Ceylon Journal of Science 55 (4) 2026: 1601-1618

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Phytochemical Profiling and *In Vivo* Antiplasmodium Activity of Fractionated *Theobroma cacao* Bark Extracts in *Plasmodium berghei* -Infected Mice

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Highlights

- Sequential extraction and column chromatography fractionated T. cacao bark bioactives.
- Ethanol extract at 100 mg/kg achieved 100% chemosuppression in infected mice.
- HPLC identified kaempferol (62.87%), quercetin, catechin, myricetin, naringin, and baicalin.
- GC-MS identified fatty acid esters, phenolics, and terpenoids in the extracts.
- Extract treatment improved PCV, reduced weight loss, and prolonged survival.

Ceylon Journal of Science 55 (4) 2026: 1601-1618
DOI: https://doi.org/10.4038/cjs.v55i4.9751

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Phytochemical Profiling and *In Vivo* Antiplasmodium Activity of Fractionated *Theobroma cacao* Bark Extracts in *Plasmodium berghei* Infected Mice

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Received: 20.04.2026 Accepted: 10.09.2026

Abstract

Malaria remains a major public health concern, particularly in sub-Saharan Africa, owing to the emergence and spread of antimalarial drug resistance. This study investigated the phytochemical profile and *in vivo* antimalarial activity of *Theobroma cacao* bark extracts. Dried bark was successively extracted with *n*-hexane, ethyl acetate, and ethanol. The resulting extracts were fractionated by column chromatography and analysed using gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) to characterize their chemical profiles. Antimalarial activity was evaluated in Swiss albino mice infected with *Plasmodium berghei* using Rane's test. Extracts were administered at doses of 100 and 500 mg/kg, with chloroquine (10 mg/kg) used as the positive control. Parasitaemia, percentage chemosuppression, parasite clearance time, packed cell volume (PCV), body weight, and survival time were assessed using standard procedures. The ethanol extract at 100 mg/kg demonstrated marked antimalarial activity, achieving 100% chemosuppression by Day 3 and complete parasite clearance by Day 5. The parasite clearance time differed significantly from that of the chloroquine-treated group ($P < 0.05$). However, the treatment did not result in prolonged survival compared with the control. GC–MS analysis identified fatty acid esters, phenolic compounds, and terpenoids, while HPLC profiling identified several flavonoids, with kaempferol constituting 62.87% of the extract. Treatment also improved PCV and reduced body weight loss compared with the control. These findings demonstrate the *in vivo* antimalarial activity of *T. cacao* bark extracts and their diverse phytochemical composition, supporting further investigation of their potential as sources of antimalarial compounds.

Keyword: Anti-malarial; Chemosuppression; Chloroquine; *Plasmodium berghei*; *Theobroma cacao*

INTRODUCTION

Although substantial progress has been made in elucidating antimalarial pharmacology from plant-derived metabolites, contemporary drug discovery demands systematic validation that extends beyond computational and *in vitro* approaches. While molecular docking and other *in silico* methods are valuable for identifying promising compounds and predicting their biological interactions, they do not ensure physiological efficacy or host compatibility. For example, the study by Lamiya et al. (2025) investigated the binding affinities of compounds from *Mangifera indica* stem bark to selected *Plasmodium falciparum* targets but did not assess their biological activity in living systems. Similarly, Sani and Kabir (2023) reported 97% *in vitro* parasite inhibition by *Jatropha curcas* extracts, yet acknowledged that such findings may not accurately reflect *in vivo* safety and therapeutic effectiveness. Likewise, the establishment of phytochemical standards, such as quercetin in *Ilex umbellulata* bark (Zothantluanga et al., 2025), does not provide insight into systemic tolerability or antiplasmodial efficacy in the absence of animal-based evaluation. Even in cases where potent antiplasmodial activity is demonstrated *in vitro*, such as the low IC_{50} value (0.63 µg/mL) reported for *Dacryodes edulis* extracts by (Dongmo et al., 2023), the lack of progression to appropriate

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in vivo models limits translational relevance. Collectively, these examples underscore that *in vivo* validation remains a critical and indispensable component in the development of plant-based antimalarial therapeutics.

Bioassay-guided fractionation has consistently demonstrated greater efficacy than crude extract screening in the serendipitous identification of potent antiplasmodial compounds, particularly from the stem bark of medicinal plants. This was exemplified by Abubakar et al. (2024), who reported that a sub-fraction of *Prosopis africana* stem bark achieved 91.09% chemosuppression in *Plasmodium berghei*-infected mice, comparable to chloroquine, while also improving hematological parameters. Nevertheless, reliance solely on bulk suppression data without comprehensive chemical characterization presents a significant limitation. For instance, Asanga et al. (2023) identified linoleic acid esters as putative bioactive constituents in a *Mangifera indica* formulation; however, the absence of direct experimental validation of their proposed enzyme inhibitory mechanisms was acknowledged. Similarly, Aboubakar et al. (2023) reported 71.10% *in vivo* suppression alongside hepatoprotective effects of *Terminalia macroptera* extracts, yet did not specify the phytochemical constituents responsible for these activities. These examples highlight the need to integrate detailed phytochemical analysis with biological evaluation. Furthermore, the selection of appropriate therapeutic models and fractionation strategies remains a critical consideration in advancing plant-based antimalarial research.

Alozieuwa and Ogbadoyi (2025) used the curative (Rane's) test with the leaf extract of *Carica papaya* found to be 76.82% effective against established infection, highlighting the need to evaluate the therapy against continuous and systemic parasitemia rather than using only 4 days suppressive test. Okom et al. (2025) reported that the antimalarial activity of crude ethanol extracts of *Phyllanthus amarus* was confirmed, albeit some studies employed the 4-day suppressive model without isolation of the responsible bioactive compounds, left vacuum that should be filled. In a similar study, Tolo et al. (2025) reported dose-dependent 78.36% suppression with the extracts of *Markhamia lutea*. However, bioactive substances were isolated for independent validation. The foregoing underscores the need for a thorough chemical characterization of the active fractions in conjunction with bioassay-guided fractionation of the agent, and that the curative *in vivo* model should be simultaneously utilized in order to obtain a valid evaluation result.

In this context, *Theobroma cacao* (cocoa) has been employed as a plant matrix, whose therapeutic activity against malaria has been validated. Most studies conducted on its therapeutic activities have focused on the beans, seeds, and pod husks, with very few studies conducted on the stem bark. Following the comprehensive review by Tušek et al. (2024), cocoa bean polyphenols have been recognized for their pronounced antioxidant and anti-inflammatory activities, which contribute to the mitigation of systemic cellular damage. Complementing this, Sitarek et al. (2024) demonstrated that flavanols and procyanidins exert direct inhibitory effects on pro-inflammatory cytokines implicated in key cellular signaling pathways, thereby potentially modulating the pathological outcomes of infectious processes. Beyond the beans, Febrina et al. (2024) evaluated methanolic extracts of cocoa pod husk, an agricultural by-product, using the brine shrimp lethality assay, revealing the presence of bioactive secondary metabolites with notable toxicological and pharmacological profiles. Similarly, GC–MS analysis of *Theobroma cacao* seed oil has identified n-hexadecanoic acid and γ-sitosterol as major constituents associated with antimicrobial and anti-inflammatory activities (Daniel & Akpabio, 2024).

Despite this substantial body of evidence supporting *T. cacao* as a rich source of biologically active phytochemicals, there remains a lack of systematic investigation into the stem bark, particularly involving sequential extraction, fractionation, and *in vivo* evaluation of antimalarial efficacy using rodent *Plasmodium* models. Addressing this gap is essential, as the stem bark is often enriched in phenolic and terpenoid defense compounds compared to other plant parts, and historically, it has served as a critical source of antimalarial leads in diverse medicinal plant species.

