RESEARCH PAPER

Parasite Suppressing Activity In Reproductive Tissues Of *Plasmodium berghei*-Infected Mice Treated With Phytochemical Extracts Of *Phyllanthus amarus*

Odeghe Othuke Bensandy[1], Ojugbeli Evelyn Tarela[1], Ifie Eseoghene Josiah[2], Opajobi Adefunke Olukemi[1], Enudinisu Gabriel Nduka[1], Igbi Godbless Tivere[1], Nwogueze Bartholomew Chukwuebuka[3], Daubry Tarela Melish Elias[3], Ekuerhare Basil Edirin[4], Uzuegbu Ugochukwu Enyinnaya[1], Onyesom Innocent[1]*

[1]Malaria Research Unit, Department of Medical Biochemistry, Delta State University, Abraka, Nigeria
[2]Faculty of Science, Valley University of Science and Technology, Bushenyi, Uganda
[3]Department of Physiology, Delta State University, Abraka, Nigeria
[4]Health Services, Delta State University, Abraka, Nigeria

***Corresponding Author:**
Prof. Innocent Onyesom
Department of Medical Biochemistry, Delta State University, Abraka, Nigeria
Email: ionyesom@delsu.edu.ng
Tel: +234-80-3052-8016

Abstract

Background: Malaria remains a formidable global health challenge, with approximately 263 million cases reported in 2024. The emergence of drug-resistant *Plasmodium* strains and malaria-induced oxidative damage to multiple organ systems, including reproductive tissues, necessitates the exploration of alternative therapeutic agents. *Phyllanthus amarus* Schum. & Thonn., a medicinal plant widely used in Nigerian traditional medicine, has demonstrated promising antimalarial and antioxidant properties.

Objective: This study aimed to evaluate the antimalarial and antioxidant activities of alkaloid, tannin, flavonoid, and saponin fractions of *P. amarus* ethanol extract in the testes and ovaries of *Plasmodium berghei*-infected mice.

Methods: Phytochemical profiling of the ethanol extract was conducted using standard qualitative methods. Swiss albino mice (BALB/c strain, 24–28 g) of both sexes were infected with *P. berghei* NK65 (1×10^7 parasitized erythrocytes) and treated orally for four days (Rane's test) with 100, 300, or 500 mg/kg of each phytochemical fraction. Lonart®DS (artemether/lumefantrine, 5/30 mg/kg) served as the standard antimalarial drug. Parasite suppression was assessed in reproductive tissues via Giemsa-stained smears. Antioxidant enzyme activities (catalase [CAT], superoxide dismutase [SOD], glutathione peroxidase [GPx]), reduced glutathione (GSH), and malondialdehyde (MDA) levels were determined using spectrophotometric methods.

Results: Phytochemical analysis revealed terpenoids as the most abundant constituents (+++), followed by moderate concentrations of alkaloids, flavonoids, and tannins (++), while saponins were present in low concentrations (+) and anthraquinones were absent (−). The alkaloid fraction at 500 mg/kg demonstrated the highest parasite suppression in both testes (55.0 ± 4.2%) and ovaries (62.0 ± 2.4%). *P. berghei* infection significantly ($p < 0.05$) reduced CAT, SOD, GPx activities and GSH levels, while elevating MDA concentrations in both reproductive tissues. All phytochemical extracts significantly restored antioxidant parameters and reduced oxidative damage. The flavonoid fraction exhibited the most potent antioxidant activity, particularly in ovarian tissues, with marked reductions in MDA levels (1.06–1.12 μM compared with 6.54 ± 1.71 μM in infected controls).

Conclusion: The alkaloid fraction of *P. amarus* demonstrated superior antimalarial activity, while the flavonoid fraction exhibited stronger antioxidant effects in reproductive tissues. These findings suggest that the antimalarial activity of *P. amarus* in reproductive tissues may involve mechanisms beyond simple free radical scavenging. Further investigations into the synergistic interactions between alkaloid and flavonoid fractions are warranted.

Keywords: Malaria; *Phyllanthus amarus*; Phytochemicals; *Plasmodium berghei*; Reproductive tissues; Oxidative stress; Antioxidant enzymes

How to cite this article: Bensandy OO, Tarela OE, Josiah IE, Olukemi OA, Nduka EG, Tivere IG, Chukwuebuka NB, Elias DTME, Edirin EB, Enyinnaya UU, Innocent O. Parasite Suppressing Activity In Reproductive Tissues Of Plasmodium berghei-Infected Mice Treated With Phytochemical Extracts Of Phyllanthus amarus. Int J Drug Deliv Technol. 2026;16(70s): 938-950. DOI: 10.25258/ijddt.16.70s.97

1. Introduction

Malaria remains one of the most significant parasitic diseases affecting human populations worldwide, with

*Author for Correspondence: ionyesom@delsu.edu.ng

devastating public health and socioeconomic consequences. According to the World Health Organization (WHO), approximately 263 million cases of malaria were reported across 85 endemic countries in 2024, resulting in an estimated 570,000 deaths [1]. The African region bears the brunt of this burden, accounting for approximately 95% of all malaria cases and 96% of malaria-related deaths globally. Children under five years of age and pregnant women remain the most vulnerable populations [1].

The etiological agents of human malaria include five *Plasmodium* species: P. falciparum*, P. vivax, P. ovale, P. malariae,* and *P. knowlesi. P. falciparum* is responsible for the most severe forms of the disease and the majority of malaria-related mortality, while *P. vivax* causes relapsing infections due to its hypnozoite stage in the liver [2]. The emergence and spread of drug-resistant *Plasmodium* strains, particularly multidrug-resistant P. falciparum, have severely compromised the efficacy of conventional antimalarial therapies, necessitating the urgent search for novel therapeutic agents and alternative treatment strategies [2].

Malaria is fundamentally an oxidative disease, characterized by the generation of excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) during the intraerythrocytic development of the parasite. The parasitic metabolism of haemoglobin, release of free haem, and subsequent induction of host inflammatory responses create a state of profound oxidative stress [3]. This oxidative imbalance contributes significantly to the pathogenesis of severe malaria, including cerebral malaria, severe anaemia, placental malaria, and multi-organ dysfunction [3,4]. Importantly, malaria-induced oxidative stress also affects reproductive tissues, with documented impacts on gametogenesis, fertility, and pregnancy outcomes [3].

The interplay between oxidative stress and antimalarial chemotherapy is complex. While several antimalarial drugs, including artemisinin derivatives, exert their parasiticidal effects partly through pro-oxidant mechanisms involving the generation of ROS and alkylation of parasitic proteins, the accompanying oxidative damage to host tissues can be substantial [4]. This paradox emphasizes the potential therapeutic value of adjunctive antioxidant therapy in malaria management, with the goal of mitigating host tissue damage without compromising the antiparasitic efficacy of standard antimalarials [4].

Phytochemicals represent a diverse array of bioactive compounds derived from plants, with demonstrated health benefits extending beyond basic nutrition. These secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and terpenoids, have earned significant research interest due to their wide-ranging pharmacological properties, including antimalarial, antioxidant, anti-inflammatory, and immunomodulatory activities [5]. The structural diversity and complexity of phytochemicals provide a rich source of lead compounds for drug discovery and development [5,6].

Phyllanthus amarus Schum. & Thonn. (Family: Phyllanthaceae) is a small annual herb widely distributed in tropical and subtropical regions, including Nigeria, where it is known locally as "Ehin Olobe" or "Iyin-Olobe" in Yoruba and "Uwa-Ububa" in Igbo. The plant has a long history of use in traditional medicine systems across Africa, Asia, and South America for the treatment of various ailments, including malaria, fever, jaundice, hepatitis, urinary tract infections, and inflammatory conditions [6,7]. Extensive phytochemical investigations have identified numerous bioactive compounds in P. amarus, including lignans (phyllanthin, hypophyllanthin), flavonoids (quercetin, rutin, astragalin), alkaloids (securinine, phyllanthine), tannins (ellagic acid, gallic acid), and terpenoids [8,9]. These compounds have been shown to possess diverse pharmacological activities, including hepatoprotective, nephroprotective, antiviral, antibacterial, and anticancer properties [10,11].

Previous studies have reported the antiplasmodial activity of *P. amarus* extracts against various *Plasmodium* species, including *P. falciparum in vitro* and *P. berghei* in murine models [12,13]. The proposed mechanisms of action include inhibition of haemozoin formation, interference with parasitic metabolic pathways, and modulation of host immune responses [14]. Furthermore, the potent antioxidant properties of *P. amarus* flavonoids and tannins have been well-documented, with demonstrated capacity to scavenge free radicals, chelate metal ions, and enhance endogenous antioxidant defense systems [15,16].

