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Qualitative phytochemical analysis and *in vitro* activity of ethanol and aqueous leaf extracts of *Tamarindus indica*

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Abstract
Background: Malaria remains a major public health burden, and declining efficacy of existing therapies underscores the need for new antiplasmodial leads. *Tamarindus indica* is widely used in traditional medicine and is rich in secondary metabolites with potential bioactivity.
Objective: To compare the qualitative phytochemical profiles and *in vitro* antiplasmodial activity of ethanol and aqueous leaf extracts of *T. indica*, and to document extraction yields.
Methods: Mature leaves of *T. indica* were authenticated, dried, pulverized, and extracted by Soxhlet using 95% ethanol and distilled water. Crude extracts were concentrated and stored at 4 °C. Standard qualitative tests screened for carbohydrates, glycosides, alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, and phenols. Antiplasmodial activity was evaluated against *Plasmodium falciparum* of positive human blood. After 24 h incubation at 37 °C with each extract, Giemsa-stained thin smears were examined (×100 oil immersion) and parasitemia (%) calculated as infected RBCs/total RBCs × 100. Artemether-lumefantrine served as positive control; solvent served as negative control. Descriptive comparisons were made between extracts.
Results: From 400 g powdered leaves, extraction yields were 4.75% (ethanol) and 2.27% (aqueous). Both extracts contained glycosides, saponins, tannins, terpenoids, steroids, and phenols. Alkaloids and flavonoids were detected only in the ethanol extract, whereas carbohydrates were detected only in the aqueous extract. Parasitemia was reduced to 1.5% with the ethanol extract and 2.5% with the aqueous extract, compared with 0.0% (positive control) and 15.0% (negative control), indicating superior activity of the ethanolic extract.
Conclusion: Leaf extracts of *Tamarindus indica* demonstrated *in vitro* inhibition of *Plasmodium falciparum*, with ethanol extraction yielding more phytochemicals associated with antiplasmodial activity and greater suppression of parasitemia than the aqueous extract. These findings support the ethnomedicinal use of *T. indica* and justify further work on compound isolation, dose-response characterization, cytotoxicity, and *in vivo* efficacy.

Keywords: *Tamarindus indica*, *Plasmodium falciparum*, antiplasmodial, phytochemical screening, Soxhlet extraction, ethanol extract, aqueous extract, parasitemia

Introduction
The search for plant-derived antimalarials remains urgent as resistance threatens first-line artemisinin-based combinations (Hamza *et al*., 2024) [5]. *Tamarindus indica* is a widely used ethnomedicinal species rich in secondary metabolites, including phenolics, flavonoids, tannins, saponins, and alkaloids, implicated in diverse pharmacological effects (Kagoro *et al*., 2022; Adeyemi *et al*., 2020) [6, 1]. Solvent polarity strongly influences extractive yield and the recovery of such bioactives; ethanol frequently provides higher yields of phenolic- and alkaloid-rich fractions relative to water (Ghaly *et al*., 2023; Bairagi *et al*., 2021) [4, 2]. *In vitro* studies have reported *T. indica* leaf extracts with notable activity against *P. falciparum* (Hamza *et al*., 2024) [5]. Building on this evidence, we evaluated qualitative phytochemicals and the *in vitro* antiplasmodial activity of ethanol and aqueous leaf extracts of *T. indica*.

Materials and Methods
Study design and setting
Experimental study evaluating phytochemicals and *in vitro* antiplasmodial activity of *Tamarindus indica* leaf extracts. Laboratory work was conducted at COMAHS-USL and the Pharmacy Board of Sierra Leone Microbiology Laboratory (February-May 2025).

Plant collection, authentication, and processing
Mature leaves of *Tamarindus indica* were harvested in the Western Area Rural District (Sierra Leone), authenticated at COMAHS, air-dried, pulverized, and sieved to uniform powder (final powdered yield: 585 g).

Extraction (Soxhlet)
Powdered leaves (200 g per run) were extracted separately with 95% ethanol or distilled water using Soxhlet apparatus (~6 h, 4-5 siphons/cycle). Concentrated crude extracts were obtained by rotary evaporation and stored at 4 °C.

Qualitative phytochemical screening
Standard tests (Sofowora; Trease & Evans; Harborne) were used to detect carbohydrates, glycosides, alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, and phenols (Adeyemi *et al*., 2020; Kagoro *et al*., 2022) [6, 1].

