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Phytochemical composition, toxicity assessment, and hepatorenal effects of methanol extracts of *Vitex doniana* Leaves in *Plasmodium berghei*-infected mice

Abstract

Background: Malaria, caused by *Plasmodium* parasites, remains a significant public health concern in tropical regions, including Nigeria. The increasing resistance of *Plasmodium* species to conventional antimalarial drugs necessitates the search for alternative therapies from medicinal plants. *Vitex doniana* is traditionally used for malaria treatment, but its biochemical effects remain underexplored. This study investigates the phytochemical composition, toxicity, and hepatorenal effects of methanol extracts of *V. doniana* leaves in *Plasmodium berghei*-infected mice.

Methods: Preliminary qualitative and quantitative phytochemical analyses were conducted to identify bioactive constituents. Acute toxicity (LD₅₀) was assessed up to 5000 mg/kg body weight. *P. berghei*-infected mice were treated with *V. doniana* extract, and hepatorenal parameters, including liver enzyme activities (ALT, AST, ALP), total bilirubin, kidney function markers (urea, creatinine), and serum electrolytes (sodium, potassium), were evaluated.

Results: Phytochemical screening revealed moderate levels of steroids, carbohydrates, glycosides, proteins, and flavonoids, while phenolics, terpenoids, and tannins were present in high concentrations. Saponins were not detected. The extract exhibited no acute toxicity up to 5000 mg/kg. Treatment significantly reduced ($p < 0.05$) parasitemia levels compared to the untreated group. Hepatorenal assessment showed a significant decrease ($p < 0.05$) in ALT, AST, ALP, and bilirubin levels, indicating hepatoprotective effects. Similarly, kidney function markers (urea and creatinine) were significantly reduced ($p < 0.05$), suggesting nephroprotective properties. Serum electrolyte analysis revealed increased sodium and potassium levels in treated groups compared to untreated controls.

Conclusion: These findings highlight the phytochemical richness of *V. doniana* leaves and their potential therapeutic benefits. The extract demonstrated no acute toxicity and exerted significant hepatorenal protective effects, reducing liver and kidney dysfunction in *P. berghei*-infected mice. Also, its modulation of serum electrolytes suggests a role in maintaining physiological homeostasis. This study provides scientific validation for the traditional use of *V. doniana* in malaria treatment and supports its potential as a safe and effective natural remedy for malaria-induced hepatic and renal dysfunction.

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Chikaodi Victoria Aniagu,¹ Victor Nwadiogbu Ogugua,² Ikechukwu Jacob Okoro,³ Stanley Obinna Ezeadichie,¹ Mima Wariso,¹ Osah Martins Onwuka^{4,5}

¹Department of Medical Biochemistry, Godfrey Okoye University, Nigeria

²Department of Biochemistry, University of Nigeria, Nigeria

³Department of Medical Biochemistry, David Umahi Federal University of Health Sciences, Nigeria

⁴International Institute for Pharmaceutical Research and Innovation, David Umahi Federal University of Health Sciences, Nigeria

⁵Department of Human Physiology, Godfrey Okoye University, Nigeria

Correspondence: Osah Martins Onwuka, Department of Human Physiology, Godfrey Okoye University, Enugu State, Nigeria

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Introduction

Medicinal plants have been integral to healthcare systems worldwide, particularly in regions with limited access to synthetic drugs. These plants contain bioactive compounds that can either be used directly or serve as precursors for pharmaceutical drug development.¹ In Africa, where a large proportion of the population depends on traditional medicine, plant-based remedies play a crucial role in disease management.² Herbal medicine is widely used for malaria treatment, including during pregnancy, despite concerns about safety and efficacy.³ Malaria, a mosquito-borne disease caused by *Plasmodium* species, remains a significant global health challenge, particularly in tropical and subtropical regions, including Africa, Asia, and the Americas.⁴ The disease affects an estimated 300 to 500 million people annually, leading to approximately 1.5 to 2.7 million deaths, with *Plasmodium falciparum* being the most virulent species.⁵ Despite advancements in antimalarial therapy, the emergence of drug-resistant *Plasmodium* strains underscores the urgent need for alternative treatments, particularly from natural sources.⁶

Natural products have historically contributed to the discovery of effective antimalarial agents.⁷ One such plant with promising medicinal potential is *Vitex doniana*, a deciduous evergreen tree widely distributed in tropical West Africa. Ethnomedicinal reports suggest that various parts of the plant possess therapeutic properties for ailments such as malaria, gastrointestinal disorders, inflammation, and epilepsy.⁸⁻¹⁰ In southeastern Nigeria, traditional healers prepare decoctions from *V. doniana* leaves for malaria treatment, yet its efficacy and biochemical effects remain largely unverified.

