate, corresponding to a doubling time of approximately 17–18 hours. Using the exponential growth model in Equation 2 the doubling time (T_d) is 17.5hours. Thus, the doubling time of the clinical isolate was approximately 17–18 hours, indicating relatively efficient parasite replication compared to typical field isolates.

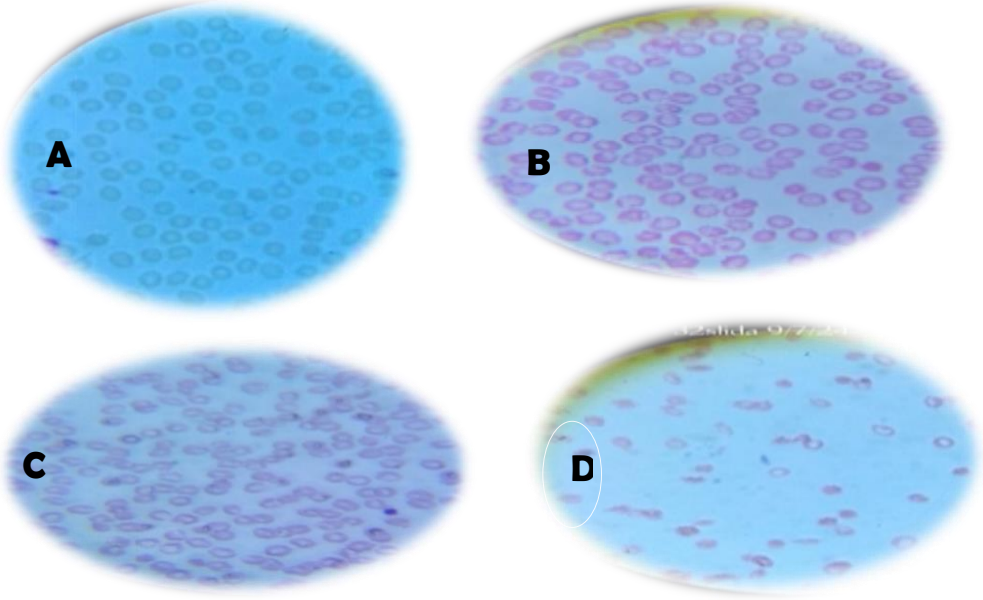

Figure 1A-D: Chronological growth phases of Asexual Stage P. falciparum over the period one cycle (48Hours) A-B lag phase C-D Exponential phase

