Research Article
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Exploring the Anti-Malarial Potential of *Terminalia brownii* Fresen: A Phytochemical and Biological Activity Study

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Abstract: *Terminalia brownii* is a widely distributed African tree traditionally used to treat ailments such as cough, malaria, hepatitis, and microbial infections. The growing resistance of *Plasmodium falciparum* to Artemisinin combination therapy and other antimalarial drugs highlights the need for new therapies with improved potency and fewer side effects. This study analyzed the phytochemical constituents and antiplasmodial activity of *T. brownii* leaf extracts. The leaves were air-dried, powdered, and macerated in 70% methanol, followed by fractionation with n-hexane, chloroform, and ethyl acetate. Qualitative analysis of the methanol crude extract (MCE) revealed tannins, flavonoids, alkaloids, triterpenes, steroids, saponins, phenols, and cardiac glycosides, but not anthraquinones. Quantitative analysis showed phenols (195.45 mg/g), tannins (156.10 mg/g), and flavonoids (135.10 mg/g) as the most abundant. The ethyl acetate fraction contained phenols (103 mg/g) and tannins (69.56 mg/g) but lacked steroids and triterpenes. Antiplasmodial activity was evaluated in *Plasmodium berghei*-infected mice. The LD50 of the crude extract exceeded 5000 mg/kg, and significant dose-dependent suppression of parasitemia ($p<0.05$) was observed at 250, 500, and 1000 mg/kg. These findings support the traditional use of *T. brownii* against malaria and encourage further studies on its bioactive fractions and compounds.

Introduction

Malaria, according to the World Health Organization (WHO), is a life-threatening disease caused by Plasmodium parasites transmitted through the bite of infected female Anopheles mosquitoes, with the WHO African Region bearing the highest proportion of the burden, accounting for approximately 94% of the globe's malaria cases as well as 95% of the deaths (1). In 2023, there were an estimated 263 million malaria cases and 597,000 malaria deaths worldwide, Nigeria alone recorded about 25.9% of the cases and 30.9% of the deaths, illustrating the disproportionate impact on vulnerable populations such as children below the age of five, which constitute about 73.7% of all malaria deaths, and pregnant women causing miscarriage and stillbirth (1). In some regions, the emergence of resistance to ACTs in the Plasmodium parasite, slow parasite clearance, and resistance to partner drugs threaten the effectiveness of current malaria control policies. While recent advances in drug discovery and development, including the licensing of malaria vaccines like RTS, S, and R21, have provided new approaches in the fight against malaria especially in infants, their rollout and coverage, along with the need for even more effective drugs to combat the evolving drug resistance pattern, still pose challenges, highlighting the pressing need for sustained investment on research and in comprehensive malaria interventions (1). While progress has been made (e.g., 12.7 million deaths averted since 2000), drug/insecticide resistance, vaccine limitations, and climatic threats hinder the eradication of malaria. The WHO African Region, specifically Nigeria, remains a hotspot, demanding scaled-up investments, innovations, and equity in interventions to meet the 2030 targets (1).

Medicinal plants are the "backbone" of traditional medicine, and approximately 3.3 billion individuals in less developed nations utilize medicinal plants regularly. These medicinal plants are rich sources of bioactive components, known as phytochemical constituents, including alkaloids, flavonoids, saponins, terpenoids, tannins, phenols, steroids, and glycosides. These constituents have potential in drug development and synthesis (2, 3). Medicinal plants harbor bioactive secondary metabolites with potent antibacterial, anticancer, anti-inflammatory, antivenin, antidiabetic, and antiplasmodial properties. An Example includes quinine, a class of alkaloids isolated from the bark of the Cinchona tree

(family Rubiaceae), which has long been used as a frontline antimalarial (4). Similarly, Artemisia annua (sweet wormwood) produces artemisinin, a sesquiterpene lactone, which is the cornerstone of modern artemisinin-based combination therapies (ACTs) and the gold standard in the treatment of malaria. These plant constituents demonstrate the medicinal utility of natural sources, highlighting their historical and current significance as integral components of global disease control (4).

