



Evaluation of In Vivo Antiplasmodial Effect of Aqueous and Ethanol Extracts of *Tamarindus indica* Leaf Against *Plasmodium berghei* in Albino Mice

Gambo J. B.; Mamoon M. A.; & Garba F. B.

Department of Applied Biology, Kaduna Polytechnic, Kaduna, Nigeria

Correspondence author: elaskagambo@yahoo.com

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Abstract

Malaria remains a significant global health concern, particularly in tropical and subtropical regions, where it causes high morbidity and mortality rates. The emergence of *Plasmodium falciparum* resistance to conventional antimalarial drugs has necessitated the search for alternative therapies, particularly from medicinal plants with known pharmacological properties. *Tamarindus indica* has been traditionally used in herbal medicine for various therapeutic purposes, including malaria treatment. This study aimed to evaluate the antiplasmodial effects of aqueous and ethanol extracts of *Tamarindus indica* leaves in *Plasmodium berghei*-infected mice using a structured methodology. Fresh leaves of *Tamarindus indica* were collected, authenticated, air-dried, and ground into a fine powder before extraction using aqueous and ethanol solvents. The extracts were concentrated, and phytochemical screening was conducted to identify bioactive compounds. Healthy mice were acclimatized and divided into five groups: uninfected control, infected untreated control, infected + standard drug (chloroquine), infected + aqueous extract, and infected + ethanol extract. Following parasite inoculation, treatments were administered for four days, and parasitaemia levels were assessed using Giemsa-stained blood smears under a microscope. Data were analyzed statistically to determine mean parasitaemia levels and percentage reduction. Results showed a significant reduction in parasitaemia in treated groups compared to the untreated control. The ethanol extract exhibited a higher percentage reduction (85.71%) than the aqueous extract (82.86%), while chloroquine showed the highest efficacy with a

95% reduction. Phytochemical screening revealed the presence of key bioactive compounds, which are likely responsible for the observed antimalarial effects. The findings support the traditional use of *Tamarindus indica* in malaria management and highlight its potential as a source of novel antimalarial compounds. However, further research is needed to isolate and characterize the active compounds, elucidate their mechanisms of action, and assess their safety and efficacy in clinical trials. This study contributes to the growing body of evidence supporting plant-based therapies as viable alternatives for malaria treatment, particularly in regions with limited access to conventional antimalarial drugs.

Keywords: Plasmodia, malaria, extract, phytochemical, *Tamarindus indica*.

INTRODUCTION

Malaria is a major public health concern leading to morbidity and mortality, which occurs in all six World Health Organization regions (WHO, 2023). In 2021, an estimated 247 million cases and 619,000 fatalities occurred worldwide, with sub-Saharan Africa accounting for the vast majority of cases and deaths (WHO, 2023). According to the WHO, 26.6% of all malaria cases and 31.3% of deaths worldwide occurred in Nigeria in 2021 (WHO, 2023). The population of people most vulnerable to malaria are pregnant women and children under the age of five. In 2021, 76.8% of all the world's malaria deaths were of children under the age of five (WHO, 2023). In 2021, an estimated 1.3 million women were exposed to malaria during pregnancy, which resulted in 961,000 children with low birth weight (WHO, 2023). The development and reemergence of parasite resistance to first-line antimalarial drugs, particularly ACTs, as well as the disruption of humanitarian

initiatives, have led to a sustained rise in malaria-related death and morbidity despite all efforts (WHO, 2023).

According to Sivakrishnan (2018), “a medicinal plant” is any plant in which one or more of its organs contain substances that can be used for therapeutic purposes or are precursors for the synthesis of effective drugs. Medicinal plants contain diverse phytochemicals with known or unknown biological activities. Plant-derived substances are believed to be more accessible, in line with patients' preferences, and safer and more cost-effective treatments. Examples of these substances include tropane alkaloids (atropine, hyoscyne, scopolamine, and hyoscyamine), opium alkaloids (papaverine, codeine, and morphine), flavonoids (stilbenes, chalcones, luteolin, quercetin, rutin, apigenin, and kaempferol), terpenoids (stigmasterol, sitosterol, betulinic acid, lupeol, and olenolic acid), and essential oils (from caraway and

peppermint) (Ahn, 2017; Kaur and Kaur, 2017). High concentrations of phytochemical constituents with antiplasmodial properties found in medicinal plants aid in treating malaria. For example, quinine from the bark of the *Cinchona* tree, a member of the Rubiaceae family of plants, is well known for treating malaria (Adebayo and Krettli, 2011; WHO, 2005). Alternatively, *Artemisia annua*, another ancient medicinal plant, is a significant source of the antimalarial drug artemisinin, a sesquiterpene lactone (Adebayo and Krettli, 2011; Czechowski *et al.*, 2020).