Belonging to the Verbenaceae family, *Vitex doniana* is a perennial shrub or tree, growing up to 15 meters, with distinctive long-stalked leaves and small purple-tinged flowers. The tree produces edible fruit between May and August, and its propagation occurs naturally through seeds or cuttings.^{11,12} Despite its widespread traditional use, scientific data on its antiplasmodial and hepatorenal effects are limited. This study aims to investigate the phytochemical composition, acute toxicity, and potential hepatorenal protective effects of *V. doniana* methanol leaf extract in *Plasmodium berghei*-infected mice. The findings will provide insight into the plant's safety

profile and therapeutic relevance in malaria-induced hepatic and renal dysfunction.

Materials and methods

Plant materials

Fresh leaves of *Vitex doniana* were collected from Mmaku town in Awgu Local Government Area, Enugu State, Nigeria. The leaves were authenticated by Mr. Alfred Ozioko at the Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu State. The collected leaves were air-dried, pulverized, and stored for further use.

Preparation of plant extract

The leaves of *Vitex doniana* were cleaned and shade-dried for several weeks. The dried leaves were then pulverized using a mechanical grinder. A known weight of 1000g of the dried leaves was macerated in 5 liters of methanol for 72 hours. The resulting mixture was filtered through muslin cloth and further filtered using Whatman No. 1 filter paper to remove fine residues. The filtrate was concentrated using a rotary evaporator to obtain crude methanol slurry-like extracts. The extract was weighed and stored in a refrigerator for further use.

Determination of the percentage yield of the methanol extract

The percentage yield of the methanol extract was determined using the formula:

$$\text{Percentage Yield} = (\text{Weight of Extract} / \text{Weight of Dried Leaves}) \times 100$$

Qualitative phytochemical analysis

Phytochemical constituents were identified using standard methods:

- Alkaloids: Presence confirmed by the formation of a reddish-brown precipitate with Wagner's reagent.
- Glycosides: Identified by a brick-red color after treatment with Fehling's solution A and B.
- Steroids: Identified by a violet color change after treatment with acetic anhydride and H_2SO_4 .
- Carbohydrates: Presence indicated by a brown ring after treatment with Molisch's solution and concentrated H_2SO_4 .
- Flavonoids: Identified by the ammonium and aluminum chloride tests.
- Tannins: Confirmed by a green precipitate with ferric chloride.
- Saponins: Indicated by foam formation upon shaking with distilled water.
- Terpenoids: Identified by a violet color change with acetic anhydride and H_2SO_4 .
- Phenolics: Identified using the Folin-Ciocalteu reagent.

Quantitative phytochemical analysis

The quantities of bioactive constituents were determined by the following methods:

- Flavonoids: Measured by absorbance at 470 nm after extraction with ethyl acetate.
- Tannins: Measured by absorbance at 750 nm after extraction with methanol.

- Alkaloids: Quantified by absorbance at 565 nm after extraction with ethanol and sulfuric acid.
- Carbohydrates: Measured by absorbance at 490 nm after extraction with water.
- Steroids: Measured by absorbance at 550 nm after extraction with ethanol.
- Terpenoids: Measured by absorbance at 700 nm after extraction with ethanol.
- Total Phenolics: Measured by absorbance at 725 nm using the Folin-Ciocalteu reagent.
- Saponins: Measured by absorbance at 430 nm after extraction with methanol.

Acute toxicity study (LD_{50})

Acute toxicity of the methanol extract of *V. doniana* was assessed using the method described by Lorke.¹³ In the first phase, three groups of mice were orally administered 10, 100, and 1000 mg/kg body weight of the extract, with observation for 24 hours for signs of toxicity. In the second phase, three additional groups received doses of 1600, 2900, and 5000 mg/kg body weight, with continuous monitoring for signs of toxicity, morbidity, and mortality. The median lethal dose (LD_{50}) was determined based on the number of deaths observed at each dose level.

Animals and experimental design

A total of forty-eight (48) male mice, aged 6 to 8 weeks and weighing 20-24g, were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The mice were acclimatized for 7 days under standard laboratory conditions, including a 12-hour light/dark cycle, with free access to commercial grower's mash and water ad libitum.

