Advancement in Medicinal Plant Research
Vol. 14(1), pp. 16-25, February 2026
ISSN: 2354-2152
Full Length Research Paper

In vitro antimalarial activity of the extracts of *Mangifera indica* L. against *Plasmodium falciparum* gotten from children under fifteen years attending Federal Medical Center Jalingo, Taraba State, Nigeria

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Accepted 29 January, 2026

ABSTRACT

Malaria remains a leading cause of morbidity and mortality among children under 15 years of age in Nigeria, highlighting the urgent need for effective and affordable antimalarial agents. *Mangifera indica* L. (mango) is traditionally used in malaria management; however, its *in vitro* efficacy against *Plasmodium falciparum* requires systematic evaluation. This study investigated the antimalarial activity of aqueous and ethanolic extracts of *M. indica* leaves and stem bark against *P. falciparum* isolates obtained from children attending the Federal Medical Centre, Jalingo, Taraba State, Nigeria. Plant materials were air-dried, pulverized, and extracted by cold maceration using distilled water and ethanol. Qualitative phytochemical screening was conducted to identify secondary metabolites, while quantitative analyses determined the concentrations of alkaloids, saponins, tannins, flavonoids, and phenols. *In vitro* antiplasmodial activity was assessed using parasitized red blood cells by measuring mean parasitized cell counts, percentage inhibition, and IC_{50} values across extract concentrations of 1.56–100 µg/mL. Data were expressed as mean ± standard error of the mean (SEM), with statistical significance set at $p \leq 0.05$. Phytochemical analysis revealed high saponin content in the aqueous extracts of both leaves and stem bark, and elevated alkaloid levels in the ethanolic stem bark extract (9.66 ± 0.27 mg/100 g). Flavonoids were most abundant in the ethanolic leaf extract (11.24 ± 0.39 mg/100 g), whereas phenols were highest in the aqueous stem bark extract (0.75 ± 0.03 mg/100 g). All extracts produced concentration-dependent reductions in parasitized cells. The ethanolic stem bark and leaf extracts showed the lowest parasitemia levels of 50.00 ± 1.96 and 40.16 ± 4.36, respectively, at 3.13 µg/mL, corresponding to high percentage inhibition (90.44 ± 2.57% and 93.22 ± 2.60%). IC_{50} values confirmed strong antiplasmodial activity, with ethanolic stem bark and leaf extracts showing IC_{50} values of 2.63 µg/mL and 2.93 µg/mL, respectively. Combination extracts demonstrated consistent synergistic inhibition across all concentrations tested. These findings indicate that *M. indica* leaves and stem bark possess potent antimalarial activity and may serve as promising sources for the development of natural antiplasmodial agents.

Keywords: Antimalarial activity, malaria, *Mangifera indica, Plasmodium falciparum*, phytochemical analysis.

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INTRODUCTION

Malaria remains a critical global public health challenge, with a disproportionately high burden in sub-Saharan Africa (World Health Organization, 2023). The disease is caused by protozoan parasites of the genus *Plasmodium*, of which *Plasmodium falciparum* is the most virulent and is responsible for the majority of severe cases and malaria-related deaths worldwide (White, 2018). Children under five years of age represent the most vulnerable population, accounting for a substantial proportion of malaria-associated morbidity and mortality. Many African

countries, including Nigeria, continue to report high infection rates within this age group (Nigeria Malaria Indicator Survey, 2021). Nigeria alone bears one of the heaviest malaria burdens globally, accounting for an estimated 27% of worldwide cases and a significant proportion of childhood deaths each year, underscoring the endemic nature of the disease and the urgent need for improved control and treatment strategies (World Health Organization, 2023).

Conventional antimalarial drugs, such as chloroquine and artemisinin-based combination therapies (ACTs), have historically formed the cornerstone of malaria treatment. However, the emergence and widespread distribution of drug-resistant *P. falciparum* strains have progressively reduced treatment efficacy in many endemic regions (Alven and Aderibigbe, 2019; Alghamdi et al., 2024). This growing resistance, together with limited access to effective chemotherapeutics and preventive measures in resource-poor settings, has intensified the need to explore alternative antimalarial agents (Saxena et al., 2013). Medicinal plants, which are rich in diverse bioactive phytochemicals, have long been used in traditional medicine in malaria-endemic communities and are increasingly being investigated as sources of novel antiplasmodial compounds (Adebayo and Krettli, 2011). *In vitro* antimalarial screening is a valuable tool for the early evaluation of plant extracts against *P. falciparum*, enabling the identification of promising candidates for drug development and potential integration into complementary treatment strategies (Gruessner and Weathers, 2021).

Mangifera indica L. (mango), a widely cultivated tropical plant in Nigeria and other parts of Africa, has a long history of ethnomedicinal use. It contains a wide range of phytochemicals, including mangiferin, flavonoids, alkaloids, and phenolic acids, which have been associated with various biological activities, including antiplasmodial effects (Mehmood et al., 2024; Udok et al., 2024). Previous studies and reviews have highlighted the potential of these constituents to interfere with parasite metabolic pathways, making *M. indica* a promising candidate for antimalarial research (Saxena et al., 2013). Experimental investigations have demonstrated that extracts from *M. indica* leaves exhibit measurable inhibitory effects on *P. falciparum* growth *in vitro*, supporting its potential as a source of bioactive antimalarial agents (Udok et al., 2024).