For this reason, the present study was carried out to exploit this gap by sequential extraction of *Theobroma cacao* bark using solvents of increasing polarity (n-hexane, ethyl acetate and ethanol) and subsequent fractionation of the most promising crude extracts by column chromatography. Volatile constituents, semi-volatile constituents, and flavonoid constituents of the resulting fractions and crude extracts were identified using GC-MS and HPLC. Importantly, all extracts and fractions were tested *in vivo* in the curative (Rane's) test in Swiss albino mice infected with *P. berghei,* where the time taken for the clearance of the parasite, packed cell volume (as an index of hemoprotection), body weight changes, and survival rate were measured. This study aimed to evaluate the antimalarial activity of *Theobroma cacao* bark and to establish a scientific basis for exploring this underutilized plant tissue as a potential source of novel antimalarial agents.

MATERIALS AND METHODS

Plant material

The fresh stem bark of *Theobroma cacao* was harvested from a farm in Isan Ekiti, Ekiti State, South-West, Nigeria, where the plant was naturally cultivated. The plant sample was appropriately identified and authenticated in the Herbarium of the Department of Plant Biology, University of Ilorin, Nigeria, and a voucher specimen was kept at the Herbarium of the Department of Plant Biology, UIL with voucher specimen n umber: UILH/001/1848. The bark sample was carefully washed with distilled water, dried at ambient temperature (25 ± 2°C) for a month, and finely

ground using an industrial grinder. The yield of the dry powder was 1508.75 g.

Sequential extraction

Theobroma cacao dried powdered stem bark (1508.75 g) was macerated cold in five liters each of n-hexane, ethyl acetate, and ethanol sequentially. The solvent was left for 72 hours with intermittent shaking, before being subjected to decantation and filtration after each extraction. The respective filtrates were concentrated under vacuum at 40°C in a rotary evaporator to obtain 5.03 g of hexane crude extract of bark (HCEB), 3.93 g of ethyl acetate crude extract of bark (ACEB), and 3.56 g of ethanol crude extract of bark (ECEB).

Column fractionations

Based on yield and phytochemistry content, HCEB and ECEB extracts were subjected to column chromatography fractionation on silica gel (Merck 60-120 mesh) using a solvent gradient of n-hexane, ethyl acetate, and ethanol. The elution of HCEB yielded 31 fractions of 100 mL each, which were pooled together based on the TLC profile carried out on a silica gel G plate and viewed under UV 254 and 365 nm or using iodine vapour visualization as appropriate. Based on the TLC profile, the 31 fractions were merged into nine combined fractions, of which two sub-fractions were further re-chromatographed on a silica gel column to afford a fraction with crystalline nature.

The crude ethanol extract (ECEB) subjected to silica gel column chromatography fractionation using solvents of increasing polarity afforded ninety-four (94) fractions of approximately 100 mL per fraction. These were merged into seven (7) fractions, and three fractions were re-chromatographed on the silica gel column chromatography to afford a compound mixture of reduced complexity.

Phytochemical testing (Qualitative and Quantitative)

Each qualitative and quantitative analysis was performed three times (n=3). Qualitative analysis was carried out to determine the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, tannins, phlobatannins, cardiac glycosides, steroids, and reducing sugars following standard procedure (Harborne, 1998; Ejikeme et al., 2014). The presence of the phytochemicals was rated according to their relative abundance using the notation: (+++) high abundance, (++) moderate abundance, (+) presence, (-) absence. Total alkaloids (precipitation with Dragendorff reagent), total flavonoids (aluminium chloride colorimetry), total phenol content (Folin-Ciocalteu reagent), and saponins and terpenoids (validated colorimetric procedures) were quantified.

Gas Chromatography Mass Spectrometry (GC-MS)

All three crude extracts (HCEB, ACEB, and ECEB) were analyzed for their volatile and semi-volatile chemical composition using an efficient GC-MS system. The GC-MS instrument was equipped with an HP-5 MS capillary column (30 m × 0.25 mm, 0.25 µm coating thickness). The temperature program was maintained as follows: Initial temperature 50°C (held for 2 min), then raised to 280°C with a heating rate of 10°C/min, and then finally held at 280°C for 10 min. Helium was used as the carrier gas, flowing steadily at the rate of 1.0 mL/min. The components eluted from the system were identified based on careful evaluation of their mass fragmentation patterns and matching with references available in the NIST/WILEY library.

High-Performance Liquid Chromatography (HPLC) analysis

The samples selected for the HPLC profiling of specific flavonoids were the crude ethanol extract (ECEB) and Fraction E, obtained from the ECEB extract, which were based on the therapeutic performance obtained in the *in vivo* study. The composition of these extracts and fractions was determined by HPLC analysis, and specific peaks were identified by matching them with the peaks of authentic samples using their absorption on a UV detector. The chromatographic separation was done on a reversed-phase C18 column of dimensions 250 mm × 4.6 mm, with particles having sizes of 5 µm. The composition of the mobile phases were solvent A which had water mixed with 0.1% formic acid and solvent B that contained acetonitrile with 0.1% formic acid in varying percentages: from 0–5 minutes, 10% B; from 5–20 minutes, 10–40% B; from 20–25 minutes, 40-90% B; and from 25–30 minutes, 90% B using a flow rate 1.0 mL/min, with detection wavelengths of 280 nm for **flavonoids and 360 nm for flavonols.**

***In vivo* Study**

Healthy male Swiss albino mice (weighing between 18 - 25g and 6-8 weeks old) were procured from the Animal House of the College of Medicine, University of Ibadan, Nigeria. After this, the mice were acclimatized for two weeks at the lab with controlled environmental conditions (temperature of 25 ± 2oC, relative humidity of 70 - 80%, and regular light/dark cycles). They were caged under good ventilation with clean plastic cages, new plastic cages with fresh wood shavings, and unobstructed access to commercial standard wood pellets (Ladokun Feeds Nigeria Ltd) and drinking water.

Ethics

All experiments performed were planned with adherence

to the guidelines of the National Institutes of Health (NIH) Guidelines for Laboratory Animals and performed in accordance with these guidelines. The Animal Use Ethical Committee of Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria reviewed and approved this research project (protocol number: CSREC/P-01/26). All possible steps were taken to avoid causing pain to animals and to minimize the number of animals used in this experiment.

Inoculation of malaria parasites

The *P. berghei* NK65 strain used for injection was a chloroquine-sensitive type collected from the MR4 Center. The maintenance and propagation of the parasite were carried out through serial passages performed weekly, whereby infected donor mice were utilized. At the start of the experiment, the donor mice with 30% parasitaemia were first euthanized using mild anesthesia. Blood collection was performed using the method of cardiac puncture, which was collected into heparinized syringes and thereafter diluted in normal saline solution. The infected red blood cells in an amount of $1x10^7$ per 0.2 mL were administered to the experimental mice using the intraperitoneal (i.p.) route of administration.

Experimental procedure

For the antimalarial activity evaluation of the drugs, a randomized block experimental design was conducted (Table 1). There were 40 mice involved in the experiments, and they were divided into eight groups (n=5). The mice were divided into the different treatment groups based on a computer-generated randomization list. The researcher analyzing the parasitemia, among other factors, was blinded to the treatment group information (the identity of the treatment group was revealed only after all analyses were completed).

***In Vivo* curative test of antimalarial agents (Rane's Test)**

The antimalarial curative (Rane's test) was carried out as described by Fidock et al. (2004) and Peters (1965). All the mice were inoculated with *P. berghei* on Day 0. The blood smear was taken from the tail vein of the mice, and the parasitemia was determined on Day 10 post-infection. For strains with malaria infection signs (30-60%), the treatment was done once daily for four days (days 10- 13) with the extract at different concentrations and chloroquine and/or vehicle (normal saline). 100 or 500 mg/kg of body weight extracts, 10 mg/kg of chloroquine, and normal saline were used.