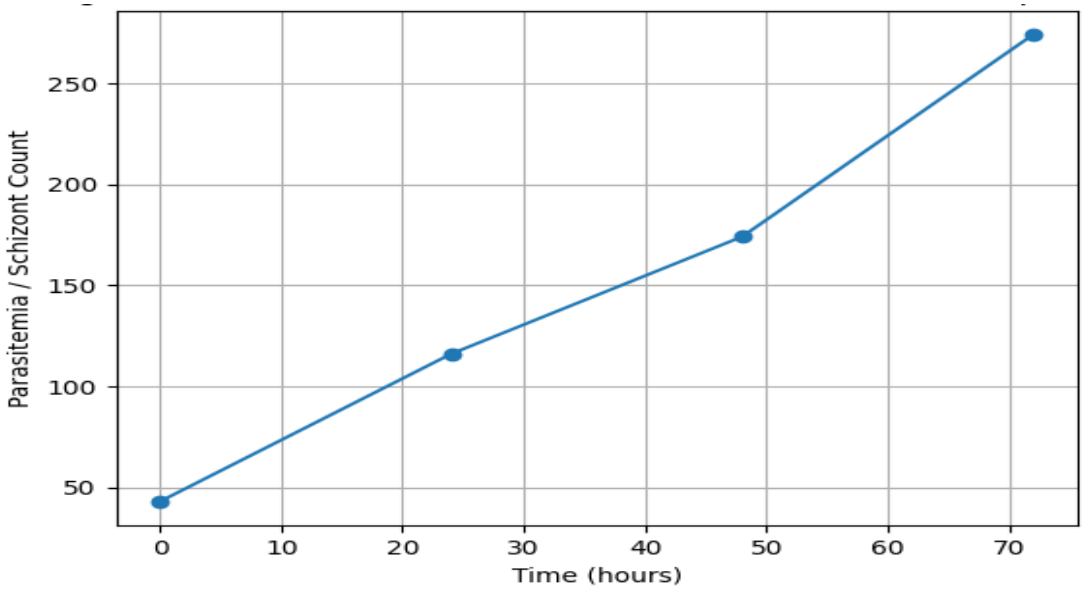

Figure 2: Parasite Growth Curve (Control and Before Extract Exposure)

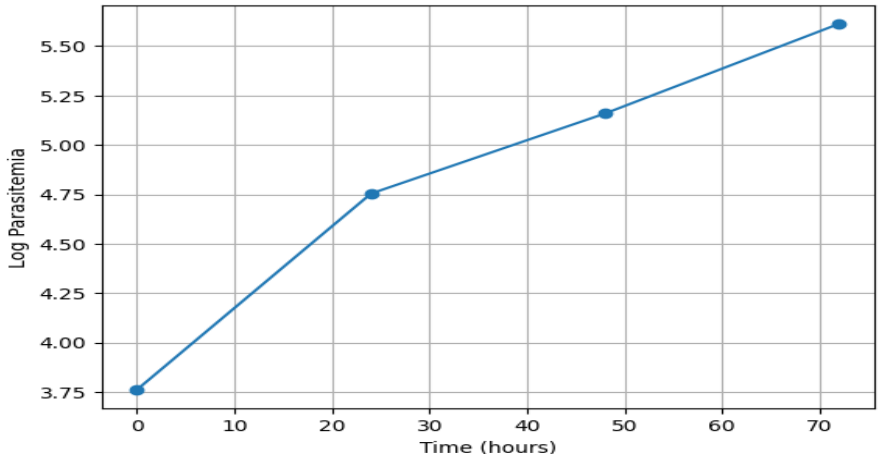

Figure 3: Log-transformed growth curve showing exponential phase and doubling time estimation

Effect of Plant Extracts on Growth Kinetics of *Plasmodium falciparum*

Treatment with plant-derived extracts significantly altered the growth kinetics of *P. falciparum* in a concentration-dependent manner, as evidenced by changes in parasitemia, inhibition rates, and phase transitions (Figures 3 - 6).

Effect of *Tamarindus indica*

Exposure to *T. indica* resulted in a pronounced suppression of parasite proliferation, with schizont counts declining sharply from 43 in the control to approximately 3.5 at the highest concentration (100 µg/mL). This corresponded to a growth inhibition of 91.94%, indicating potent anti-plasmodial activity (Figure 4). The extract effectively disrupted the exponential growth phase, leading to a near-complete arrest of parasite replication. The steep decline observed in the growth curve (Figure 4) confirms its strong inhibitory effect on parasite multiplication.

Effect of *Hibiscus sabdariffa*

Treatment with *H. sabdariffa* exhibited a moderate but significant reduction in parasite growth. Maximum inhibition reached 85.76% at 100 µg/mL as presented in Figure 5, indicating substantial but comparatively lower efficacy than *T. indica*. The growth curve (Figure 5) demonstrates a gradual decline in parasitemia, suggesting a slower suppression of replication kinetics. Unlike *T. indica*, the extract did not completely abolish the exponential phase but significantly reduced its slope.

Effect of Combined Extract (*T. indica* + *H. sabdariffa*)

The combined extract produced a marked disruption of parasite growth kinetics, as presented in Figure 6, with parasitemia reduced to approximately 9.5 schizonts at the highest concentration. Growth inhibition reached 91.85%, comparable to that of *T. indica* alone and approaching standard antimalarial efficacy. Notably, the combination caused a collapse of the exponential growth phase and induced an early transition into the stationary/decline phase (Figure 6). This suggests possible synergistic or additive interactions between the bioactive compounds, leading to enhanced interference with parasite replication mechanisms.

