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Schizont Maturation and β-Hematin Inhibition Properties of the n-Butanol Fraction of Methanol Root Extract of *Cissus cornifolia* (Baker)

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Abstract

Cissus cornifolia Baker, (Vitacea) is traditionally used in the treatment of many ailments including malaria. This study aimed to assess the in vitro antiplasmodial activity of the n-butanol fraction (NBF) of methanol root extract of *Cissus cornifolia* using the schizont maturation inhibition model. The root was extracted using cold maceration with diluted methanol and further fractionated in n-butanol and ethyl acetate. Phytochemical analysis and quantification were carried out to determine the secondary metabolites. The median lethal dose of the methanol root extract was determined by the OECD method and that of the n-butanol fraction (BF) by Lorke's method. Schizont inhibition was carried out using the candle jar anaerobic continuous culture method. Colorimetric assay method for β-hematin inhibition was performed using a 96-well plate. Inhibition of both schizont and β-hematin was determined Phytochemical screening identified saponins, flavonoids, tannins, alkaloids, terpenoids, steroids, and cardiac glycosides. The oral LD_{50} of methanol root extract (ME) was above 5000 mg/kg while that of the BF was 2154 mg/kg. The BF significantly ($P < 0.05$) reduced the mean number of schizonts compared to the negative control. The NBF exhibited significant antimalarial activity with IC_{50} (3.70 µg/mL) similar to that of chloroquine with IC_{50} (3.60 µg/mL). These results suggest that the plant may be useful against malarial parasite and justify the claim of its effectiveness in folkloric medicine

Keywords: Antiplasmodial, *Cissus cornifolia*, schizonts, β-hematin. methanol extract, *Plasmodium falciparum*

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Introduction

Malaria is one of the major diseases affecting people in developing countries (WHO, 2023). It is one of the leading causes of avoidable death, especially in children and pregnant women (WHO, 2023). In 2022 WHO estimated that 233 million cases of malaria occurred worldwide with 580,000 recorded deaths (WHO, 2023). Despite health intervention by the affected countries and organizations, there is high prevalence of the disease in South-East Asia and Africa (WHO, 2023). The first line treatment for uncomplicated malaria is Artemisinin Combination Therapy (ACT). However, emerging challenges with ACTs, such as delayed hemolysis following Artesunate and oral ACTs (Kurth *et al*., 2016), raise concerns about their future in malaria chemotherapy. The side effect profile of some antimalarial drugs limits their clinical use. For example, cardiotoxicity is associated with quinine, halofantrine, and mefloquine; hemolytic anemia with primaquine; and kidney damage with quinine (Atsushi *et al*., 2010). Remedies of natural origin are believed to be safe and pose low risk; however, some plants are inherently toxic, leading to adverse effects (Doma and Yaro, 2024).

Traditional medicine is the sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO, 2018). In developing countries traditional medicines still play important role in health care systems. WHO in 2008 estimated that 80% of African population relied on herbal medication for their health care, with possible rise 88% in 2018 (Hawa *et al*. 2021). In Nigeria, 76.65% of the population still relies on traditional medicines (Afisulahi *et al*. 2022). For example, in Kano and Jigawa States of northern Nigeria, the majority of the population relies on traditional plant medicine to meet their primary health care needs (Adoum, 2016).

Cissus cornifolia Baker-Planch (Family -Vitaceae) is an annual, sub-erect herb found mainly in the rocky environment and Savannah regions of Ghana and Northern Nigeria. In Nigeria, more especially in the northern part of the country *Cissus cornifolia* root has been used for years as a remedy of malaria and other illnesses (Burkill, 2000, Maitama *et al*., 2025). The herb is locally called *Ewe Akoko* in Yoruba, *Ugolo* in Igbo, *Rigarbiri* or "*Duwawun biri*" among the Hausa speaking people of Northern Nigeria (Burkill, 2000). The plant parts are used in African ethnomedicine for wide variety of illness such as gonorrhoea, malaria, pharyngitis and as a sedative in cases of mental derangement (Burkill, 2000). This study was undertaken to assess the possible mechanism of the antiplasmodial activity of the n-butanol fraction of *Cissus cornifoli.*

Materials and Methods

Collection and identification of plant materials

The root of *Cissus cornifolia* was collected from Basawa village, Sabon Gari Local Government Authority Kaduna State, Nigeria in Febuary 2022. The authenticity of the plant material was confirmed by Botanist in the herbarium section of Plant Biology Department, Bayero University, Kano (BUK) with a voucher specimen number (BUKHAN 0491) assigned.