Terminalia brownii Fresen, commonly known as red pod Terminalia, is a medicinal plant within the Combretaceae family first labeled by Robert Brown in 1810 within the order Myrtales (5). The plant is found in tropical and subtropical regions of North Nigeria, Kenya, Tanzania, Ethiopia, Sudan, and the Democratic Republic of Congo (6). It is used extensively for the management of rheumatic and back pain, hepatitis, malaria, bacterial and fungal infections, gastric ulcers, and sexually transmitted diseases (7). Traditionally, in Tanzania, it is utilized by healers specifically for treating diarrhea, colic, and heartburn (8). Its aromatic properties also make it valuable as a local perfume for women (9). The previous Pharmacological activities include high antimicrobial activity against Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Candida albicans (10). The plant exhibits significant anti-inflammatory, antinociceptive, radical scavenging, and anticancer properties, with the stem wood displaying extremely high anticancer activity (9). Kareru *et al*. (2008) reported the susceptibility of the plant leaf and bark extracts to Escherichia coli and Staphylococcus aureus (11). Phytochemical investigations have revealed some bioactive compounds responsible for these pharmacological effects. Previous investigations revealed isolated secondary metabolites like sitosterol, stigmasterol, betulinic acid, arjugenin, and methyl ellagic acid derivatives from the bark of the stem (9). Other analyses detected gallic acid, dihydroxyflavone, and kaempferol 7-methoxy-3-sulfate in the methanolic extracts of the leaves (10). Most recently, Tijani *et al*. (2025) isolated a geranylated chalcone from the ethyl acetate fraction of the leaves, contributing to the known bioactive compounds of the medicinally important plant (11). These findings collectively suggest the immense potential of Terminalia brownii as a medicinal agent, solidifying its value as a source for traditional and modern drug discovery studies. The presence of bioactive constituents and the pharmacological effects of the plant make it a bountiful material for additional pharmaceutical investigations. The qualitative and quantitative analysis of the plant aids in the extraction, purification, and identification of bioactive metabolites, facilitating their easy application (2). In the present work, the phytochemical profile and antiplasmodial activity of *Terminalia brownii* extract were evaluated *in vivo* using mice, given its extensive acceptance as a material solution in most of Nigeria.

Experimental Section

Materials Collection, Identification, and Preparation of Plant Material

The leaf part of *Terminalia brownii* was collected from Maraba Yakawada Giwa local government, Zaria, Kaduna State, Nigeria, in March 2022. The plant material was authenticated at the Herbarium Section of the Botany department, Ahmadu Bello University, Zaria, by matching with herbarium reference (ABU0406). The leaves were washed, air-dried, and size-reduced with a pestle and mortar to form a powdered plant material.

Chemicals

All solvents and reagents utilized for extraction, fractionation, and phytochemical screening were laboratory grade (Merck Millipore). The standard chloroquine powder was from Sigma Aldrich, St. Louis, MO, USA.

Extraction and Fractionation

The leaf powder (1500 g) plant was extracted in methanol (70%) using a cold maceration procedure for 72 h with occasional shaking. The solvent was removed with a rotary evaporator, and a concentrated methanol crude extract (MCE) was obtained. About 280 g of the MCE was suspended in distilled water to obtain water-soluble and water-insoluble fractions. The aqueous insoluble water was also separated into n-hexane, chloroform, ethyl acetate, and n-butanol fractions, respectively, to obtain fractions named HF (hexane fraction), CF (chloroform fraction), EAF (ethyl acetate fraction), and Butanol fraction, which were subjected to further studies.

Preliminary Phytochemical Screening

Qualitative phytochemical analysis was performed on the crude methanol extract and its fractions according to standard procedures (12, 13). The quantitative analysis was carried out on the methanol crude extract and ethyl acetate fraction according to standard procedures (14-21).

Experimental Animals

Locally bred adult Swiss Albino mice of both genders, 8-10 weeks old and weighing 19-30 g, were supplied from the Department of Pharmacology and Therapeutics' animal house in Ahmadu Bello University, Zaria. They were maintained on a regular diet and water, and under room conditions in propylene cages containing sawdust bedding. The research protocols were approved by Ahmadu Bello University, Zaria, Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2023/061.

Malaria Parasite Preparation

The study employed chloroquine-sensitive Plasmodium berghei NK-65 parasites, which were supplied by the Animal House Facility of the Department of Pharmacology and Therapeutics at Ahmadu Bello University, Zaria, Nigeria. The parasite was maintained by periodic intraperitoneal passage in mice in the same facility. For infection, 0.2 mL of blood, with a rough estimate of 1×10^7 infected erythrocytes, was taken from donor mice and then administered intraperitoneally to the test animals.