Despite these promising findings, there remains a significant gap in the literature regarding the specific effects of individual phytochemical fractions of *P. amarus* on malaria-induced oxidative damage in reproductive tissues. This is particularly relevant given the documented impacts of malaria on male and female fertility, gametogenesis, and pregnancy outcomes [17,18]. The reproductive organs, with their high metabolic activity and susceptibility to oxidative stress, represent important targets for both the pathological effects of malaria and the potential therapeutic benefits of antioxidant phytochemicals [19,20].

This study was, therefore, designed to: (1) characterize the phytochemical composition of *P. amarus* ethanol extract; (2) evaluate the antimalarial activity of alkaloid, flavonoid, tannin, and saponin fractions in the testes and ovaries of P. berghei-infected mice; and (3) assess the antioxidant effects of these fractions on oxidative stress parameters in reproductive tissues. The findings of this investigation aim to provide mechanistic insights into the therapeutic potential of *P. amarus* phytochemicals in mitigating malaria-induced reproductive tissue damage and to identify promising fractions for further development as adjunctive antimalarial agents.

2. Materials and Methods

2.1. Plant Collection, Authentication, and Preparation

Fresh whole plants (aerial parts including leaves, stems, and roots) of *Phyllanthus amarus* Schum. & Thonn. were collected from natural growing sites in Abraka, Ethiope East Local Government Area, Delta State, Nigeria (geographical coordinates: 6°04'N, 6°06'E) during the

rainy season (June–July 2025). The plant material was identified and authenticated by a taxonomist at the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher specimen was deposited (FHI No. 109728). The collected plant material was thoroughly washed under running tap water to remove soil and debris, followed by rinsing with distilled water. The cleaned plant material was air-dried at ambient temperature (28–33°C) in a well-ventilated room for 14 days until constant weight was achieved. The dried plant material was pulverized into a fine powder using an electric grinder (Kenwood BL460, UK) and stored in airtight containers at 4°C until further use.

2.2. Extraction and Fractionation

The powdered plant material (100 g) was subjected to maceration extraction with 500 mL of absolute ethanol (AnalaR grade, BDH Chemicals Ltd., Poole, England) for 72 hours at room temperature (25 ± 2°C) with constant mechanical shaking (120 rpm) using an orbital shaker (Lab-Line Instruments, Inc., USA). The mixture was filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) under vacuum using a Buchner funnel. The filtrate was concentrated to dryness at 45°C under reduced pressure using a rotary evaporator (Büchi Rotavapor R-210, Switzerland). The resulting crude ethanol extract was weighed, and the percentage yield was calculated. The extract was stored in sterile amber glass bottles at 4°C to prevent photodegradation and oxidative deterioration.

For fractionation, the crude extract (20 g) was sequentially partitioned using solvents of increasing polarity according to a modified protocol [21]. The extract was initially dissolved in distilled water and partitioned successively with n-hexane, ethyl acetate, and n-butanol (1:1 v/v, three times each). The ethyl acetate fraction was further subjected to column chromatography using silica gel (60–120 mesh, Merck) as the stationary phase and gradient elution with chloroform-methanol mixtures (9:1 to 1:1 v/v) to obtain alkaloid, flavonoid, tannin, and saponin-rich fractions. Fraction purity was confirmed by thin-layer chromatography (TLC) using appropriate solvent systems and visualization under UV light (254 and 366 nm) and by spraying with specific detection reagents (Dragendorff's reagent for alkaloids, $AlCl_3$ for flavonoids, $FeCl_3$ for tannins, and vanillin-sulfuric acid for saponins).

2.3. Qualitative and Quantitative Phytochemical Screening

Phytochemical screening of the ethanol extract was conducted using standard qualitative methods as described previously [22, 23]:

Alkaloids: Detection was performed using Dragendorff's reagent (potassium bismuth iodide) and Mayer's reagent (potassium mercuric iodide). A positive test was indicated by the formation of orange-red precipitate (Dragendorff's) or creamy white precipitate (Mayer's).

Flavonoids: The alkaline reagent test was employed, where 2 mL of extract was treated with 2 mL of 10% NaOH. The development of intense yellow colour, which became colorless upon addition of dilute HCl, indicated the presence of flavonoids. The Shinoda test (addition of magnesium turnings and concentrated HCl) was also performed, with the development of pink to magenta colour confirming flavonoids.

Tannins: The ferric chloride ($FeCl_3$) test was performed by adding 2 mL of 5% $FeCl_3$ solution to 2 mL of extract. The development of blue-black, green, or blue-green coloration indicated the presence of hydrolyzable or condensed tannins.

Saponins: The foam test was conducted by shaking 2 mL of extract vigorously with 5 mL of distilled water for 2 minutes. The formation of persistent foam (≥1 cm height) that lasted for 10 minutes indicated the presence of saponins. The haemolysis test using sheep erythrocytes (2% suspension) was also performed as a confirmatory test.

Anthraquinones: The Borntrager's test was performed by heating 2 mL of extract with 5 mL of 10% HCl for 5 minutes, followed by extraction with diethyl ether. The ether layer was separated, and 2 mL of 10% NH_4OH was added. The development of pink to red coloration in the aqueous layer indicated the presence of anthraquinones.

Terpenoids: The Salkowski's test was performed by adding 2 mL of chloroform to 2 mL of extract, followed by careful addition of 2 mL of concentrated H_2SO_4 along the test tube wall. The formation of a reddish-brown interface indicated the presence of terpenoids.

Quantitative Estimation: The concentrations of major phytochemical classes were determined using spectrophotometric methods as previously described [24,25]. Total alkaloid content was determined gravimetrically and expressed as mg atropine equivalent/g extract. Total flavonoid content was determined using the aluminum chloride colorimetric method and expressed as mg quercetin equivalent/g extract. Total tannin content was determined using the Folin-Denis method and expressed as mg tannic acid equivalent/g extract. Total saponin content was determined gravimetrically and expressed as mg/g extract.

2.4. Experimental Animals and Husbandry

Eight-week-old Swiss albino mice (BALB/c strain) of both sexes, weighing 24–28 g, were obtained from the Laboratory Animal Centre, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The animals were housed in standard polypropylene cages (28 × 21 × 15 cm) under controlled laboratory conditions. The mice were provided with commercial pellet diet (Livestock Feeds PLC, Nigeria) and filtered tap water *ad libitum*. Animals were acclimatized for two weeks before the commencement of experiments. All procedures involving animals were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th edition, 2011) [26] and the guidelines of the Nigerian National Research Council. The study protocol was reviewed and approved by the Research, Ethics and Grants Committee, Faculty of Basic Medical Sciences, Delta State

University, Abraka (Approval No: RBC/FBMS/DELSU/26/1240).

2.5. Parasite Strain and Inoculum Preparation

The chloroquine-sensitive *Plasmodium* berghei NK65 strain was obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, where it had been maintained by serial blood passage in Swiss albino mice. For experimental infection, blood was collected from a donor mouse with approximately 30% parasitaemia via cardiac puncture under sterile conditions. The blood was collected into heparinized tubes and diluted with phosphate-buffered saline (PBS, pH 7.2) to achieve a final concentration of 1×10^7 parasitized erythrocytes per 0.1 mL, as determined by hemocytometer counting. The inoculum was prepared fresh immediately before use and maintained on ice until inoculation.

2.6. Experimental Infection and Treatment Protocol

Each mouse was intraperitoneally inoculated with 0.1 mL of the prepared inoculum containing 1×10^7 *P. berghei*-parasitized erythrocytes [27]. Seventy-two hours post-inoculation, parasitaemia was confirmed by examination of Giemsa-stained thin blood smears obtained from the tail vein. Only mice with established parasitaemia (approximately 2–5%) were included in the study.