*Equipmen*t

Electronic balance, Microscope, Microscope slides, Syringes, Mortar and pestle, Animal cages, Spatula, Syringes, Membrane filter 0.45 and 0.22, Weighing balance, Pippete, Heparinized Capillary tube, K-EDTA disposable plastic sample bottles, Incubator, Candle desiccator, Candle, Micro plate reader.

Drugs and chemicals

Solvents used were methanol, ethylacetate, N-butanol (Sigma Aldrich, USA), reagent immersion oil (Agary Ltd, India),10%giemsa solution, dextrose (Juhel Ltd,Nigeria), Phosphate buffer, RPMI solution (Sigma Aldrich, USA), Chloroquine phosphate (West coast India), Artesunate (Incepta India) Pyrimethamin (Incepta India), Yeast, and Aspirin (Bayer, Germany).

Plasmodium falciparum sample

The parasitized blood group O^+ erythrocyte infected with 5% *Plasmodium falciparum* was obtained from Murtala Muhammad general hospital, Kano as clinical isolate. The sample collected was immediately transferred into ethylenediamine tetra-acetic acid (EDTA) disposable plastic sample bottles with tightly fitted plastic corks and mixed thoroughly and then transported to the Pharmacology Laboratory in a thermos flask containing water maintained at 40 degrees.

Ethical clearance

Ethical clearance was obtained from Animal Care and Use Research Ethics Committee; BUK aanimals use protocol (AUP) Number: BUK/ACCUREC/CAP/PG10

Extraction of plant extract

The roots were washed with distilled water to remove dirt and dried at room temperature until it attained constant weight. The dried material was ground into fine powder using mortar and pestle. A portion (2 kilogram) of the powder was extracted using cold maceration with 10 litters of 70% methanol for 7 days with occasional stirring. The mixture was filtered using Whatman No. 1 filter paper (1mm mesh size) and then concentrated in water bath maintained at 50^0C until greenish black residues was obtained.

Fractionation of crude extract

The crude methanol extract (ME) was subjected to liquid-liquid partition to separate the extract into different fractions. Five hundred milliliters (500 ml) of the reconstituted extract was placed in a separating funnel and 500 ml of ethyl acetate and n-butanol were added sequentially as a 1:1 (v/v) solution with frequent shaking. The sample was left to stand for 30 minutes for each solvent in the funnel until a fine separation line appears, separating the supernatant from the sediment before desorption. The process was repeated thrice in order to get enough quantity for each fraction. The ethyl acetate fractions (EAF), n-butanol fraction (BF) as well as the aqueous residues were concentrated over a water bath maintained at 50 degrees Celsius. The concentrated fractions were kept in sealed containers and refrigerated at 21 degrees Celsius for further use. Further investigations were done with n-butanol extract (BF)

Phytochemical Screening

The phytochemical screening for the determination of the secondary metabolites in the methanol extract (ME) was carried out as described by Trease and Evans (2002).

Acute toxicity Studies

Acute oral toxicity studies (LD_{50}) of ME were conducted in mice using Organization for Economic Co-operation and Development guide lines 420 (OECD, 2001). In this method, two groups each of three animals were fasted prior to dosing. The ME were administered in a single oral dose. A start dose of 2000 mg/kg was used for each animal in the first phase. The animals were observed for 48 hours. In the second phase, the same procedure was used but at a dose of 5000 mg/kg. The animals were observed individually at least once during the first 30 minutes after dosing and periodically during the first 24 hours and then daily for 14 days. The acute toxicity study of BF was conducted using Lorke (1983) method. In the first phase, 9 mice were divided into three groups of three mice each and then treated orally with the n-butanol fraction of *Cissus cornifolia* at doses of 10, 100 and 1000 mg/kg. The mice were observed within 24 hours for signs of toxicity including death. In the second phase, four groups of one mouse each were treated with the selected doses of 1600, 2900, and 5000 mg/kg based on the result of the first phase. The mice were observed for signs of toxicity including death. The median lethal dose (LD_{50}) was calculated as the geometric mean of the lowest dose that caused death and the highest dose at which all the animals survived.