The dose of 100mg/kg was selected to represent a low to moderate dose range found in bioassay-guided antimalarial studies, while that of 500 mg/kg represents a dose range that does not exceed the acute toxicity limits observed from similar plant extract studies (Alozieuwa & Ogbadoyi, 2025; Okom et al., 2025).

Table 1: Experimental groups, treatment doses and administration schedules used to evaluate the antimalarial activity of *Theobroma cacao* bark extracts

Group	Mice/ group (n)	Dose (mg/kg/day)	Route of Administration	Treatment Regimen
Group 1 Negative Control	5	0.2 mL/day	Oral	Infected + Vehicle (Normal Saline)
Group 2 Positive Control	5	10 mg/kg/day	Oral	Infected + Chloroquine
Group 3	5	100 mg/kg/day	Oral	Infected + n-Hexane Extract (HCEB)
Group 4	5	500 mg/kg/day	Oral	Infected + n-Hexane Extract (HCEB)
Group 5	5	100 mg/kg/day	Oral	Infected + n-Hexane Extract (ACEB)
Group 6	5	500 mg/kg/day	Oral	Infected + n-Hexane Extract (ACEB)
Group 7	5	100 mg/kg/day	Oral	Infected + Ethanol Extract (ECEB)
Group 8	5	500 mg/kg/day	Oral	Infected + Ethanol Extract (ECEB)

The completely randomized experimental design was employed in this study with five mice in each group (n = 5). All experiments in this study were repeated three times (independently repeated three times), and results from the replicates were combined. The negative and positive controls included: (i) Infected-untreated, (ii) Infected + Chloroquine.

Assessment of parasitemia and other physiological parameters

Testing of parasitemia and parasite clearance time

Blood smears were made from each mouse belonging to each group in the tail vein as thin blood films on designated days as follows: day 10 (before treatment, considered as baseline), day 11, 12, 13, 17, and 23 post-infection. Slides were left to dry in the air, soaked in absolute methanol for 15 minutes, and then stained with fresh Giemsa solution in phosphate buffer (pH 7.2) for 15 minutes. The stained blood films were examined under a light microscope using 100x magnification oil-immersion objective lens. Two blood films per mouse per group were blinded and analyzed for percentage parasitemia and percentage inhibition according to the formulas below:

$$\text{Percentage Parasitemia} = \left(\frac{\text{Number of parasitized RBCs}}{\text{Total number of RBCs counted}}\right) \text{x } 100$$

$$\% \text{ Inhibition} = \left(\frac{Pc - Pt}{Pc}\right) \text{x } 100$$

Where:

P_c = Mean parasitemia of the negative control group.

P_t = Mean parasitemia of the extract/drug-treated test group.

PCT, or Parasite Clearance Time, refers to the total number of days it took to achieve parasite clearance (0% parasitemia). The mean survival time was estimated at 28 days post-infection.

Measurement of Packed Cell Volume (PCV) and Body Weight

To ascertain the efficacy of the extracts against the two conditions associated with malaria, namely hemolysis and cachexia, the measurement of packed cell volume and body weight was done thus:

Measurement of Packed Cell Volume (PCV)

Blood samples were collected from the tail veins of the mice using heparinized capillary tubes, and the amount of blood in each capillary tube was filled to three-fourths and then spun using a Hawksley microhematocrit centrifuge at 11,000 rpm for five minutes. The result was analyzed for PCV before treatment (on day 10) and after treatment (on day 14).

Blood weight measurement

Body weight of each mouse was measured using an electronic digital balance before treatment (on day 10) and after treatment (on day 14).

Statistical analysis

Quantitative data are presented as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism version 9.0. Differences among treatment groups in percentage parasitaemia, body weight, and packed cell volume (PCV) were analysed using one-way analysis of variance (ANOVA), followed by an appropriate multiple-comparisons post hoc test. Survival was expressed as the percentage of mice surviving on Days 4, 7 and 14, and mean survival time (MST) was compared among treatment groups using one-way ANOVA followed by Tukey's multiple-comparisons post hoc test. A P value < 0.05 was considered statistically significant. Phytochemical analyses were performed in triplicate (n = 3). For the *in vivo* experiments, five mice were included in each treatment group (n = 5 per group), and the experiment was independently replicated three times.

RESULTS AND DISCUSSION

Phytochemical composition of *Theobroma cacao* bark extracts

Phytochemical screening of cocoa bark extracts revealed a substantial presence of secondary metabolites (Tables 2 and 3) with potential antimalarial properties. Notably, there is a clear relationship between composition, concentration, and solvent polarity. In particular, ethanol extraction produced a crude extract rich in alkaloids and phenols (++), compounds widely recognized for their antimalarial activity. Very high concentrations of phenols (42.20 mg/100g in ECEB) are shown to play an important role because of their role in the destruction of the membrane of parasites and inhibition of hemozoin biosynthesis. All the extracts contained terpenoids, flavonoids, and tannins, which suggests that terpenoids are involved in the anti-malarial activity through the inhibition of mitochondrial metabolism, flavonoids are involved in the anti-malarial activity through the biosynthesis of fatty acids, and tannins are involved in the anti-malarial activity through ion trapping. All the extracts were, however, found to contain terpenoids, flavonoids, and tannins, suggesting possible involvement in one or more of the pathways such as mitochondrial metabolism, fatty acid biosynthesis, and ion sequestration. There are no phlobatannins detected in the extracts obtained by ethyl acetate (ACEB) and ethanol (EFCB) extractions, which suggests that these are not likely the compounds that demonstrate the antimalarial activity of these two samples.

Table 2: Phytochemical constituents and concentrations of secondary metabolites in *Theobroma cacao* bark extracts and fractions

Sample	Alkaloid	Tannin	Phlobatannin	Saponnin	Terpenoid	Cardiac Glycosides	Steroid	Reducing Sugar	Flavonoid	Phenol
HCEB	+	+	+	+	+	+	+	+	+	+
ECEB	++	+	+	++	+	+	+	+	+	++
ACEB	+	+	-	+	+	+	+	+	+	+
EFCB (60-83)	+	+	-	+	+	+	+	+	+	+

Index of scoring: (+++) highly prevalent, (++) fairly prevalent, (+) prevalent, (–) not prevalent. HCEB = Crude Hexane Extract of Bark; ECEB = Crude Ethanol Extract of Bark; ACEB = Crude Ethyl Acetate Extract of Bark; EFCB = Column Fractions of Ethanol (60-83).

Table 3: Quantification of secondary metabolites (mg/100 g) in crude extracts and fractions of *Theobroma cacao* bark

Code	Alkaloid	Tannin	Phlobatannin	Saponin	Terpenoid	Cardiac Glycosides	Steroid	Reducing Sugar	Flavonoid	Phenol
HCEB	55.53±0.17	38.67±0.09	25.33±0.09	38.56±0.02	35.85±0.05	30.42±0.13	25.85±0.05	20.40±0.12	35.31±0.06	30.49±0.17
ECEB	53.53±0.03	40.80±0.08	25.46±0.09	30.60±0.03	35.23±0.02	38.41±0.18	30.16±0.03	25.40±0.00	30.22±0.03	42.22±0.02
ACEB	44.87±0.36	30.31±0.04	-	30.44±0.02	25.77±0.03	32.45±0.25	22.33±0.02	27.43±0.01	35.44±0.07	30.45±0.19
EFCB	50.29±0.08	25.25±0.03	-	40.44±0.03	32.81±0.01	39.46±0.22	30.33±0.02	26.41±0.01	35.44±0.07	30.49±0.12

Values obtained from triplicate analysis (n = 3). Alkaloids estimated through Dragendorff's test; flavonoids estimated through aluminium chloride colorimetry; total phenols estimated using Folin-Ciocalteu reagent. (–) = Below

GC-MS Results

The main chemical compounds identified in the n-hexane extract include Linoleic acid, Palmitic acid methyl ester, Ethyl linoleate, and ethyl palmitate, mostly fatty acid esters (Table 4). However, the amount of each compound detected varies: linoleic acid at 29.08% and ethyl linoleate at 17.86%. Though fatty acid esters are not commonly known for their antimalarial properties, there are fatty acid derivatives and fatty acids that show the capability to incorporate themselves in Plasmodium's lipid bilayer membrane and change the lipid structure to make it more permeable to other antimalarials. On the other hand, the results from the GC-MS for the ethyl acetate extract indicate compounds such as Guerbet hexadecanol, 2-octyldodecanol, palmitic acid methyl ester, and methyl henicosanoate, among others (Table 5). Several compounds indicate a lipophilic nature of bioactivity, thus enhancing bioavailability. In all cases, the most polar solvent, ethanol, contains compounds that are important for antimalarial activity (Table 6). Methoxyphenols occur frequently in antimalarial compounds since they inhibit electron transport and redox activity in parasites.

