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In Vivo Antiplasmodial and Schizont Inhibition Activity of Methanol Stem Bark Extract of *Ficus Vallis-Choude*

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Abstract
Ficus vallis-choudae has been traditionally utilized for treating malaria, pain, jaundice, headaches, and convulsions. This study aimed to assess the antiplasmodial activity of the plant's methanol stem bark extract (FvMSE) both in vitro and in vivo. Preliminary phytochemical analysis was conducted, and the oral median lethal dose (LD50) was estimated following the OECD protocol. The in vitro antiplasmodial activity was evaluated using the schizont inhibition model as outlined by Trager and Jensen. For the in vivo assessment, mice infected with chloroquine-sensitive Plasmodium berghei were used in curative, suppressive, and prophylactic experimental models. Phytochemical screening identified the presence of saponins, flavonoids, tannins, alkaloids, terpenoids, steroids, and cardiac glycosides. The oral LD50 of FvMSE was found to be above 5000 mg/kg. The extract exhibited significant chemosuppressive activity ($p<0.05$) at doses of 1000, 500, and 250 mg/kg, reducing parasite density compared to the distilled water-treated group. In the prophylactic model, the extract significantly decreased ($p<0.05$) parasite counts in all treated groups compared to the distilled water-treated group, although its activity was lower than that of the standard drug pyrimethamine. The curative study results demonstrated that FvMSE significantly reduced ($p<0.05$) parasite densities compared to the distilled water-treated group. Additionally, FvMSE significantly ($p<0.05$) reduced the mean number of schizonts compared to the negative control, similar to the effects observed with standard drugs chloroquine and artesunate. These findings support the traditional use of Ficus vallis-choudae in treating malaria.

Keywords: Antiplasmodial, *Ficus vallis-choudae*, *In vitro*, *Plasmodium berghei*

INTRODUCTION
Malaria is considered one of the deadliest parasitic diseases, with a high incidence of 247 million clinical cases and 600 million deaths reported annually (WHO, 2022). The World Health Organization (WHO) has reported that infectious diseases such as malaria kill a child every three seconds worldwide (WHO, 2019). The African Region carries an overwhelmingly large portion of the global malaria burden. In 2019, this region was responsible for 94% of all malaria cases and deaths worldwide. Total funding for malaria control and elimination reached an estimated US$ 3 billion in 2019, with contributions from governments of endemic countries amounting to US$ 900 million, representing 31% of total funding (WHO, 2020).

The recommended clinical 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).

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Remedies from natural origins are believed to be harmless and pose low risk; however, some plants are inherently toxic, leading to adverse effects (Abdullahi *et al*., 2020). The ongoing spread of *Plasmodium falciparum* resistance to antimalarial drugs, coupled with the toxicity and high cost of these drugs, represents a significant challenge to malaria control efforts. Resistance to various antimalarial drugs (quinolones and antifolate family), including artemisinin derivatives, has been reported and documented (Noedl *et al*., 2008; WHO, 2010), creating a major impediment in the fight against malaria and its associated complications.

Medicinal plants have a long history of extensive use and form the foundation of ethno-medical practices worldwide (Cragg and Newman, 2013; Mondal *et al*., 2019). They are considered vital sources of medicine, particularly in Africa, where they are an important component of the health care delivery system (Cragg and Newman, 2013). This is because medicinal plants contain various compounds with diverse pharmacological activities and are deemed safer compared to conventional therapies (Seo *et al*., 2018; De Olivera *et al*., 2019). Herbal products have been extensively used in managing feverish conditions, pain, and inflammation. Recently, there has been a resurgence of interest in medicinal plants that could be used against such ailments (Solati *et al*., 2017; Oguntibeju, 2018).

In developing countries, traditional medicine continues to play a crucial role in local health care systems. The WHO estimates that up to 80% of the population in some Asian and African countries depend on traditional medicine for their health care needs (WHO, 2010; Zhenji and Xu, 2011). In Nigeria, a significant portion of the population still relies on traditional medicine (Adefolaju, 2011). Malaria treatment using medicinal plant extracts has a long and successful tradition (Kaur et al. 2009). For instance, quinine was isolated from Cinchona (Rubiaceae) and artemisinin from Qinghaosu (Asteraceae) (Rathore *et al*., 2005). The global community is encouraged to embark on research in traditional medicine (WHO, 2012). Additionally, plants are extensively used in traditional medicine for disease treatment and are cost-effective (Mokuolu *et al*., 2016; Oguntibeju, 2018).