Despite these encouraging findings, there remains a significant gap in region-specific research, particularly studies evaluating the activity of *M. indica* extracts against *P. falciparum* isolates obtained from clinically infected pediatric populations in endemic Nigerian settings (Evbuomwan et al., 2023). Assessing the *in vitro* antimalarial activity of *M. indica* against *P. falciparum* isolates from children attending healthcare facilities such as the Federal Medical Centre, Jalingo, Taraba State, is therefore essential. Such investigations will provide valuable insights into the therapeutic relevance of this plant, support evidence-based ethnopharmacological practices, and potentially inform the development of adjunct or alternative antimalarial agents adapted to local parasite profiles and resistance patterns.

MATERIALS AND METHODS

Study area

The study was conducted in Jalingo, Taraba State, Nigeria, which covers a total area of 60,291.82 km². Jalingo lies approximately between latitudes 6°30′ and 9°36′ N and longitudes 9°10′ and 7°50′ E (Figure 1). The study spanned twelve months, from November 2022 to October 2023, with intermittent breaks due to the seasonal variation in malaria transmission, which peaks during the rainy season when parasite density is highest.

Collection and extraction of plant materials

Fresh leaves and stem bark of *Mangifera indica* were collected in January 2023 from Jalingo, Taraba State, Nigeria. The plant was authenticated by a taxonomist in the Department of Biological Sciences, Taraba State University, Jalingo. The plant materials were air-dried under shade, pulverized using a laboratory grinder, and stored until extraction.

One hundred grams (100 g) of each powdered sample were extracted separately with 1 L of distilled water and ethanol using the cold maceration method for 72 h. The resulting extracts were filtered and concentrated to dryness on a water bath, weighed, and stored in airtight containers at 4 °C until use to protect them from light and moisture (Coelho et al., 2019).

Qualitative phytochemical screening

Phytochemical screening was performed to detect the presence of alkaloids, carbohydrates, flavonoids, saponins, tannins, glycosides (cardiac and steroidal), terpenes/terpenoids, fatty acids, and resins using standard methods described by Sofowora (2008) and Trease and Evans (2009).

Test for alkaloids

Six milliliters (6 mL) of extract were mixed with 6 mL of 1% HCl in a steam bath and filtered. One milliliter of Mayer's reagent was added. The appearance of turbidity indicated the presence of alkaloids, which was further confirmed by the formation of an emulsion after adding a few drops of olive oil.