The animals were randomly divided into six groups, with eight mice in each group:

- Group 1 (Control): Non-infected, untreated mice administered normal saline and normal feed.
- Group 2 (Positive Control): *P. berghei*-infected, untreated mice.
- Group 3 (Standard Control): *P. berghei*-infected mice treated with 5 mg/kg chloroquine.
- Group 4: *P. berghei*-infected mice treated with 200 mg/kg *V. doniana* extract.
- Group 5: *P. berghei*-infected mice treated with 400 mg/kg *V. doniana* extract.
- Group 6: *P. berghei*-infected mice treated with 600 mg/kg *V. doniana* extract.

All groups, except the control group, were inoculated intraperitoneally with 0.2 ml of *P. berghei* parasitized erythrocytes, and malaria infection was confirmed 72 hours post-inoculation. The treatment with *V. doniana* extract or chloroquine was administered for 14 consecutive days.

Treatment protocol

The *V. doniana* extract was administered orally to the mice in Groups 4, 5, and 6 at doses of 200, 400, and 600 mg/kg, respectively, daily for 14 days. The chloroquine treatment for Group 3 was administered at 5 mg/kg body weight per day for the same duration.

The control group received normal saline and normal feed without any treatment.

Hepatorenal parameters evaluation

At the end of the treatment period, the animals were euthanized, and blood samples were collected for the evaluation of hepatorenal parameters. The following markers were assessed:

- a) Liver function: ALT, AST, ALP, and total bilirubin.
- b) Kidney function: Urea, creatinine.
- c) Serum electrolytes: Sodium and potassium.

All biochemical analyses were performed using standard methods.^{14,15}

Statistical analysis

The data obtained were expressed as mean ± standard deviation (SD) and analyzed using Statistical Product and Service Solutions (SPSS), version 16. Tests of statistical significance were carried out using both one-way Analysis of Variance (ANOVA) and t-test. A p-value of < 0.05 was considered statistically significant.

Percentage yield of methanol extract of *Vitex doniana* leaves

The percentage yield of the methanol extract from *Vitex doniana* leaves was calculated after extraction. The total weight of the dried crude leaf powder used was 1000 g, and the weight of the obtained extract was 60.81 g, yielding a percentage of 6.08% (Table 1). This result indicates the efficiency of the methanol extraction process.

Table 1 Percentage yield of methanol extract of *Vitex doniana* leaves

Weight of crude sample (g)	Weight of extract (g)	Percentage yield (%)
1000.00	60.81	6.08

Qualitative phytochemical composition of methanol extract of *Vitex doniana* leaves

The qualitative analysis of the methanol extract of *Vitex doniana* leaves revealed the presence of various phytochemicals. Alkaloids, flavonoids, and terpenoids were present in moderate to high concentrations, while steroids, glycosides, and carbohydrates were found in lower amounts. Notably, saponins were absent in the extract (Table 2).

Table 2 Qualitative phytochemical constituents of methanol extract of *Vitex doniana* leaves

Phytochemical Constituents	Qualitative
Alkaloid	++
Flavonoids	++
Terpenoids	+++
Steroids	+
Phenolics	+++
Saponin	N.D
Tannin	+++
Glycosides	+
Protein	++
Carbohydrate	+
Acidic compounds	+

+ = slightly present; ++ = moderately present; +++ = highly present; N.D = Not Detected

Quantitative phytochemical constituents of methanol extract of *Vitex doniana* leaves

The quantitative analysis revealed the following concentrations of phytochemicals in the methanol extract (mg/100 g); highlight that the extract is rich in phenolics, terpenoids, and alkaloids, which are often associated with beneficial biological activities, such as antioxidant, anti-inflammatory, and antimicrobial effects (Table 3).

Table 3 Quantitative phytochemical constituents of methanol extract of *Vitex doniana* leaves

Phytochemical Constituents	Concentration (mg/100 g)
Alkaloid	433.22 ± 7.51
Flavonoids	302.06 ± 6.21
Terpenoids	1055.87 ± 140.44
Steroids	2.65 ± 0.08
Total Phenolics	1806.45 ± 234.18
Glycosides	5.138 ± 0.17
Tannin	1039.60 ± 181.43
Carbohydrate	29.93 ± 7.23
Protein	406.19 ± 19.97

Results are expressed in Means ± SD (n = 3)

Acute toxicity of methanol extract of *Vitex doniana* leaves

The acute toxicity testing of the methanol extract of *Vitex doniana* leaves showed no mortality or adverse reactions even at the highest dose tested (5000 mg/kg body weight). This indicates that the extract is non-toxic at these high concentrations (Table 4).