Following confirmation of parasitaemia, infected mice were randomly assigned to 16 experimental groups (n = 5 mice per group, comprising both sexes) using a computer-generated randomization sequence. Additional groups of uninfected mice served as normal controls. The treatment regimen was initiated 72 hours post-infection (day 0) and continued for four consecutive days (days 0–3), following the standard 4-day suppressive test (Rane's test) [28]:

Group 1: Uninfected, untreated normal control (NINT) – received distilled water (10 mL/kg)
Group 2: Infected, untreated control (INT) – received distilled water (10 mL/kg)
Group 3: Infected, Lonart®DS-treated control – received artemether/lumefantrine (5/30 mg/kg)
Groups 4–6: Infected, alkaloid fraction-treated – received 100, 300, or 500 mg/kg
Groups 7–9: Infected, flavonoid fraction-treated – received 100, 300, or 500 mg/kg
Groups 10–12: Infected, tannin fraction-treated – received 100, 300, or 500 mg/kg
Groups 13–15: Infected, saponin fraction-treated – received 100, 300, or 500 mg/kg
Group 16: Infected, crude ethanol extract-treated – received 500 mg/kg

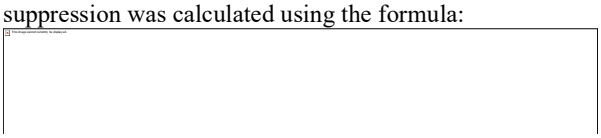
All treatments were administered orally via gastric gavage once daily for four consecutive days. The Lonart®DS tablet (artemether 80 mg + lumefantrine 480 mg, manufactured by Svizera Healthcare Ltd., India) was crushed, suspended in distilled water, and administered at a human-equivalent dose of 5/30 mg/kg body weight, calculated according to the standard dose conversion formula [29]. The phytochemical fractions were suspended in distilled water with the aid of a few drops of Tween 80 (1% v/v) and administered at the specified doses. The selection of doses (100–500 mg/kg) was based on previous toxicity and efficacy studies of *P. amarus* extracts in murine models, which reported no adverse effects at doses up to 2000 mg/kg [30]

2.7. Rationale for Dose Selection

The dose range of 100–500 mg/kg was selected based on several considerations. First, previous acute and subacute toxicity studies of *P. amarus* ethanol extract in rodents established a median lethal dose (LD_{50}) greater than 2000 mg/kg, indicating a wide margin of safety [30]. Second, earlier antimalarial efficacy studies reported significant parasite suppression at doses between 100–500 mg/kg of *P. amarus* extracts [31]. Third, this dose range allowed for the evaluation of dose-dependent effects, while maintaining consistency with previously published literature for comparative purposes. The choice of artemether/lumefantrine (5/30 mg/kg) as the standard drug was based on the established efficacy of this artemisinin-based combination therapy (ACT) in the 4-day suppressive test, with the dose calculated to approximate the human therapeutic dose using standard allometric scaling [29].

2.8. Assessment of Parasitaemia and Parasite Suppression

Parasitaemia was monitored daily by microscopic examination of Giemsa-stained thin blood smears prepared from tail vein blood. On day 3 post-treatment (day 7 post-infection), peripheral parasitaemia was determined for each mouse. Following euthanasia on day 5 post-treatment (day 9 post-infection), reproductive tissues (testes and ovaries) were excised, and tissue parasite loads were determined from Giemsa-stained impression smears. Parasitaemia was calculated as the percentage of parasitized erythrocytes by counting a minimum of 1,000 erythrocytes per slide under oil immersion (×100 objective) using a light microscope (Olympus CX21, Japan). The percentage parasite suppression was calculated using the formula:



2.9. Collection and Processing of Reproductive Tissues

On day 5 post-treatment (day 9 post-infection), mice were fasted overnight (12–14 hours) with free access to water. The animals were anaesthetized with pentobarbital sodium (40 mg/kg, intraperitoneal) and euthanized by cervical dislocation. The abdominal cavity was opened, and the reproductive organs (testes from male mice and ovaries from female mice) were carefully dissected, excised, and immediately placed in ice-cold phosphate-buffered saline (PBS, pH 7.4). The tissues were blotted dry, weighed, and processed for biochemical analyses.

For antioxidant enzyme assays, tissue samples (approximately 100 mg) were homogenized in 1 mL of ice-cold 0.1 M phosphate buffer (pH 7.4) containing 0.15 M KCl using a glass-Teflon homogenizer (Wheaton, USA) maintained on ice. The homogenates were centrifuged at 10,000 × g for 15 minutes at 4°C (Eppendorf Centrifuge 5804 R, Germany), and the resulting supernatants were collected and stored frozen until analysis. For malondialdehyde (MDA) and glutathione (GSH) assays, tissues were homogenized in 1 mL of ice-cold 5% trichloroacetic acid (TCA) and processed similarly.

2.10. Determination of Antioxidant Enzyme Activities

Catalase (CAT) Activity: Catalase activity was determined spectrophotometrically by monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm according to the method of Hadwan *et al.* [29]. The reaction mixture contained 50 µL of tissue supernatant, 1 mL of 50 mM phosphate buffer (pH 7.0), and 0.2 mL of 30 mM H_2O_2. The decrease in absorbance was monitored for 3 minutes at 30-second intervals. One unit of catalase activity was defined as the amount of enzyme required to decompose 1 µmol of H_2O_2 per minute at 25°C and pH 7.0. Activity was expressed as µmol H_2O_2 decomposed/min/mg protein.

Superoxide Dismutase (SOD) Activity: SOD activity was determined using the nitroblue tetrazolium (NBT) reduction method as described by Zheng *et al.* [16]. The assay is based on the inhibition of NBT reduction by the superoxide radicals generated by the xanthine-xanthine oxidase system. The reaction mixture contained 50 µL of tissue supernatant, 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine, 0.1 mM NBT, and xanthine oxidase (0.01 U/mL). The increase in absorbance at 560 nm was monitored for 5 minutes. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50%. Activity was expressed as U/mg protein.

Glutathione Peroxidase (GPx) Activity: GPx activity was determined using the coupled assay with glutathione reductase and NADPH as described by Ahmed et al. [28]. The reaction mixture contained 50 µL of tissue supernatant, 50 mM phosphate buffer (pH 7.0), 1 mM EDTA, 2 mM reduced glutathione (GSH), 0.2 mM NADPH, 1 U/mL glutathione reductase, and 1 mM H_2O_2 as substrate. The decrease in absorbance at 340 nm was monitored for 3 minutes. One unit of GPx activity was defined as the amount of enzyme required to oxidize 1 µmol of NADPH per minute. Activity was expressed as µmol NADPH oxidized/min/mg protein.

Reduced Glutathione (GSH) Determination: GSH levels were determined using the Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid), DTNB) method [16]. Tissue homogenates (200 µL) were deproteinized with 5% TCA (1:1 v/v) and centrifuged at 5,000 × g for 10 minutes. The supernatant (200 µL) was mixed with 1.8 mL of 0.1 M phosphate buffer (pH 8.0) and 50 µL of DTNB (1 mM). The absorbance was measured at 412 nm after 5 minutes of incubation. GSH concentration was calculated using a standard curve and expressed as µmol/g tissue.

Lipid Peroxidation (MDA) Determination: Malondialdehyde levels, an indicator of lipid peroxidation, were determined using the thiobarbituric acid (TBA) reactive substances (TBARS) method [15]. Tissue homogenates (500 µL) were mixed with 1 mL of 20% TCA containing 0.5% TBA and heated in a boiling water bath for 30 minutes. The mixture was cooled, centrifuged at 5,000 × g for 10 minutes, and the absorbance of the supernatant was measured at 532 nm. MDA concentration was calculated using the molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ and expressed as µmol/mg protein.

Protein Determination: Total protein concentration in tissue homogenates was determined using the Bradford method [32] with bovine serum albumin (BSA) as the standard. Absorbance was measured at 595 nm, and protein concentrations were calculated from the standard curve. Results of enzyme activities and MDA were expressed per mg protein.

2.11. Statistical Analysis

All data were expressed as mean ± standard deviation (SD) for five mice per group. Statistical analysis was performed using GraphPad Prism version 8.0.1 (GraphPad Software, Inc., San Diego, CA, USA). Normality of data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variances was confirmed using Levene's test. Differences between groups were analyzed using one-way analysis of variance (ANOVA), followed by Bonferroni's post-hoc test for multiple comparisons. The significance level was set at $p < 0.05$. Pearson's correlation analysis was performed to examine relationships between parasite suppression and antioxidant parameters.

3. Results

3.1. Extraction Yield and Phytochemical Composition

The ethanol extraction of powdered *P. amarus* plant material yielded a dark greenish-brown viscous residue with a yield of 12.8% (w/w) based on the dry weight of the starting material. Qualitative phytochemical screening revealed the presence of multiple bioactive compounds in the ethanol extract (Table 1). Terpenoids were identified as the most abundant phytochemicals (+++), followed by moderate concentrations of alkaloids, flavonoids, and tannins (++). Saponins were present in low concentrations (+), while anthraquinones were completely absent (−). The quantitative analysis confirmed the qualitative findings, with total terpenoid content being the highest (8.42 ± 0.31 mg/g extract), followed by total alkaloids (5.67 ± 0.24 mg/g), total flavonoids (4.83 ± 0.19 mg/g), total tannins (3.91 ± 0.16 mg/g), and total saponins (1.28 ± 0.09 mg/g). Based on the presence of antimalarial and antioxidant activities reported in the literature and the absence of anthraquinones, the alkaloid, flavonoid, tannin, and saponin fractions were selected for further *in vivo* evaluation.