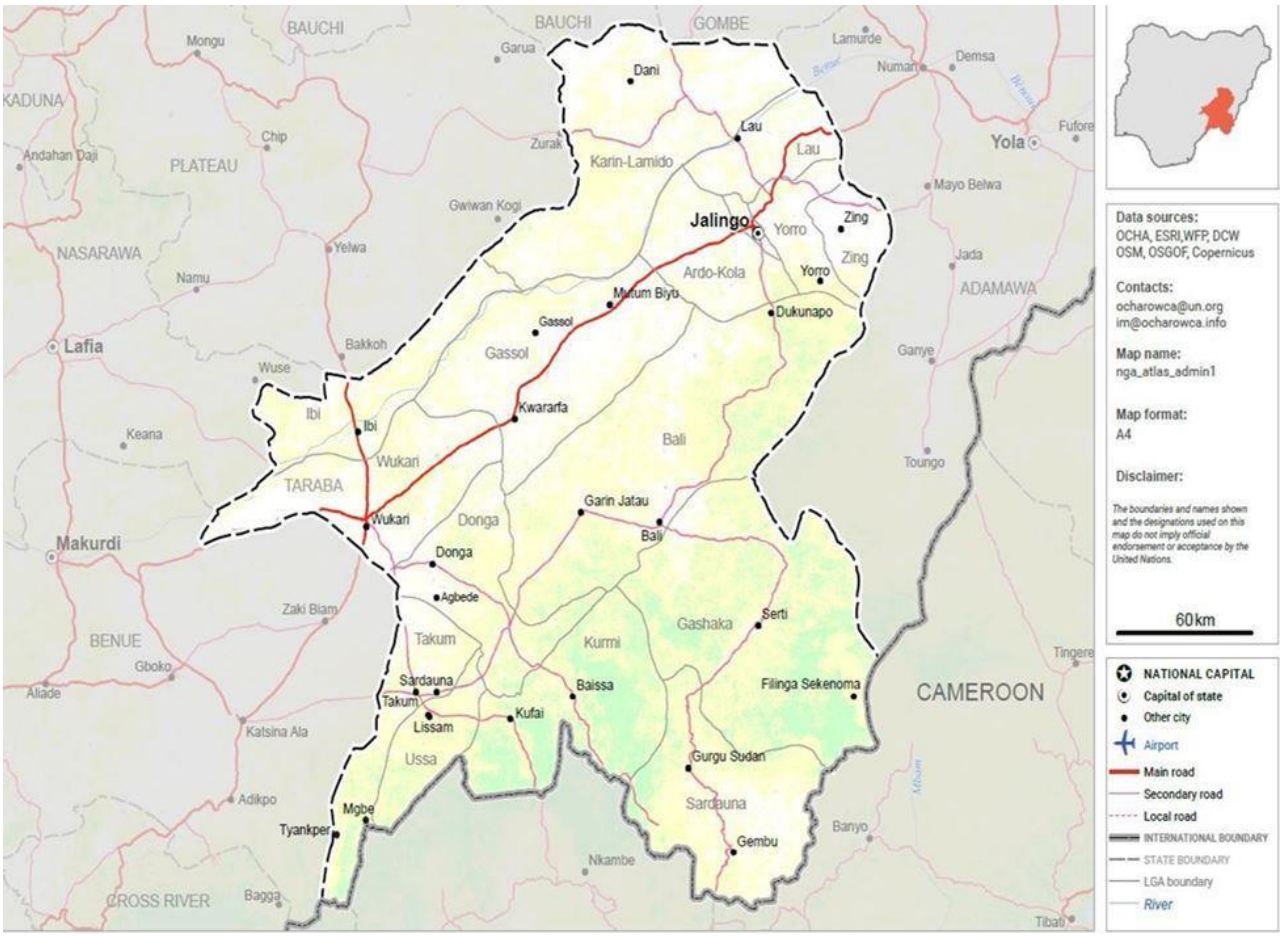

Figure 1. Map of Taraba State showing the study area. Source: OCHA, ESRI, WFP im@ocharowca.info

Test for saponins

Half a gram (0.5 g) of extract was dissolved in 5 mL of distilled water and shaken vigorously. The formation of a stable froth indicated the presence of saponins. The addition of six drops of olive oil producing an emulsion confirmed this result.

Test for tannins

Half a gram (0.5 g) of extract was dissolved in 10 mL of distilled water and a few drops of 1% ferric chloride solution were added. A brownish-green or blue-black coloration indicated the presence of tannins.

Test for anthraquinones

Bornträger's test was used. Briefly, 0.5 g of extract was mixed with 5 mL of chloroform and shaken for 5 min. The mixture was filtered and the filtrate was shaken with an equal volume of 100% ammonia solution. A pink, violet, or red coloration in the ammoniacal layer indicated the presence of free anthraquinones.

Test for cardiac glycosides

One hundred milligrams (100 mg) of extract were dissolved in 70% ethanol and filtered. Three drops of lead sub-acetate were added, followed by filtration. The filtrate was extracted with 10 mL of chloroform and concentrated to dryness. The residue was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride and carefully underlaid with 1 mL of concentrated sulfuric acid. A brown ring at the interface indicated the presence of deoxysugars characteristic of cardenolides.

Test for steroids

About 100 mg of extract was dissolved in 2 mL of chloroform, and concentrated sulfuric acid was carefully added to form a lower layer. A reddish-brown color at the interface indicated the presence of steroids.

Test for terpenes

A small quantity of extract was dissolved in chloroform, followed by the addition of 1 mL of acetic anhydride and two drops of concentrated sulfuric acid. A pink coloration that changed to bluish-green indicated the presence of terpenes.

Test for flavonoids

Five milliliters of dilute ammonia were added to 5 mL of extract, followed by 5 mL of concentrated sulfuric acid. The formation of a yellow color indicated the presence of flavonoids.

Test for carbohydrates

One gram (1 g) of extract was dissolved in 10 mL of distilled water and boiled with Fehling's solutions A and B. The appearance of a brick-red precipitate indicated the presence of reducing sugars.

Quantitative phytochemical analysis

Determination of total flavonoid content

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (Zainol et al., 2019). One milliliter of extract was mixed with 4 mL of methanol. Subsequently, 0.3 mL of 5% sodium nitrite was added, followed after 5 min by 0.3 mL of 10% aluminum chloride. After 6 min, 2 mL of 1 M sodium hydroxide was added, and the volume was adjusted to 10 mL with methanol. Absorbance was measured at 510 nm. Results were expressed as mg rutin equivalents (RE)/g dry weight.

Determination of total phenolic content

Total phenolics were determined using the Folin–Ciocalteu method (Hagerman et al., 2004). Extract (0.5 g) was mixed with 1 mL of 80% ethanol and centrifuged at 12,000 rpm for 15 min. The supernatant was collected and dried in a water bath, then reconstituted to 3 mL with distilled water. Two milliliters of 20% Na_2CO_3 and 0.5 mL of Folin-Ciocalteu reagent were added. After 5 min, an additional 2 mL of 20% Na_2CO_3 was added, and the mixture was heated in boiling water. Absorbance was measured at 650 nm using catechol as the standard.

Determination of total tannin content

Five hundred milligrams (500 mg) of extract were mixed with 50 mL of distilled water and shaken for 1 h. The mixture was filtered, and 5 mL of the filtrate was mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. Absorbance was measured at 120 nm (Adegbusi et al., 2022).