Result of HPLC fingerprinting analysis

The identification of potent antimalarial flavonoids (Figure 1) from the ethanol fraction CB (EFCB) using HPLC fingerprinting provides evidence of the antimalarial activity of the bark. The presence of an assembly of flavonoids having antimalarial activity in the HPLC spectrum of EFCB can be seen. Kaempferol stands out among the various components of the extract, accounting for as much as 62.87% of all the peaks with UV absorption. In previous studies, kaempferol was found to possess antimalarial properties based on its ability to inhibit the fatty acid biosynthesis pathway in the malaria parasite as well as induce oxidative stress. Kaempferol, however, was not individually isolated for testing in the current experiment, and its biological properties cannot therefore be attributed to it alone.

Other flavonoids identified include quercetin (6.11%), catechin (6.78%), myricetin (7.66%), naringin (9.55%) and baicalin (7.03%) (Figure 1). The simultaneous presence of the flavonoids having proven antimalarial activity in one sample is strong evidence of their potential ability to exert such activity. Flavonoids are capable of exerting their activity in a synergistic manner, with potent effects being observed in many cases. They could target different stages of the life cycle of Plasmodium or multiple pathways important for its survival. The target pathways could include the maintenance of the integrity of the cell membrane, mitochondrial functioning, redox balance, and hemozoin production. The peaks correspond to different UV absorption profiles recorded at the various retention times, thus supporting the preponderance of antiplasmodial flavonoids, namely, kaempferol (62.87%), naringin (9.55%), myricetin (7.66%), baicalin (7.03%), catechin (6.78%), and quercetin (6.11%).

***In vivo* Antimalarial Potency of *Theobroma cacao* Bark Extracts: Chemosuppression and Parasite Eradication**

Using the murine model described in Table 7, the potential of the tested cocoa bark extracts to cure infections in malaria parasites was assessed. It was found that the control group of untreated malaria-infected mice presented an unchecked increase in parasitaemia to 62.92% at Day 3, which signifies a severe infection. The extract of the ethanol fraction (ECEB) at the lower dose concentration of 100 mg/kg showed excellent antimalarial efficacy, with 100% chemosuppression at Day 3 and parasite clearance (0.00 ± 0.00%) until Day 5.

Table 4: Chemical profiles of the n-hexane extract of *Theobroma cacao* bark (HCEB)

S/N	Compound Name	Molecular Formula	% Composition
1.	Linoleic acid	$C_{18}H_{32}O_2$	29.08
2.	Methyl palmitate	$C_{17}H_{34}O_2$	23.48
3.	Ethyl linoleate	$C_{20}H_{36}O_2$	17.86
4.	Ethyl palmitate	$C_{18}H_{36}O_2$	15.06
5.	Methyl stearate	$C_{19}H_{38}O_2$	2.80
6.	Ethyl 9-octadecenoate	$C_{20}H_{38}O_2$	2.79
7.	Methyl (9Z)-12-hydroxy-9-octadecenoate	$C_{19}H_{36}O_3$	3.67
8.	Methyl margarate	$C_{18}H_{36}O_2$	4.57
9.	Methyl (10E)-10-nonadecenoate	$C_{20}H_{38}O_2$	1.46
10.	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	0.84
11.	Ethyl heptadecanoate	$C_{19}H_{38}O_2$	0.46
12.	Methyl pentadecanoate	$C_{16}H_{32}O_2$	0.27
13.	Methyl myristate	$C_{15}H_{30}O_2$	0.25

Table 5: Chemical profile of ethyl acetate extract (ACEB) of *Theobroma cacao*

S/N	Compounds Name	Molecular Formula	% Composition
1.	Guerbet hexadecanol	$C_{16}H_{34}O$	11.87
2.	2-Octyldodecanol	$C_{20}H_{42}O$	10.98
3.	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	10.95
4.	Methyl henicosanoate	$C_{22}H_{44}O_2$	10.28
5.	Methyl behenate	$C_{23}H_{46}O_2$	8.76
6.	Ethyl docosanoate	$C_{24}H_{48}O_2$	7.59
7.	n-Hexadec-1-ene	$C_{16}H_{32}$	7.16
8.	9,12-Octadecenoic acid, methyl ester	$C_{19}H_{34}O_2$	7.11
9.	Ethyl n-heptadecanoate	$C_{19}H_{38}O_2$	5.69
10.	(9Z)-9-Tetradecenal	$C_{14}H_{26}O$	5.84
11.	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	5.77
12.	Caprylone	Caprylone	4.41
13.	Octadecyl aldehyde	$C_{18}H_{36}O$	3.59

Table 6: Chemical profiles of ethanol bark extract (ECEB) of *Theobroma cacao*

S/N	Compound's Name	Molecular Formula	% Composition
1.	Butyl oxitol	$C_6H_{14}O_2$	20.44
2.	Nitroisobutylglycerol	$C_4H_9NO_5$	12.40
3.	3,4,5-Trimethoxyphenol	$C_9H_{12}O_4$	11.53
4.	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	10.40
5.	Palmitic acid, ethyl ester	$C_{18}H_{36}O_2$	9.07
6.	3,4-Altrosan	$C_6H_{10}O_5$	8.96
7.	Sambucinol	$C_{15}H_{22}O_4$	6.70
8.	Linoleic acid	$C_{18}H_{32}O_2$	6.51
9.	3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	$C_6H_8O_4$	3.89
10.	Emery oleic acid ester	$C_{19}H_{36}O_2$	1.84
11.	2-Ethyl-1-dodecanol	$C_{14}H_{30}O$	1.78
12.	Ethyl 9-heptadecenoate	$C_{19}H_{36}O_2$	1.65
13.	Guerbet hexadecanol	$C_{16}H_{34}O$	1.64
14.	Palmitaldehyde	$C_{16}H_{32}O$	1.61
15.	2,3,7-Trimethyloctane	$C_{11}H_{24}$	1.58