Comparative Growth Kinetic Phase Modulation

A comparative analysis of growth phases revealed substantial alterations under extract-treated conditions presented in Table 3.

Table 3 shows that typical control condition, parasites exhibited typical exponential growth characterized by a steep increase in parasitaemia (Figure 2), further confirmed by a linear trend in the log-transformed plot (Figure 3), indicating true exponential replication with a doubling time of approximately 17-18 hours. In contrast, extract-treated cultures displayed significant kinetic alterations. The exponential phase was markedly shortened and suppressed, while the lag phase was extended, indicating delayed parasite adaptation. Furthermore, an early onset of the stationary phase followed by a rapid decline phase was observed, particularly in the combined extract group (Figure 6). The observed reduction in parasite growth (Figure 5) demonstrates a clear hierarchy of efficacy, while, the Control indicated 100% growth with no inhibition *H. Sabdariffa* and *T. Indica* extracts indicated *85.76%* and 91.94% inhibition respectively. The combination of the two extracts however, showed 91.85% inhibition (Figure 6).

The steep slope observed in the control growth curve (Figure 3) reflects rapid replication kinetics, whereas

the flattened curves under treatment (Figure 4) indicate strong suppression of parasite proliferation. The combined extract, in particular, induced a kinetic collapse characterized by early growth arrest and reduced parasitemia.

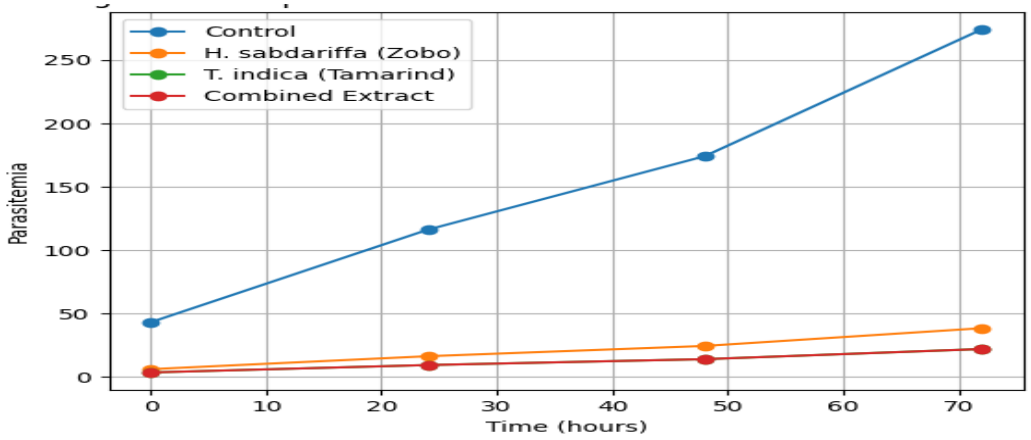

Figure 4: Comparative Growth Kinetics Under Extract Treatments (Non treated, *H. Sabdarifa, T. indica* and Combined extracts

