COMPARATIVE *IN VITRO PLASMODIUM FALCIPARUM* CLEARING ACTIVITY OF *FICUS ASPERIFOLIA* AND *FICUS VALLIS-CHOUDAE* IN PARASITES INFECTED RED BLOOD CELLS (RBCS)

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Abstract: Malaria still constitutes a major global health challenge due its high morbidity and mortality. *Ficus asperifolia* and *Ficus vallis-choudae* (Moraceae) have been traditionally used in the treatment of malaria among other ailments. The aim of this study was to evaluate and compare the *in vitro* antiplasmodial activity of the methanol leaf extract (FaMLE), stem bark extract (FaMSE) of *Ficus asperifolia* and methanol leaf extract (FvMLE), stem bark extract (FvMSE) of *Ficus vallis-choudae*. Plants secondary metabolites were determined using standard protocols and oral median lethal dose (LD_{50}) of the extracts were estimated using the OECD guidelines. The *in vitro* antiplasmodial activity was evaluated using the percentage parasite clearing activity model as described by Trager. Flavonoids, tannins, saponins, alkaloids, terpenoids, cardiac glycosides and steroids were seen as the major metabolites. The oral LD_{50} of the extracts were found to be above 5000 mg/kg. FaMLE, FvMLE, FaMSE and FvMSE showed significant ($p<0.05$) parasite reduction when compared to the negative control as was observed with Chloroquine and Artesunate (10 µg/ml). The extracts in a concentration dependent manner (6.25, 12.5, 25.0, 50.0 and 100 µg/ml) exhibited the reduction with varying percentage of parasite clearance. The FaMLE had 37.96, 55.02, 66.89, 75.92 and 80.94% respectively while the FvMLE revealed 24.04, 45.06, 57.96, 63.06 and 75.96 as percentage parasite clearance respectively. The percentage parasite clearance for FaMSE and FvMSE was found to be 20.49, 35.07, 52.09, 60.06 68.04 and 33.93, 56.12, 62.97, 70.96, 78.95 respectively. It was observed that FaMLE and FvMSE showed better *in vitro* parasite clearing activity and were recommended for the *in vivo* studies using laboratory animals to further confirm their antiplasmodial potentials.

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

Introduction

Malaria is a significant global health issue, contributing to high rates of illness and death, particularly in tropical and subtropical regions (NIAID, 2007). This infectious disease is caused by single-celled microorganisms from the *Plasmodium* group. There are five *Plasmodium* species known to infect humans. *Plasmodium falciparum* is the deadliest and the primary cause of malaria cases. While most fatalities are due to *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium ovale*, and *Plasmodium malariae* usually result in less severe malaria. *Plasmodium knowlesi* is a rare cause of malaria in humans (WHO, 2020). Malaria, a life-threatening parasitic disease, is mainly transmitted by the bite of an infected female Anopheles mosquito. The mosquito injects the parasites from its saliva into a person's bloodstream, where they travel to the liver to mature and reproduce. Diagnosis of malaria is typically done by examining blood samples under a microscope using blood films or through antigen-based rapid diagnostic tests. Polymerase chain reaction (PCR) methods to detect the parasite's DNA exist but are not commonly used in malaria-endemic areas due to their high cost and complexity (Nadjm and Behrens, 2012).

Sub-Saharan Africa bears a significantly large portion of the global malaria burden. In 2021, this region accounted for 95% of malaria cases and 96% of malaria-related deaths (WHO, 2022). In Nigeria, like many other developing countries, malaria prevalence is notable, with the country contributing about 31.3% of the global malaria death toll (WHO, 2022). A study conducted in Kano, northwestern Nigeria, reported a malaria infection prevalence of 60.06% (Dawaki *et al*., 2016). The disease severely impacts Africa's economy, being a major factor in economic slowdown due to loss of income and lives (Maigemu and Hassan, 2015). According to Chima *et al.* (2003), the economic costs of malaria are divided into direct and indirect costs. Direct costs are associated with prevention and treatment expenditures, while indirect costs arise from lost productivity and time due to malaria morbidity and mortality (Niringiye and Douglason, 2010). Consequently, there has been a renewed focus on research and innovation in diagnostic methods, drugs, vaccines, and control measures aimed at eradicating the disease. This renewed effort has led to a global reduction in malaria incidence rates by 30% and a 34% reduction in Africa between 2000 and 2013 (Dawaki *et al*., 2016).

Medicinal plants have been used extensively throughout history and are fundamental to ethno-medical practices worldwide (Cragg and Newman, 2013; Mondal *et al*., 2019). They are considered essential sources of medicine and play a crucial role in the healthcare systems of Africa (Cragg and Newman, 2013). This is attributed to their diverse pharmacological compounds and perceived safety compared to conventional treatments (Seo *et al*., 2018; De Olivera *et al*., 2019). Herbal products have been widely used to manage fever, pain, and inflammation, and there is a growing renewed interest in medicinal plants for treating these conditions (Solati *et al*., 2017; Oguntibeju, 2018).