Various parts of the plant *Ficus vallis-choudae* have been used to treat stomach pain, paralysis, convulsion, epilepsy, jaundice, nausea, bronchial and gastrointestinal troubles, and malaria (Burkill, 1985; Adekunle *et al*., 2005; Olowokudejo *et al*., 2008; Bello *et al*., 2017). Previous studies have reported that the stem bark extract of *Ficus vallis-choudae* possesses antifungal and anticonvulsant activities (Adekunle *et al*., 2005; Malami *et al*., 2010) as well as anti-inflammatory and anti-nociceptive effects (Lawan *et al*., 2008). Additionally, a survey of the bioactive properties of plant species has reported the antiplasmodial potentials of the Ficus species (Sabrina *et al*., 2006). It is also reported that the ripe figs of the plant possess *in vitro* antiplasmodial activity (Chouna *et al*., 2022).

However, the reviewed literature did not reveal any report indicating the scientific evaluation of the antiplasmodial activity of the stem bark extract of *Ficus vallis-choudae* using both the *in vitro* and *in vivo* antimalarial screening models. Hence conducting this research becomes pertinent especially to validate the folkloric claim that the stem bark extract is effective in treating malaria.

MATERIALS AND METHODS

MATERIALS

Experimental Animals

Swiss albino mice (weighing 16-24 g) and Wistar strain rats (weighing 170-200 g), of both sexes, were sourced from the animal house facility of the Department of Pharmacology and

Therapeutics at Ahmadu Bello University Zaria, Nigeria. These animals were housed in standard propylene cages and maintained under a natural day-night cycle at the Department of Pharmacology and Therapeutics, Bayero University, Kano. They were provided with standard mouse feed (Vital Feeds, Jos, Nigeria) and had unrestricted access to water. The animals were given at least three days to acclimate to the laboratory environment before being used in experiments.

Malaria Parasites
Chloroquine-sensitive *Plasmodium berghei berghei* was obtained from the National Institute of Medical Research (NIMR) Yaba, Lagos, Nigeria and was maintained by continuous intraperitoneal inoculation every four days in fresh mice.

Drugs and Chemicals
The drugs and chemicals used for the studies include: Ethyl acetate (MERCK Eurolab); N-Butanol (KESHI, USA); Chloroform (Sigma Aldrich, St. Louis Mo, USA); Hydrochloric acid, Sulphuric acid (May and Baker, UK), Ferric chloride anhydrous (Avishkar, India), ammonia (Loba chemie, India). Agappe diagnostic kit (Switzerland), Randox diagnostic kit (UK), Distilled Water, Giemsa solution, Immersion Oil, HEPES, Pyrimethamine, RPMI 1640, DMSO, Chloroquine (Fluka, Germany), Artesunate (Cusnat, China), Pyrimethamine (SKG, Nigeria), Methanol (JHD Sci-Tech. Co. Ltd, China) Elisa kit (Wuhan Fine Biotech) etc.

Apparatus
Water Bath (HH-4 ENGLAND Lab science), Electric EDTA bottles, Filter paper (1mm mesh size), Vacutainer syringe, Heparanised capillary tubes, Plasticine, Flat bottom test tubes, CPDA tubes, 96 well Micro plates, Eppendorf micro pipettes, Desiccator, Candle jar, Centrifuge (England), Thermostat oven (DHG-9101-ISA), Micro-hematocrit reader (Hawksley-15006, England), Biobase auto hematological analyser (BK 6300) etc.

Plant Collection and Authentication
The fresh stem barks of *Ficus vallis-choudae* were collected from the Toro district in the Toro Local Government Area of Bauchi State, Nigeria. The plants were identified and authenticated by Baha'uddeen Said Adam from the herbarium unit of the Department of Biological Sciences at Bayero University, Kano, Nigeria. A voucher specimen for Ficus vallis-choudae was assigned the number BUKHAN 0447.

Preparation of Plant Extract
Fresh stem bark of Ficus vallis-choudae was gathered, washed with clean water, and dried in the shade. Once dried, the plant material was ground into a fine powder using a porcelain mortar and pestle, and then sifted. A total of 2 kg of this powdered plant material was soaked in 7 liters of 70% v/v methanol at room temperature for 7 days, with occasional stirring. After the extraction, the crude methanol extract was filtered through Whatman's filter paper (1 mm mesh size) and concentrated in a water bath at 45°C until a brownish residue formed. This residue was then kept in a desiccator.

METHODS
Preliminary Phytochemical Screening
Preliminary phytochemical screening was carried out on the crude methanol stem bark extract of *Ficus vallis-choudae* (FvMSE) as described by Trease and Evans (2009). They were tested for the presence of biologically active secondary metabolites such as alkaloids, flavonoids, saponins, cardiac glycosides, tannins, anthraquinones, steroids and triterpenes.

Acute toxicity study in mice (LD_{50})

LD_{50} determination was conducted using Organization for Economic Co-operation and Development guide lines 420 (OECD, 2001) in rats and mice.