Determination of Plasmodium Parasite from O^+ Erythrocyte

Using a clean capillary tube, a drop of blood sample was placed at the centre of a clean glass slide. With a cover slip placed at angle 45 degrees in front of each drop a thin smear was made. The thin smear was immersed in 30% methanol contained in petri dish for 15 minutes. Giemsa stain was dropped on each smear and allowed for 10 minutes. The smear was dried and observed using electronic microscope x 100 using immersion oil after which an average parasitemia was determined by reading three microscopic fields.

Determination of schizont inhibition

Equal volume (10 µl) of different concentrations (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml) of the NBF solution and (10 µl) of culture media (RPMI) were added to wells in a microtiter plate and labeled. For each well, 5 µl of the clinical isolate containing malaria-positive erythrocytes was added and gently mixed to ensure even distribution. The microtiter plate was placed in a bell jar with a burning candle. The jar was covered until the candle extinguished, creating an environment with approximately 95% nitrogen, 2% oxygen, and 3% carbon dioxide (Trager and Jensen, 1976). The setup was then incubated at 37°C for 24 to 48 hours. A control group with only culture media and positive erythrocytes (negative control) and another with culture media, positive erythrocytes, and antimalarial agents artesunate and chloroquine (positive control) were incubated separately alongside the test concentrations.

The microplate was removed from the candle jar after the expiration of the incubation period. The contents were processed by discarding the supernatant and using the remaining red cells to prepare duplicate thick smears on clean microscope slides. The thick films were left to dry and then stained with 10% Giemsa solution, diluted in phosphate buffer (pH 7.2), for 15 minutes before being rinsed with distilled water. The slides were allowed to dry completely and then examined microscopically under a 100x magnification objective lens to assess schizont growth in each well. The schizont growth in the test samples and standard drugs was compared to that in the negative control to calculate the percentage of schizont inhibition as described below:

$$\% \, inhibition = Average\ schizont\ count\ in\ negative\ control - Average\ schizont\ count\ in\ test\ sample\ X\ 100$$

A graph plot of percentage schizont inhibition against log concentration was plotted to obtain the IC_{50} for the test samples and those of the standard drugs used in the experiment.

β -hematin inhibition assay

Inhibition of β-hematin synthesis was conducted according to the method described by Baelmans *et al*. (2000). A 50 µL solution of hemine chloride in DMSO (Dimethylsulfoxide) (5.2 ug/mL) was distributed in 96-well micro-plates. Different amount of the NBF was dissolved in DMSO by double dilution to produce a concentration of 2, 4, 8, 16 and 32 ug/mL and was added in triplicates to the test wells. Controls contained 5.2 ug/ml DMSO only (negative) and chloroquine (2 to 32 ug/mL) (positive). β-hematin synthesis was initiated by the addition of 100 µL of 0.2 M sodium acetate buffer at pH 4.4. Plates were incubated at 37 OC for 48 h. The incubated plates were then centrifuged at 4000 rpm for 15 min. After discarding the supernatant, the pellet was washed three times with DMSO (200 uL) and finally dissolved in 200 ul, 0.2N NaOH solution. The solubilized aggregates were further diluted at 1:2 with NaOH solution (0.1 N) and absorbance recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as percentage inhibition of β-hematin synthesis compared to negative control. The effective concentrations of sample required to inhibit the β-hematin synthesis by 50 % (IC50 value) was then determined.

Data Presentation and Analysis

Data were presented as tables and charts where applicable and expressed as Mean±SEM. Statistical significance testing was done using the one-way analysis of variance (ANOVA) followed by post-hoc analysis-Values of less than or equal to 0.05 were considered statistically significant.

Results

Extraction Yields

The yield of the crude methanol extract was 192 g (39%), while the yield of the ethyl acetate fraction was 9.5 g (5%), n-butanol fraction 75 g (39%) and residual aqueous fraction 85 g (45%)

Phytochemical constituents of the crude extract and fractions of cissus cornifolia root

Preliminary phytochemical screening of the methanol root extract revealed the presence of alkaloids, flavonoids, steroids/terpenoids, cardiac glycosides, carbohydrates, phenols, anthraquinone and tannins. The Ethyl acetate fraction had steroids, alkaloids, flavonoids, cardiac glycosides, phenols, saponins and anthraquinones while the n-butanol fraction contained alkaloids, terpenoids, carbohydrate, saponins, steroids, tannins, anthraquinones, saponins and flavonoids. The residual aqueous fractions had cardiac glycoside, alkaloids, flavonoids, tannins, saponins, anthraquinones, phenols, and carbohydrate (Table 1).