Tamarindus indica, commonly known as tamarind, is a tropical tree belonging to the Fabaceae family. Traditionally, various parts of the plant, including its leaves, bark, and fruit pulp, have been used to treat a range of ailments such as fever, diarrhoea, jaundice, and infections (Muganga *et al.*, 2010). Several studies have reported the pharmacological potential of *T. indica*, including its antioxidant, antimicrobial, and anti-inflammatory properties (Alrasheid *et al.*, 2019). However, there is limited evidence on the in vivo antimalarial effects of these extracts, particularly in animal models. Evaluating their efficacy in vivo is critical to understanding their potential as alternative treatments for malaria.

This study seeks to investigate the antiplasmodial effect of aqueous and ethanol extracts of *T. indica* leaves against *Plasmodium berghei* in mice. The findings could contribute to the identification of cost-effective, plant-based antimalarial agents that could complement existing therapies, particularly in resource-limited settings where access to conventional drugs is challenging.

MATERIALS AND METHODS

Materials used for the experiment

Equipment	Model	Manufacturer
Glass wares	Pyrex	England
Hot Air-drying Oven	DHG	Xian Heb Biotechnology Company Ltd
Centrifuge Machine	TDL-4	B. Bran Scientific and Instrument Company, England
Water bath	GD 100	Grant Scientific Tech. Germany.
Spectrophotometer	SP 300	Optima Germany
Micropipette	Diapette	TECO Diagnostic, USA
Rotary evaporator	RE-52A	Shangai, Biochemistry Instrument Factory, China

Sample collection

Leaf collection and identification

Pure *Tamarindus indica* leaves were collected from Rigasa, Igabi Local Government Kaduna, placed in a polythene bag, and transported to the Biological Science Department at Kaduna State University for identification. It was assigned a reference number KASU/BSH/436.

Sample Preparation

The *Tamarindus indica* leaves were thoroughly washed with clean water and air-dried under shade for several days until they were completely dry. The dried leaves were grounded into a fine powder using mortar and pistle. The powdered material was stored in a plastic container and kept at room temperature (25°C) prior to extraction.

Extraction of *Tamarindus indica* Leaf Using Aqueous and Ethanol

Four hundred grams (400g) of the powdered *Tamarindus indica* leaf was subjected to cold maceration in 3.5L each of ethanol and water for 72 hours with occasional shaking. After maceration, the mixtures were filtered using Whatman filter paper, and each extract was concentrated to dryness using a water bath at 45°C under controlled temperature and reduced pressure. The extracts were then kept in a clean plastic container and stored in a refrigerator until use.

Phytochemical Screening of Crude ethanol and aqueous Extracts of *Tamarindus indica* leaf

1. Test for Alkaloids

About 0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath; 1ml each of the filtrate was treated with a few drops of Mayer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was observed as preliminary evidence for the presence of alkaloids in the extracts (Harborne, 1984; Evans, 1989).

2. Test for Saponins

About 0.5g of each plant extract was shaken with water in a test tube. Frothing which persists on warming was accepted as preliminary evidence for the presence of saponins (Evans, 1989).

3. Test for Tannins

About 0.5g of each plant extract was stirred with 1ml of distilled water, filtered, and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was evidence of the presence of tannins (Evans, 1989).

4. Test for Anthraquinones

About 0.5g of each of the extracts was taken in a dry test tube and 5ml of chloroform was added and shaken for 5 minutes. The extract was filtered, and the filtrate shaken with an equal volume of 100% ammonia solution. A pink violet or red colour in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones (Evans, 1989).

5. Test for Glycosides

One hundred grammes of each extract were taken in a test tube and 2.5ml of dilute sulphuric acid was added and boiled in a bath of water for 15 minutes. This was cooled and neutralized with 20% potassium hydroxide solution. Five ml of a mixture of Fehlings solution A and B added and boiled for 3 minutes. A brick red precipitate indicated the hydrolysis of a reducing sugar, an indication of glycoside (Harborne, 1984).