Antiplasmodial assay
Sterile stock solutions (100 µg/mL) of each extract were prepared and incubated (1 mL *P. falciparum*-positive human blood + 100 µL extract; 37 °C, 24 h). Thin smears were Giemsa-stained and examined (×100 oil immersion). Parasitemia (%) = infected RBCs / total RBCs × 100. Positive control: artemether-lumefantrine (0.0% parasitemia). Negative control: solvents. Methods harmonize with published *in vitro* protocols (Chandel & Bagai, 2011; Hamza *et al*., 2024) [5, 3].

Results
Powdered Plant Parts of *Tamarindus indica* Leaf
The table below presents the calculated weight of the powdered Tamarindus indica leaf. The powdered yield was determined using the formula:

Powdered Weight (g) = Weight of Bottle + Sample − Weight of Bottle

Plant Part	Weight of Bottle (g)	Weight of Bottle + Sample (g)	Powdered Weight (g)
Leaf	215	800	585

Crude Extract Yield by Solvent
The percentage yield of the 95% ethanol and distilled water extracts of *Tamarindus indica* was calculated based on the initial weight of the powdered plant material (400g) and the weight of the crude extracts obtained after solvent evaporation. The total extract yield was 28.09 g, with 19.00 g from ethanol extraction and 9.09 g from aqueous extraction.
The percentage yield was calculated using the formula:

Percentage Yield (%) = (Weight of crude extract / Initial weight of plant material) × 100

Ethanol extract: (19.00 g / 400 g) × 100 = 4.750%

Aqueous extract: (9.09 g / 400 g) × 100 = 2.272%

Total yield: (28.09 g / 400 g) × 100 = 7.022%

The table below summarizes the percentage yields of the extracts:

Solvent Used	Weight of Crude Extract (g)	Percentage Yield (%)
95% Ethanol	19.00	4.750
Distilled Water	9.09	2.272

Phytochemical Screening of *Tamarindus indica* Leaf Extracts

Phytochemicals	95% Ethanol Extract	Distilled Water Extract
Carbohydrate (Molisch, Fehling, Benedict)	-	+
Glycosides	+	+
Alkaloids	+	-
Flavonoids	+	-
Saponins	+	+
Tannins	+	+
Terpenoids	+	+
Steroids	+	+
Phenols	+	+

+= present and - = absent

Parasitemia in Treated plant extracts and Control Groups

Sample / control group	Infected RBCs	Total RBCs Counted	Parasitemia (%)
Ethanol Extract (T. indica)	3	200	(3/200) × 100 = 1.5%
Aqueous Extract (T. indica)	5	200	(5/200) × 100 = 2.5%
Positive Control (Artemether-Lumefantrine)	0	200	(0/200) × 100 = 0.0%
Negative Control (distilled water)	30	200	(30/200) × 100 = 15.0%

Discussion
Ethanol afforded higher crude yield (4.750%) than water (2.272%), consistent with the broader solvation of semi-polar phenolics and alkaloids (Ghaly *et al*., 2023; Bairagi *et al*., 2021) [4, 2]. Phytochemical screening confirmed glycosides, saponins, tannins, terpenoids, steroids, and phenols in both extracts, with alkaloids and flavonoids restricted to the ethanol extract classes repeatedly linked to antiplasmodial mechanisms (Adeyemi *et al*., 2020; Chandel & Bagai, 2011) [1, 3]. *In vitro*, the ethanol extract reduced *P. falciparum* parasitemia to 1.5% (vs. 2.5% for aqueous), mirroring reports of potent leaf-extract activity (Hamza *et al*., 2024) [5]. These data support the ethnopharmacological rationale for *Tamarindus indica* as a candidate for antimalarial lead discovery. Key limitations include single-strain testing, qualitative phytochemistry (no quantitation), and absence of compound isolation or cytotoxicity profiling.

Conclusion
Leaf extracts of *Tamarindus indica* demonstrated notable *in vitro* antiplasmodial effects against *Plasmodium falciparum* with ethanol outperforming water likely due to enriched alkaloid and flavonoid content. The findings justify advanced characterization (HPLC/LC-MS), *in vivo* validation, dose-response determination, and safety assessment to progress *T. indica*-derived antimalarial leads.

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