Antimalarial Studies (*In vivo*)

Inoculation of mice

A mouse infected with *Plasmodium berghei berghei*, showing 34% parasitemia, served as the source of the parasites. Blood was collected from the mouse's retro-orbital sinus and transferred to a container with EDTA. To prepare the inoculum, the percentage of parasitemia and the erythrocyte count were measured, and the blood was then diluted with an isotonic solution. This diluted blood was adjusted so that a 0.2 ml dose administered intraperitoneally would contain approximately 1×10^7 parasitized erythrocytes (Kaltra *et al*., 2006; Okokon and Nwafor, 2009).

Parasitemia determination

Thin blood smears were made on microscope slides, fixed with absolute methanol for 10 minutes, and then stained with 10% Giemsa stain for 30 minutes. The slides were gently rinsed with running water and allowed to air-dry at room temperature. Using a light microscope with a 100x oil immersion objective lens, parasitized red blood cells were counted. An average count from three different fields on each slide was noted (according to Laychiluh, 2011). The percentage of parasitemia and suppression were then calculated as follows:

$$\% \text{ parasitemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of RBC counted}} \times 100$$

$$\% \text{ Suppression} = \frac{Po-Pt}{Po} \times 100$$

Where Po is the average parasitemia of the control group and Pt is the average parasitemia of the test group.

Antiplasmodial activity against early infection (Suppressive test)

The Peter's 4-day suppressive test was carried out to evaluate the effectiveness against chloroquine-sensitive *Plasmodium berghei berghei* NK 65 infection in mice (Peters, 1967). Albino mice, weighing between 22 g and 24 g, were infected intraperitoneally with a standard dose of P. berghei containing 1×10^7 infected erythrocytes. The mice were randomly assigned to six groups, each with six mice, and treated orally for 4 consecutive days with different doses of extracts. Two control groups were included: the positive controls received daily treatments of 5 mg/kg chloroquine and 2 mg/kg artesunate, while the negative control group was given 10 ml/kg distilled water. On the fifth day, blood samples were collected from the tail of each mouse, and smears were prepared (Khan *et al*., 2015). Methanol was used to fix the blood films; while10% Giemsa at pH 7.2 was applied for staining and allowed to dry. The slides were then examined microscopically to determine parasitemia. The percentage suppression of parasitemia for each dose was calculated by comparing the parasitemia levels in the treated mice to those in the infected control group (Peter *et al*., 1975). The average percentage suppression was then computed as follows:

$$\text{Average \% suppression} = \frac{A-B}{A}$$

Where A = Average percentage *parasitaemia* in negative control group, and B = Average percentage *parasitaemia* in test group

Prophylactic (repository) test

The prophylactic activity of the extract was assessed using the method described by Peters (1967). Random selection was used to divide the experimental mice into five groups of six animals each. The first group which was considered as negative control was given 10 ml/kg of distilled water, the second, third and fourth groups were given 250, 500 and 1000 mg/kg of the leaf extract respectively while the fifth group also known as the positive control was given 1.2 mg/kg of pyrimethamine orally. The animals were treated for a five day period and 24 hours later inoculated with *Plasmodium berghei berghei*. After three days, the tails of the mice were bruised to collect blood for parasitemia determination.

Antiplasmodial activity against established infection (Rane or Curative test)

Evaluation of the schizontocidal activity of the extract against established infection was carried out as described by Ryley and Peters (1970). The mice were inoculated with *Plasmodium berghei* during the first day of the experiment and was considered day zero (D_0). Three days later (D_3), random selection was used to divide the experimental mice into six groups of six animals each. The first group which was considered as negative control was given 10 ml/kg of distilled water, the second, third and fourth groups were given 250, 500 and 1000 mg/kg of the leaf extract respectively while the fifth and sixth group also known as the positive control were orally administered 5 mg/kg of chloroquine and artesunate respectively for five consecutive days (D_3–D_7). Three days after parasite inoculation and seven days after treatment, the tails of the mice were bruised to collect blood for parasitemia determination.

Determination of mean survival time

The death of experimental animals was observed and recorded as mortality. Mortality was tracked daily, recording the number of days from the inoculation of the parasite to the death of each mouse in both the treatment and control groups during the follow-up period. The mean survival time (MST) for each group was calculated as described by Mengistie *et al* (2012) as follows:

$$\text{MST} = \frac{\text{Sum of survival time (days) of all mice in a group}}{\text{Total number of mice in that group}}$$

The mean survival time (MST) of the mice in each treatment group was determined over a period of 28 days (D_0–D_{27}) by finding the average survival time (days) of the mice (post inoculation).