6. Test for Cardiac Glycocides (Keller Kiliani test)

About 0.5g of extract diluted to 5ml in water and 2 ml of glacial acid was added containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Harborne, 1984; Evans, 1989).

7. Lieberman's Test Steroids and Terpenes

A little quantity of each extract was dissolved in chloroform and 1ml of acetic anhydride was added, then 2 drops of conc. sulphuric acid were added. A pink colour which changes to bluish green on standing is indicative of the presence of steroid and terpenes (Evans, 1989).

8. Test for Flavonoids

Two grammes of the extract were completely detained with acetone. The residue was extracted in warm water after evaporating on water bath. The mixture was filtered while hot. The filtrate was cooled and used for the following tests (Evans, 1989).

Animals Procurement

Fifteen (15) apparently healthy albino mice of both sexes were obtained from the National Institute of Trypanosomiasis (NITR), Kaduna State. The animals were kept in a rat cage in a well-ventilated environment and hygienic laboratory conditions.

Acclimatization

The animals were housed in the animal house of the Applied Biology Departmental Garden, Kaduna Polytechnic, Kaduna enclosed in steel cages, and fed with standard feed pellets and clean water *ad libitum* at standard room temperature under a 12-hour light-dark cycle for seven weeks as a preliminary acclimatization period. All experimental protocols were in compliance with the Ethical Committee of Kaduna Polytechnic Kaduna. Additionally, Ethics on Research in Animals and internationally accepted principles for laboratory animal use and care were strictly adhered to.

Parasite Inoculation

Donor mice infected with *P. berghei* ANKA were used to prepare the inoculum. Blood was collected via cardiac puncture, diluted with PBS to obtain 1×10^7 parasitized erythrocytes/mL. Mice in the experimental groups were inoculated intraperitoneally with 0.2 mL of the inoculum.

Experimental Design

The mice were randomly divided into 4 groups (n = 3 per group):

- **Group 1:** Uninfected control
- **Group 2:** Infected, untreated control.
- **Group 3:** Infected + standard drug (artesunate, 5 mg/kg).
- **Group 4:** Infected + aqueous extract (100 mg/kg).
- **Group 5:** Infected + ethanol extract (100 mg/kg).

Treatments were administered orally once daily for 4 consecutive days (D0–D3) post-infection.

Parasitemia Monitoring

Thin blood smears were prepared from tail vein blood of each mouse on days 1, 2, 3, and 4 post-infections. The blood smears were fixed with methanol, stained with 10% Giemsa, and examined under a light microscope ($\times 100$ magnification) to determine parasitemia.

- **Parasitemia (%)** was calculated as:

$$\text{Parasitemia (\%)} = \left(\frac{\text{Number of infected RBCs}}{\text{Total number of RBCs}} \right) \times 100$$

Percentage Suppression

The antiparasmodial activity of the extracts was evaluated by calculating the percentage suppression of parasitemia on day 4 relative to the negative control using the formula:

$$\text{Suppression (\%)} = \left(\frac{\text{Parasitemia in control group} - \text{Parasitemia in treated group}}{\text{Parasitemia in control group}} \right) \times 100$$

Statistical Analysis

Statistical analysis was performed using SPSS. Data were expressed as mean $\pm$ standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to determine significant differences between groups, followed by Tukey's post hoc test for pairwise comparisons. A p-value of <0.05 was considered statistically significant.

RESULTS

Percentage Yield of Aqueous and Ethanol Extracts of *T. indica* leaf

The *Table 1* below shows the percentage yield of extracts obtained from 200 g of *T. indica* leaf. The ethanol extract produced the highest yield at 13.50%, corresponding to 27.00 g of extract, while the aqueous extract yielded 11.20%, equivalent to 22.40 g. This indicates that the ethanol extraction method was more efficient in recovering bioactive compounds from the *T. indica* leaf compared to aqueous extraction. These differences in yield may reflect the solubility of the plant's phytochemicals in water versus ethanol, with ethanol being more effective for this specific *T. indica* leaf.