Table 4 Phase I and II of the acute toxicity testing of *Vitex doniana*

Phase/Groups	Dosage of extract (mg/kg b.w)	Mortality rate
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1600	0/3
	2900	0/3
	5000	0/3

n = 3

Effects of methanol extract of *Vitex doniana* leaves on liver marker enzymes and bilirubin concentration in malaria-infected mice

The methanol extract of *Vitex doniana* leaves significantly influenced the activities of liver marker enzymes and total bilirubin concentration in malaria-infected albino mice. The activities of AST, ALT, and ALP enzymes were reduced in the test groups compared to the positive control (group 2, infected and untreated mice). Additionally, total bilirubin levels were significantly lower in groups treated with the extract (400 mg/kg and 600 mg/kg) compared to the untreated positive control (Table 5).

Group 1: Control (normal saline and normal feed), Group 2: Positive control (*Plasmodium berghei*-infected mice, untreated), Group 3: Standard control (*Plasmodium berghei*-infected mice treated with 5 mg/kg chloroquine), Group 4: *Plasmodium berghei*-infected mice treated with 200 mg/kg *Vitex doniana* methanol leaf extract, Group 5: *Plasmodium berghei*-infected mice treated with 400 mg/kg *Vitex doniana* methanol leaf extract, Group 6: *Plasmodium berghei*-infected mice treated with 600 mg/kg *Vitex doniana* methanol leaf extract.

Table 5 Effect of methanol extract of *Vitex doniana* leaves on the activities of liver marker enzymes and total bilirubin concentration in malaria-infected albino mice

Treatment Group	AST Activity (IU/L)	ALT Activity (IU/L)	ALP Activity (IU/L)	Total Bilirubin Concentration (mg/dl)
Group 1	64.00 ± 3.16a	29.20 ± 4.82a	71.80 ± 2.28a	2.84 ± 0.61a
Group 2	87.40 ± 5.08b	63.80 ± 3.35d	94.40 ± 3.65b	6.58 ± 0.20d
Group 3	66.40 ± 1.67a	35.80 ± 3.35b	72.20 ± 3.83a	3.54 ± 0.68a,b
Group 4	65.20 ± 6.06a	43.40 ± 4.56c	75.40 ± 8.82a	4.80 ± 0.84c
Group 5	67.80 ± 3.03a	39.40 ± 3.44bc	76.80 ± 5.07a	4.18 ± 0.46b,c
Group 6	64.80 ± 4.09a	34.20 ± 3.03b	72.40 ± 2.70a	1.28 0.55a

Results are expressed in Means ± SD (n = 5), Mean values with considered significant at p < 0.05.

Effect of methanol extract of *Vitex doniana* leaves on kidney function parameters and serum electrolyte (sodium and potassium) concentrations of malaria-infected mice

The methanol extract of *Vitex doniana* leaves showed a notable influence on kidney function parameters and serum electrolyte concentrations in malaria-infected albino mice. Specifically, the extract significantly reduced urea and creatinine concentrations in treated groups, especially at higher dosages (200, 400, and 600 mg/kg), indicating a potential nephroprotective effect. Additionally, the extract modulated sodium and potassium levels, with significant increases in these electrolytes in the treated groups compared to the positive control, suggesting the extract's ability to restore electrolyte balance disrupted by malaria infection (Table 6).

Table 6 Effect of Methanol Extract of *Vitex doniana* Leaves on Kidney Function Parameters and Serum Electrolyte Concentrations in Malaria-Infected Albino Mice

Treatment Group	Urea Conc (mg/dl)	Creatinine Conc (mg/dl)	Sodium (mg/dl)	Potassium (mg/dl)
Group 1	5.80 ± 0.18a	5.42 ± 0.62 b,c	8.22 ± 0.81 e	6.36 ± 0.66 d
Group 2	7.80 ± 0.24b	7.04 ± 0.38 c	2.20 ± 0.40 a	2.35 ± 0.81 a
Group 3	5.60 ± 0.32a	5.23 ± 0.52 b	6.10 ± 0.78 c,d	5.17 ± 0.99 c
Group 4	6.02 ± 0.92a	5.43 ± 0.36 b,c	5.98 ± 0.23 c	3.44 ± 0.76 b
Group 5	8.00 ± 0.48b	5.36 ± 0.16 b	6.12 ± 0.52 c,d	4.63 ± 0.58 c
Group 6	5.40 ± 0.38a	5.32 ± 0.54 b	6.13 ± 0.43 c,d	5.31 ± 0.54 c

Results are expressed in Means ± SD (n = 5), Mean values with different letters considered significant at p < 0.05.

