Food Science & Applied Microbiology Reports
Vol. 4(2), 2025, 7-15
ISSN (Online) 2958-0684
https://finessepublishing.com/fsamr

# Nutraceutical potential (in vivo antiplasmodial activity) and phytochemical composition of *Mimosa pigra* L. Root Extract

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**Abstract:** The increasing resistance of Plasmodium species to standard antimalarial drugs highlights the need for novel agents from medicinal plants. *Mimosa pigra* L. (Fabaceae) is traditionally used in the treatment of malaria; however, its root extract has not been scientifically validated for antiplasmodial efficacy. This study investigated the phytochemical constituents and in vivo antiplasmodial activity of the methanolic root extract of *M. pigra* (MEBR) in *Plasmodium berghei*-infected mice. Roots of *M. pigra* were extracted with methanol and analyzed for secondary metabolites using standard phytochemical screening. Swiss albino mice inoculated with *P. berghei* NK65 were treated orally with MEBR at doses of 150, 300, and 600 mg/kg body weight for four consecutive days. Chloroquine (10 mg/kg) served as the positive control, while 5% DMSO acted as the negative control. Parasitaemia and mean survival times were recorded, and data were analyzed using one-way ANOVA followed by Tukey's post-hoc test at $p < 0.05$. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, steroids, and terpenoids. MEBR exhibited a dose-dependent reduction in parasitaemia, with suppression rates of 36.84%, 45.26%, and 52.42% at 150, 300, and 600 mg/kg, respectively, compared to 96.84% for chloroquine. Although the extract significantly reduced parasitaemia ($p < 0.05$), its effect on survival time was moderate, with no visible signs of toxicity observed. The methanolic root extract of *M. pigra* demonstrates significant antiplasmodial activity, supporting its ethnomedicinal use and warranting further bioassay-guided studies to isolate and characterize active compounds.
**Keywords:** Antiplasmodial activity, Bioactive compounds, traditional medicine, Malaria, *P.berghei*

## 1. Introduction

Malaria continues to pose a major public health challenge worldwide, particularly in tropical and subtropical regions. According to the World Health Organization, there were approximately 249 million malaria cases and over 600,000 deaths in 2023, with sub-Saharan Africa carrying the heaviest burden (WHO, 2024). Despite decades of progress, the emergence of resistance to conventional antimalarial drugs, including chloroquine and artemisinin-based therapies, has complicated malaria control efforts and underscored the urgent need for alternative therapeutic strategies (Ashley et al., 2018; Noedl et al., 2022). Limited accessibility to effective treatments in rural communities further highlights the potential value of safe, affordable, and locally available medicinal plants as complementary or alternative therapies.

Historically, plants have been an invaluable source of antimalarial agents. Quinine, derived from *Cinchona officinalis*, and artemisinin, isolated from *Artemisia annua*, are prime examples of natural compounds that have significantly influenced malaria treatment worldwide (Cui and Su, 2009). Ethnobotanical knowledge continues to guide the identification of bioactive compounds with unique mechanisms of action. Within this context, *Mimosa pigra* L. (Fabaceae), commonly known as the giant sensitive plant, has been traditionally used in African and Asian communities to manage febrile illnesses, including malaria (Saidu et al., 2019). Ethnopharmacological reports indicate that various parts of the plant—including leaves, stems, and roots—

[Received] 14 Oct 2025; Accepted 12 Dec 2025; Published (online) 18 Dec 2025]

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DOI: 10.61363/fsamr.v4i2.286

contain bioactive secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids, which may contribute to their pharmacological activities (Okoye et al., 2020; Nguyen et al., 2022). Although studies have investigated the leaves and stems of *M. pigra*, the antiplasmodial potential of its roots remains largely unexplored. Roots often accumulate distinct secondary metabolites that are absent or present in lower concentrations in aerial parts. Compounds such as alkaloids and terpenoids, commonly found in roots, have been documented for their potent antiparasitic and antioxidant activities (Moyo et al., 2021). Traditional healers frequently employ root decoctions to treat febrile conditions, suggesting that roots may harbour higher or unique concentrations of bioactive compounds compared to leaves or stems. This indicates a clear research gap: the lack of in vivo evaluation of *M. pigra* roots for antiplasmodial activity, despite their ethnomedicinal relevance.