Acute Toxicity Study

Acute oral toxicity of methanol leaf extract was evaluated as per OECD Guideline 425 (2008) (22) to determine the median lethal dose (LD_{50}). Six- to eight-week-old Swiss Albino mice were fasted overnight and weighed before the test. A limit test procedure was carried out at doses of 2000 mg/kg and 5000 mg/kg body weight. In the initial 2000 mg/kg test, two mice were administered the extract orally, with a 2-hour food restriction following dosing. No mortality was observed after 24 h; four additional mice were dosed at the same level. The animals were all under strict observation for 14 days, with careful attention paid to changes in skin and fur, Ocular and mucous membrane changes, Respiratory

changes, Behavioral changes, and Mortality. The same experimental protocol was then adopted for the higher dose limit test (5000 mg/kg) to assess the safety profile of the extract.

Preparation of Parasite Inoculation

For parasite preparation, a donor mouse with approximately 30% parasitemia was killed humanely under diethyl ether anesthesia. Blood was obtained via cardiac puncture into a Vacutainer tube containing a heparin anticoagulant of 0.5% trisodium citrate. The collected blood was then diluted with sterile physiological saline (0.9% NaCl) to achieve an inoculum concentration of approximately 1×10^7 infected erythrocytes per milliliter. The ready-to-use inoculum was immediately stored at a refrigeration temperature (4 °C) to maintain parasite viability for subsequent experimental use (4).

In Vivo Antimalarial Activity

Animal Grouping and Dosing

The experimental design involved thirty mice (of mixed gender) distributed among the study groups. For suppressive and curative tests, the following control groups were established: Group IV (negative control) received distilled water (2 mL/kg, p.o.), while Group V (positive control) received chloroquine (5 mg/kg, p.o.). All the treatments were given by oral gavage.

Test for Early Malaria Infection

The study followed the standard procedure described by Peter *et al*. (1965) (23). Twenty-five mice were randomly distributed into five treatment groups (n = 5 per group three hours post-infection. Groups 1–3 received oral administration of the test extract at 250, 500, and 1000 mg/kg body weight, respectively, once daily for four consecutive days. Group 4 (positive control) received chloroquine (5 mg/kg, p.o.), while Group 5 (negative control) received distilled water (2 mL/kg, p.o.). On day 5, caudal vein blood samples were taken to prepare thin blood smears, which were then air-dried, methanol-fixed, and stained with 3% Giemsa (pH 7.2) for microscopic examination. The suppression of parasitemia (%) was calculated using **Equation 1**.

$$\%\text{Suppression} = \frac{\%\text{P}_{\text{negative}} - \%\text{P}_{\text{treated}}}{\%\text{P}_{\text{negative}}} \times 100$$

Equation 1 | %P = parasitemia percentage.

Test on Established Infection (Curative Test)

This study employed the established protocol of Ryley and Peter (1970) (24) with modifications. Following parasite inoculation, mice were maintained for 72 h to allow for parasitemia development before being randomized into five treatment groups (n = 5 per group). Groups 1-3 received oral administration of the test extract at doses of 250, 500, and 1000 mg/kg body weight, respectively, administered once daily for four consecutive days. Control groups consisted of Group 4 (positive control, chloroquine 5 mg/kg) and Group 5 (negative control, distilled water 2 mL/kg). On day 5 post-infection, parasitemia was assessed through microscopic evaluation of Giemsa-stained (3%, pH 7.2) thin blood smears prepared from caudal vein samples following standard protocols of methanol fixation and air-drying.

Statistical Analysis

Values were given as Mean ± SEM and as percentages. ANOVA was used for data analysis, followed by a post hoc Dunnett test for multiple comparisons. Differences at $p \leq 0.05$ were considered significant.

Results

Qualitative phytochemical constituents of the crude methanol extract and partitioned fractions of *Terminalia brownii.*

Preliminary Phytochemical screening of *Terminalia brownii* crude methanol extract and partitioned fractions contained various bioactive constituents such as tannins, terpenoids, triterpenes, saponins, flavonoids, carbohydrates, cardiac glycosides, and alkaloids, but without anthraquinones (**Table 1**). Quantitative estimation revealed the concentration of the said phytochemicals in the crude extract as well as the ethyl acetate water-insoluble fraction in mg per gram of dry weight (**Table 2**).

Acute Toxicity Test of *Terminalia brownii*

The acute oral toxicity of the methanol crude extract of Terminalia brownii was evaluated in mice following OECD guidelines (Test No. 425). Results showed an LD_{50} value above 5000 mg/kg body weight, indicating low toxicity. During the 14-day observation, no treatment-related adverse effects were recorded. Monitoring revealed no alterations in physiological parameters, including pupillary responses, skin/fur condition, or secretory functions (lacrimation, salivation). Likewise, no changes were observed in excretory patterns (defecation/urination frequency or characteristics), confirming the absence of overt toxicity at tested doses.