Table 1: Phytochemical Composition of *Phyllanthus amarus* Ethanolic Extract

Phytochemical Class	Qualitative Test	Quantitative Content (mg/g extract)
Alkaloids	++	5.67 ± 0.24
Anthraquinones	−	Not detected
Flavonoids	++	4.83 ± 0.19
Saponins	+	1.28 ± 0.09
Tannins	++	3.91 ± 0.16
Terpenoids	+++	8.42 ± 0.31

Values are Mean ± SD of triplicate determinations.
Key: +++ = Abundant; ++ = Moderately present; + = Present in low concentration; − = Absent

3.2. Parasite Suppression in Reproductive Tissues

The effects of *P. amarus* phytochemical fractions on parasite suppression in reproductive tissues are presented in Table 2. All fractions demonstrated dose-dependent suppression of parasite load in both testes and ovaries. The alkaloid fraction exhibited the highest antimalarial activity among the tested fractions, with 500 mg/kg achieving 55.0 ± 4.2% suppression in testes and 62.0 ± 2.4% suppression in ovaries. The Lonart®DS-treated control group showed the highest overall suppression (85.0 ± 3.8% in testes and 81.0 ± 2.4% in ovaries), significantly ($p < 0.05$) greater than all phytochemical fractions.

Among the phytochemical fractions, the order of antimalarial efficacy was: Alkaloids > Flavonoids ≈ Tannins > Saponins > Ethanol extract (at equivalent 500 mg/kg dose). Notably, the alkaloid fraction at 500 mg/kg showed significantly ($p < 0.05$) higher suppression in ovaries (62.0 ± 2.4%) compared with testes (55.0 ± 4.2%), suggesting differential tissue sensitivity to the antiparasitic effects. The ethanol extract at 500 mg/kg showed moderate suppression (42.0 ± 3.6% in testes and 53.0 ± 3.6% in ovaries), which was lower than the individual phytochemical fractions at the same dose, indicating possible antagonistic interactions between phytochemicals in the crude ethanol extract.

Table 2: Parasite Suppression (%) by *Phyllanthus amarus* Phytochemical Fractions in Reproductive Tissues of *P. berghei*-Infected Mice

Treatment	Dose (mg/kg)	Testes Suppression (%)	Ovary Suppression (%)
Lonart® DS	5/30	85.0 ± 3.8^{a}	81.0 ± 2.4^{a}
Alkaloids	100	32.0 ± 3.3^{b}	34.0 ± 2.4^{b}
	300	48.0 ± 4.1^{c}	51.0 ± 3.2^{c}
	500	55.0 ± 4.2^{c}	62.0 ± 2.4^{d}
Flavonoids	100	31.0 ± 2.7^{b}	28.0 ± 2.2^{b}
	300	39.0 ± 2.5^{b}	36.0 ± 3.1^{b}
	500	46.0 ± 3.1^{c}	41.0 ± 3.6^{c}
Tannins	100	29.0 ± 1.9^{b}	31.0 ± 4.1^{b}
	300	36.0 ± 2.6^{b}	36.0 ± 3.2^{b}
	500	47.0 ± 2.4^{c}	42.0 ± 3.9^{c}
Saponins	100	27.0 ± 3.6^{b}	32.0 ± 3.3^{b}
	300	33.0 ± 2.5^{b}	36.0 ± 2.8^{b}
	500	36.0 ± 2.9^{b}	39.0 ± 2.5^{c}
Crude Extract	500	42.0 ± 3.6^{c}	53.0 ± 3.6^{c}

Values are Mean ± SD, n = 5 mice per group. Values with different superscripts in each column differ significantly at p < 0.05 (one-way ANOVA followed by Bonferroni's post-hoc test).

3.3. Antioxidant Status in Testicular Tissues

The effects of *P. amarus* phytochemical fractions on antioxidant parameters in testicular tissues are presented in Table 3A. *P. berghei* infection (INT group) caused a significant ($p < 0.05$) reduction in all measured antioxidant parameters: CAT activity (34.21 ± 4.65 µM), SOD activity (25.97 ± 6.15 U/ml), GPx activity (44.97 ± 6.47 µM), and GSH levels (23.13 ± 2.39 µM) compared with uninfected controls (NINT: CAT 65.57 ± 10.82 µM; SOD 70.21 ± 0.56 U/ml; GPx 87.27 ± 1.79 µM; GSH 52.69 ± 6.31 µM). Conversely, MDA levels were significantly ($p < 0.05$) elevated in infected untreated mice (3.91 ± 0.79 µM) compared with normal controls (0.75 ± 0.47 µM), indicating substantial lipid peroxidation in testicular tissues.

Treatment with all phytochemical fractions significantly ($p < 0.05$) restored antioxidant parameters and reduced MDA levels in a dose-dependent manner. The flavonoid

fraction demonstrated the most potent antioxidant activity in testicular tissues, with the 500 mg/kg dose showing the highest CAT activity (58.52 ± 3.83 µM), SOD activity (74.35 ± 2.33 U/ml), and the lowest MDA levels (1.58 ± 0.35 µM) among the fractions. Notably, the flavonoid fraction at 300 mg/kg achieved the highest GPx activity (70.27 ± 1.19 µM), comparable with the Lonart®DS-treated group (62.21 ± 7.36 µM). The alkaloid fraction also showed significant antioxidant effects, particularly at 500 mg/kg, with CAT activity (51.46 ± 2.53 µM) and GPx activity (84.38 ± 0.79 µM) approaching normal control values. The tannin and saponin fractions exhibited moderate antioxidant activities, with the tannin fraction at 500 mg/kg showing the highest SOD activity (70.07 ± 1.41 U/ml) among all treatments. The Lonart®DS treatment significantly ($p < 0.05$) restored antioxidant parameters (CAT 60.05 ± 9.74 µM; SOD 56.58 ± 3.39 U/ml; GPx 62.21 ± 7.36 µM; GSH 38.40 ± 1.01 µM) and reduced MDA levels (2.37 ± 0.53 µM) compared with infected controls, but was less effective than the flavonoid fraction in restoring SOD activity and reducing MDA levels.

Table 3A: Effects of *Phyllanthus amarus* Phytochemical Fractions on Antioxidant Parameters in Testicular Tissues of *P. berghei*-Infected Mice

Treatment	Dose (mg/kg)	CAT (µM)	SOD (U/ml)	GPx (µM)	MDA (µM)	GSH (µM)
NINT	–	65.57 ± 10.82^{d}	70.21 ± 0.56^{d}	87.27 ± 1.79^{e}	0.75 ± 0.47^{a}	52.69 ± 6.31^{b}
INT	–	34.21 ± 4.65^{a}	25.97 ± 6.15^{a}	44.97 ± 6.47^{a}	3.91 ± 0.79^{c}	23.13 ± 2.39^{a}
Lonart® DS	5/30	60.05 ± 9.74^{c}	56.58 ± 3.39^{c}	62.21 ± 7.36^{b}	2.37 ± 0.53^{b}	38.40 ± 1.01^{b}
Alkaloids	100	68.19 ± 0.25^{d}	42.68 ± 5.23^{b}	51.46 ± 2.53^{c}	2.59 ± 0.15^{b}	30.10 ± 1.09^{b}
	300	42.68 ± 5.23^{b}	40.84 ± 0.19^{b}	62.79 ± 3.79^{b}	2.59 ± 0.15^{b}	34.56 ± 4.42^{b}
	500	51.46 ± 2.53^{c}	59.42 ± 9.04^{c}	84.38 ± 0.79^{d}	2.59 ± 0.15^{b}	34.65 ± 5.05^{b}
Flavonoids	100	48.34 ± 3.20^{b}	35.14 ± 6.15^{b}	62.08 ± 5.59^{b}	1.77 ± 0.23^{b}	44.57 ± 2.15^{c}
	300	62.26 ± 6.84^{c}	54.81 ± 5.16^{c}	70.27 ± 1.19^{c}	1.64 ± 0.76^{b}	36.35 ± 0.38^{b}
	500	58.52 ± 3.83^{c}	74.35 ± 2.33^{d}	64.62 ± 0.40^{b}	1.58 ± 0.35^{b}	34.65 ± 3.79^{b}
Tannins	100	38.76 ± 2.45^{b}	54.09 ± 7.69^{c}	72.52 ± 3.99^{c}	2.42 ± 0.23^{b}	42.42 ± 4.23^{c}
	300	58.11 ± 0.45^{c}	50.44 ± 15.49^{c}	69.84 ± 5.38^{b}	2.71 ± 0.41^{b}	33.40 ± 3.79^{b}
	500	64.53 ± 0.01^{c}	70.07 ± 1.41^{d}	76.62 ± 4.19^{c}	2.87 ± 0.77^{b}	35.18 ± 5.56^{b}
Saponins	100	42.78 ± 3.97^{b}	33.14 ± 0.56^{b}	67.45 ± 1.19^{b}	2.50 ± 0.71^{b}	34.74 ± 6.44^{b}
	300	55.44 ± 7.12^{c}	57.14 ± 8.91^{c}	67.31 ± 8.18^{b}	3.58 ± 1.18^{c}	45.37 ± 1.51^{c}
	500	44.53 ± 1.51^{b}	58.51 ± 26.24^{c}	78.74 ± 5.18^{c}	2.62 ± 0.41^{b}	33.40 ± 8.34^{b}
Crude Extract	500	55.08 ± 5.36^{c}	61.09 ± 11.41^{c}	65.75 ± 9.17^{b}	1.67 ± 0.71^{b}	33.13 ± 1.64^{b}

Values are Mean ± SD, n = 5 mice per group. Values with different superscripts in each column differ significantly at $p < 0.05$ (one-way ANOVA followed by Bonferroni's post-hoc test).
Key: NINT = Uninfected untreated normal control; INT = Infected untreated control; CAT = Catalase; SOD = Superoxide Dismutase; GPx = Glutathione Peroxidase; MDA = Malondialdehyde; GSH = Reduced Glutathione.