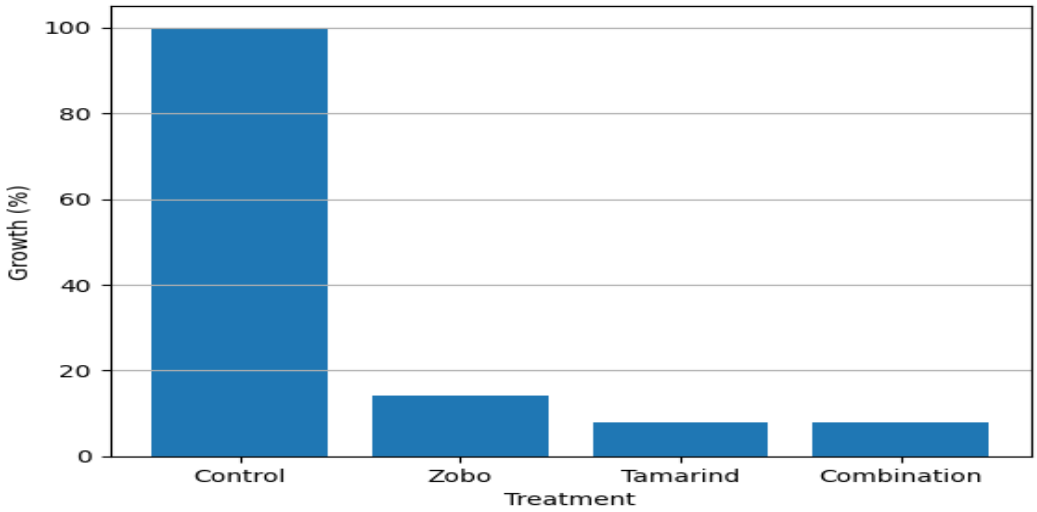

Figure 5: Growth Reduction across the Non-treatment and Treatment groups

Table 3: Comparative Growth Kinetic Phase Modulation of *P. falciparum* in control and Extract Treatment Conditions

Growth Phase	Control	Extract-Treated Cultures
Lag phase	Short (rapid adaptation)	Prolonged
Exponential phase	Strong multiplication	Severely suppressed
Stationary phase	Delayed	Early onset
Decline phase	Minimal	Evident at higher concentrations

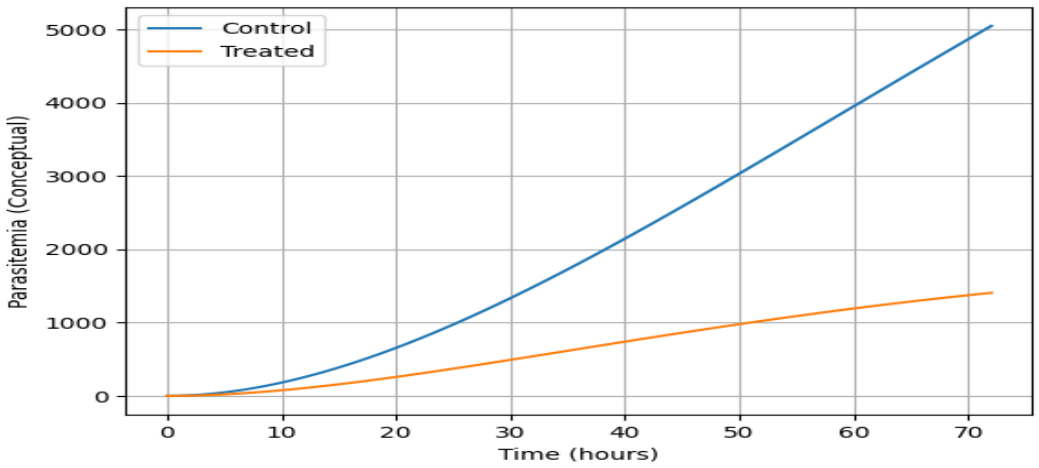

Figure 6: Growth Kinetic Phase Shift of *P. falciparum* clinical Isolate under extracts Treatment

Anti-plasmodial Activity of Individual Extracts

The schizont count of *P. falciparum* decreased progressively with increasing concentrations of all tested extracts, demonstrating a clear dose-dependent anti-plasmodial effect (Table 4). At baseline (0 µg/mL), the highest parasite burden was observed in the untreated control (274.0 ± 1.41), while comparatively lower baseline counts were recorded for *H. Sabdariffa* (Zobo) (52.5 ± 0.70), *T. Indica* (Tamarind) (43.0 ± 1.41), and their combination (116.0 ± 5.66)**.** Among the treatments, Tamarind extract exhibited the strongest inhibitory activity, achieving the lowest schizont count at the highest concentration tested (3.5 ± 2.12 at 100 µg/mL), followed by Zobo (7.5 ± 3.54) (Table 4). The combined extract (Zobo + Tamarind) also demonstrated substantial activity but did not show a clear synergistic advantage over Tamarind alone, suggesting a potentially additive or mildly antagonistic interaction. The reference drug (Quinine) showed a marked reduction in parasite count across concentrations, confirming assay validity and serving as a positive control benchmark (Table 4).