Table 1. Qualitative phytochemical screening of the partitioned fractions of Terminalia brownii.

Constituents	Test	Observation	Inference			
			HF	CF	EA	BF
Tannins	Lead acetate	Cream	+	+	+	-
Triterpenes	Liebermann-Burchard	Brown ring at the interface	+	+	-	-
Steriods	Salkowski	Reddish color	+	+	-	-
Saponins	Frothing	Froth persists for 15 minutes	-	-	+	+
Flavonoids	NaOH	Yellow color	-	-	+	+
Anthraquinones	Bontrager	Pinkish color	-	-	-	-
Alkaloids	Dragendoff	Orange-brownish	+	+	+	+
Cardiac glycoside	Keller-Killiani	Red color	-	-	+	+

Note: (+) = Present, (-) = Absent, HF = Hexane fraction, CF = Chloroform fraction, EF = Ethylacetate fraction, BF = Butanol fraction

Table 2. Quantitative phytochemical constituents of the crude methanol extract and ethylacetate fraction of *Terminalia brownii*.

Phytochemical constituents	Crude Methanol Extract Concentration (mg/g)	Ethylacetate Concentration (mg/g)
Tannins	156.10	69.56
Triterpenes	85.05	0
Steriods	90.78	0
Saponins	115.20	22.60
Flavonoids	135.06	62.38
Alkaloids	58.12	34.96
Cardiac glycoside	119.90	19.41
Phenols	195.45	103.32

Table 3. Anti-plasmodial activity of crude methanol leaf extract of *Terminalia brownii* in established infection (suppressive test).

Treatment (mg/kg	Mean ± SEM Parasitemia	% Suppression
Infected + CME 250	16.24 ± 0.47*	23.09
Infected + CME 500	12.20 ± 0.37*	42.70
Infected + CME 1000	10.16 ± 0.32*	56.00
Infected + CQ 5	4.04 ± 0.25*	84.54

Note: Data presented as mean ± SEM; n=5, (*, $p < 0.05$, Dunnett post hoc test) shows a significant difference compared to the negative control group; N/S = normal saline; CME = crude methanol extract; CQ = chloroquine.

Table 4. Antiplasmodial activity of crude methanol leaf extract of *Terminalia brownii* in established infection (curative test).

Treatment (mg/kg)	Mean ± SEM Parasitemia	% Curative
Infected + CME 250	13.24±0.47*	33.09
Infected + CME 500	10.20±0.37*	48.00
Infected + CME 1000	9.16±0.30*	58.00
Infected + CQ 5	4.04±0.25*	84.54

Note: Data presented as mean ± SEM; n=5, (*, $p < 0.05$, Dunnett post hoc test) shows a significant difference compared to the negative control group; N/S = normal saline; CME = crude methanol extract; CQ = chloroquine.

In Vivo Antimalarial Studies

Four-day Suppressive Test in Mice

The suppressive activity test revealed that the crude methanol leaf extract of Terminalia brownii exhibited significant ($p < 0.05$) dose-dependent suppression of parasitemia at the tested concentrations. Suppression of 23.09%, 42.70%, and 56.00% was achieved with treatments of 250, 500, and 1000 mg/kg body weight, respectively, reflecting a clear concentration-response relationship. The chloroquine control (5 mg/kg) was more effective with 84.54% suppression, as shown in **Table 3**. The findings confirm the concentration-dependent antimalarial activity of the extract and provide its pharmacological activity profile in comparison to the reference treatment.

Established an Infection Test in Mice (Curative Test)

In the curative test, *Terminalia brownii* methanol leaf extract demonstrated dose-dependent and statistically significant ($p < 0.05$) reduction in parasitemia levels. A treatment with 250, 500, and 1000 mg/kg body weight resulted in the clearance of 33.09%, 48.00%, and 58.00% of parasites, respectively, demonstrating a progressive rise in efficacy with rising doses. In comparison to the control, chloroquine (5 mg/kg) demonstrated 84.54% curative activity, as shown in **Table 4**. The findings demonstrate the remarkable antimalarial activity of the extract, which warrants its use in therapeutic applications.

Discussion

Table 1 presents the qualitative phytochemical screening of the partitioned fractions of *Terminalia brownii*. The table presents the presence of various bioactive constituents, including tannins, triterpenes, steroids, saponins, flavonoids, alkaloids, and cardiac glycosides, in multiple fractions (hexane, chloroform, ethyl acetate, and butanol). Of interest, tannins and alkaloids were present in most fractions, while anthraquinones were absent in all. The ethyl acetate and butanol fractions exhibited higher phytochemical richness,

including flavonoids and cardiac glycosides, suggesting that they are promising sources of bioactive compounds worthy of further investigation.