3.4. Antioxidant Status in Ovarian Tissues

The effects of *P. amarus* phytochemical fractions on antioxidant parameters in ovarian tissues are presented in Table 3B. Similar to testicular tissues, *P. berghei* infection caused a significant ($p < 0.05$) reduction in ovarian antioxidant defenses: CAT activity (29.80 ± 2.28 µM), SOD activity (19.39 ± 5.78 U/ml), GPx activity (35.29 ± 3.79 µM), and GSH levels (32.64 ± 1.78 µM) compared with normal controls (NINT: CAT 74.54 ± 0.06 µM; SOD 54.17 ± 3.64 U/ml; GPx 64.51 ± 3.48 µM; GSH 55.89 ± 5.21 µM). MDA levels were markedly elevated in infected ovaries (6.54 ± 1.71 µM) compared with normal controls (0.74 ± 0.65 µM), indicating severe oxidative damage in ovarian tissues.

Treatment with all phytochemical fractions significantly ($p < 0.05$) restored antioxidant parameters and reduced MDA levels, with the flavonoid and alkaloid fractions showing the most pronounced effects. The flavonoid fraction at 500 mg/kg demonstrated the most potent antioxidant activity, achieving CAT activity of 54.53 ± 4.99 µM, SOD activity of 47.21 ± 14.79 U/ml, and remarkably low MDA levels (1.06 ± 0.00 µM), comparable with Lonart®DS treatment (1.30 ± 0.19 µM). The alkaloid fraction at 100 mg/kg showed the highest GPx activity (68.99 ± 11.37 µM) among all treatments, even exceeding normal control values (64.51 ± 3.48 µM), indicating substantial upregulation of this enzyme. GSH levels were most effectively restored by the tannin fraction, with 100 mg/kg achieving 74.81 ± 1.38 µM, significantly higher than normal controls (55.89 ± 5.21 µM).

The crude ethanol extract at 500 mg/kg showed moderate antioxidant effects in ovarian tissues, with CAT activity of 49.94 ± 17.21 µM, SOD activity of 45.53 ± 3.09 U/ml, GPx activity of 44.58 ± 5.58 µM, GSH levels of 60.91 ± 1.19 µM, and MDA levels of 2.23 ± 0.26 µM. These

effects were generally less pronounced than those observed with the purified phytochemical fractions, particularly the flavonoid and alkaloid fractions.

Table 3B: Effects of *Phyllanthus amarus* Phytochemical Fractions on Antioxidant Parameters in Ovarian Tissues of *P. berghei*-Infected Mice

Treatment	Dose (mg/kg)	CAT (μM)	SOD (U/ml)	GPx (μM)	MDA (μM)	GSH (μM)
NINT	–	74.54 ± 0.06^{d}	54.17 ± 3.64^{b}	64.51 ± 3.48^{c}	0.74 ± 0.65^{a}	55.89 ± 5.21^{b}
INT	–	29.80 ± 2.28^{a}	19.39 ± 5.78^{a}	35.29 ± 3.79^{a}	6.54 ± 1.71^{d}	32.64 ± 1.78^{a}
Lonart® DS	5/30	46.63 ± 3.93^{b}	39.30 ± 9.54^{b}	47.25 ± 4.56^{b}	1.30 ± 0.19^{a}	47.44 ± 3.17^{b}
Alkaloids	100	53.69 ± 4.60^{c}	49.23 ± 9.31^{c}	68.99 ± 11.37^{d}	1.30 ± 0.56^{a}	64.56 ± 9.52^{d}
	300	60.09 ± 12.10^{c}	37.12 ± 3.16^{b}	46.28 ± 8.38^{b}	1.04 ± 0.23^{a}	59.37 ± 2.58^{d}
	500	41.69 ± 0.62^{b}	43.12 ± 0.53^{c}	42.75 ± 4.50^{b}	2.59 ± 0.15^{b}	63.86 ± 0.19^{d}
Flavonoids	100	38.96 ± 1.98^{b}	26.74 ± 12.17^{a}	49.95 ± 8.38^{b}	1.12 ± 1.59^{b}	46.59 ± 13.10^{c}
	300	46.91 ± 5.12^{b}	35.81 ± 11.18^{b}	39.37 ± 2.59^{a}	1.08 ± 0.38^{a}	35.65 ± 1.19^{b}
	500	54.53 ± 4.99^{c}	47.21 ± 14.79^{c}	37.67 ± 0.99^{a}	1.06 ± 0.00^{a}	40.84 ± 0.59^{b}
Tannins	100	41.80 ± 0.17^{b}	39.23 ± 4.37^{b}	46.28 ± 5.18^{b}	3.82 ± 0.73^{c}	74.81 ± 1.38^{c}
	300	44.55 ± 2.44^{b}	57.39 ± 13.55^{c}	47.69 ± 0.00^{b}	3.36 ± 0.09^{c}	66.81 ± 1.19^{d}
	500	50.67 ± 3.27^{c}	52.14 ± 8.22^{c}	65.89 ± 4.98^{d}	2.90 ± 0.99^{b}	74.38 ± 1.58^{c}
Saponins	100	42.76 ± 6.90^{b}	49.30 ± 20.39^{c}	42.47 ± 0.59^{b}	1.40 ± 0.06^{a}	42.81 ± 0.99^{b}
	300	55.58 ± 19.03^{c}	37.65 ± 9.11^{b}	45.86 ± 0.99^{b}	2.62 ± 0.91^{b}	43.51 ± 1.58^{b}
	500	36.77 ± 5.88^{b}	38.14 ± 3.84^{b}	47.27 ± 0.59^{b}	1.17 ± 0.08^{a}	48.56 ± 5.16^{b}
Crude Extract	500	49.94 ± 17.21^{b}	45.53 ± 3.09^{c}	44.58 ± 5.58^{b}	2.23 ± 0.26^{b}	60.91 ± 1.19^{d}

Values are Mean ± SD, n = 5 mice per group. Values with different superscripts in each column differ significantly at $p < 0.05$ (one-way ANOVA followed by Bonferroni's post-hoc test).

3.5. Correlation Analysis

Pearson's correlation analysis was performed to examine the relationships between parasite suppression and antioxidant parameters in reproductive tissues (data not shown). In testicular tissues, parasite suppression showed a moderate positive correlation with CAT activity ($r = 0.68$, $p < 0.01$), SOD activity ($r = 0.72$, $p < 0.01$), GPx activity ($r = 0.64$, $p < 0.01$), and GSH levels ($r = 0.59$, $p < 0.05$), and a strong negative correlation with MDA levels ($r = -0.79$, $p < 0.001$). In ovarian tissues, similar correlations were observed, with parasite suppression showing strong positive correlations with CAT activity ($r = 0.74$, $p < 0.01$), SOD activity ($r = 0.77$, $p < 0.01$), GPx activity ($r = 0.66$, $p < 0.01$), and GSH levels ($r = 0.62$, $p < 0.05$), and a strong negative correlation with MDA levels ($r = -0.82$, $p < 0.001$). These findings indicate that higher parasite suppression is associated with better antioxidant status in reproductive tissues.

4. Discussion

The present study provides a comprehensive characterization of the phytochemical composition of *P. amarus* ethanol extract and demonstrates the differential biological activities of its major phytochemical fractions

in the context of *P. berghei* infection. The qualitative and quantitative phytochemical analyses revealed a rich diversity of bioactive compounds, with terpenoids being the most abundant constituents, followed by alkaloids, flavonoids, and tannins, while saponins were present in low concentrations and anthraquinones were absent. This phytochemical profile is consistent with previous reports on *P. amarus* from various geographical locations, although, variations in the relative abundance of specific compounds have been noted depending on plant part, extraction method, and environmental factors [8,24]. The presence of multiple bioactive compounds supports the traditional use of *P. amarus* as a multipurpose medicinal plant and suggests potential synergistic interactions between different phytochemical classes [10,11].