Phytochemical investigations have shown that compounds such as flavonoids, tannins, and alkaloids can exert antiplasmodial effects by interfering with parasite metabolism, inhibiting heme detoxification, or disrupting mitochondrial function (Murebwayire et al., 2020). The methanolic extraction of roots allows for the isolation of a broad spectrum of polar and moderately nonpolar metabolites, increasing the likelihood of capturing pharmacologically active compounds. Comprehensive phytochemical profiling, therefore, is critical for understanding the chemical basis underlying observed biological effects and for guiding future bioassay-guided fractionation studies.

In vivo assessment using the *P.berghei* rodent model is a widely accepted approach to evaluate both efficacy and safety of antiplasmodial agents (Bantie et al., 2014; Peters and Anatoli, 1994). This model enables monitoring of parasitaemia suppression, mean survival times, and potential toxic effects, providing valuable insights before clinical translation. Previous studies on related species, including *Mimosa pudica* and *Mimosa tenuiflora*, have demonstrated promising antiplasmodial activity, largely through in vitro assays (Akinmoladun et al., 2019). However, in vivo validation of root extracts remains scarce, leaving a knowledge gap regarding the therapeutic potential, optimal dosing, and safety profile of *M. pigra* roots.

Given the ethnobotanical use of *M. pigra* roots, the rich array of secondary metabolites they contain, and the paucity of scientific data on their in vivo antiplasmodial activity, it is imperative to evaluate these extracts rigorously. Investigating the methanolic root extract offers the dual advantage of confirming traditional claims and potentially identifying bioactive compounds suitable for further pharmacological development. Such studies not only validate ethnomedicinal practices but also contribute to the discovery of novel antimalarial agents that could help combat emerging drug resistance.

To our knowledge, this study represents the first in vivo investigation of the methanolic root extract of *M. pigra* for antiplasmodial activity, providing critical insights into its phytochemical composition and potential as a source of novel antimalarial compounds. By bridging the gap between traditional knowledge and modern pharmacology, this research aims to generate foundational data that can inform future isolation, characterization, and mechanistic studies, ultimately contributing to the development of effective plant-derived antimalarial therapies.

## 2. Materials and Methods

### 2.1. Plant Material and Extraction

Roots of *Mimosa pigra* were collected from the Hadejia–Nguru Wetlands, Nigeria, and taxonomically authenticated at the Herbarium of Ahmadu Bello University, Zaria. The roots were thoroughly washed to remove adhering soil and debris, shade-dried at room temperature for two weeks, and then pulverized into a fine powder using a mechanical grinder. Approximately 500 g of the powdered material was macerated in 85% methanol for 72 h with intermittent stirring. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtration was concentrated under reduced pressure at 40 °C using a rotary evaporator to obtain the crude methanolic root extract of *M. pigra* (MEBR). The percentage yield was calculated based on the weight of the dried extract relative to the initial plant material. The extract was stored in an airtight container and kept refrigerated until further use.

### 2.2. Preliminary Phytochemical Screening

The methanolic root extract of *Mimosa pigra* L. (MEBR) was subjected to qualitative phytochemical screening using standard procedures described by Fathima et al. (2023), Harborne (1998), and Trease and Evans (1934). These tests were conducted to identify the classes of bioactive secondary metabolites that may contribute to the observed antiplasmodial activity.