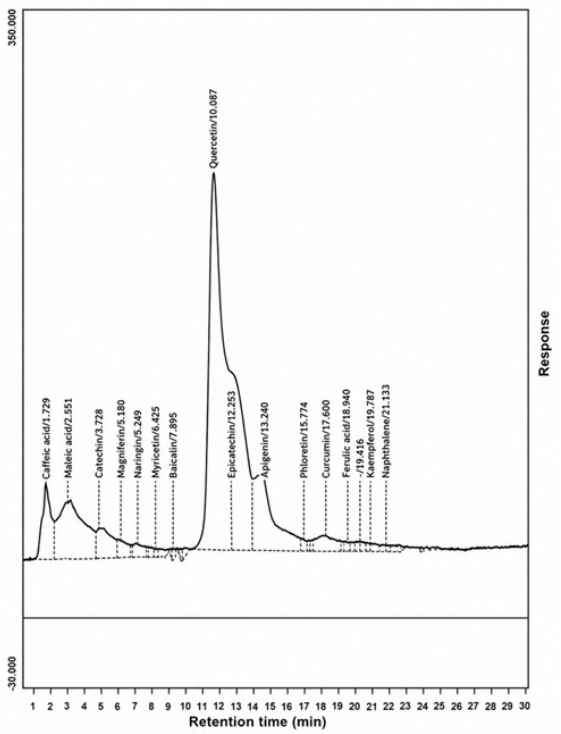

Figure 1: HPLC fingerprint profile of bioactive ethanol fraction (EFCB) of *Theobroma cacao* bark extract. X-axis: Retention Time (min); Y-axis: Absorbance (mAU) at 280 nm

However, chemosuppression for the antimalarial property was 100%, while chloroquine (CQ) had chemosuppression of 95.81% at the dose level of 10 mg/kg (Figure 2, Table 7). In terms of the parasite clearance time, which was 3.00 ± 0.01 days for ECEB (100 mg/kg), it was shorter than that of CQ (4.00 ± 0.01 days) for the initial treatment (Figure 3, Table 8). But all mice administered ECEB died after 14 days, with an average survival time of 12.00 ± 0.01 days, while those mice given CQ survived for 28.00 ± 0.01 days at 14 days with 80% survival. Thus, it is not possible to claim any superiority

On the other hand, the efficacy of ECEB at 500 mg/kg was relatively low (only 67.93% parasite suppression at Day 7), whereas the parasite suppression rate at Day 5 was 78.36%. This implies that the antiplasmodial activity of this fraction depends on the dose, where high doses lead to increased efficacy, possibly via stimulation of the immune response of the host.

Parasites in other extracts showed good chemosuppression rates on Day 5: the ethyl acetate extract (ACEB) at 100 mg/kg and 500 mg/kg had 88.25% and 90.96%, respectively, and the hexane extract (HEEB) showed its maximum efficacy at 500 mg/kg with 81.07% suppression (Table 7).

Table 7: Effects of *Theobroma cacao* bark extracts at 100 and 500 mg/kg on parasitemia and percentage chemosuppression in *Plasmodium berghei*-infected mice via curative (Rane's) test

Treatment Groups	Day 1 Average Parasitaemia	% Suppression	Day 2 Average Parasitaemia	% Suppression	Day 3 Average Parasitaemia	% Suppression
Infected-untreated	3.97± 1.86	NCS	62.92±21.69	NCS	35.91 ± 0.01	NCS
ECEB (100 mg/kg)	11.60±5.46	NCS	0.00± 0.00	100*	0.00 ± 0.00	100*
ECEB (500 mg/kg)	1.06±0.44	73.30*	7.35 ± 2.62	67.93*	7.11 ± 0.80	78.36*
HCEB (100 mg/kg)	2.34 ± 1.31	41.06*	23.95 ± 1.40	NCS	20.13 ± 8.63	38.74*
HCEB (500 mg/kg)	2.74 ± 0.60	30.98*	89.54 ± 8.44	NCS	6.22 ± 0.02	81.07*
ACEB (100 mg/kg)	5.50 ± 3.59	NCS	83.76 ± 9.87	NCS	3.86 ± 0.92	88.25*
ACEB(500 mg/kg)	5.21 ± 2.19	NCS	49.25±18.45	NCS	2.97 ± 1.58	90.96*
CQ (10 mg/kg)	2.67±0.76	32.75*	0.96±0.38	95.81*	0.00	100*

Results are expressed as Mean ± SEM (n = 5). Analysis was performed via one-way ANOVA with post hoc Tukey's test.* $p < 0.05$ compared to the control-infected group. NCS = no chemosuppression. ECEB = crude ethanol extract; HCEB = crude hexane extract; ACEB = crude ethyl acetate extract; CQ = chloroquine.

Table 8: Mean Parasite Clearance Time (PCT) and recrudescence interval of mice infected with *Plasmodium berghei* orally treated with *T. cacao* bark extracts (Rane's curative test)

Treatment groups	Parasite clearance time (days)	Recrudescence time (days)
Infected-untreated	NPC	NPC
ECEB (100 mg/kg)	3.00±0.01*	-
ECEB (500 mg/kg)	NPC	NPC
HCEB (100 mg/kg)	NPC	NPC
HCEB (500 mg/kg)	NPC	NPC
ACEB (100 mg/kg)	NPC	NPC
ACEB (500 mg/kg)	NPC	NPC
CQ (10 mg/kg)	4.00 ± 0.01*	0

Values represent Mean ± SEM (n = 5). One-way ANOVA followed by Tukey's post-hoc test. *p < 0.05 compared with infected and untreated control. NPC = no parasite clearance**.**

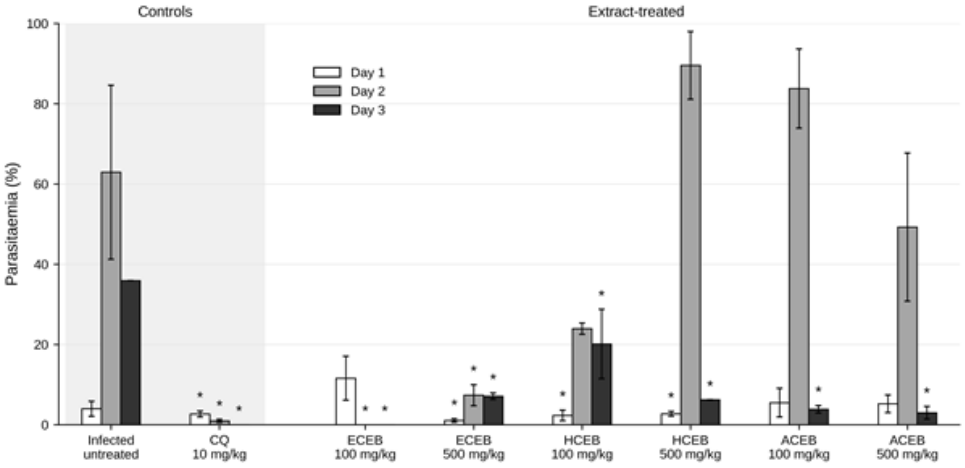

Figure 2: Assessment of *in vivo* blood-stage antiplasmodial activity of *T. cacao* bark extracts. Data are mean parasitaemia (%) ± SEM (n = 5) on Days 1, 2 and 3 of treatment. *p < 0.05 compared with the infected untreated control (one-way ANOVA followed by Tukey's post hoc test); bars without * showed no significant chemosuppression (NCS). ECEB, (Crude Ethanol Extracts of Barks); HCEB, (Crude Hexane Extracts of Barks); ACEB, (Crude Ethyl Acetate Extracts of Barks); CQ, chloroquine

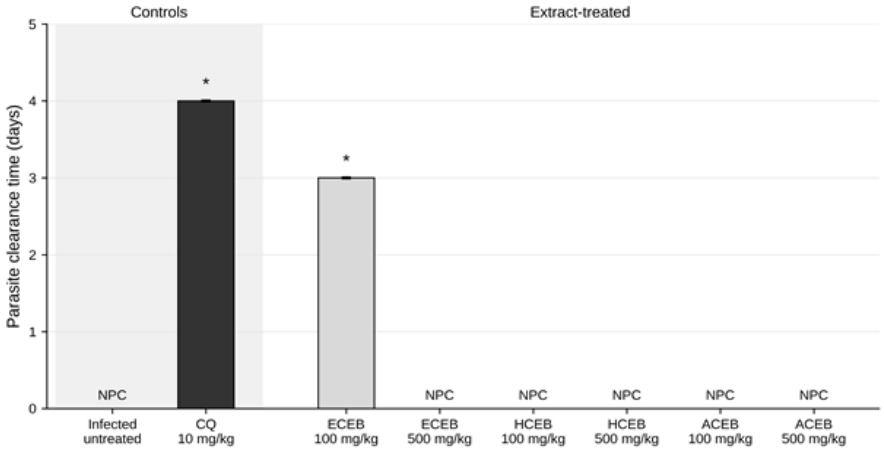

Figure 3: Parasite clearance time (days) in *P. berghei*-infected mice treated with *T. cacao* bark extracts. Data are mean ± SEM (n = 5). *p < 0.05 compared with the infected untreated control (one-way ANOVA followed by Tukey's post hoc test). NPC, no parasite clearance. ECEB, (Crude Ethanol Extracts of Barks); HCEB, (Crude Hexane Extracts of Barks); ACEB, , (Crude Ethyl Acetate Extracts of Barks); CQ, chloroquine