Measurement of packed cell volume (PCV)

The PCV of each mouse was determined before parasite inoculation and four days after they were infected. For this purpose, blood was collected from the retro orbital sinus of each mouse in heparinized micro haematocrit capillary tubes up to $3/4^{th}$ of their length. The tubes were sealed by crystal seal and place in a micro hematocrit centrifuge (Hettich haematokrit) with the sealed ends out wards. The blood was centrifuged at 12,000 rpm for 5 minutes. Then the tubes were taken out from the centrifuge and the result was read using micro haematocrit reader using the modified Win Trobe's Method (Munzer *et al*. 1980). The volume of the total blood and the volume of erythrocytes were measured and the PCV calculated as;

$$\text{PCV} = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} \times 100$$

***In vitro* studies**

Parasite sample collection

Ethical approval was obtained from the hospital, Federal Medical Centre (FMC) Nguru, Yobe State for the collection of clinical isolates. Blood group O Rhesus positive (Rh +ve) sample was collected by standard veins puncture, using 70% v/v alcohol after the vein was cleaned. A vein, visible from the skin surface was located and 4ml of blood drawn with needle and syringe was delivered into a citrate phosphate dextrose adenine (CPDA) bottle. Rapid diagnostics test (RDT) was done prior to determination of parasitaemia. Malaria positive samples collected were stored at 4^0c and vaccine carrier was used for the transportation of samples from Nguru to the laboratory unit of the Department of Pharmacology, Bayero University Kano.

Determination of schizont inhibition

Same amount of extract solution (10 µl) and culture media (RPMI) were added to wells in a microtiter plate and labeled with concentrations (100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml) in triplicates. For each concentration, 5 µl of malaria-positive erythrocytes was added and gently mixed to ensure even distribution. The microtiter plate was then 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 desiccator after the expiration of the incubation peiod. 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:

$$\% \text{ inhibition} = \frac{\text{Aver schizont count in negative control} - \text{Aver schizont in test sample}}{\text{Average schizont in negative control}} \times 100$$

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.

Data Presentation and Analysis

Data were presented in tables and charts where appropriate and expressed as Mean ± SEM or percentage inhibition. Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. P-values of 0.05 or less were considered statistically significant

RESULTS AND DISCUSSION

Preliminary phytochemical constituents

The results of the preliminary phytochemical screening of *Ficus vallis-choudae* methanol stem bark extract (FvMSE) showed the presence of various phytochemicals such as cardiac glycosides, tannins, flavonoids, alkaloids, saponins, steroids and terpenoids.

The acute toxicity study

The orally evaluated median lethal dose (LD_{50}) being an index of acute toxicity, of FvMSE in mice and rats was found to be above 5000 mg/kg (Table 1).

Table 1: The Oral Median Lethal Dose (LD_{50}) of FvMSE

Extract	Experimental Animal	Amount (mg/kg)
FvMSE	Mice	Above 5000
FvMSE	Rats	Above 5000

FvMSE = Methanol stem bark extract of *Ficus vallis-choudae*

In vivo* antiplasmodial activity of *Ficus vallis-choudae

Chemo suppressive activity

The mean percentage parasitemia values in the FvMSE-treated groups were significantly reduced ($p<0.05$) compared to the distilled water group. The FvMSE extract demonstrated dose-dependent percentage suppression at 1000, 500, and 250 mg/kg, achieving suppression rates of 67.5%, 50.6%, and 54.87%, respectively (Table 2). In contrast, the standard drugs chloroquine at 5 mg/kg and artesunate at 2 mg/kg achieved suppression rates of 78.9% and 85.9%, respectively (Table 2). Additionally, the packed cell volume (PCV) determination after a 4-day suppression test revealed no statistically significant difference between the FvMSE-treated groups and the negative control (Table 3).

Table 2: Chemo suppressive Activity of Methanol Stem bark extract of *Ficus vallis-choudae* in Mice Infected with *Plasmodium berghei*

Treatment	Dose (mg/kg)	Average % Parasitemia (Day Four)	Percentage Suppression
D/W	10 ml/kg	22.19±1.08	_
FvMSE	(1000)	7.22±0.72*	67.5
FvMSE	(500)	10.97±0.89*	50.6
FvMSE	(250)	10.04±0.67*	54.8
CQ	(5)	4.69±0.24*	78.9
ART	(2)	3.11±0.06*	85.9

Key: * indicates a significant difference from the negative control at $p<0.05$, determined using one-way ANOVA and Dunnett's Post Hoc tests. FvMSE stands for Methanol stem bark extract of *Ficus vallis-choudae*, DW stands for Distilled water, ART stands for Artesunate, and CQ stands for Chloroquine Phosphate.

Table 3: Observed Percentage Changes in PCV Measurement of Mice Infected with *Plasmodium berghei* and Treated with Methanol Stem bark Extract of *Ficus vallis-choudae*

Dose (mg/kg)	Average PCV (%)		% Difference
	Day Zero	Day Four	
D/W 10ml/kg	55.31±2.10	51.63±1.08	- 6.7
FvMSE (1000)	52.77±1.72	51.23±0.92	- 2.9
FvMSE (500)	58.50±1.19	53.67±1.09	- 8.3
FvMSE (250)	57.33±1.67	54.50±1.23	- 4.9
CQ (5)	54.62±1.24	52.00±1.21	- 4.8
ART (2)	53.89±1.06	52.14±1.06	- 3.2

There is no significant difference from the control at $p<0.05$, based on one-way ANOVA and Dunnett's Post Hoc tests. FvMSE refers to Methanol leaf extract of *Ficus vallis-choudae*, ART stands for Artesunate, and CQ denotes Chloroquine Phosphate.