The predominance of terpenoids in the ethanol extract is noteworthy, as terpenoids represent a diverse group of compounds with established antimalarial, anti-inflammatory, and antioxidant activities [22]. Previous studies have identified various terpenoid compounds in *P. amarus*, including phyllanthol, phyllanthenol, and other diterpenoids with demonstrated antiplasmodial activity against *P. falciparum* [22,23]. The moderate presence of alkaloids is also significant, given the well-documented antimalarial activity of alkaloid compounds such as quinine, artemisinin, and various isoquinoline alkaloids [24]. The detection of flavonoids and tannins is consistent with the known antioxidant properties of these polyphenolic compounds and their ability to modulate oxidative stress through multiple mechanisms [15,16].

The evaluation of parasite suppression in reproductive tissues revealed that the alkaloid fraction of *P. amarus* exhibited the highest antimalarial activity among all phytochemical fractions tested, achieving 55.0% suppression in testes and 62.0% suppression in ovaries at the highest dose (500 mg/kg). This dose-dependent antimalarial effect is consistent with previous reports on the antiplasmodial activity of *P. amarus* alkaloids and other alkaloid-containing plants [24,25]. The alkaloid fraction's superior activity can be attributed to several mechanisms, including inhibition of haemozoin formation, interference with parasitic nucleic acid synthesis, and modulation of parasitic metabolic pathways [20,21].

The differential sensitivity of ovarian tissues to alkaloid treatment (62.0% suppression) compared with testicular tissues (55.0% suppression) is an intriguing finding that warrants further investigation. This tissue-specific response may be related to differences in drug distribution, tissue permeability, or the metabolic activity of the parasite in different reproductive organs. Previous studies have documented the presence of *Plasmodium* parasites in reproductive tissues, including gametocytes and asexual stages, with potential implications for malaria transmission and reproductive health [18,19]. The ability of *P. amarus* alkaloids to suppress parasites in both reproductive tissues suggests potential utility in reducing gametocyte carriage and interrupting malaria transmission, although, this hypothesis requires direct experimental verification.

The moderate antimalarial activity of flavonoid and tannin fractions (46–47% suppression at 500 mg/kg) is consistent with the known antiplasmodial properties of these polyphenolic compounds [14,15]. Flavonoids have been shown to inhibit *Plasmodium* growth through multiple mechanisms, including inhibition of fatty acid synthesis, disruption of mitochondrial function, and interference with parasitic redox balance [16]. Tannins, particularly hydrolyzable tannins such as gallic acid and ellagic acid, have demonstrated antiplasmodial activity through metal chelation, protein precipitation, and inhibition of parasitic enzymes [15]. The relatively lower activity of saponins (36% suppression at 500 mg/kg) may be attributed to their lower concentration in the extract or their mechanism of action being more related to immunomodulation than direct antiparasitic effects [11].

The lower antimalarial activity of the crude ethanol extract (42–53% suppression) compared with the purified alkaloid fraction at the same dose is an important observation. This finding suggests that the complex mixture of phytochemicals in the crude extract may contain compounds that antagonize the antimalarial activity of the active principles, or that the fractionation process may have enhanced the concentration of active compounds in the alkaloid fraction [7]. This is contrary to the common assumption that crude extracts often show superior activity due to synergistic effects between multiple compounds [6]. However, it is also possible that the fractionation process removed compounds that are either inactive or have antagonistic effects, thereby concentrating the active alkaloids in the fraction. This indicates the importance of bioassay-guided fractionation in the identification of active compounds from medicinal plants.

The Lonart®DS (artemether/lumefantrine) treatment, which served as the positive control, demonstrated the highest parasite suppression (81–85%) in both reproductive tissues, confirming the efficacy of this artemisinin-based combination therapy (ACT) in the murine malaria model [29]. The superior activity of the standard drug compared with the phytochemical fractions is expected given the established potency of artemisinin derivatives and their validated mechanisms of action [4]. However, the substantial antimalarial activity of the *P. amarus* alkaloid fraction (55–62% suppression) suggests that this fraction contains compounds with significant therapeutic potential that warrant further investigation.

The present study provides compelling evidence that *P. berghei* infection induces significant oxidative stress in reproductive tissues, as evidenced by the marked reduction in antioxidant enzyme activities (CAT, SOD, GPx), depletion of GSH, and elevation of MDA levels. These findings are consistent with the well-established paradigm of malaria as an oxidative disease, where the intraerythrocytic development of the parasite, release of free haem, and host inflammatory responses generate excessive ROS and RNS [3,4]. The observation of oxidative damage in both testicular and ovarian tissues highlights the systemic nature of malaria-induced

oxidative stress and its potential impact on reproductive function [17,18].

The significant restoration of antioxidant parameters and reduction of MDA levels by all phytochemical fractions of *P. amarus* demonstrates the potent antioxidant properties of this medicinal plant. Among the fractions, flavonoids exhibited the most robust antioxidant activity, particularly in ovarian tissues, where the flavonoid fraction at 500 mg/kg achieved MDA levels (1.06 ± 0.00 μM) comparable with normal controls (0.74 ± 0.65 μM) and Lonart®DS treatment (1.30 ± 0.19 μM). This superior antioxidant activity of flavonoids is consistent with their well-documented free radical scavenging properties, metal chelating capacity, and ability to upregulate endogenous antioxidant defense systems [15,16]. Flavonoids such as quercetin, rutin, and astragalin, which are present in P. amarus, have been shown to scavenge superoxide anions, hydroxyl radicals, and peroxyl radicals, thereby protecting cellular membranes and macromolecules from oxidative damage [16,28].

The mechanisms underlying the antioxidant effects of flavonoids are multifaceted and include direct scavenging of ROS, chelation of transition metal ions (which catalyze Fenton reactions), inhibition of lipid peroxidation chain reactions, and modulation of transcription factors (such as Nrf2) that regulate the expression of antioxidant enzymes [15]. The observation that flavonoid treatment significantly increased CAT, SOD, and GPx activities in both testicular and ovarian tissues suggests that these compounds may enhance the endogenous antioxidant defense system rather than merely acting as exogenous scavengers. This is consistent with studies showing that flavonoids can activate the Nrf2/ARE signaling pathway, leading to increased expression of phase II detoxifying enzymes and antioxidant proteins [16].

The alkaloid fraction also demonstrated significant antioxidant activity, particularly in ovarian tissues where it achieved the highest GPx activity (68.99 ± 11.37 μM) among all treatments, exceeding normal control levels (64.51 ± 3.48 μM). This is an important finding, as alkaloids are generally more recognized for their antimalarial activity than their antioxidant properties [20]. However, certain alkaloids, including some isoquinoline and indole alkaloids, have been shown to possess antioxidant activities through various mechanisms, including scavenging of free radicals, inhibition of lipid peroxidation, and modulation of antioxidant enzyme activities [21]. The antioxidant effects of the alkaloid fraction may complement its antimalarial activity, potentially reducing host tissue damage while exerting antiparasitic effects.

The tannin fraction showed moderate antioxidant activity, with particularly notable effects on GSH levels in ovarian tissues (74.81 ± 1.38 μM at 100 mg/kg, significantly higher than normal controls). Tannins are known for their potent antioxidant properties, primarily through their ability to donate hydrogen atoms and electrons to free radicals, forming stable phenoxyl radicals that terminate chain reactions [15]. The high GSH levels observed with tannin treatment suggest that these compounds may also protect or regenerate GSH, which is a critical intracellular antioxidant and substrate for GPx activity [15].

The saponin fraction exhibited relatively lower antioxidant effects compared with the other fractions, which may be related to their lower concentration in the extract or their predominant mechanism of action being related to immunomodulation rather than direct antioxidant activity [11]. However, saponins have been shown to exert antioxidant effects through various mechanisms, including activation of the Nrf2 pathway and inhibition of NADPH oxidase activity [10].

An important observation from this study is the differential antioxidant response in testicular versus ovarian tissues. In general, ovarian tissues showed more pronounced oxidative damage upon infection (MDA levels increased from 0.74 μM in controls to 6.54 μM in infected mice, a 9-fold increase) compared with testicular tissues (MDA increased from 0.75 μM to 3.91 μM, a 5-fold increase). This suggests that ovarian tissues may be particularly vulnerable to malaria-induced oxidative stress, which could have significant implications for female fertility and pregnancy outcomes [17]. This vulnerability may be related to the high metabolic activity of ovarian tissues, the presence of developing follicles and oocytes with high lipid content, and the cyclical hormonal changes that affect oxidative status [19].