Table 9: Protective efficiency of *Theobroma cacao* bark extracts (Hexane, Ethyl Acetate, and Ethanol)

	Weights (g)		
Treatment	**Day 0**	**Day 4 (%)**	**Weight Difference**
Infected-untreated	22.40±1.40	19.33±0.84	13.71
ECEB (100 mg/kg)	20.00±0.89	16.00±0.82	20.00*
ECEB (500 mg/kg)	21.60±0.75	17.00±2.53	21.30*
HCEB (100 mg/kg)	23.00±1.52	16.00±0.01	30.43*
HCEB (500 mg/kg)	21.20±1.59	16.75±1.70	20.99*
AECB (100 mg/kg)	24.00±0.55	17.75±1.49	26.04*
AECB (500 mg/kg)	23.60±1.63	20.25±0.01	14.19*
CQ (10 mg/kg)	23.80±0.86	19.25±1.03	15.57*

Values represent Mean ± SEM (n = 5). ANOVA one-way test with post-hoc Tukey's test was done. $p < 0.05$ than infected-control group

Table 10: Hemoprotective action of the *T. cacao* bark extracts with changes in PCV of mice infected with *P. berghei* (Rane's curative test)

	PCV (%)	PCV (%)	
Treatment Groups	**Day 0**	**Day 4**	**PCV Difference (%)**
Infected-untreated	58.40±6.15	45.67±9.28	21.80
ECEB (100 mg/kg)	58.60±2.25	50.00±1.23	14.68*
ECEB (500 mg/kg)	56.60±4.48	50.00±1.53	11.68*
HCEB (100 mg/kg)	53.60±3.75	41.00±0.01	23.51*
HCEB (500 mg/kg)	52.40±3.54	46.75±2.98	10.78*
AECB (100 mg/kg)	52.40±1.50	45.50±2.22	13.17*
AECB (500 mg/kg)	55.40±4.67	47.50±2.63	14.26*
CQ (10 mg/kg)	50.00±4.72	53.25±1.80	6.50*

Values represent Mean ± SEM (n = 5). Two-way ANOVA followed by Tukey's test. $p < 0.05$ compared with infected and untreated control

Table 11: Mice survival and mean survival time (MST) of *P. berghei*-infected mice orally treated with *T. cacao* bark extracts

Treatment Groups	Day 4	Day 7	Day 14	Mean Survival Time (Days)
Infected-untreated	100	80	0	10.00 ± 0.01
ECEB (100 mg/kg)	100	100	0	12.00 ± 0.01*
ECEB (500 mg/kg)	100	100	0	12.00 ± 0.01*
HCEB (100 mg/kg)	100	100	0	8.00 ± 0.01*
HCEB (500 mg/kg)	100	100	0	12.00 ± 0.01*
AECB (100 mg/kg)	100	100	0	12.00 ± 0.01*
AECB (500 mg/kg)	100	100	0	12.00 ± 0.01*
CQ (10 mg/kg)	100	100	80	28.00 ± 0.01*

Values represent Mean ± SEM (n = 5). One-way ANOVA followed by Tukey's post-hoc test. $p < 0.05$ compared with infected and untreated control

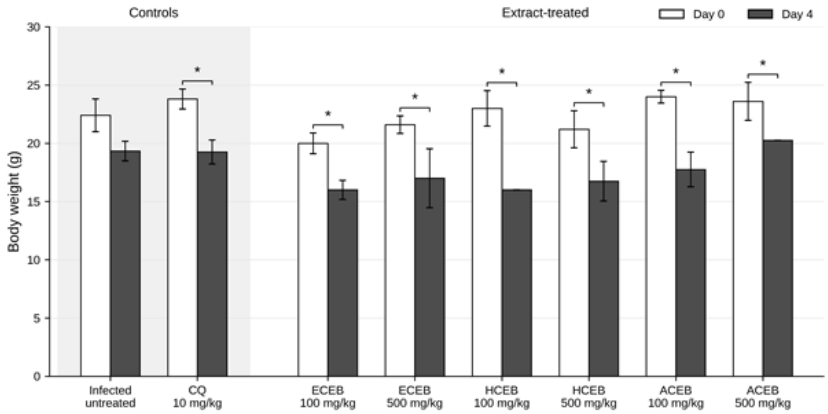

Figure 4: Body weight (g) of *P. berghei*-infected mice on Day 0 and Day 4 of treatment with *T. cacao* bark extracts. Data are mean ± SEM (n = 5). *p < 0.05 for the change in body weight between Day 0 and Day 4 (one-way ANOVA followed by Tukey's post hoc test). ECEB, (Crude Ethanol Extracts of Barks); HCEB, (Crude Hexane Extracts of Barks); ACEB, , (Crude Ethyl Acetate Extracts of Barks); CQ, chloroquine

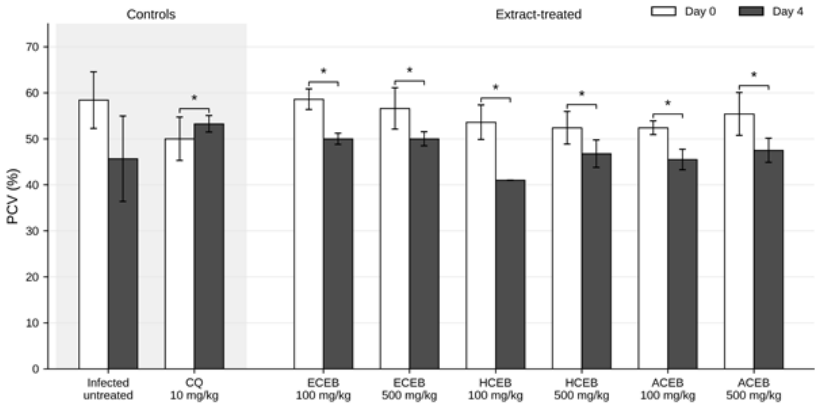

Figure 5: Packed cell volume (PCV, %) of *P. berghei*-infected mice on Day 0 and Day 4 of treatment with *T. cacao* bark extracts. Data are mean ± SEM (n = 5). *p < 0.05 for the change in PCV between Day 0 and Day 4 (one-way ANOVA followed by Tukey's post hoc test). ECEB, (Crude Ethanol Extracts of Barks); HCEB, (Crude Hexane Extracts of Barks); ACEB, (Crude Ethyl Acetate Extracts of Barks); CQ, chloroquine