Evaluation of prophylactic activity

In the prophylactic activity screening test, the mean percentage parasitemia values in the FvMSE-treated groups were significantly reduced ($p<0.05$) compared to the distilled water control group. The FvMSE extract demonstrated dose-dependent prophylactic activity at 1000, 500, and 250 mg/kg, with activities of 62.4%, 51.0%, and 30.2%, respectively. In comparison, the standard drug pyrimethamine at 1.2 mg/kg achieved a prophylactic activity of 80.1% (Table 4)

Table 4: Prophylactic Activity of Methanol Stem bark extract of *Ficus vallis-choudae* in Mice Infected with *Plasmodium berghei*

Treatment	Dose (mg/kg)	Average percentage Parasitemia (Day seven)	% Prophylaxis
D/W	10 ml/kg	21.88 ± 2.91	_
Pyrimethamine	(1.2)	4.35 ± 0.92*	80.1
FvMSE	(1000)	8.23 ± 1.23*	62.4
FvMSE	(500)	10.70 ± 1.93*	51.0
FvMSE	(250)	15.27 ± 2.58	30.2

Key: * significantly different from negative control at $p<0.05$ using one-way ANOVA and Dunnett's Post Hoc tests. FvMSE stands for methanol stem bark extract of *Ficus vallis-choudae*.

Evaluation of curative activity

The observed reduction in the mean percentage parasitaemia values of the FvMSE treated groups compared to the distilled water control group, was statistically significant ($p<0.05$). The FvMSE extract at doses of 1000, 500, and 250 mg/kg demonstrated dose-dependent parasite clearance rates of 76.8%, 62.0%, and 58.2%, respectively. In comparison, the standard drugs chloroquine at 5 mg/kg and artesunate at 2 mg/kg achieved clearance rates of 80.5% and 81.8%, respectively (Table 5). Additionally, the FvMSE extract prolonged the mean survival of the animals to 25, 24, and 20 days compared to the untreated group, while the standard drug-treated groups (Artesunate at 2 mg/kg and Chloroquine at 5 mg/kg) each had an average survival of 27 days (Table 5).

Table 5: Curative Activity of Methanol Stem bark Extract of *F. vallis-choudae* in Mice Infected with *Plasmodium berghei*

Treatment	Dose (mg/kg)	Average % Parasitemia		% Clearance	Mean Survival (Days)
		Pre (Day four)	Post (Day seven)		
D/W	10ml/kg	16.00±2.65	26.17±1.79	_	14.81±2.60
ART	(2)	17.00±1.41	4.77±0.09*	81.8	27.13±1.67*
CQ	(5)	17.38±1.70	5.10±0.25*	80.5	27.67±0.33*
FvMSE	(1000)	18.78±1.65	6.06±0.41*	76.8	25.89±1.33*
FvMSE	(500)	15.17±1.70	9.95±0.64*	62.0	24.31±1.00*
FvMSE	(250)	13.88±1.21	10.68±0.98*	58.2	20.33±2.78

Key: * significantly different from negative control at $p<0.05$ using one-way ANOVA and Dunnett's Post Hoc tests. FvMSE stands for methanol stem bark extract of *Ficus vallis-choudae.* D/W stands for distilled water, ART stands for artesunate, CQ stands for chloroquine Phosphate, % stands for percentage.

FvMSE activity on *Plasmodium falciparum* Schizont Maturation

The FvMSE significantly reduced the mean number of schizonts compared to the negative control, showing similar effects to the standard drugs chloroquine and artesunate. The extract demonstrated concentration-dependent inhibition of schizont maturation with percentages of 53.88%, 63.01%, 74.89%, and 90.41% at concentrations of 6.25, 12.5, 25.0, 50.0 and 100 µg/ml respectively(Table 6). Similarly, the standard drugs chloroquine and artesunate also showed concentration-dependent inhibition, with percentages of 33.80%, 48.40%, 59.36%, 74.42%, and 89.04% for chloroquine, and 39.73%, 44.75%, 62.56%, 78.08%, and 91.32% for artesunate at the same concentrations (Tables 7 and 8). The IC_{50} (µg/ml) values were determined to be 11.663 for FvMSE, 14.390 for chloroquine, and 12.831 for artesunate (Figures 1 and 2).