Parasite Viability

Parasite viability exhibited distinct and contrasting trends across the tested extracts. A progressive dose-dependent reduction in viability was observed for Tamarind, the combined extract (Zobo + Tamarind), and the standard drug (Quinine), whereas Zobo extract showed an unexpected dose-dependent increase in parasite viability (Table 5). Specifically, Tamarind demonstrated a strong inhibitory effect, reducing parasite viability from 59.31 ± 0.31 at 1.56 µg/mL to 8.06 ± 4.67 at 100 µg/mL. A similar trend was observed for the combined extract, with viability decreasing from 76.19 ± 4.21 to 8.15 ± 1.43 across the same concentration range. Quinine exhibited the most consistent and potent suppression, reducing viability to 6.21 ± 0.55 at 100 µg/mL. In contrast, Zobo extract showed a marked increase in parasite viability, rising from 107.17 ± 1.65 at 1.56 µg/mL to 785.00 ± 360.62 at 100 µg/mL, suggesting a potential parasite growth-promoting or protective effect rather than inhibition (Table 5).

Percentage Inhibition

A clear dose-dependent increase in parasite inhibition was observed across all treatments (Figure 7), confirming significant anti-plasmodial activity. At the lowest concentration (1.56 µg/mL), Tamarind (40.69 ± 0.31%) and Quinine (46.53 ± 1.05%) demonstrated substantially higher inhibition compared to Zobo (6.68 ± 1.44%), while the combined extract showed intermediate activity (23.81 ± 4.21%) (Table 6). As concentration increased, Tamarind consistently exhibited greater inhibitory potency than Zobo, achieving 91.94 ± 4.67% inhibition at 100 µg/mL, closely approaching the effect of Quinine (93.79 ± 0.55%). The combined extract (Zobo + Tamarind) demonstrated improved efficacy relative to Zobo alone, reaching 91.85 ± 1.43% Even though the inhibition increased steadily from 6.68% to 85.76%, indicating moderate but concentration-dependent anti-plasmodial potential (Table 6).

Table 4: Effect of *H. Sabdariffa*, *T. indica* and Their Combination on Schizont Count of *P. falciparum*

Concentration (µg/mL)	Zobo (Mean ± SD)	Tamarind (Mean ± SD)	Zobo + Tamarind (Mean ± SD)	Quinine Control (Mean ± SD)
0.00	52.5 ± 0.70	43.0 ± 1.41	116.0 ± 5.66	274.0 ± 1.41
1.56	49.0 ± 1.41	25.5 ± 0.71	88.5 ± 9.19	146.5 ± 2.12
3.13	45.0 ± 1.41	22.5 ± 0.71	70.0 ± 1.41	116.5 ± 2.12
6.25	37.5 ± 2.12	17.5 ± 2.12	49.0 ± 4.24	91.5 ± 2.12
12.50	28.0 ± 2.83	13.5 ± 0.71	31.0 ± 1.41	51.5 ± 2.12
25.00	22.0 ± 2.83	11.5 ± 0.71	26.5 ± 3.54	41.5 ± 2.12
50.00	15.0 ± 2.83	8.0 ± 1.41	17.0 ± 2.83	27.0 ± 1.41
100.00	7.5 ± 3.54	3.5 ± 2.12	9.5 ± 2.12	17.0 ± 1.41

Note: Values are expressed as Mean ± Standard Deviation (SD) of triplicate determinations.