Determination of saponin content

Two grams (2 g) of extract were mixed with 10 mL of 20% ethanol and heated at 55 °C for 4 h with continuous stirring. The mixture was filtered and re-extracted. The combined extract was reduced to 4 mL, transferred to a separatory funnel, and partitioned with diethyl ether and n-butanol. The butanol fraction was washed with 5% NaCl, evaporated, dried, and weighed. Saponin content was expressed as a percentage.

Determination of alkaloid content

Alkaloids were determined using Harborne (1973) gravimetric method. Five grams of extract were soaked in 200 mL of 10% acetic acid in ethanol for 4 h, filtered, and concentrated. Ammonium hydroxide was added to precipitate the alkaloids, which were filtered, dried, and weighed. Results were expressed as mg/100 g dry weight.

Collection of blood samples for *in vitro* sensitivity test

Blood samples for in vitro sensitivity testing were obtained at the Federal Medical Center Jalingo, Taraba State, Nigeria, between November 2022 and October 2023. Eligible subjects were febrile children ≤15 years who had fever within the preceding 24 h, axillary temperature ≤37.5 °C, no antimalarial intake in the previous two weeks, and provided oral or written informed consent through the patient, parent, or legal guardian. Initial malaria screening was performed using finger-prick peripheral blood to prepare Giemsa-stained (10 %, pH 7.3 phosphate buffer, 10 min) thick and thin smears for microscopic detection of parasites (Olasehinde et al., 2014). Only children with mono-infection of *Plasmodium falciparum* and parasitemia between 2,000/µL and ≤80,000/µL qualified for in vitro susceptibility testing (Labaran et al., 2022).

For the in vitro assay, ten fresh *P. falciparum* isolates were obtained from confirmed symptomatic cases aged 0–15 years by collecting 2.5 mL of venous blood via standard antecubital venipuncture into EDTA tubes. Samples were centrifuged at 2,000 rpm for 10 min, plasma was discarded, and erythrocyte pellets were washed three times in RPMI-1640 medium (Gibco, USA) before culture (Mwangi, 2022). Children whose samples did not meet the inclusion criteria were subsequently

treated with Artemether-Lumefantrine (COARTEM) as prescribed by a pediatrician.

Preparation of culture medium and extract solution

The RPMI 1640 powder (Gibco), 6 g of HEPES, and 2 g of $NaHCO_3$ (Sigma Aldrich) were dissolved in 1 liter of distilled-deionized water to create the culture medium. A 0.22 µm membrane filter was then used to filter the medium. Next, 0.5 ml of gentamycin (from a 50 mg/ml stock) was added, and 45 ml aliquots were stored at 4ºC. 5 milliliters of 5% Albumax II were added to each aliquot before it was cultivated (Bankole *et al*., 2016). A 2 mg/ml solution of each was obtained by dissolving the water extracts in distilled water first and the ethanol extracts in methanol. Both extracts were then diluted in distilled deionized water. Then, 200µg/ml stock solutions were made by further diluting this 2 mg/ml solution in the malaria culture medium (Thiengsusuk et al., 2013). A final concentration range of 1.56– 100µg/ml was achieved in 96-well microtiter plates (Becton Dickinson Labware, USA) after the extracts were tested in six consecutive two-fold dilutions.

In vitro antimalarial assay

Parasite cultures were prepared in RPMI-1640 medium supplemented with Albumax II and gentamycin. Extracts were serially diluted to concentrations of 1.56–100 µg/mL. Parasite cultures were incubated at 37 °C in a candle jar for 24–30 h (James, 2019). Schizont inhibition was assessed microscopically after Giemsa staining.

The percentage inhibition per concentration was calculated using the formula (Usman *et al.,* 2022):

$$\% \text{ inhibition} = \frac{[1 - \text{ parasite density in test wells})}{\text{parasite density in control wells}} \text{ x } 100$$

The IC_{50} values, the concentration required to inhibit schizont growth by 50%, were determined by linear interpolation from the schizont growth inhibition curves (Log of concentration versus percent inhibition) generated from each parasite-extract interaction (Mustofa *et al*., 2007)

Thresholds for *in vitro* antimalarial activity

The criteria for assessing the *in vitro* antimalarial effectiveness of the plant extracts were established based on Jeruto et al. (2015). The classification is as follows: an extract with an IC_{50} below 10µg/ml is deemed very effective, one ranging from 10 to 50µg/ml is deemed moderate, and any extract over 50µg/ml is regarded as having low activity.

Statistical analysis

All experiments were conducted in triplicate. Data were analyzed using GraphPad Prism version 8.2. Differences between means were evaluated using two-way ANOVA followed by Duncan's Multiple Range Test (DMRT), with significance set at $p < 0.05$.

RESULTS

Qualitative phytochemical composition of aqueous and ethanolic extracts of *Mangifera indica* leaves and stem bark

The qualitative phytochemical profiles of the aqueous and ethanolic extracts of *Mangifera indica* leaves and stem bark are presented in Table 1. Both aqueous and ethanolic stem bark extracts showed a strong presence of alkaloids (++), whereas leaf extracts contained alkaloids at lower levels, being slightly present (+) in the aqueous extract and moderately present (++) in the ethanolic extract. Saponins were abundant (+++) in the aqueous extracts of both leaves and stem bark but were reduced to moderate levels (++) in the ethanolic extracts.