Table 6: Schizont Maturation Activity of Methanol Leaf Extract of *Ficus vallis-choudae*

Treatment	Conc (µg/ml)	Mean no. of Schizonts	Percentage Inhibition	IC_{50} (µg/ml)
NControl	-	21.9±2.07	-	
FvMSE	6.25	13.6±1.98	37.90	
FvMSE	12.50	10.1±1.09*	53.88	
FvMSE	25.00	8.1±1.03*	63.01	**11.663**
FvMSE	50.00	5.5±0.46*	74.89	
FvMSE	100.00	2.1±0.18*	90.41	

Key: * indicates a significant difference from the negative control, analyzed using one-way ANOVA with $p<0.05$ followed by Dunnett's post hoc test. NControl stands for negative control, and FvMSE refers to the methanol stem bark extract of *Ficus vallis-choudae*.

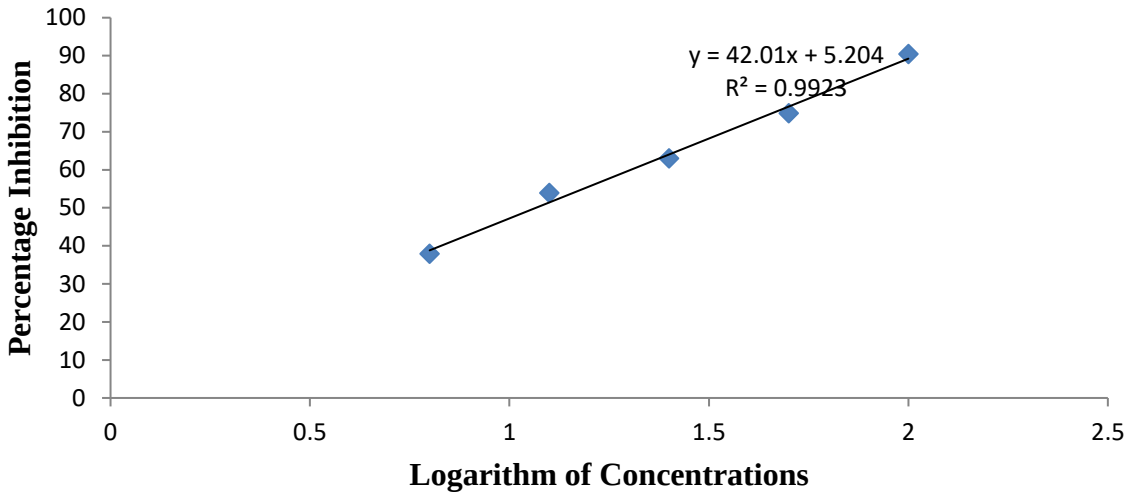

Figure 1: Calculation of IC_{50} from the plot of percentage inhibition against the logarithm of concentrations in the activity of the methanol stem bark extract of *Ficus vallis-choudae* on schizont inhibition; which was calculated to be 11.663.

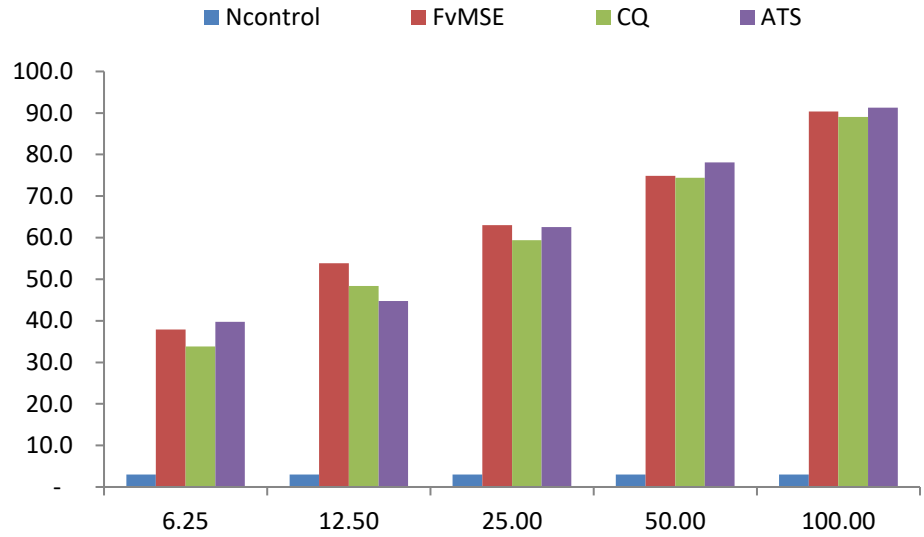

Figure 2: Comparative presentation of the activity of methanol stem bark extract of *Ficus vallis-choudae* (FvMSE) on schizont inhibition and that of the standard drugs used. Ncontrol = negative, CQ = Chloroquine, ATS = Artesunate.