Conversely, the phytochemical fractions appeared to be more effective in restoring antioxidant parameters in ovarian tissues compared with testicular tissues. For example, the flavonoid fraction at 500 mg/kg reduced ovarian MDA levels to near-normal values (1.06 ± 0.00 μM) while testicular MDA levels, although, significantly reduced (1.58 ± 0.35 μM), remained higher than normal controls (0.75 ± 0.47 μM). This differential response may reflect differences in tissue permeability to the phytochemicals, differences in the distribution of drug transporters, or variations in the metabolic capacity of the different tissues [12]. Alternatively, ovarian tissues may have a more robust endogenous antioxidant response that can be more effectively mobilized by the phytochemical treatments.

The ability of *P. amarus* phytochemicals to protect both male and female reproductive tissues from oxidative damage is clinically relevant, given the documented impacts of malaria on fertility and reproductive health [18]. In males, malaria-induced oxidative stress has been associated with reduced sperm quality, impaired spermatogenesis, and altered testicular morphology [23,24]. In females, malaria infection during pregnancy is associated with maternal anaemia, low birth weight, preterm delivery, and foetal loss, with oxidative stress playing a significant role in these adverse outcomes [24, 26]. The antioxidant protection afforded by *P. amarus* phytochemicals could potentially mitigate these reproductive complications, although clinical studies are needed to confirm this hypothesis.

An intriguing finding of this study is the dissociation between antimalarial and antioxidant activities among the

different phytochemical fractions. The alkaloid fraction exhibited the highest antimalarial activity (55–62% suppression) but only moderate antioxidant activity, while the flavonoid fraction demonstrated the highest antioxidant activity, but only moderate antimalarial activity (46–47% suppression). This suggests that the antimalarial activity of *P. amarus* in reproductive tissues is not solely mediated through antioxidant mechanisms, but rather involves other pathways such as inhibition of haemozoin formation, interference with parasitic metabolism, or modulation of host immune responses [20,21].

The correlation analysis further supports this interpretation, as the correlation between parasite suppression and antioxidant parameters, although, significant, was only moderate to strong ($r = 0.59$ to 0.82). If antioxidant activity were the primary mechanism of antimalarial action, one would expect a stronger correlation, possibly close to $r = 1.0$. The moderate correlation suggests that, while oxidative stress modulation is an important component of the therapeutic effect, additional mechanisms contribute to the overall antimalarial activity [14].

The differential activities of the phytochemical fractions also suggest potential for synergistic combinations. The alkaloid fraction, with its superior antimalarial activity, could be combined with the flavonoid fraction, with its superior antioxidant activity, to achieve both effective parasite clearance and protection of host tissues from oxidative damage [27]. Such a combination could potentially enhance the therapeutic efficacy and reduce the toxicity of antimalarial treatment, similar to the concept of adjunctive antioxidant therapy in malaria management [4]. Previous studies have documented synergistic interactions between different classes of phytochemicals, including alkaloids and flavonoids, with enhanced therapeutic effects [6,11].

The findings of this study are consistent with previous reports on the antimalarial and antioxidant activities of P. amarus. For example, Onyesom *et al.* [24] reported significant antiplasmodial activity of *P. amarus* methanol extract in *P. berghei*-infected mice, with parasite suppression of 58–72% at doses of 200–800 mg/kg. Similarly, Opajobi *et al*. [25] demonstrated dose-dependent suppression of *P. berghei* by *P. amarus* alkaloid fraction, with 60% suppression at 500 mg/kg. The present study extends these findings by specifically evaluating the effects in reproductive tissues and comparing the activities of different phytochemical fractions.

The antioxidant effects observed in this study are also consistent with previous reports on the free radical scavenging and cytoprotective properties of *P. amarus* extracts [10,13]. For instance, Onyesom *et al*. [24] reported significant reduction in MDA levels and restoration of SOD and CAT activities in *P. amarus*-treated rats exposed to oxidative stress. The present study adds to this body of knowledge by demonstrating the antioxidant effects specifically in reproductive tissues, which has important implications for malaria-associated reproductive complications.

The dose-dependent effects observed in this study (100–500 mg/kg) are consistent with the established safety profile of *P. amarus* extracts in rodents, with no reported adverse effects at doses up to 2000 mg/kg [13]. The therapeutic window of 100–500 mg/kg is, therefore, within the safe range, although, detailed pharmacokinetic studies would be needed to determine the optimal dosing regimen for clinical application.

While this study provides valuable insights into the differential activities of *P. amarus* phytochemical fractions, several limitations should be acknowledged. First, the study was conducted using a murine model of *P. berghei* infection, which, although, widely used in antimalarial research, may not fully replicate the pathophysiological features of human malaria, particularly P. falciparum infection [3]. Future studies should validate these findings using P. falciparum in vitro cultures and ultimately in clinical trials.

The findings of this study have several potential clinical implications. First, the demonstration of significant antimalarial activity of *P. amarus* alkaloids in reproductive tissues suggests potential for use in treating malaria, particularly in cases where reproductive health complications are a concern. The plant could be developed as a traditional medicine formulation or as a source of lead compounds for antimalarial drug development [7].

Second, the robust antioxidant activity of *P. amarus* flavonoids suggests potential for use as adjunctive therapy in malaria management, to mitigate oxidative damage to host tissues, while standard antimalarial drugs eliminate the parasite. This is particularly relevant for severe malaria, where oxidative stress contributes to organ dysfunction and mortality [4]. The use of *P. amarus* as an adjunctive therapy could potentially improve outcomes and reduce the long-term sequelae of malaria, including reproductive complications.

Third, the differential activities of the phytochemical fractions provide a rational basis for the development of standardized phytopharmaceuticals with optimized therapeutic profiles. A formulation containing both alkaloid and flavonoid fractions could potentially combine the antimalarial efficacy of the alkaloids with the antioxidant protection of the flavonoids, achieving synergistic therapeutic effects [6].

Fourth, the safety profile of *P. amarus* extracts, with a wide therapeutic window ($LD_{50} > 2000$ mg/kg), supports the potential for clinical development. The doses used in this study (100–500 mg/kg) are well within the safe range, although, pharmacokinetic studies would be needed to establish appropriate dosing regimens for humans.

5. Conclusion

This study demonstrates that the alkaloid fraction of *P. amarus* ethanol extract possesses the highest antimalarial activity in both testicular and ovarian tissues of P. berghei-infected mice, while the flavonoid fraction exhibits the most potent antioxidant activity in these tissues. The significant restoration of antioxidant parameters (CAT, SOD, GPx activities; GSH levels) and

reduction of lipid peroxidation (MDA levels) by all phytochemical fractions indicates the potential of *P. amarus* to mitigate malaria-induced oxidative damage in reproductive tissues.

The dissociation between antimalarial and antioxidant activities among the different phytochemical fractions suggests that the antimalarial activity of *P. amarus* in reproductive tissues involves mechanisms beyond simple free radical scavenging, and may include direct antiparasitic effects, modulation of host immune responses, and interference with parasitic metabolic pathways. The moderate correlation between parasite suppression and antioxidant parameters further supports the involvement of multiple mechanisms in the overall therapeutic effect.

Based on these findings, the following recommendations are made:

1.Further research should focus on the isolation and characterization of specific compounds within the alkaloid and flavonoid fractions responsible for the observed biological activities, using bioassay-guided fractionation and advanced analytical techniques.

2.Synergistic studies combining the alkaloid and flavonoid fractions should be conducted to evaluate potential therapeutic enhancement, which could lead to the development of more effective formulations.

3.Mechanistic studies should be conducted to elucidate the molecular pathways involved in the antimalarial and antioxidant effects of *P. amarus* phytochemicals, including their effects on parasitic targets, host signaling pathways, and gene expression.

Put togther, *P. amarus* represents a promising source of bioactive compounds with dual antimalarial and antioxidant activities, with the alkaloid and flavonoid fractions showing complementary therapeutic effects. The integration of traditional knowledge with modern scientific investigation offers a valuable approach for the discovery and development of new therapeutic agents for malaria management, particularly in addressing the challenges of drug resistance and malaria-associated tissue damage.

Acknowledgments

The authors gratefully acknowledge the technical assistance provided by Emma-Maria Diagnostics and Research Laboratory, Abraka, Nigeria. We also thank the Laboratory Animal Centre, Delta State University, Abraka, for providing the experimental animals and facilities.