Quantitative analysis of secondary metabolites

The result indicated that the largest quantity of alkaloids (41.50 mg/g), Triterpenes (24.35 mg/g) and steroids (38.53 mg/g) were found in ethyl acetate fraction. N-butanol fraction was found to have highest number of flavonoids (217.75 mg/g), saponins (38.78 mg/g), phenols (135.52 mg/g), while the residual aqueous fraction was rich in tannins (105.86 mg/g), carbohydrates (237.58 mg/g), and cardiac glycosides (61.33 mg/g) (Table 2).

Median lethal dose

The oral median lethal dose of methanol crude extract of *Cissus cornifolia* in mice and rat was estimated to be greater than 5000 mg/kg body weight. However, the LD_{50} of BF was estimated to be 2154 mg/kg.

Schizonts maturation inhibition activity of n- butanol fraction

The standard drug chloroquine significantly reduced ($p < 0.01$) the mean number of schizonts in concentration-dependent manner with schizonts growth inhibition percentage of 45.77, 51.55, 61.14, 78.10, and 86.15% at concentration of 6.25, 12.50, 25.00, 50.00 and 100 µg/mL respectively. Similarly, Artesunate exhibited concentration-dependent inhibition with percentage inhibition of 56.83, 68.41, 71.78, 80.41 and 84.95% at same concentrations, while the n- butanol fraction exhibited concentration-dependent inhibition with percentage inhibition of 29.84, 32.23, 50.56, 60.10 and 78.60. The IC_{50} (µg/mL) values were 4.4 (Chloroquine), 2.4 (Artesunate) and 5.0 (n-butanol fraction) (Table 3).

Effect of n-Butanol Fraction on β-Hematin Inhibition

The n-butanol fraction showed significant ($P < 0.05$) reduction in mean absorbance values as a result of inhibition of β-hematin formation when compared to negative control. The fraction at concentrations of 2, 4, 8, 16, and 32 µg/ml exhibited varying degree of β-hematin inhibition of 53.69, 59.80, 60.60, 84.40 and 92.40% respectively, with IC_{50} of 3.7 µg/ml (Table 4).

Chloroquine significantly ($P < 0.001$) inhibited of β-hematin formation with concentrations of 2, 4, 8, 16 and 32 µg/ml exhibiting reduction of β-hematin of 39.68, 61.67,

84.83, 86.10 and 96.40% respectively, with IC_{50} of 3.6 µg/ml (Table 5).

Table 1: Qualitative Phytochemical Constituents of Crude Extract and Fractions of *Cissus cornifolia* Root

Constituents	ME	EAF	BF	RAF
Anthraquinones	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Tannins	+	+	+	+
Cardiac glycosides	+	+	+	+
Alkaloids	+	+	+	+
Phenols	+	+	+	+

Key: + = Present, ME=Methanol root extract, BF=n-butanol fraction, EAF=Ethyl acetate fraction RAF= Residual aqueous fraction

Table 2: Quantitative Phytochemical Constituents of *Cissus cornifolia*

Constituents	EAF (mg/g)	BF (mg/g)	RAF C(mg/g)
Alkaloids	41.50±0.02	19.81±0.05	19.56±0.13
Flavonoids	42.08±0.08	217.75±0.02	32.54±0.34
Saponins	15.84±0.02	38.78±0.04	12.42±0.06
Phenols	36.04±0.06	135.52±0.07	25.65±0.14
Tannins	9.98±0.08	71.12±0.02	105.86±0.04
Carbohydrates	130.46±0.81	184.54±0.04	237.58±0.09
Steroids	38.53±0.01	21.35±0.17	18.38±0.04
Triterpenes	24.35±0.01	23.07±0.02	18.23±0.01
Cardiac glycosides	20.33±0.01	23.90±0.01	61.33±0.02

Key: BF: n-Butanol fraction EAF: Ethyl acetate fraction RAF: Residual aqueous fraction

Table 3: Schizonts Maturation Inhibitory Activity of N- Butanol Fraction of *Cissus cornifolia*