Table 7: Activity of the Standard drug Chloroquine on *Plasmodium falciparum* Schizont Maturation

Treatment	Conc (µg/ml)	Mean no. of Schizonts	Percentage inhibition	IC_{50} (µg/ml)
NControl	-	21.9±2.07	-	
CQ	6.25	14.5±1.98	33.80	
CQ	12.50	11.3±1.06*	48.40	
CQ	25.00	8.9±1.03*	59.36	**14.390**
CQ	50.00	5.6±0.86*	74.42	
CQ	100.00	2.4±0.41*	89.04	

Key: * indicates a significant difference from the negative control, analyzed using one-way ANOVA with $p<0.05$ followed by Dunnett's post hoc test. NControl stands for negative control, CQ for chloroquine and FvMSE refers to the methanol stem bark extract of *Ficus vallis-choudae*.

Table 8: Activity of the Standard drug Artesunate on *Plasmodium falciparum* Schizont Maturation

Treatment	Conc (µg/ml)	Mean no. of Schizonts	Percentage Inhibition	IC_{50} (µg/ml)
Control	-	21.9±2.07	-	
ATS	6.25	13.2±1.27	39.73	
ATS	12.50	12.1±1.04*	44.75	
ATS	25.00	8.2±1.12*	62.56	**12.831**
ATS	50.00	4.8±1.01*	78.08	
ATS	100.00	1.9±0.88*	91.32	

Key: * indicates a significant difference from the negative control, analyzed using one-way ANOVA with $p<0.05$ followed by Dunnett's post hoc test. NControl stands for negative control, ATS for artesunate and FvMSE refers to the methanol stem bark extract of *Ficus vallis-choudae*.

DISCUSSION

Medicinal plants have been acknowledged and utilized throughout history for their therapeutic benefits. They are considered the primary source of biologically active compounds, with many documented species scientifically validated for their medicinal applications (Faustino *et al*., 2010). The Ficus genus, commonly known as figs, includes several species that have been researched for their medicinal properties, notably their potential antiplasmodial activity (Muregi *et al*., 2001; Ajaiyeoba *et al*., 2013; Folade *et al*., 2014; Mukhtar *et al*., 2019). It is crucial to recognize that the effectiveness of these species can vary, underscoring the importance of pharmacological activity studies. *Ficus vallis-choudae* is traditionally employed in treating malaria, pain, jaundice, headache, and convulsions. This research aimed to evaluate the antiplasmodial effects of the methanol stem bark extract (FvMSE) of *Ficus vallis-choudae*, both *in vitro* and *in vivo*.

Preliminary phytochemical screening offers essential insights into the various classes of secondary metabolites present in a plant and its medicinal significance (Shabbir *et al*., 2013). During the initial screening of the leaf and stem bark extracts of *Ficus vallis-choudae*, the presence of alkaloids, flavonoids, saponins, terpenoids, steroids and cardiac glycosides were detected, which may individually or collectively contribute to the observed antiplasmodial activity. This finding aligns with the study by Lawan *et al*., (2008). Secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, and steroids are known to be responsible for the biological activities of plants (Edewor-Kuponiyi, 2013; Rungsung *et al*., 2015). Many plants contain a diverse array of phytochemicals as their bioactive compounds and are reported to have antiplasmodial properties (Alshawsh *et al*., 2007; Matur *et al*., 2009). While some research has linked the antiplasmodial activity of plants to their alkaloid, flavonoid, and terpenoid content (Akuodor *et al*., 2010; Philip, 2020; Tajjuddeen *et al*., 2021), other studies have shown antiplasmodial activity associated with saponins (Akanbi *et al*., 2018; Nafiu *et al*., 2021) and glycosides (Yun *et al*., 2021).

The oral LD_{50} value for the methanol stem bark extract of *Ficus vallis-choudae* (FvMSE) was found to be above 5000 mg/kg, suggesting it is relatively safe. This finding is consistent with the studies by Lawan *et al*., (2008) and Malami et al. (2010). Although LD50 is a useful indicator for determining a substance's safety margin, it should not be viewed as an absolute measure or a complete evaluation of its properties (Cassarette et al. 1996). The Organization for Economic Cooperation and Development (OECD) in Paris, France, has recommended chemical labeling and classification for acute systemic toxicity based on oral median lethal dose values: very toxic if ≤ 5 mg/kg, toxic if > 5 mg/kg but ≤ 50 mg/kg, harmful if > 50 mg/kg

but ≤ 500 mg/kg, and non-toxic or not harmful if > 500 mg/kg but ≤ 2000 mg/kg (Walum, 1998). According to this classification, the oral median lethal dose for FvMSE being above 5000 mg/kg indicates its relative safety when taken orally.

Screening of substances using *in vivo* experimental models provides a thorough approach to evaluating the effectiveness of drugs within a biological system. However, it is important to understand that *in vivo* studies are affected by multiple host factors, including the drug's distribution within the body and its natural antiparasitic properties, which can influence the outcomes. Therefore, the efficacy of a drug *in vivo* does not necessarily ensure ideal disposition characteristics. For instance, artemisinin shows exceptional efficacy *in vivo* despite having poor disposition in animals (White, 2008; Asanga *et al*., 2017). This underscores that the success of an antimalarial drug cannot be solely determined by its disposition properties. In the context of in vivo screening for antimalarial drugs, Plasmodium berghei is commonly used due to its accessibility and widespread acceptance in drug discovery research (Fidock *et al*., 2004). However, it is important to note that certain genes specific to Plasmodium berghei, which has adapted to rodents, may have significantly diverged from those found in the human pathogen Plasmodium falciparum (Asanga *et al*., 2017).