Tannins and flavonoids were detected in all extracts. Tannins showed a slight presence (+) across all solvents and plant parts, while flavonoids were moderately present (++) in aqueous extracts but reduced (+) in ethanolic extracts. Carbohydrates were absent (–) in all extracts. Phenols were highly present (+++) in aqueous extracts of both leaves and stem bark but only slightly present (+) in ethanolic extracts. Steroids and anthraquinones were moderately present (++) in the aqueous extracts but absent (–) in the ethanolic extracts. Cardiac glycosides were strongly present (+++) in the aqueous extracts and absent (–) in the ethanolic extracts of both plant parts. In contrast, terpenes showed the opposite pattern, being absent (–) in aqueous extracts but highly present (+++) in ethanolic extracts.

Extraction yields were higher for aqueous extracts (14.75% for leaves and 10.70% for stem bark) than for ethanolic extracts (8.78% for leaves and 5.20% for stem bark).

Quantitative phytochemical contents of aqueous and ethanolic extracts of *Mangifera indica*

The quantitative phytochemical composition of the extracts is shown in Table 2. Significant differences ($P \leq 0.05$) were observed among plant parts and extraction solvents. Alkaloid and saponin contents increased from leaves to stem bark and from aqueous to ethanolic extracts, with the ethanolic stem bark extract recording the highest concentrations (9.66 ± 0.27 mg/100 g and 8.48 ± 0.22 mg/100 g, respectively). The aqueous leaf

Table 1. Qualitative phytochemical contents of aqueous and ethanolic extracts of *Mangifera indica* leaves and stem bark.

Constituents	Leaves		Stem	
	Aqeuous	Ethanolic	Aqeuous	Ethanolic
Alkaloids	+	++	++	++
Saponins	+++	++	+++	++
Tannins	+	+	+	+
Flavanoids	++	+	++	+
Carbohydrates	-	-	-	-
Phenols	+++	+	+++	+
Steriods	++	-	++	-
Anthraquinones	++	-	++	-
Cardiac glycoside	+++	-	+++	-
Terpenes	-	+++	-	+++
Percentage yield	14.75	8.78	10.70	5.20

Were - = absent, + = slightly present, ++ = more present, +++ = highly present.

Table 2. Quantitative phytochemical contents of aqueous and ethanolic extracts of *Mangifera indica* leaves and stem bark.

Phytochemical (mg/100 g)	Leaves		Stem Bark	
	Aqueous	Ethanolic	Aqueous	Ethanolic
Alkaloids	2.98 ± 0.12^{d}	5.12 ± 0.20^{c}	7.85 ± 0.31^{b}	9.66 ± 0.27^{a}
Saponins	3.24 ± 0.15^{d}	5.48 ± 0.19^{c}	7.62 ± 0.28^{b}	8.48 ± 0.22^{a}
Tannins	0.43 ± 0.03^{a}	0.81 ± 0.04^{a}	0.96 ± 0.05^{a}	1.10 ± 0.06^{a}
Flavonoids	10.48 ± 0.41^{b}	11.24 ± 0.39^{a}	6.14 ± 0.33^{d}	6.86 ± 0.29^{c}
Phenols (TPC)	0.11 ± 0.01^{a}	0.21 ± 0.01^{a}	0.63 ± 0.02^{a}	0.75 ± 0.03^{a}
L.S.D	0.62			
P-Value	≤0.0001			

At P≤0.05 there was a significant difference in the quantitative phytochemical contents of aqueous and ethanolic extracts of *Mangifera indica* Leaves and Stem Bark. Values are presented as mean±standard error of means. Ranking was done across the extracts and values with the same super script are not significant.

extract contained the lowest levels of these metabolites.

Tannins and phenols occurred at relatively low concentrations in all extracts, with no statistically significant differences among the means; however, the ethanolic stem bark extract showed the highest values (1.10 ± 0.06 mg/100 g for tannins and 0.75 ± 0.03 mg/100 g for phenols). Flavonoid content displayed a distinct pattern, being significantly higher in leaves than in stem bark. The ethanolic leaf extract exhibited the highest flavonoid concentration (11.24 ± 0.39 mg/100 g), while the aqueous stem bark extract showed the lowest value (6.14 ± 0.33 mg/100 g), indicating differential distribution of metabolites within the plant.

Mean number of parasitized red blood cells following treatment with *M. indica* Extracts

The mean parasitized red blood cell counts following exposure to aqueous and ethanolic extracts of *M. indica* leaves and stem bark are presented in Table 3. All extracts produced a concentration-dependent reduction in parasitemia, with statistically significant differences observed ($P \leq 0.05$). The control group consistently recorded the highest parasitized cell count (95.76 ± 2.51), confirming the absence of antiplasmodial activity in untreated cultures.