Table 5: Effect of *H. sabdariffa*, *T. indica* their Combination and Quinine on Parasite Viability of *P. falciparum*

Concentration (µg/mL)	Zobo (Mean ± SD) %	Tamarind (Mean ± SD)%	Zobo + Tamarind (Mean ± SD) %	Quinine Control (Mean ± SD)%
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1.56	107.17 ± 1.65	59.31 ± 0.31	76.19 ± 4.21	53.47 ± 1.05
3.13	116.70 ± 2.10	52.33 ± 0.08	60.39 ± 1.73	42.52 ± 0.99
6.25	140.17 ± 6.04	40.64 ± 3.60	42.20 ± 1.60	33.40 ± 0.95
12.50	188.33 ± 16.50	31.39 ± 0.61	26.73 ± 0.08	18.79 ± 0.68
25.00	240.45 ± 27.70	26.73 ± 0.77	22.80 ± 1.94	15.15 ± 0.85
50.00	355.88 ± 62.39	18.56 ± 2.68	14.61 ± 1.73	9.85 ± 0.47
100.00	785.00 ± 360.62	8.06 ± 4.67	8.15 ± 1.43	6.21 ± 0.55

Note: Values are expressed as Mean ± Standard Deviation (SD) of triplicate determinations

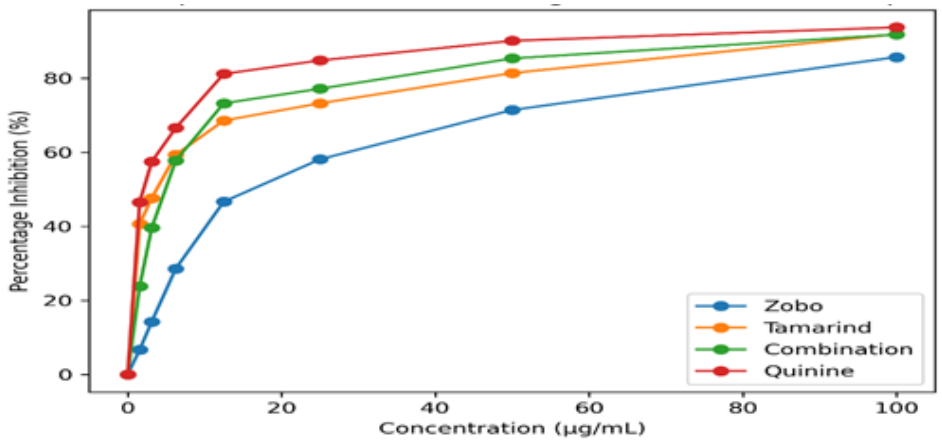

Figure 7: Dose – Response Curve of the Control, Extract and its Combination Against *P. falciparum*

Table 6. Effect of *H. sabdariffa*, *T. Indica*, Their Combination, and Quinine on Parasite Inhibition of *P. falciparum*

Concentration (µg/mL)	Zobo (Mean ± SD)%	Tamarind (Mean ± SD)%	Zobo + Tamarind (Mean ± SD) %	Quinine Control (Mean ± SD) %
0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
1.56	6.68 ± 1.44	40.69 ± 0.31	23.81 ± 4.21	46.53 ± 1.05
3.13	14.29 ± 1.54	47.67 ± 0.08	39.61 ± 1.73	57.48 ± 0.99
6.25	28.59 ± 3.08	59.36 ± 3.60	57.80 ± 1.60	66.60 ± 0.95
12.50	46.70 ± 4.67	68.61 ± 0.61	73.27 ± 0.08	81.21 ± 0.68
25.00	58.13 ± 4.82	73.27 ± 0.77	77.20 ± 1.94	84.85 ± 0.85
50.00	71.46 ± 5.00	81.44 ± 2.68	85.39 ± 1.73	90.15 ± 0.47
100.00	85.76 ± 6.54	91.94 ± 4.67	91.85 ± 1.43	93.79 ± 0.55

Note: Values represent Mean ± Standard Deviation (SD) of triplicate experiments.