Group 1: Control (normal saline and normal feed), Group 2: Positive control (*Plasmodium berghei*-infected mice, untreated), Group 3: Standard control (*Plasmodium berghei*-infected mice treated with 5 mg/kg chloroquine), Group 4: *Plasmodium berghei*-infected mice treated with 200 mg/kg *Vitex doniana* methanol leaf extract, Group 5: *Plasmodium berghei*-infected mice treated with 400 mg/kg *Vitex doniana* methanol leaf extract, Group 6: *Plasmodium berghei*-infected mice treated with 600 mg/kg *Vitex doniana* methanol leaf extract

Discussion

The results of this study highlight the pharmacological potential of *Vitex doniana* leaves, particularly in the context of malaria treatment. The percentage yield of the methanol extract from *Vitex doniana* leaves (6.08%) indicates the efficiency of the extraction process, with

a significant number of bioactive compounds being isolated from the crude leaf material. This yield is comparable to other plant extracts, suggesting that methanol is an effective solvent for extracting pharmacologically active substances from *Vitex doniana* leaves.¹⁶

The qualitative phytochemical screening revealed the presence of bioactive compounds, such as alkaloids, flavonoids, terpenoids, phenolics, and tannins. These compounds are widely known for their antioxidant, anti-inflammatory, antimicrobial, and hepatoprotective properties, making *Vitex doniana* a promising candidate for further therapeutic exploration.¹⁷ The absence of saponins in the extract suggests that some common plant secondary metabolites were not present, which could influence the extract's therapeutic properties or reduce the risk of unwanted effects associated with saponins. The concentration of phenolics (1806.45 mg/100 g) is particularly notable, as phenolic compounds are well-documented for their strong antioxidant activity, which could help mitigate oxidative stress induced by malaria infection.¹⁸

In the acute toxicity evaluation, the extract displayed no signs of toxicity, even at the highest dose tested (5000 mg/kg), which is promising for its potential safe use in therapeutic applications.¹⁹ The non-toxic nature of the extract is a positive indication that *Vitex doniana* leaves could be used in the management of malaria and potentially in long-term therapeutic settings. Regarding liver function, the methanol extract of *Vitex doniana* leaves demonstrated significant improvements in the activities of liver marker enzymes (AST, ALT, ALP) and total bilirubin levels. These findings suggest that the extract may possess hepatoprotective properties, which could be beneficial for individuals suffering from malaria, a condition often accompanied by liver dysfunction.²⁰ The reduction in the activities of AST, ALT, and ALP in treated groups is indicative of a protective effect against liver damage, which is commonly associated with *Plasmodium* infection.²¹

The extract also exhibited nephroprotective effects, as evidenced by the significant reduction in serum urea and creatinine levels in treated groups. Elevated levels of urea and creatinine are typically indicators of kidney dysfunction, which is common in malaria-infected individuals.²² The ability of the methanol extract to normalize these parameters, especially at higher doses (400 and 600 mg/kg), suggests that *Vitex doniana* leaves may be useful in protecting renal function during malaria infection. Additionally, the extract's ability to restore electrolyte balance, as shown by the modulation of sodium and potassium levels, further supports its potential as a therapeutic agent in managing the complications of malaria.²³ The increased sodium and potassium concentrations in treated groups reflect the extract's capacity to regulate electrolyte homeostasis, which can be disrupted in malaria-infected individuals.²⁴

The results from this study underscore the therapeutic potential of *Vitex doniana* as a multifaceted remedy for malaria, particularly due to its ability to protect liver and kidney function, restore electrolyte balance, and offer antioxidant and anti-inflammatory benefits.^{25,26} Further investigations are warranted to isolate and identify the specific bioactive compounds responsible for these therapeutic effects, as well as to explore the underlying mechanisms of action. Also, clinical studies are needed to verify the efficacy and safety of *Vitex doniana* leaves in humans, paving the way for its potential inclusion in malaria management protocols.

Conclusion

In conclusion, the methanol extract exhibited notable bioactive compounds, including alkaloids, flavonoids, and phenolics, which contribute to its antioxidant, anti-inflammatory, and hepatoprotective

properties. The extract demonstrated no acute toxicity, suggesting a safe profile for further exploration in malaria treatment. Furthermore, *Vitex doniana* showed promising hepatoprotective and nephroprotective effects, as evidenced by its ability to normalize liver enzymes, bilirubin levels, and renal function markers in malaria-infected rats. The extract's role in restoring electrolyte balance further highlights its potential in managing complications associated with malaria. These findings support the continued investigation of *Vitex doniana* as a natural therapeutic agent, and clinical studies are necessary to validate its efficacy and safety in humans. Future research should focus on isolating specific bioactive compounds and elucidating the mechanisms of action to fully harness the medicinal potential of *Vitex doniana* in the treatment of malaria and related complications.

Conflict of interest

None.

Funding

None.

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