The quantification of phytochemical constituents of crude methanol extract and ethyl acetate fraction of *Terminalia brownii* is presented in **Table 2**. The crude extract had the highest content of phenols (195.45 mg/g), followed by tannins (156.10 mg/g) and flavonoids (135.06 mg/g), and the ethyl acetate fraction was rich in cardiac glycosides (103.32 mg/g) and flavonoids (62.38 mg/g). The ethyl acetate fraction contained no traces of triterpenes and steroids, illustrating the selective enrichment of specific compounds during fractionation. The results reflect the richness of the extract with bioactive molecules that may be responsible for the pharmacological activities.

The acute toxicity test provides a short-term evaluation of potential risks associated with a single dose of a test substance, typically conducted in accordance with OECD regulations. Perhaps the most critical parameter during such tests is the median lethal dose (LD50), which is the dose that results in 50% mortality in a test population within an established duration (25). LD50 values have also been employed as a standard measurement of acute toxicity, where declining values express higher toxicity and vice versa. The methanol crude extract, with an LD50 of ≥ 5000 mg/kg, is considered safe for oral administration, according to Lorke (1983) (26). During this study, the methanol crude extract from Terminalia brownii leaves showed an oral LD50 of≥ 5000 mg/kg, with no mortality or damage observed during the assessment. These findings suggest that the extract is practically non-toxic and considered safe when administered orally in malaria treatment.

Table 3 demonstrates the suppressive activity against the crude methanol extract in the 4-day suppressive test. The extract revealed dose-dependent parasitemia suppression, with a maximum dose of 1000 mg/kg eliciting 56.00% suppression, compared to 84.54% suppression by the standard drug chloroquine at 5 mg/kg. The significant suppression in parasitemia at all tested dose levels ($p < 0.05$) is testimony to the potential of the extract as an antimalarial; its suppressive effectiveness was lower than that of the comparative drug.

Table 4 measures the curative effect of the crude methanol extract on existing malaria infection. Similar to the suppressive test, the extract showed dose-dependent activity, with the highest dose (1000 mg/kg) achieving 58.00% clearance of the parasites, compared to 84.54% for chloroquine. The rise in curative effect with increasing dosage suggests that higher doses or purified fractions of the extract can enhance its therapeutic value. These findings warrant further study of *Terminalia brownii* for antimalarial drug development.

The observed parasitemia inhibition in the suppressive and curative phases might be due to the presence of secondary metabolites in the plant, acting singly or in synergy to inhibit the parasite. Literature data have shown that secondary metabolites such as tannins, triterpenes, steroids, saponins, flavonoids, alkaloids, and cardiac glycosides possess antimalarial activity (4).

Suppressive and curative antimalarial act in the erythrocytic stage (the parasite's life cycle responsible for the clinical signs), of which chloroquine is a potent suppressive and curative antiplasmodial agent. From this, it's speculated that the crude extract also acts within these stages in the parasite's life cycle (4). An extract is reported to exhibit moderate, good, or very good *in vivo* antiplasmodial activity if it shows≥50% chemosuppression at daily doses of 250, 500, and 1000 mg/kg body weight, respectively (4). Based on this, the methanol crude extracts of Terminalia brownii exhibited good suppression and curative activities at doses of 250 and 500 mg/kg.

Conclusion

The study establishes *Terminalia brownii* methanol extract as rich in bioactive compounds, including phenols, flavonoids, alkaloids, and cardiac glycosides, which are most likely responsible for its impressive antiplasmodial activity. The extract inhibited dose-dependently (56%). It cured (58%) in rodent models, alongside an extremely low toxicity profile (LD50 ≥ 5000 mg/kg), indicating its safety and effectiveness as a natural alternative for the treatment of malaria. The findings from this study justify the ethnomedicinal claim of the plant in the management of malaria by various communities. Purifying and isolating its active compounds through additional studies should be carried out on the fractions, elucidating mechanisms of action against *Plasmodium* stages, and *in silico* studies on the isolated compounds, which could make it even more clinically effective.

Abbreviations

OECD = Organization for Economic Cooperation and Development; LD_{50} = Lethal Dose 50; ACTs = Artemisinin-based combination; RTS,S = Repeat T-cell Surface.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

The research protocols were approved by Ahmadu Bello University, Zaria, Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2023/061.

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Additional Information

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