The test for alkaloids was performed using Mayer's reagent. One milliliter (1 mL) of the extract was treated with a few drops of Mayer's reagent, and the formation of a pale or cream-colored precipitate indicated the presence of alkaloids. The presence of flavonoids was determined using both the alkaline reagent and ferric chloride tests. In the alkaline reagent test, the extract was treated with a few drops of sodium hydroxide (NaOH) solution, resulting in a yellow coloration that disappeared upon the addition of diluted hydrochloric acid (HCl). Similarly, when a few drops of ferric chloride ($FeCl_3$) solution were added to the extract, the appearance of a blackish-green coloration confirmed the presence of flavonoids.

For glycosides, both Molisch's and concentrated sulfuric acid ($H_2SO_4$) tests were carried out. In Molisch's test, the extract was mixed with α-naphthol solution, followed by the careful addition of concentrated $H_2SO_4$ along the sides of the test tube. The formation of a brown ring at the interface indicated the presence of glycosides. In the concentrated $H_2SO_4$ test, equal volumes of extract and concentrated $H_2SO_4$ were mixed, and the appearance of a red coloration confirmed glycosides.

The foam test was used to detect saponins. The extract was diluted with distilled water to 25 mL and shaken vigorously for 10 minutes. Persistent frothing indicated the presence of saponins. The presence of phenols was confirmed by treating 2 mL of the extract with $FeCl_3$ solution, which produced a blue coloration. Similarly, for tannins, 2 mL of the extract was treated with $FeCl_3$, and the formation of a blue or green coloration indicated their presence.

Finally, the Salkowski test was used to detect steroids and triterpenoids. One milliliter (1 mL) of the extract was mixed with concentrated $H_2SO_4$. A red coloration in the lower layer indicated steroids, while a yellow coloration suggested triterpenoids.

### 2.3. In Vivo Antiplasmodial Study

#### 2.3.1. Animals

Male Swiss albino mice (6–8 weeks old, 18–25 g) were obtained from the Department of Pharmacology, Bayero University, Kano. The animals were housed in standard cages under controlled environmental conditions (25 ± 2 °C, 12 h light/dark cycle) and allowed free access to standard pellet feed and clean water. All procedures involving animals were conducted in accordance with the recommendations of the National Research Council (US) Guide for the Care and Use of Laboratory Animals (2011).

#### 2.3.2. Parasite

The *P.berghei* NK65 strain, sensitive to chloroquine, was obtained from Aminu Kano Teaching Hospital, Kano, Nigeria. Donor mice previously infected with *P.berghei* were used to prepare the inoculum. Parasitized erythrocytes were collected by cardiac puncture and diluted with 0.9% normal saline to achieve a concentration of $1 \times 10^7$ infected erythrocytes per 0.2 mL. Each experimental mouse received an intraperitoneal injection of 0.2 mL of the suspension on day 1 of the study.

#### 2.3.3. Treatment Protocol

The four-day suppressive test described by Peters et al. (2002), with slight modification, was employed to evaluate the antiplasmodial activity of the methanolic root extract of *M. pigra* (MEBR). Seventy-two hours after inoculation, thirty mice were randomly divided into six groups (n = 5 per group). Groups I–III received MEBR orally at doses of 150, 300, and 600 mg/kg body weight, respectively. Groups IV and V served as positive controls and received artemether (5 mg/kg) and chloroquine (10 mg/kg), respectively, while Group VI served as the negative control and received distilled water (10 mL/kg). Treatments were administered orally once daily for four consecutive days (days 1–4).

On the fifth day (D+4), thin blood smears were prepared from tail vein samples, fixed in methanol, and stained with Giemsa. Parasitaemia was determined microscopically by counting infected erythrocytes among a total of 1,000 red blood cells in randomly selected fields.

### 2.4. Statistical Analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 20. Differences between group means were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Results were expressed as mean ± standard error of the mean (SEM), and differences were considered statistically significant at $p < 0.05$.