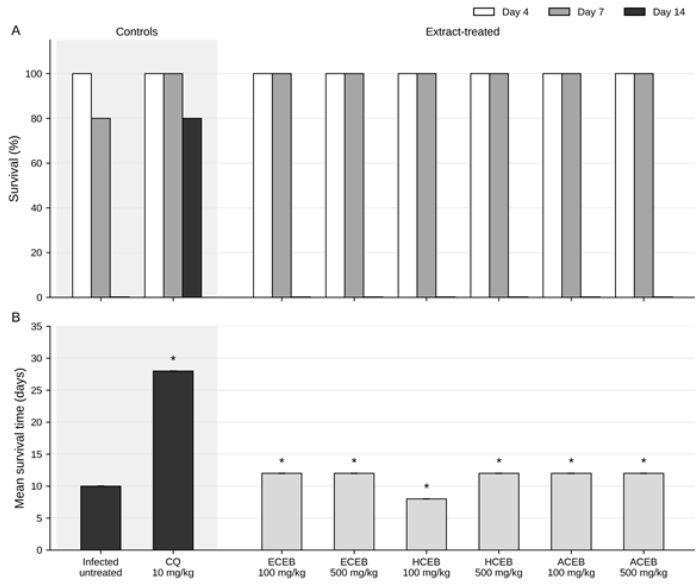

Figure 6: Survival of *P. berghei*-infected mice treated with *T. cacao* bark extracts. (A) Percentage survival on Days 4, 7 and 14. (B) Mean survival time (days), mean ± SEM (n = 5). *p < 0.05 compared with the infected untreated control (one-way ANOVA followed by Tukey's post hoc test).). ECEB, (Crude Ethanol Extracts of Barks); HCEB, (Crude Hexane Extracts of Barks); ACEB, (Crude Ethyl Acetate Extracts of Barks); CQ, chloroquine

Thus, the relative antiplasmodial activities of extracts in decreasing order were: CQ > ECEB 100 > ACEB 500 > ACEB 100 > HCEB 500 > ECEB 500 > HCEB 100. In conclusion, the ethanol extract at 100 mg/kg is most effective, although most extracts have considerable potency based on the varied polarities.

Hematological and physiological parameters

The effect of the hemolytic nature of malaria can be shown from the infected group that did not receive any treatment, which displayed a 21.80% decrease in PCV. All the extracts were able to show significant results ($p < 0.05$) in reducing hemolytic anemia induced by malaria when compared to the control group (Figure 5, Table 10). HECB (500 mg/kg) proved to be more effective in reducing PCV to 10.78%, followed by ECEB (500 mg/kg), which managed to reduce PCV to 11.66%. This shows the importance of the non-polar fractions in helping prevent membrane destruction or reducing the parasite load due to sequestration of the parasites along with the polar fractions in their antimalarial activities. Weight loss was observed among the infected mice due to cachexia induced by malaria. Though none of the extracts could prevent weight loss, ACEB (500 mg/kg) caused minimum weight loss, 14.19%, compared to the CQ group, which had 15.57% weight loss (Figure 4, Table 9). This suggests an improved tolerance, possibly accompanied by reduced metabolic stress, which could be attributed to the anti-inflammatory nature of the compounds contained in ACEB. Although none of the extracts matched chloroquine (CQ) in sustaining long-term survival, all treatment groups except HECB at 100 mg/kg produced a statistically significant increase in mean survival time (MST) compared to the control. MST rose from 10.00 days in untreated control mice to 12.00 days (Figure 6, Table 11).

DISCUSSION

The in vivo results correspond to the chemical data and confirm high antimalarial activity of the ethanol extract due to the combined effect of high amounts of flavonoids, mainly kaempferol (62.87%). Parasite elimination within 3 days is significantly faster than with chloroquine treatment (4 days) during the first stage. This observation is supported by previous research on Prosopis africana, with 91.09% inhibition (Abubakar et al., 2024), and *Carica papaya,* with 76.82% inhibition (Alozieuwa & Ogbadoyi, 2025). Nevertheless, the present study has managed to achieve 100% chemosuppression at a smaller dose and with more rapid parasite clearance compared to the cited literature.

Noteworthy is the fact that kaempferol, quercetin, catechin, myricetin, naringin, and baicalin have been detected in the ethanol extract. The mentioned substances inhibit the synthesis of fatty acids and provoke oxidative stress in *Plasmodium* organisms (Asanga et al., 2023; Okom et al., 2025). The ethyl acetate extract had a moderately high chemosuppression rate (up to 90.96%) and weight maintenance, which might be related to the detected fatty acids and esters in the sample, presumably due to its anti-inflammatory properties. The hexane extract demonstrated a hemoprotective effect due to the lipophilicity of the main constituents (linoleic acid, ethyl linoleate) that preserve red blood cell integrity.

Chemosuppression at 100% by the ethanol extract at 100 mg/kg dose is significantly higher than that of various traditional antimalarial plants: *Terminalia macroptera* (71.10%), *Feretia apodanthera* (68.45%), *Erythrina sigmoidea* (77.01%), and the polyherbal extract (84.4%) (Aboubakar et al., 2023; Boni et al., 2025; Jemimah Sandra et al., 2024; Odediran et al., 2025).

The novelty of the study includes the first bioassay-guided fractionation and complete HPLC-based investigation of T. cacao bark. Previously, only cocoa pods, leaves, or beans were studied (Febrina et al., 2024; Komlaga et al., 2021; Tušek et al., 2024). Thus, it was shown that the bark of *T. cacao* is an abundant source of antiplasmodial flavonoids; furthermore, chemosuppression within the first days is superior to that of most known plants from Western Africa.

However, one of the crucial limitations of the study is the poor long-term survival rate, as all extract-treated mice were dead by the end of day 14, whereas the chloroquine group had a mean survival period of 28 days. It suggests that parasite recrudescence and absence of sustained cure occurred in the case of *T. cacao* bark extracts. A special section is devoted to the safety issues. Therefore, despite being a rich source of lead molecules, *T. cacao* bark extract is not recommended as monotherapy.

Mechanistic approach to the low-dose effect

One of the observations made during the course of this study was the complete chemosuppressive effect produced by the 100 mg/kg dose of the ethanolic extract of *Theobroma cacao* bark, in addition to a shorter time period required to clear parasites (3.00±0.01 days) relative to that achieved with the use of chloroquine (4.00±0.01 days). It is important to understand the mechanism responsible for producing such an effect, as it differs from that induced by the 500 mg/kg dose. As far as the non-linear dose responses are concerned, they have also been observed with other types of plants studied in terms of their efficiency in fighting malaria. In particular, Chaniad et al. (2022; 2023) stated that high dosages of certain plant extracts do not cause a proportional increase in efficacy due to some pharmacological/physiological considerations connected with the nature of the dose. In a similar way, Alozieuwa and Ogbadoyi (2025) concluded that although the plant Carica papaya shows great curative ability, the highest dosages were ineffective. According to HPLC analysis, kaempferol is present in the largest quantity in comparison to other flavonoids, contributing to 62.87% of all UV peaks found in the ethanolic extract. As flavonoids have proven antiplasmodial activity according to literature data, it can be speculated that at a relatively low biological concentration, such an interaction of active

ingredients can lead to saturation of some specific parasite targets (fatty acid synthesis pathway and mitochondrial respiration chain) without provoking a defense mechanism in the parasite or host organism. On the contrary, in case of higher concentration (that is, 500 mg/kg) a series of factors that reduce the effect's efficiency (reduction of pathogenic burden up to 67.93% by day three) can be considered: arget saturation caused by too many bioactive components of the extract which cease being efficient but get quickly metabolized or get entrapped in cells; adverse effects associated with the impact on the functioning of host cells (e.g., changing the permeability of red blood cell membrane, inhibiting digestion and/or absorption); decreased efficiency caused by increased toxicity toward the host cells, despite good acute tolerability (no mortality) noted, the processes related to reticulocyte formation and spleen clearance cannot be completely excluded.

The presence of additional flavonoids, such as naringin (9.55%), myricetin (7.66%), baicalin (7.03%), catechin (6.78%), and quercetin (6.11%), indicates a great synergistic and polypharmacological efficiency of this natural substance. Besides, it can be assumed that all polyphenols mentioned are known for their capability to interfere with the activity of oxidative stress pathways.