Table 1: Percentage Yield of Aqueous and Ethanol Extracts of *T. indica* leaf

Extract Type	Weight of <i>T. indica</i> leaf (g)	Weight of Extract (g)	Percentage Yield (%)
Aqueous Extract	200	22.40	11.20
Ethanol Extract	200	27.00	13.50

Result for Phytochemical Screening of Aqueous and Ethanol Extracts

The phytochemical screening revealed the presence of various bioactive compounds in both aqueous and ethanol extracts. The ethanol extract showed a higher concentration of flavonoids and steroids, while the aqueous extract had higher levels of tannins and saponins. Both extracts exhibited a similar presence of phenols and terpenoids, indicating that these compounds are abundant regardless of the solvent used.

Table 2: Phytochemical Screening of Aqueous and Ethanol Extracts of *T. indica* leaf

Phytochemical Constituents	Aqueous Extract	Ethanol Extract
Alkaloids	+	++
Flavonoids	++	+++
Saponins	++	+
Tannins	+++	++
Phenols	+++	+++
Glycosides	+	+
Terpenoids	++	++
Steroids	-	+

Key:+++ : Highly Present ++ : Moderately Present + : Slightly Present - : Absent

Effect of Treatments on Parasitaemia Levels

The effect of treatments on parasitaemia Levels is depicted in Table 3. The result outlines the progression of parasitaemia levels across four days for various treatment groups. The uninfected control group consistently maintained a parasitaemia level of 0.00 ± 0.00 throughout, confirming the absence of infection. In contrast, the infected, untreated control

group exhibited a significant increase in parasitaemia from 11.67 ± 1.53 on Day 1 to 23.00 ± 2.65 on Day 4, indicating rapid disease progression. The group treated with the standard drug showed a remarkable parasitaemia reduction of 95.00%, with levels dropping from 11.00 ± 2.00 on Day 1 to 0.55 ± 0.58 on Day 4, demonstrating its high efficacy. The aqueous extract achieved an 82.86% reduction, reducing parasitaemia to 2.00 ± 1.00 by Day 4, while the ethanol extract performed slightly better with an 85.71% reduction to 1.67 ± 0.58 . These results highlight the potential effectiveness of the extracts, with ethanol extract showing slightly higher antiplasmodial activity.

Table 3: Effect of Treatments on Parasitaemia Levels

Group	Day 1	Day 2	Day 3	Day 4
Uninfected Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Infected, Untreated Control	11.67 ± 1.53	15.00 ± 1.00	19.67 ± 2.52	23.00 ± 2.65
Infected + Standard Drug	11.00 ± 2.00	5.67 ± 1.53	4.00 ± 1.00	0.55 ± 0.58
Infected + Aqueous Extract	11.67 ± 2.52	7.33 ± 2.08	3.67 ± 1.53	2.00 ± 1.00
Infected + Ethanol Extract	11.67 ± 1.53	4.00 ± 1.00	2.67 ± 0.58	1.67 ± 0.58

DISCUSSION

Percentage Yield of Aqueous and Ethanol Extracts of *Tamarindus indica* Leaf

The results presented in *Table 1* show that the ethanol extract of *Tamarindus indica* leaf yielded 13.50% from 200 g of plant material, while the aqueous extract yielded 11.20%. This suggests that ethanol extraction was slightly more efficient in extracting bioactive compounds from the leaf compared to water. These differences in yields can be attributed to the solubility of the phytochemicals present in the plant material. Ethanol, being a polar solvent, is often more effective in extracting a wide range of bioactive compounds, including alkaloids, flavonoids, and terpenoids, which are known to be present in *T. indica* (Brahmachari, 2011; Dhingra & Tiwari, 2015). The higher yield of ethanol extract may therefore be a result of its superior ability to dissolve these compounds (Mohammad *et al.*, 2019).

In contrast, aqueous extraction is typically favored for extracting water-soluble compounds, including saponins and some flavonoids, which could explain the relatively lower yield compared to ethanol. This is consistent with previous studies that have shown lower yields with aqueous extraction due to its limited ability to extract non-polar compounds (Sahoo & Bhutia, 2019).

While ethanol extraction yielded more, it is important to note that the aqueous extract still provided a substantial amount of bioactive material, which could be advantageous for

therapeutic purposes in cases where water-based preparations are preferred. The difference in yields further emphasizes the importance of selecting the appropriate solvent for the extraction of specific bioactive compounds based on their solubility properties.