Among the treatments, the ethanolic leaf (MILE) and stem bark (MIBE) extracts showed the most pronounced reductions in parasitized cells. At 3.13 µg/mL, MILE and MIBE recorded mean parasitized cell counts of 40.16 ± 4.36 and 50.00 ± 1.96, respectively. At 100 µg/mL, these values increased to 87.15 ± 8.21 and 85.67 ± 8.14, reflecting variations in parasite response across concentrations. Aqueous extracts of leaves (MILA) and bark (MIBA) also produced significant reductions, although their effects were generally weaker than those of the ethanolic extracts.

Combination extracts (MILA/MIBA and MILE/MIBE) produced intermediate responses, with MILE/MIBE showing a marked reduction to 19.82 ± 2.60 at the lowest tested concentration, suggesting possible synergistic

Table 3. Mean number of parasitized red blood cells using the aqueous and ethanolic extracts of *M. indica* leaves and stem bark.

Extracts	Concentration (µg/mL)						
	1.56	3.13	6.25	12.5	25	50	100
MILA	13.30±1.40^{e}	47.00±2.22^{c}	55.15±2.41^{d}	63.73±1.98^{c}	65.94±0.63^{c}	71.43±1.74^{cd}	74.72±1.71^{c}
MILE	28.16±3.71^{d}	40.16±4.36^{d}	62.35±1.85^{bc}	72.00±1.68^{b}	79.00±1.08^{b}	81.17±1.22^{b}	87.15±8.21^{b}
MIBA	48.73±1.51^{b}	58.65±1.08^{b}	65.53±8.10^{b}	70.25±8.60^{b}	77.43±9.91^{b}	82.14±8.40^{b}	87.43±7.05^{b}
MIBE	38.00±8.70^{c}	50.00±1.96^{bc}	57.25±1.56^{c}	74.93±8.33^{b}	76.94±8.40^{b}	82.17±1.16^{b}	85.67±8.14^{b}
MILA/MIBA	30.51±1.66^{d}	47.16±3.31^{c}	59.21±1.87^{c}	64.91±2.06^{c}	67.38±2.25^{c}	69.95±2.31^{d}	73.73±2.45^{c}
MILE/MIBE	19.82±2.60^{e}	53.02±1.62^{b}	57.54±1.53^{cd}	62.53±1.81^{c}	67.18±1.91^{c}	77.35±1.77^{c}	88.00±7.70^{b}
Control	95.76±2.51^{a}	95.76±2.51^{a}	95.76±2.51^{a}	95.76±2.51^{a}	95.76±2.51^{a}	95.76±2.51^{a}	95.76±2.51^{a}
L.S.D	6.21						
P-Value	≤0.0001						

Keys: **MILA** - *M. indica* leaf aqueous; **MIBA** - *M. indica* bark aqueous; **MILE** - *M. indica* leaf ethanol; **MIBE** - *M. indica* bark ethanol.
At P≤0.05 there was a significant difference in the Mean number of parasitized red blood cells at various concentrations of aqueous and ethanolic extracts of *Mangifera indica* Leaves and Stem Bark. Values are presented as mean±standard error of means. Ranking was done across the extracts and values with the same super script are not significant.

interactions.

Percentage inhibition of *Plasmodium falciparum*

The percentage inhibition of *P. falciparum* by the extracts is shown in Table 4. All extracts exhibited concentration-dependent increases in parasite inhibition, with statistically significant differences (P ≤ 0.05). The aqueous leaf–bark combination (MILA/MIBA) produced the highest overall inhibition, reaching 91.32 ± 2.59% at 100 µg/mL, indicating synergistic activity.

The aqueous leaf extract (MILA) also showed strong inhibition at concentrations ≥12.5 µg/mL. Ethanolic extracts of leaves (MILE) and stem bark (MIBE) demonstrated variable inhibition at lower concentrations but achieved comparable efficacy at higher concentrations (50–100 µg/mL). The lowest inhibition (19.84 ± 0.96%) was recorded for the ethanolic leaf–bark combination (MILE/MIBE) at 1.56 µg/mL. The control group showed no inhibition (0.00%).

Antimalarial activity and IC_{50} values

The antimalarial potency of the extracts, expressed as IC_{50} values, is presented in Table 5. All extracts showed notable antiplasmodial activity. Ethanolic extracts were the most potent, with the ethanolic stem bark extract exhibiting the lowest IC_{50} value (2.63 µg/mL), followed closely by the ethanolic leaf extract (2.93 µg/mL).

Aqueous extracts of stem bark and leaves also displayed strong activity, with IC_{50} values of 3.51 µg/mL and 4.90 µg/mL, respectively. Combination extracts showed intermediate potency, with IC_{50} values of 3.07 µg/mL for MILA/MIBA and 3.82 µg/mL for MILE/MIBE. These results indicate that *M. indica*, particularly its ethanolic extracts, possesses strong *in vitro* antimalarial activity and that combining plant parts does not produce antagonistic effects.

Table 4. Percentage inhibition (%) of *P. falciparum* by aqueous and ethanolic extracts of *M. indica* leaves and stem bark.