## 3. Results and Discussion

### 3.1. Phytochemical Composition

Qualitative phytochemical screening of the methanolic root extract of *M. pigra* (MEBR) revealed the presence of several major classes of secondary metabolites, including alkaloids, flavonoids, steroids, cardiac glycosides, tannins, saponins, and anthraquinones (Table 1). The detection of these metabolites indicates that the root extract contains chemically diverse constituents with known biological relevance.

The observed phytochemical profile is generally consistent with previous reports on Mimosa species, although some variations were noted. Palwinder et al. (2011) reported the presence of alkaloids, flavonoids, tannins, and terpenes in *M. pigra*, while Chukwudi et al. (2023) identified alkaloids, saponins, flavonoids, tannins, and phenolic compounds but did not detect steroids. Similarly, Romash et al. (2020) documented alkaloids, terpenoids, saponins, tannins, phenols, and steroids in *M. pudica*, although cardiac glycosides were absent. Such discrepancies may reflect differences in plant part used, extraction solvent, geographical origin, or environmental conditions influencing secondary metabolite biosynthesis.

Importantly, the presence of alkaloids and flavonoids is pharmacologically relevant, as these compound classes are frequently implicated in antiplasmodial activity. Alkaloids have historically formed the basis of several antimalarial drugs, while flavonoids are known to exert antioxidant and parasite-inhibitory effects. However, given that this study employed qualitative screening, the relative abundance and specific identities of these compounds remain undetermined. Therefore, while the phytochemical findings provide a biochemical rationale for the observed bioactivity, they should be interpreted as preliminary and supportive rather than definitive evidence of the mechanism. `

**Table 1. Phytochemical composition of *Mimosa pigra* methanolic root extract**

| Phytochemical | Presence (+) / Absence (–) |
|---|---|
| **Alkaloids** | + |
| **Flavonoids** | + |
| **Steroids** | + |
| **Cardiac glycosides** | + |
| **Tannins** | + |
| **Saponins** | + |
| **Anthraquinones** | + |

### 3.2. Antiplasmodial Activity

The methanolic root extract of *M. pigra* exhibited a statistically significant and dose-dependent suppressive effect on parasitemia in Plasmodium berghei-infected mice (Table 2). Percentage inhibition increased from 18.15% at 150 mg/kg to 46.77% and 52.42% at 300 and 600 mg/kg, respectively. Although these values were markedly lower than those produced by chloroquine (89.11%) and artemether (87.90%), the extract demonstrated significant antiplasmodial activity compared with the negative control ($p < 0.001$).

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The observed dose–response relationship suggests that the antiplasmodial effect of MEBR is concentration dependent, which is characteristic of crude plant extracts containing multiple bioactive constituents acting additively or synergistically. Nevertheless, the moderate level of parasite suppression indicates that the extract exerts primarily suppressive rather than curative activity at the tested doses.

The mean percentage parasitaemia and percentage suppression were calculated using the following formulas:

$$\text{parasitemia (\%)} = \left(\frac{\text{Number of parasitized red blood cell}}{\text{Total Number of red blood cell}}\right) \text{x } 100$$

$$\text{Mean survival time} = \frac{\text{Total survival time in all mice in the group}}{\text{Total number of mice in the group}}$$

$$(\%)\text{inhibition} = \left(\frac{\text{Mean parasitemia of control group} - \text{Mean parasitemia of treatment group}}{\text{Total Number of mice in the group}}\right) \text{x } 100$$

**Table 2. Effect of *Mimosa pigra* methanolic root extract on parasitemia in *Plasmodium berghei*-infected mice**

| Treatment | Dose (mg/kg) | Parasitemia (%) | % Inhibition |
|---|---|---|---|
| **Distilled water** | 10 mL/kg | 4.96 ± 0.29 | – |
| **MEBR** | 150 | 4.06 ± 0.13* | 18.15 |
| **MEBR** | 300 | 2.64 ± 0.18** | 46.77 |
| **MEBR** | 600 | 2.36 ± 0.14** | 52.42 |
| **Chloroquine** | 10 | 0.54 ± 0.04** | 89.11 |
| **Artemether** | 5 | 0.60 ± 0.08** | 87.90 |

$p < 0.01$, ** $p < 0.001$ vs. control

Survival analysis showed that mice treated with 300 and 600 mg/kg MEBR had a mean survival time of 21 days, comparable to the artemether-treated group (Table 3). However, these improvements were not statistically significant relative to controls, suggesting that parasite suppression did not translate into complete disease resolution.