It has been reported that some plant extracts exert their antiplasmodial effects by inhibiting protein synthesis in parasites (Kirby, 1997). Additionally, alkaloids have been implicated in blocking protein synthesis in *Plasmodium falciparum* (Nergiz, 1993). Given that alkaloids are present as common constituents of FvMSE, it is plausible that their relative abundance in the extract may contribute to the observed activity. FvMSE can be considered to have good chemo suppressive activity. In vivo studies report that the antimalarial activity of an extract is considered very good if suppression is ≥ 50% at a 100 mg/kg dose, good if suppression is ≥ 50% at a 250 mg/kg dose, and moderate if suppression is ≥ 50% at a 500 mg/kg dose (Deharo *et al*., 2001; Tajbakhsh *et al*., 2021).

The observed prophylactic activity in the treatment groups revealed a significant reduction in parasite density in the FvMSE-treated groups compared to the negative control. This indicates that the extract possesses varying levels of repository activity, suggesting that consuming the plant before malaria infection might help mitigate the onset and severity of malaria symptoms. The result was consistent with the report by Nkafamiya *et al*., (2010) on plants nutritious values which may equally serve as immune boasters in preventing diseases such as malaria. Therefore, the prophylactic efficacy of the extract is indicative of non-selectivity of action on the different stages of malaria parasite, since prophylactic drugs work either by the disruption of the initial development of malaria parasite in the liver or by suppressing the emergent asexual blood stages or even by the prevention of the relapses induced by hypnozoites (Hill *et al*., 2006).

Malaria is known as a complicated syndrome involving many inflammatory responses which may enhance cell-to-cell interaction, cell stimulation involving malaria derived antigens/toxins and the host derived factors such as cytokines (Boampong *et al*., 2013). Malaria-induced inflammatory conditions are characterized by the generation of free radicals and the activation of phospholipase, leading to the production of prostaglandins and tumor necrosis factors. Consequently, the curative properties observed in FvMSE may be attributed to its ability to inhibit the production and/or release of these inflammatory mediators associated with malaria or through a direct cytotoxic effect on the parasites. In addition, there were significant increases in the mean survival time (MST) of all the treatment groups when compared with that of the negative control group indicating that the treatment is efficacious, as MST is used as an indicator for drug efficacy in antimalaria drug researches (Udobre *et al*.,

2013). The outcome of the assessment of mean survival time (MST) from the curative model indicated that mice treated with chloroquine, artesunate, and the extracts had significantly longer survival times compared to the negative control group. The extended survival of the mice was reflected in the higher parasitemia clearance achieved by both the standard drugs and the extracts. This might be due to the presence of secondary metabolites that prevent the overall pathologic effect of the parasite in the infected mice, like antioxidant, anti-inflammatory effects in addition to good parasites suppression (Toma *et al*., 2015).

Most anti-malarial drugs, such as chloroquine, quinine, mefloquine, halofantrine, pyrimethamine, sulfadoxine, sulfones, tetracyclines, act on the erythrocytic stage of parasite during the course of infection, which is the primary symptomatic phase of infection, thereby terminating the clinical attacks of malaria and addressing the constant threat of drug resistance (Fidock, 2010). Erythrocytic stages in culture of *Plasmodium falciparum* under *in vitro* conditions is practically feasible with easier manipulative steps in the laboratory and found to be a major initial tool to screen schizontocidal compounds (Trager and Jensen 1976). Though this morphological microscopic method is cumbersome and labour intensive, it has been established because of its reproducibility and simplicity (Bhattacharya *et al*., 2009; Sinha *et al*., 2017). The schizont inhibition test is used both as a screening model for antiplasmodial activity and to determine potential mechanisms of action. Presently, the FvMSE exhibited notable antiplasmodial activity with an IC_{50} of 11.663 µg/ml, which was better compared to chloroquine (14.390 µg/ml) and artesunate (12.831 µg/ml). It is reported that for *in vitro* studies, the antiplasmodial activity of an extract is considered very good, if IC_{50} is ≤ 5 µg/ml good, if it is 5-10 µg/ml, and is considered moderate if it is 10-20 µg/ml (Deharo *et al*., 2001).

CONCLUSION

The methanol stem bark extract of *Ficus vallis-choudae* exhibited both *in vitro* and *in vivo* antiplasmodial effect through prophylactic, suppressive, curative and schizont inhibition activity. This justify the claim for it use in traditional medical practice for the treatment of malaria.

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