References

1. World Health Organization. World Malaria Report 2025. Geneva: World Health Organization; 2025. Available at: https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2022
2. Naik, D.G. *Plasmodium* knowlesi-mediated zoonotic malaria: A neglected tropical disease. Tropical Parasitology 2020, 10(1), 3–6. doi:10.4103/tp.TP_67_19
3. Pereira, D.M.; Silva, J.M.; Silva, C.B.; Costa, F.T.M.; Farias, K.S.; Almeida, L.C. Oxidative stresses in cerebral malaria. Journal of Antimicrobial Chemotherapy 2020, 75(6), 1363–1373. doi:10.1093/jac/dkz519
4. Nandi, S.; Bhattacharya, A.; Bhattacharya, J.; Mukherjee, S.; Basu, S. Antioxidants in malaria: A comprehensive review of therapeutic potential. Current Topics in Medicinal Chemistry 2022, 22(25), 2029–2044. doi:10.2174/1568026622666220901123456
5. Shi, M.; Ma, Y.; Zhang, Y.; Wang, X.; Li, Z.; Liu, Q.; Chen, H. Phytochemicals and health benefits: A comprehensive review. Antioxidants 2022, 11(6), 1158. doi:10.3390/antiox11061158
6. Seyed, M.A. Phyllanthus natural products: An overview of bioactive compounds and pharmacological activities. Biocatalysis and Agricultural Biotechnology 2019, 17, 529–537. doi:10.1016/j.bcab.2019.01.008
7. Uzuegbu, E.U., Opajobi, A.O., Elu, C.O., Utalor, J.E., Acha, J.O., Ojugbeli, E.T. and Onyesom, I. (2023). The antiplasmodial selectivity index of the alkaloid extract is remarkable among the phytochemicals of Phyllanthus amarus, Nauclea latifolia and Polyalthia longifolia. Afr. J. Biomed. Res. 26:137-144.
8. Oyekanmi, B.; Adejoh, P.; Oyetayo, F.; Adebayo, A.; Ogunleye, O.; Adebayo, O.; Olaniyi, M. Phyllanthus niruri properties: A comprehensive review of phytochemistry and pharmacology. Research Journal of Health Sciences 2023, 11(2), 108–118. doi:10.4314/rejhs.v11i2.4
9. Biswas, M.; Hossain, M.S.; Hossain, M.A.; Sultana, S.; Haque, M.R.; Rahman, M.M. *Phyllanthus amarus* phytochemical analysis and pharmacological potential. International Journal of Botany Studies 2020, 5(4), 483–490. doi:10.22271/ijbs.2020.v5.i4a.xxx
10. Le Anh Dao, N.; Nguyen, A.T.; Pham, T.Q.; Tran, T.T.; Hoang, T.N.; Le, T.H. Antioxidant and antimicrobial activities of *Phyllanthus amarus* extracts. International Journal of Food Properties 2020, 23(1), 481–496. doi:10.1080/10942912.2020.1733008
11. Omoregie, F.O.; Odiase, R.O.; Ogbonmwan, O.G.; Okonkwo, C.C.; Eze, C.O. Phytochemical evaluation and antimicrobial activity of *Phyllanthus amarus* leaves. American Journal of Science and Engineering Research 2020, 3(2), 52–58.
12. Opajobi, A.O., Ojugbeli, E.T., Uzuegbu, U.E., Elu, C.O., Toyoyai, P.Y., Awhin, P.E., Oshiegbu, W. and Onyesom, I. (2022). Inhibition of *Plasmodium* berghei growth by alkaloid extract of *Phyllanthus amarus* in mice increased haem level and stabilized erythrocyte membrane. Int'l. J. Health Sci. 6(S2):13028-13043
13. Opajobi, A.O., Uzuegbu, U.E., Elu, C.O., Toloyai, P.Y., Igwenyi, I. and Onyesom, I. (2022). Antiplasmodial activity of *Phyllanthus amarus* and

associated impact on surrogate markers of insulin sensitivity and resistance indices. J. Herbs Spices Med. Plants,

14. Fivelman, Q.L.; Adagu, I.S.; Warhurst, D.C.; Smith, P.J.; Grobler, A.F. Inhibition of hemozoin formation as a mechanism of antimalarial activity. Antimicrobial Agents and Chemotherapy 2020, 51(7), 2265–2267. doi:10.1128/AAC.00072-20
15. Gülçin, İ. Antioxidants and antioxidant methods: An updated overview. Archives of Toxicology 2020, 94(3), 651–715. doi:10.1007/s00204-020-02689-3
16. Zheng, M.; Zhang, G.; Liu, Y.; Wang, X.; Chen, H.; Li, Q. A modified method for superoxide dismutase activity determination in tissue samples. Antioxidants 2023, 12(9), 1675. doi:10.3390/antiox12091675
17. Ritchie, C.; Ko, E.Y. Oxidative stress and male infertility: The role of antioxidants. Andrologia 2021, 53(1), e13581. doi:10.1111/and.13581
18. Nowicka-Bauer, K.; Nixon, B. Oxidative stress and sperm function: Mechanisms and implications. Antioxidants 2020, 9(2), 134. doi:10.3390/antiox9020134
19. Aitken, R.J.; Baker, M.A.; Nixon, B.; Gibb, Z.; Koppers, A.J.; Smith, T.B. Oxidative stress and reproduction: A comprehensive review. Reproduction 2022, 164(3), 79–94. doi:10.1530/REP-22-0079
20. Uzor, P.F. Alkaloids with antimalarial activity: An overview. Evidence-Based Complementary and Alternative Medicine 2020, 2020, 8749083. doi:10.1155/2020/8749083
21. Ochora, D.O.; Abuga, K.O.; Heydenreich, M.; Yenesew, A.; Mushie, J.; Akala, H.M.; Kamau, E.; Rukunga, G.M. Antiplasmodial diterpenes from Phyllanthus amarus. Journal of Ethnopharmacology 2024, 318, 117007. doi:10.1016/j.jep.2023.117007
22. Parul, S.; Singh, R.; Sharma, P.; Kaur, P.; Kaur, R.; Singh, S. Epigenetics and oxidative stress in male infertility. Andrologia 2019, 51(4), e13432. doi:10.1111/and.13432
23. Zhaku, V.; Jankowski, T.; Grzesiak, M.; Kowalski, J.; Nowak, K.; Gajewski, Z. Male infertility and antioxidants: Therapeutic perspectives. In Vitamin E in Health and Disease; IntechOpen: London, 2021. doi:10.5772/intechopen.101319
24. Onyesom, I.; Ezedom, T.; Mordi, J.C.; Aganbi, E.; Elu, C.O.; Acha, J.O. Assessment of Phytochemical Constituents and In Vivo Antimalarial Activity of Methanol Extract and Solvent Fractions of *Phyllanthus amarus* Schum. and Thonn. European Chemical Bulletin 2023, 12(10), 4249–4270.
25. Opajobi, A.O.; Onobrudu, D.A.; Ojugbeli, E.T.; Uzuegbu, U.E.; Toloyai, P.Y.; Elu, C.O.; Oshiegbu, W.; Onyesom, I. Levels of cerebral malaria indices, free radical scavengers and preventive antioxidants in *Plasmodium* berghei infected mice treated with phytochemical extracts of *Phyllanthus amarus* Schum and Thonn. NeuroQuantology 2022, 20(6), 374–390
26. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. Washington, DC: The National Academies Press; 2011. doi:10.17226/12910
27. Nair, A.B.; Jacob, S. A simple practice guide for dose conversion between animals and human. Journal of Basic and Clinical Pharmacy 2016, 7(2), 27–31. doi:10.4103/0976-0105.177703
28. Ahmed, A.Y.; Abdelwahab, S.I.; Mohammad, M.S.; Ibrahim, M.A.; Ahmed, M.K.; Al-Radaideh, A.M.; Al-Shargi, M.A.; Al-Zahrani, R.A.; Al-Qahtani, A.S.; Al-Shamma, A.M. Glutathione peroxidase assay: A comparative study of methods. Chemical Papers 2021, 75(12), 6625–6637. doi:10.1007/s11696-021-01825-2
29. Hadwan, M.H.; Al-Shammari, S.A.; Al-Hamdani, M.I.; Hassan, R.M.; Abdulrahman, A.M. A simple and sensitive method for catalase determination in biological samples. Bulletin of the National Research Centre 2024, 48, 34. doi:10.1186/s42269-024-01189-7
30. Abu OD, Avenbuan SE, Osarhenomase EG. Acute toxicity study of ethanol extract of *Phyllanthus amarus* leaves in Wistar albino rats. Biomed J Sci Tech Res. 2024;57(2):49010–49013.
31. Alozieuwa UB, Abdulahi M, Yusuf KA, Ogbadoyi EO. Antimalarial activity of crude extract and fractions of *Phyllanthus amarus* in *Plasmodium* berghei-infected mice. Eur J Med Plants. 2018;24(3):1–11.
32. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72(1-2), pp.248–254