IC_{50} Values of Extracts

The extracts demonstrated significant, concentration-dependent inhibition of *P. falciparum*, as shown in Table 7 with *T. indica* exhibiting the highest potency among plant treatments (IC_{50} ≈ 3.8 µg/mL), approaching the efficacy of quinine 2.5 µg/mL. The combination therapy enhanced activity relative to *H. sabdariffa* alone with value 13.5 µg/mL, suggesting additive phytochemical interactions.

Dose -response analysis and Synergistic Interaction Analysis

The interaction between *T. indica* and *H. sabdariffa* extracts was evaluated using fixed-ratio combination assays and quantified through the Fractional Inhibitory Concentration Index (FICI) and Combination Index (CI) analysis. The combined extract demonstrated a dose-dependent inhibitory effect against *P. falciparum*, with increasing concentrations resulting in progressive reductions in schizont counts and parasite viability. At the highest concentration tested (100 µg/mL). The dose-response analysis presented in Table 8 demonstrated excellent model fitting, with all treatments showing strong linearity on log-transformed concentration (R^2 > 0.94). *T. Indica* and Quinine exhibited moderate Hill slopes of 27.90 and 26.74 respectively, consistent with classical pharmacodynamic behavior, indicating gradual and controlled inhibition of *P. falciparum* (Table 8). In contrast, Zobo displayed a markedly steeper slope of 45.21, while the combination showed an intermediate slope of 37.38 suggesting a delayed onset of activity and or partial interactions. The interaction between *T. indica* and *H. sabdariffa* was evaluated using the Combination Index (CI) method as presented in Table 9. Combination index analysis CI = 1.61 revealed an antagonistic interaction between *H. sabdariffa* and *T. indica*, suggesting that co-administration does not enhance efficacy beyond individual extract performance.

Table 7: IC_{50} Values of Extracts *H. Sabdariffa* and *T. Indica*, Combination of (*H. Sabdariffa* and *T. Indica*) and Quinine

Extracts Treatment	IC_{50} (µg/mL)	Interpretation
Zobo	13.5± 0.99 µg/mL	Moderate activity
Tamarind	3.8 ± 0.96µg/mL	High potency
Zobo + Tamarind	5.2 ± 0.99 µg/mL	Improved vs Zobo (additive effect)
Quinine	2.5 ± 0.74µg/mL	Highest potency (reference drug)

Average ± Standard Deviation

Table 8: Dose -Response analysis of the Control and Extracts showing the R^2 Values

Extract Treatment	Hill Slope (approx)	R^2	Interpretation
Zobo	45.21	0.994	Very steep, strong dose dependence
Tamarind	27.90	0.992	Typical pharmacological response
Zobo + Tamarind	37.38	0.948	Moderate slope, interaction present
Quinine	26.74	0.953	Standard drug-like response

Table 9: Combination Index (CI) – Drug Interaction Analysis

CI Value	Interpretation
< 1	Synergy
= 1	Additive
> 1	Antagonism

CI = 1.61 → Antagonistic Interaction

DISCUSSION

The current study provides a detailed evaluation of the anti-plasmodial potential of *T. indica* and *H. sabdariffa,* integrating growth kinetics, dose-response analysis, and drug interaction modeling, thereby offering mechanistic insights into plant-based antimalarial therapy. The observed rapid parasite replication observed in control cultures, having a doubling time of approximately 17-18 hours, aligns with established growth dynamics of *P. falciparum* under optimal *in vitro* conditions (Ashley *et al.*, 2021). The calculated parasite multiplication rate (PMR ≈ 6.3-fold per 48 hours) further confirms the viability and adaptability of clinical isolates, consistent with reports on field strains which exhibited moderate-to-high replication efficiency (Roux *et al.*, 2023).