Phytochemical Screening of Aqueous and Ethanol Extracts of *Tamarindus indica* Leaf

The results from *Table 2* demonstrate the presence of various phytochemical constituents in both aqueous and ethanol extracts of *Tamarindus indica* leaf. Aqueous extract showed the presence of alkaloids, flavonoids, saponins, tannins, phenols, glycosides, terpenoids, but no steroids, whereas ethanol extract exhibited alkaloids, flavonoids, saponins, tannins, phenols, glycosides, terpenoids, and steroids.

The higher presence of flavonoids in the ethanol extract compared to the aqueous extract is consistent with the ability of ethanol to extract flavonoid compounds more efficiently due to its higher polarity. Flavonoids, known for their antioxidant, anti-inflammatory, and anti-cancer properties, are often more soluble in organic solvents like ethanol (Gul *et al.*, 2018). This suggests that ethanol extraction is more effective in extracting compounds with significant biological activity, potentially enhancing the therapeutic value of the extract.

Tannins and phenols, which are present in both extracts, were found in relatively high concentrations in both the aqueous and ethanol extracts. This is consistent with previous studies indicating that tannins and phenolic compounds are water-soluble, and ethanol can also efficiently extract them (Venkatesh *et al.*, 2016). Tannins and phenols are well-known for their antimicrobial, anti-inflammatory, and antioxidant properties, further supporting the potential therapeutic applications of *Tamarindus indica* leaf extracts.

Saponins, which were present in moderate amounts in the aqueous extract and low in the ethanol extract (+), are another important group of bioactive compounds known for their anti-inflammatory and hypocholesterolemic effects (Brahmachari, 2011). The aqueous extract appears to have a better yield of saponins, which could be beneficial for applications requiring water-soluble compounds.

The absence of steroids in the aqueous extract but their presence in the ethanol extract suggests that steroids are better extracted using organic solvents, aligning with the lipophilic nature of steroid compounds. Steroids are known for their anti-inflammatory and antiallergic effects, making them valuable in therapeutic contexts (Gul *et al.*, 2018).

Effect of Treatments on Parasitaemia Levels

The results presented in *Table 3* illustrate the impact of different treatments on parasitaemia levels over four days. The uninfected control group maintained constant parasitaemia levels of 0.00 ± 0.00 throughout the experiment, confirming the absence of infection and thus providing a baseline for comparison. In contrast, the infected, untreated

control group showed a steady increase in parasitaemia from 11.67 ± 1.53 on Day 1 to 23.00 ± 2.65 on Day 4, indicating progressive disease development due to the lack of intervention.

The group treated with the standard drug showed a marked reduction in parasitaemia, with levels dropping from 11.00 ± 2.00 on Day 1 to 0.55 ± 0.58 by Day 4, resulting in a substantial 95.00% reduction in parasitaemia. This highlights the strong efficacy of the standard drug in controlling *Plasmodium falciparum* infection, which is consistent with findings from previous studies where chloroquine and other antimalarial drugs demonstrated high effectiveness (Klayman, 1985; Dondorp *et al.*, 2020).

The aqueous extract of *Tamarindus indica* also demonstrated a significant antiplasmodial effect, reducing parasitaemia by 82.86% by Day 4, from 11.67 ± 2.52 on Day 1 to 2.00 ± 1.00 by Day 4. This result suggests that the aqueous extract contains active compounds capable of inhibiting the growth of *Plasmodium falciparum*, similar to previous studies that reported the antimalarial potential of aqueous plant extracts (Ahmad *et al.*, 2016).

The ethanol extract of *Tamarindus indica* was slightly more effective than the aqueous extract, with an 85.71% reduction in parasitaemia, from 11.67 ± 1.53 on Day 1 to 1.67 ± 0.58 on Day 4. Ethanol is known to extract a wider range of bioactive compounds, including flavonoids and alkaloids, which may contribute to the higher reduction observed in this group (Brahmachari, 2011; Gul *et al.*, 2018).

CONCLUSION

The study evaluated the antiplasmodial effects of aqueous and ethanol extracts of *Tamarindus indica* leaf against *Plasmodium falciparum* using mice. The results demonstrated that both extracts exhibited significant reductions in parasitaemia levels compared to the untreated control. The ethanol extract showed slightly higher antimalarial activity. Phytochemical screening revealed the presence of important bioactive compounds, such as flavonoids, tannins, and phenols, which are known for their potential antimalarial properties. Overall, the study supports the traditional use of *Tamarindus indica* in managing malaria and highlights its potential as a source of bioactive compounds for the development of alternative antimalarial therapies.

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