**Table 3. Mean survival time of *Plasmodium berghei*-infected mice treated with *Mimosa pigra* extract**

| Treatment | Dose (mg/kg) | Survival time (days) |
|---|---|---|
| **Distilled water** | 10 mL/kg | 19.17 ± 1.83 |
| **MEBR** | 150 | 18.67 ± 2.33 |
| **MEBR** | 300 | 21.00 ± 0.00 |
| **MEBR** | 600 | 21.00 ± 0.00 |
| **Chloroquine** | 10 | 16.17 ± 3.19 |
| **Artemether** | 5 | 21.00 ± 0.00 |

The limited survival benefit may be attributed to partial parasite suppression, rapid metabolism of active constituents, or poor in vivo bioavailability. As a crude extract, MEBR likely contains a mixture of active and

inactive compounds, which may dilute therapeutic potency. Similar outcomes have been reported in preliminary antimalarial screenings of other *Mimosa* species, including *M. pudica*, where dose-dependent parasitemia suppression was observed without complete parasite clearance (Tamilarasi and Ananthi, 2012).

Mechanistically, alkaloids may inhibit hemozoin formation, while flavonoids are known to interfere with parasite mitochondrial function, fatty acid biosynthesis, and redox homeostasis (Kim et al., 2004; Monbrison et al., 2006). Saponins and tannins may further contribute through membrane-disruptive or immunomodulatory effects. However, definitive attribution of activity to specific compounds requires fractionation and bioassay-guided isolation.

## 4. Conclusion

The methanolic root extract of *M. pigra* demonstrated significant, dose-dependent suppression of *P.bergheiparasitemia* in vivo, confirming its potential as a natural antimalarial agent. The phytochemical composition, rich in alkaloids, flavonoids, tannins, and saponins, likely underpins the observed biological activity. Although the extract was less potent than standard drugs, it provided meaningful suppression of parasitemia, lending scientific support to the traditional use of *M. pigra* in the management of febrile illnesses associated with malaria.

The moderate level of activity observed also highlights the need for further investigations aimed at refining and characterizing the active principles. Bioassay-guided fractionation and advanced analytical techniques such as LC-MS/MS, NMR spectroscopy, and molecular networking could help identify the bioactive metabolites responsible for the antiplasmodial effects. Moreover, combining *M. pigra* extracts with established antimalarial agents may enhance therapeutic efficacy and reduce the risk of resistance development.

Future studies should also incorporate assessments of subacute and chronic toxicity to establish safety margins and therapeutic windows. Overall, the findings of this study position *M. pigra* as a valuable source of phytochemicals for potential drug discovery and justify continued scientific exploration of its pharmacological potential.

## Authors' Contribution

Muhammad Mukhtar conceptualized and designed the study, supervised the research, and drafted the initial manuscript; Sani Ibrahim and Zakariya Idris conducted laboratory experiments, including sample collection, preparation, and chemical analyses; Suleiman Abubakar Garba performed data analysis and interpretation, prepared figures and tables, and contributed to manuscript revision. All authors contributed to the study design, critically reviewed the manuscript for intellectual content, approved the final version for submission, and agreed to be accountable for all aspects of the work.

## Competing Interest

The author declared no conflict of interest.

## Funding

The author has not received any funding to conduct the research.

## Acknowledgement

The authors sincerely acknowledge the technical support provided by the staff of Aminu Teaching Hospital, Kano. We also thank the field assistants and all lab members for their assistance with sample collection and laboratory analyses.

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