Extracts	Concentration (µg/mL)						
	1.56	3.13	6.25	12.5	25	50	100
MILA	51.62 ± 1.45^{b}	59.73 ± 1.62^{b}	74.83 ± 2.14^{b}	86.42 ± 2.05^{a}	87.47 ± 2.11^{a}	90.74 ± 2.33^{a}	91.85 ± 2.41^{a}
MILE	20.28 ± 1.02^{d}	46.11 ± 1.54^{d}	69.76 ± 1.97^{c}	85.16 ± 2.18^{a}	87.12 ± 2.29^{a}	88.03 ± 2.37^{a}	93.22 ± 2.60^{a}
MIBA	20.27 ± 0.97^{d}	40.14 ± 1.36^{d}	71.33 ± 2.05^{bc}	77.24 ± 2.09^{b}	82.32 ± 2.22^{b}	87.14 ± 2.34^{a}	89.45 ± 2.48^{a}
MIBE	27.24 ± 1.18^{c}	49.43 ± 1.61^{c}	52.23 ± 1.74^{d}	79.28 ± 2.15^{b}	81.01 ± 2.24^{b}	83.35 ± 2.38^{b}	90.45 ± 2.57^{a}
MILA/MIBA	57.34 ± 1.52^{a}	69.03 ± 1.87^{a}	80.41 ± 2.09^{a}	87.67 ± 2.32^{a}	88.02 ± 2.41^{a}	89.35 ± 2.48^{a}	91.32 ± 2.59^{a}
MILE/MIBE	19.84 ± 0.96^{e}	39.27 ± 1.33^{e}	75.12 ± 2.18^{b}	79.15 ± 2.24^{b}	81.59 ± 2.30^{b}	89.38 ± 2.54^{a}	90.02 ± 2.61^{a}
Control	0.00 ± 0.00^{f}	0.00 ± 0.00^{f}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}
L.S.D	5.20						
P-Value	≤0.0001						

Keys: **MILA** - *M. indica* leaf aqueous; **MIBA** - *M. indica* bark aqueous; **MILE** - *M. indica* leaf ethanol; **MIBE** - *M. indica* bark ethanol.
At P≤0.05 there was a significant difference in the Percentage Inhibition (%) of *P. falciparum* by aqueous and ethanolic extracts of *M. indica* Leaves and Stem Bark. Values are presented as mean±standard error of means. Ranking was done across the extracts and values with the same super script are not significant.

Table 5. Antimalarial activity and inhibition concentration (IC_{50}) of *M. indica*.

Extract	IC_{50}(µg/ml)	Antimalarial activity (%)
Leaf Aqueous	4.90	91.85
Bark Aqueous	3.51	89.45
Leaf Ethanol	2.93	93.22
Bark Ethanol	2.63	90.44
Leaf/Bark Aqueous	3.07	91.32
Leaf/Bark Ethanol	3.82	90.01

DISCUSSION

The qualitative phytochemical analysis of aqueous and ethanolic extracts of *Mangifera indica* leaves and stem bark revealed a broad spectrum of secondary metabolites, with clear variations between plant parts and extraction solvents. The strong presence of alkaloids in the stem bark and their moderate occurrence in the leaves are consistent with previous reports identifying alkaloids as key bioactive constituents of mango tissues (Diso et al., 2017; Mohammed et al., 2025). The higher abundance of saponins and phenolic compounds in the aqueous extracts aligns with established evidence that polar solvents preferentially extract hydrophilic compounds such as saponins, phenolics, and tannins (Anhwange et al., 2004; Olaleye and Akinmoladun, 2017). In contrast, terpenes were absent in aqueous extracts but abundant in ethanolic extracts, confirming that semi-polar solvents such as ethanol are more effective at solubilizing lipophilic secondary metabolites (Sasidharan et al., 2011). The differential distribution of flavonoids, steroids, and anthraquinones between aqueous and ethanolic extracts further highlights the role of solvent polarity in metabolite extraction. The absence of carbohydrates in all extracts is consistent with earlier phytochemical surveys of *M. indica*, in which primary metabolites are typically not detected using standard qualitative screening methods (Mohammed et al., 2025). The higher extraction yields obtained with aqueous solvents also agree with previous findings that water extracts larger quantities of polar phytochemicals than ethanol (Anhwange et al., 2004).

Quantitative phytochemical analysis further demonstrated significant variation in metabolite concentrations across plant parts and extraction solvents. Alkaloids and saponins increased progressively from leaves to stem bark and from aqueous to ethanolic extracts, with the highest levels recorded in the ethanolic stem bark extract. This pattern supports earlier reports that ethanol enhances the extraction of these compounds compared with aqueous solvents (Anhwange et al., 2004; Mohammed et al., 2025). The relatively low concentrations of tannins and phenols across all extracts, with no significant differences among them, are also consistent with previous studies showing that these metabolites occur at lower levels in *M. indica* compared with flavonoids and saponins (Diso et al., 2017). Flavonoids were more abundant in the leaves, particularly in ethanolic extracts, which aligns with evidence that these compounds accumulate preferentially in aerial tissues rather than in woody stem bark (Olaleye and Akinmoladun, 2017).