Treatment	Conc.(µg/mL)	Mean no. of Schizonts	Inhibition	IC_{50} (µg/mL) (%)
DW	10.00	28.90±0.58	–	
CQ	6.25	15.67±0.88	45.77	
CQ	12.50	14.00±0.88	51.55	
CQ	25.00	11.23±0.33*	61.14	4.4
CQ	50.00	6.33±0.67*	78.10	
CQ	100.00	4.03±1.53*	86.15	
ART	6.25	25.33±0.33	56.83	
ART	12.50	11.33±0.88	68.41	
ART	25.00	8.00±0.88*	71.78	2.4
ART	50.00	5.20±1.53*	80.41	
ART	100.00	4.13±1.53*	84.95	
BF	6.25	14.67±0.88	29.84	
BF	12.5	13.33±0.33*	32.23	
BF	25	13.33±0.33*	50.56	5.0
BF	50	9.67±1.15*	60.10	
BF	100	6.33±0.67*	78.60	

Values are mean ± S.E.M. * = P < 0.05, as compared to DW group – One-way ANOVA followed by Bonferroni's post hoc test, n = 3, DW = Distilled water, ART =Artesunate, CQ =Chloroquine, BF= n-Butanol fraction,

Table 4: Effect of the n-Butanol Fraction β-Hematin on Inhibition

Treatment	Conc. (µg/ml)	Av. Absorbance	% Inhibition	IC50 (µg/ml)
DW	-	0.501±0.00	-	
BF	2	0.232±0.28	53.69	
BF	4	0.201±0.38*	59.80	
BF	8	0.197±0.27*	60.60	3.7
BF	16	0.078±0.06*	84.40	
BF	32	0.028±0.02*	92.4 0	

Values are Mean ± S.E.M. *= P < 0.001 compared to distilled water control, one-way ANOVA followed by Dunnetts post hoc test. BF= n-Butanol fraction; DW = Distilled water;

Table 5: Effect of Chloroquine on β-Hematin inhibition

Treatment	Conc. (µg/ml)	Av. Absorbance	% Inhibition	IC50 (µg/ml)
DW	-	0.501±0.00	-	
CQ	2	0.308±0.24	39.68	
CQ	4	0.192±0.32*	61.67	
CQ	8	0.076±0.21*	84.83	3.6
CQ	16	0.078±0.04*	86.10	
CQ	32	0.018±0.02*	96.40	

Values are Mean ± S.E.M. *= P < 0.001 compared to distilled water control, one-way ANOVA followed by Dunnetts post hoc test. CQ=Chloroquine; DW = Distilled water;

Discussion

Traditional medicine has played a vital role in the treatment and management of various diseases, including malaria. In Nigeria, more especially in the northern part of the country *Cissus cornifolia* root has been used for years as a remedy of malaria and other illnesses (Burkill, 2000). The present study investigated *in vitro* antimalarial effect of *C. cornifolia* root extract and fraction and provided scientific evidence to validate its traditional claim and also highlighted its potential as a source of novel antimalarial compounds.

The percentage yield of the extract and fractions resulted into varying yield. The different yields of the extract and fractions might be due to differences in solvents polarities which plays crucial role in increasing the solubility of phytochemicals (Felhi *et al.*, 2017). The qualitative phytochemical screening provides preliminary information about different classes of secondary metabolites present in a plant and the medicinal importance of such plant which may lead to drug discovery and development (Shabbir *et al*., 2013; Karande *et al*., 2016). The result of preliminary phytochemical screening of the methanol root extract of *Cissus crnifolia* revealed the presence of alkaloids, flavonoids, tannins, steroids, phenols, terpenoids, saponins and cardiac glycosides. These metabolites have also been reported in the same plant by other authors (Musa *et al*., 2008; Fulata *et al*., 2017).

Phytochemicals are bioactive compounds produced by plants, and they play a vital role in the medicinal properties of plants. Secondary metabolites such as alkaloids, flavonoids, phenols, cardiac glycosides, terpenoids and saponins are reported to possess antimalarial activities (Abdullahi *et al*., 2024).

Alkaloids are diverse group of naturally occurring nitrogen containing compounds that exhibit antimalarial properties. Many alkaloids have been isolated from medicinal plants and found to possess efficacy against Plasmodium by targeting different stages of parasite life cycle leading to its deaths (Abdullahi *et al*., 2024). Flavonoid compounds extracted from *Artemesia annua* have been reported to exhibit strong antimarial activity (Kwambe *et al*., 2019).