Comparative context in regional ethnopharmacology research

The results correspond to current trends of African ethnopharmacological studies where antiplasmodial effects have been associated with particular flavonoid profiles identified in extracts of native species using the method of bio-guided fractionation. It is a novel discovery in relation to the existing body of *Theobroma* research. Previously, research had mainly focused on analyses of *Trinitario cacao* leaf and seed materials as sources of general antimicrobial or antioxidant activity (Komlaga et al., 2021). On the contrary, the current research represents the first success in performing bio-guided fractionation of the studied bark tissue along with HPLC profiling of the flavonoid profile therein. The obtained result of 100% early chemosuppression is more significant than the suppression rates of numerous widely known West African anti-malaria plants. By comparison with the results of experiments, *Terminalia macroptera* stem bark provided suppression at 71.10% (Aboubakar et al., 2023), *Feretia apodanthera* root bark at 68.45% (Boni et al., 2025), and *Erythrina sigmoidea* stem bark at 77.01% (Jemimah Sandra et al., 2024). Moreover, the best polyherbal mixture developed by Odediran et al. (2025) exhibited only 84.4% suppression. Consequently, the *T. cacao* bark ethanol extract is among the strongest antiplasmodial extracts so far discovered using the mouse model of *Plasmodium berghei,* in terms of parasitemia clearance, although the survival rate is low.

Previous studies focused mainly on the identification of antibacterial and other antimicrobial compounds or antioxidant effects in parts of *T. cacao* plant leaves and seeds (Komlaga et al., 2021); the present study is the first attempt to perform bio-guided fractionation of the bark tissue along with its HPLC fingerprint analysis to reveal flavonoids' content. Suppression level of 100% exceeds the levels demonstrated for several known antimalarials from native West African flora. Namely, experiments have shown that the stem bark of *Terminalia macroptera* provides 71.10%, while the root bark of *Feretia apodanthera* provides 68.45%, and the stem bark of *Erythrina sigmoidea* offers 77.01% suppression (Aboubakar et al., 2023; Boni et al., 2025; Jemimah Sandra et al., 2024). The value of suppression obtained by Odediran et al. (2025) for the optimal composition of four plant species is 84.4%. Hence, the ethanol extract of the *T. cacao* bark being investigated turns out to be one of the most efficacious of those tested previously.

Implications of recrudescence dynamics for therapy

While the bark extract given at a dose of 100 mg/kg achieved full initial inhibition of parasitemia, the drug could not arrest recrudescence once parasitemia had been eliminated. All mice died on the 28th day post-treatment, with an average survival rate of 12.00 ± 0.01 days compared to 28.00 ± 0.01 days seen in the chloroquine control group. This mode of clinical presentation is typical for biologically active molecules that selectively inhibit the development of parasitemia at the stage of asexual erythrocytic schizogony but do not have effects against dormant parasites in tissues or vascular reservoirs. Such recrudescent dynamics were reported earlier in several plant species, such as *C. papaya* (Alozieuwa & Ogbadoyi, 2025) and *V. amygdalina* (Onifade et al., 2024). Thus, it can be concluded that *T. cacao* bark extract cannot be considered as monotherapy since its application alone does not guarantee positive results. On the contrary, this is a good source of high-quality kaempferol for further lead optimization.

Safety and toxicity issues related to high mortality following initial chemosuppression

Despite achieving 100% chemosuppression on day 3 with the ethanol extract, at a 100 mg/kg dosage, all the treated mice died on day 14 with an average survival time of only 12 days, unlike those receiving chloroquine, who survived up to 28 days (Gozalo et al., 2024; Murin et al., 2025). Such a trend of increased mortality despite initial success in parasitemia clearance through chemosuppressive activities has been observed in previous studies using other natural extracts against malaria (Alozieuwa & Ogbadoyi, 2025; Okom et al., 2025). It thus becomes pertinent to consider why there was a high mortality rate despite the success in eliminating the parasites.

One explanation could be that while the extract effectively cleared the blood-stage parasites, it may be highly toxic to the vital internal organs of the body, like the liver and kidneys, in a dose-dependent manner. Various studies have established that some plant extracts, which exhibit anti-malarial activities, cause biochemical and histopathological changes in the livers, kidneys, and other organs at higher

dosages (Ajayi & Akhigbe, 2012; Febrina et al., 2024). In particular, cocoa-based products have been observed to contain bioactive molecules with the capacity to cause cytotoxicity in toxicity screening tests (Febrina et al., 2024). Alternatively, the extract may suppress the host's immune system, making the organism prone to recrudescence or infections. For instance, administration of *Eleutherine plicata* led to modulation of the pro-inflammatory cytokines IFN-g and IL-10 in *P. berghei*-infected mice (de Oliveira et al., 2024).

Another reason why the cocoa-based extract may be harmful to the host despite initial efficacy in parasitemia clearance is because of the induction of high oxidative stress in the host following repeated exposure to the extract. Although plant-based products usually boost the antioxidant system (Adegoke et al., 2023), at certain doses, they may lead to oxidative damage following repeated administration. One of the major problems of most plant extracts evaluated for their anti-malarial effects is the lack of biochemical evaluation of the animals used during experiments (Alozieuwa & Ogbadoyi, 2025; Chaniad et al., 2022; Okom et al., 2025). The alterations in PCV values and body weight variations recorded in this experiment give some idea about the stress experienced physiologically, although complete biochemical analysis would need to be done to understand the mode of toxicity.

CONCLUSIONS

This study demonstrated *in vivo* antimalarial activity of *Theobroma cacao* bark extracts in *Plasmodium berghei*-infected mice, with the ethanol extract at 100 mg/kg showing the most pronounced reduction in parasitaemia. However, despite the initial reduction in parasitaemia, parasite recrudescence occurred and the treatment did not provide a sustained survival benefit. Thus, the ethanol extract did not demonstrate a curative effect under the experimental conditions used and was less effective than chloroquine in terms of sustained disease control. The phytochemical analyses revealed flavonoids, phenolic compounds, terpenoids and fatty acid esters in the extracts, suggesting that the observed activity may be associated with multiple bioactive constituents. However, the contribution of individual compounds to the antimalarial activity requires further investigation.

The different extracts showed distinct effects on parasitaemia, packed cell volume, body weight and survival, indicating that their biological activities may vary according to their phytochemical composition. The findings provide preliminary evidence supporting further investigation of *T. cacao* bark as a potential source of antimalarial compounds. Future studies should identify and isolate the compounds responsible for the observed activity, establish their mechanisms of action, evaluate dose–response relationships and assess their efficacy and safety using appropriate experimental models. Further investigation is also required to determine whether these compounds have activity against drug-resistant *Plasmodium* parasites.

ACKNOWLEDGMENT

he authors thank the Tertiary Education Trust Fund (TETFund), Nigeria, for providing financial support for this research. The authors also acknowledge the management of Ekiti State Polytechnic for providing the facilities and support necessary to conduct the study. The authors thank Mr. Bamisaye Victor for his valuable contribution to the completion of the manuscript.

DECLERATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FUNDING INFORMATION

This work was partly supported by the Tertiary Education Trust Fund (TETFund), Nigeria, under Grant No. TETF/ES/UNI/ABUAD/2024/VOL.1. The funder had no role in the study design, data collection, data analysis, interpretation of the results, or preparation of the manuscript.

AUTHOR CONTRIBUTION

Conceptualization: **J.O.A., A.E., O.A.;** Data collection: **J.O.A., A.E., I.O.T., M.A.U**.; Statistical analysis: **I.O.T., M.A.U., O.A.;** Data interpretation: **O.S.O., O.A.;** Writing – original draft: **J.O.A., A.E.;** Writing – review & editing: **O.S.O., O.A.;** Supervision & critical review: **O.A.**

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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