The *in vitro* antiplasmodial assays demonstrated a clear concentration-dependent reduction in parasitized red blood cells across all extracts. The untreated control consistently exhibited the highest parasitemia, confirming that parasite suppression was attributable to the plant extracts. Ethanolic leaf (MILE) and stem bark (MIBE) extracts produced the most pronounced reductions, supporting previous observations that ethanol extracts higher levels of bioactive compounds such as alkaloids, flavonoids, and terpenoids, which are associated with antiplasmodial activity (Diso et al., 2017; Mohammed et al., 2025). Although aqueous extracts of leaves (MILA) and stem bark (MIBA) also significantly reduced parasitemia, their effects were generally weaker, reflecting the lower extraction efficiency of non-polar antiplasmodial compounds in water (Anhwange et al., 2004). The intermediate or enhanced effects observed with combined extracts suggest possible synergistic interactions between phytochemicals from leaves and stem bark, a phenomenon previously reported for combined plant preparations with antiplasmodial activity (Olaleye and Akinmoladun, 2017).

The percentage inhibition results further confirmed the concentration-dependent antiplasmodial activity of *M. indica* extracts. The aqueous leaf–bark combination (MILA/MIBA) consistently produced the highest inhibition, reaching over 90% at 100 µg/mL, which supports the hypothesis of synergistic interactions between bioactive constituents from different plant parts (Olaleye and Akinmoladun, 2017; Mohammed et al., 2025). The strong activity of the aqueous leaf extract at higher concentrations is consistent with its high content of saponins, flavonoids, and phenols, which are known to disrupt parasite growth and survival (Anhwange et al., 2004; Diso et al., 2017). Ethanolic extracts showed comparatively lower inhibition at the lowest concentrations but matched the aqueous extracts at higher doses, reflecting the contribution of moderately non-polar compounds such as alkaloids and terpenoids that become more effective as concentration increases (Sasidharan et al., 2011).

The IC_{50} values provide further evidence of the potent

antiplasmodial activity of *M. indica*. Ethanolic stem bark and leaf extracts exhibited the lowest IC_{50} values (2.63 and 2.93 µg/mL, respectively), indicating strong activity at relatively low concentrations. This finding supports the view that ethanol efficiently extracts key antimalarial constituents, including alkaloids, flavonoids, and terpenoids, which are known to interfere with parasite metabolic pathways (Diso et al., 2017; Sasidharan et al., 2011). Although aqueous extracts also demonstrated substantial activity, their slightly higher IC_{50} values suggest that polar compounds such as saponins and phenolics, while active, may be less potent than the non-polar or semi-polar constituents. The intermediate IC_{50} values of the combined extracts, together with their high inhibition percentages, indicate additive or synergistic effects rather than antagonism, further supporting the therapeutic potential of combining leaf and stem bark extracts of *M. indica* for antimalarial applications.

CONCLUSION

The findings of this study demonstrate that *Mangifera indica* leaves and stem bark are rich sources of bioactive phytochemicals. Both qualitative and quantitative analyses showed that ethanolic extracts, particularly those derived from the stem bark, contained higher concentrations of alkaloids, flavonoids, and other moderately non-polar compounds, while aqueous extracts preferentially extracted polar constituents such as saponins and phenols. These phytochemical profiles were strongly associated with significant *in vitro* antiplasmodial activity, as reflected by concentration-dependent reductions in parasitized red blood cells and corresponding increases in the percentage inhibition of *Plasmodium falciparum*. Ethanolic extracts exhibited the highest efficacy, supported by their low IC_{50} values. Collectively, these results confirm the potent antimalarial potential of *M. indica* and highlight the importance of both plant part selection and extraction solvent in maximizing the recovery of bioactive compounds and therapeutic effectiveness.

DECLARATIONS

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Health Research Ethics Committee (HREC) of the Taraba State Ministry of Health (SMOH), with protocol number: TRSHREC/2023/023. All participants or their parents/legal guardians were fully informed about the study procedures, assured of data confidentiality, and provided written or oral informed consent prior to participation.

Authors' Contributions

EOK served as the principal investigator and was responsible for the study design, laboratory analyses, sample and data collection, and data interpretation. KKS and EOS supervised the study, provided technical and academic guidance during laboratory work, assisted in data organization, and critically reviewed the findings. TBL contributed to manuscript review and editing. All authors read and approved the final version of the manuscript.

Acknowledgements

The authors gratefully acknowledge the technical support provided by the Medical Laboratory staff of the Federal Medical Centre, Jalingo, Nigeria. We also thank Mr. Sunshine Ochala of the Department of Pharmacognosy, University of Jos, for his assistance with the phytochemical analyses. Our sincere appreciation is extended to Dr. Matthew Esona for his support in procuring some of the materials and culture media used in this study.

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Citation: Kolawole EO, Sha'a KK, Sambo EO, Lamidi TB, **2026**. *In vitro* antimalarial activity of the extracts of *Mangifera indica* L. against *Plasmodium falciparum* gotten from children under fifteen years attending Federal Medical Center Jalingo, Taraba State, Nigeria. Adv Med Plant Res, 14(1): 16-25.