Flavonoids act by increasing bioavalability and efficacy of artemesinins or help in the conversion of artemesinins into its active form by modulating oxidative stress and iron metabolism within the parasite (Lieu *et al*., 2018). Flavonoids act as pro- oxidants inside Plasmodium infected red blood cells thereby generating reactive oxygen spicies which increase oxidative stress and damage the parasite proteins, DNA and membrane leading to apoptosis like cells death (Ferreira, 2019). Flavonoids also inhibit plasmodium parasites enzymes and metabolism e.g Dihydrofolate reductase (DHFR) in folate metabolism for DNA synthesis, ATPases and protease for survival and replication, Glutathione reductase for survival and replication (Bhattacharya, 2021). Cardiac glycosides are reported to disrupt parasite ion transport and Ph regulation preventing nutrient uptake (Mehlhorn, 2018). They also induce oxidative stress and apoptosis-like cell death or inhibit synthesis of protein by binding to ribosomal sub unit thereby preventing parasite growth (Noulin, 2019).

Furthermore, phenolic compounds exhibit their antimalarial activity by inhibition of heme polymerization which results to heme accumulation that subsequently becomes toxic to the parasite that leads to its death (Akandahri *et al.*, 2022).

They also act by either generation of reactive oxygen species, inhibition of plasmodia enzyme, disruption of mitochondrial function and modulation of host immune response (Mamede *et al*., 2020). Therefore, the availability of these secondary metabolites in *C. cornifolia* root could be responsible for its antimalarial properties.

Acute toxicity studies are essential for evaluating the safety of plant derived substances, especially those intended for medicinal use. The findings help determine safe dosage ranges and identify any early toxicological symptoms. Administration of *C. cornifolia* root extract and its fractions did not exhibit any sign of toxicity throughout the study. These findings corroborate with that of Yaro *et al*., (2015).
In vitro Plasmodium falciparum research models are essential tools for studying the antimalarial properties of medicinal plants. They provide a controlled environment for high-throughput screening and can accelerate the discovery of new antimalarial agents (Abdullahi *et al*., 2024). Mechanistic studies of antimalarial drugs are crucial for understanding how drugs exhibit its biological action, identifying potential resistance and developing new drugs. This model provides easy manipulation and serves as primary screening tool for schizonticidal compounds (Trager and Jensen, 1976). Some antimalarial drugs such as Chloroquine, Quinine, Mefloquine, Halofentrine, Sulfones etc. act on the erythrocytic stage of parasite which is very critical for its survival. It has been reported that for a substance to be considered to be effective its IC_{50} value should be less than or equal to 5 µg/mL (Doma *et al*., 2024).

Plasmodium parasite relies on host heamoglobin as an essential source of amino acid for its growth for intra erythrocytic stage i.e. rings, tropozoites, schizonts (Goldberg *et al*., 1993). The parasite ingests more than 75% of the host cells hemoglobin within limited time due to their nutritional demands in blood stage (Goldberg *et al*., 1993). The parasite food vacuole contains specialized enzymes, aspartic acid and cysteine proteases, which breakdown hemoglobin protein components into amino acids (Olliaro and Goldberg, 1995). These amino acids are then used by the parasites for protein synthesis. However, the remaining heme component is toxic to the parasites, causing membrane damages and lysis (Sigala and Goldber, 2014). To counter this, the parasites covert remaining heme components into harmless crystalline material called heamozoin (Beta hematin). Antimalarial drugs like Chloroquine, Amodiaquine, Quinine, Mefloquine, etc; inhibit this conversion process (Dorn *et al*., 1998). As a result, toxic heme accumulates to lethal level, ultimately killing the parasites. The heamozoin formation pathway has been extensively studied as a key mechanism of action for antimalarial drugs which was used in this study.
Beta hematin formation pathway served as an *in vitro* antiplasmodial screening model and was used to evaluate the mechanism of action of n-butanol fraction. The ability of the n-butanol fraction to inhibit beta hematin synthesis was probably due to the presence of secondary metabolites such as flavonoids, cardiac glycosides, phenols and terpeneoids which were reported to inhibit beta hematin synthesis (Akandahri *et al.* 2022).

Conclusion

The n-butanol fraction of methanol root extract of *Cissus cornifolia* was found to possess *in vitro* antimalarial activity as a result of inhibition of schizont maturation. The antimalarial mechanism of action of n-butanol fraction may be due to inhibition of the parasite ability to convert toxic haem to insoluble unreactive crystalline heamozoin or β-heamatin which will result to build up of haem to the level that it becomes irreversibly toxic to the parasite. The study suggests that the roots of this plant which are used traditionally for the treatment of malaria may possess significant antimalarial activity and justify its used for the treatment of malaria.

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Conflict of interest

No conflict of interest declared regarding the research and its publication.

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