Treatment with *T. indica* indicated a marked suppression of the parasite *P. falciparum* growth, disrupting the exponential phase and inducing early

maturation growth arrest. This is a very strong inhibitory activity and may be attributed to its rich phytochemical composition, particularly flavonoids and polyphenols that have been reported to interfere with heme detoxification and induce oxidative stress in malaria parasites (Bero *et al*., 2022; Onguéné *et al*., 2024). The IC_{50} value (3.8 µg/mL) of *T. indic*a places the plant within the category of highly active extracts, comparable to control drug Quinine and other promising ethnopharmacological candidates (Tona *et al*., 2*020). Hibiscuss sabdariffa on the other hand* demonstrated contrasting moderate anti-plasmodial activity, but notably exhibited an unexpected dose-dependent increase in parasite viability, suggesting a potential paradoxical activity effect. This phenomenon may be linked to the antioxidant properties of anthocyanins identified with the plant, which could mitigate oxidative stress within infected erythrocytes and inadvertently support parasite survival under certain conditions (Tsumbu *et al.,* 2021; Ndzi *et al*., 2022). Similar dual effects of plant-derived antioxidants have been reported, where low to moderate concentrations is reported to inhibit parasites, while higher concentrations provide protective cellular environments (Amoa-Bosompem *et al.*, 2023).

The combination of both plant extracts yielded much improved activity, but relative to *H. sabdariffa* only alone, yet failed to surpass the efficacy of *T. indica.* Importantly, the combination index (CI = 1.61) indicated antagonism, suggesting phytochemical interference rather than synergy. This finding contrasts with the general assumption that plant combinations enhance efficacy and highlights the complexity of phytochemical interactions. Antagonistic effects may arise from competitive binding, metabolic interference, or opposing redox activities among bioactive compounds (Lehár *et al*., 2020; Fidock *et al*., 2022). Growth kinetic analysis further revealed that extract-treated parasites exhibited prolonged lag phases, suppressed exponential growth, and early onset of decline phases, indicating disruption of parasite metabolic adaptation and replication cycles. These kinetic shifts are consistent with mechanisms that are involved in the inhibition of DNA replication, mitochondrial dysfunction, and interference with nutrient uptake pathways (Cowman *et al.*, 2021). The dose response modeling showed high linearity (R^2 > 0.94), confirming the reliability of the pharmacodynamic data. The relatively moderately hill slope for *T. indica* suggests a controlled inhibitory effect, whereas the steep slope observed for *H. sabdariffa* may indicate delayed but abrupt pharmacological action, possibly due to threshold-dependent activity of its phytoconstituents (Zofou *et al.*, 2023).

These findings collectively, reinforce the therapeutic potential of *T. indica* as a candidate for antimalarial drug development while emphasizing the need for caution in combining plant extracts without mechanistic validation. The study further reveals the importance of integrating growth kinetics and interaction indices in evaluating antimalarial agents, since such parameters provide deeper insights beyond conventional IC_{50} measurements.

CONCLUSION

This current study demonstrates that *T. indica* possesses very strong and consistent anti-plasmodial activity against clinical isolates of *P. falciparum*, with efficacy that is compatible to standard antimalarial drugs. However, in contrast, *H. sabdariffa* exhibits moderate activity and may strongly exert paradoxical effects on parasite viability at higher concentrations. However, it worth to note that although the combination of both extracts improves activity but relative to only *H. sabdariffa*, it does not produce synergistic effects and instead shows antagonistic interaction. Growth kinetic analysis on the other hand confirms that the extracts disrupt parasite replication by suppressing exponential growth and inducing early decline phases. These findings strongly highlighted *T. indica* as a promising candidate for further antimalarial drug development, while emphasizing on the importance of mechanistic validation in plant-based combination therapies.

Recommendations

Further studies should focus on isolating and identifying the specific phytochemicals in *T. indica* responsible for its potent anti-plasmodial activity. Molecular investigations are needed to elucidate the exact mechanisms of action, particularly the effects on heme detoxification, oxidative stress pathways, and parasite metabolism.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this study. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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