Traditional medicine continues to play a significant role in the healthcare systems of developing countries. According to the WHO, up to 80% of the population in some Asian and African countries relies on traditional medicine for their healthcare needs (WHO, 2010; Zhenji and Xu, 2011). In Nigeria, a large portion of the population still depends

on traditional medicine (Adefolaju, 2011). In the northern Nigerian states of Kano and Jigawa, the majority of the population relies extensively on traditional plant medicines for their primary healthcare needs (Adoum, 2016).

The plants *Ficus asperifolia* and *Ficus vallis choudae* have been ethnomedicinally employed in the treatment of diseases including malaria (Burkill, 1997; Ojo *et al*., 2016; Bello *et al*., 2017). 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).

Materials and methods

Materials

Malaria Parasites

Malaria positive clinical isolate (Blood) was obtained from the Department of Pathology Federal Medical Centre (FMC) Nguru, Yobe State.

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, 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.

Equipment

Water Bath (HH-4 ENGLAND Lab science), Electric Weighing Balance (FA2104A, Gulfed Medical and Scientific England), Digital (DB-1A, PEC MEDICAL USA), Animals weighing balance (SF-400), Animal cages, Pestle and mortar, Syringes (1 ml, 2 ml, 5 ml and 10 ml), What man's Filter Paper No. 1, Crucibles, Separating funnel, Conical flask, Beakers and Retort stand. Electronic balance, Microscope, Microscope slides, syringes, Mortar and Pestle, Animal cages, Spatula, EDTA bottles, Whatman No. 1 filter paper (1mm mesh size), Vacutainer syringe, Flat bottom test tubes, CPDA tubes, 96 well Micro plates, Eppendorf micro pipettes, Desiccator, Candle jar, Centrifuge (England), Thermostat oven (DHG-9101-ISA), etc.

Authentication of the collected plant

Fresh stem barks of *Ficus vallis-choudae* were gathered from the Toro district in the Toro Local Government Area of Bauchi State, Nigeria. The plant was 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

The collected plant material was rinsed with clean water, and shade-dried. The dried plant material was ground into a fine powder using a porcelain mortar & pestle, and then sieved. A total of 2 kg of the powdered plant material was macerated with 7 L of 70% v/v methanol at room temperature for 7 days, with occasional stirring. After the extraction period, the crude methanol extract was filtered through Whatman's filter paper (1 mm mesh size) and concentrated using a water bath set at 45°C until a brownish residue was obtained. This residue was then stored in a desiccator.

Methods

Phytochemical evaluation of the extracts

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 screened for the presence of 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 mice.

In vitro Studies

Parasite sample collection

A blood sample was collected through standard venipuncture, using 70% v/v alcohol to disinfect the cleaned vein. After locating a visible vein, 4 ml of blood was drawn with a needle and syringe and transferred into a citrate phosphate dextrose adenine (CPDA) bottle. Rapid diagnostic tests (RDT) were performed before measuring parasitaemia. Malaria-positive samples were stored at 4°C and transported using a vaccine carrier.

Preparation of erythrocytes (RBCs) for culture

Blood of group O, Rhesus positive was collected in a tube without anticoagulant and centrifuged at 1000 rpm for 20 minutes. The plasma and buffy coat were then removed with a sterile Pasteur pipette. Normal saline was added, and the sample was centrifuged again at 1500 rpm for 10 minutes, with the supernatant removed. This process was repeated three times. An equal volume of complete medium was added to the RBCs to achieve a 50% hematocrit, and the mixture was stored at 4°C until needed.

Estimation of the percentage of erythrocytes infected (parasitaemia) with Plasmodium falciparum

Thick blood film and Thin blood film was made to determine the level of parasitaemia and study *Plasmodium falciparum* morphology, density and the condition of the cells. While Giemsa Staining Method applied to examine the erythrocytes and the percentage of parasitaemia was calculated by multiplying, the number of parasitized RBCs times 100 and dividing by the total number of RBC's index.

$$\%\ of\ parasitaemia = \frac{\text{number of parasitized RBCs} \qquad \text{x100}}{\text{Total RBCs counted}}$$

Evaluation of parasite clearance

Equal volumes of the extract solution (0.5 ml) and culture media were added to flat-bottomed test tubes and labeled with concentrations of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.25 µg/ml. For each concentration, 0.1 ml of malaria-positive erythrocytes was added and gently mixed to ensure even distribution. The test tubes were placed in a bell jar containing a burning candle, and the jar was sealed until the candle's flame extinguished. This created an atmosphere of approximately 95% nitrogen, 2% oxygen, and 3% carbon dioxide (Trager, 1971). The setup was then incubated at 37°C for 24 to 48 hours. Alongside the test concentrations, a control group with culture media and positive erythrocytes (negative control) and a control group with culture media, positive erythrocytes, and anti-malarial agents Artesunate and chloroquine (positive control) were also incubated. After 24 hours, a thin smear was prepared from each test tube, fixed in absolute methanol, and stained with Giemsa's stain. The smears were examined under a microscope using oil immersion to count the number of infected erythrocytes. This procedure was repeated after 48 hours of incubation to assess the activity. The percentage activity of the extracts was calculated as the percentage of parasite elimination after 24 and 48 hours using the formula: $\% = (N/Nx) \times 100$, where % represents the percentage activity of the extracts, N is the total number of cleared RBCs, and Nx is the total number of parasitized RBCs (Mukhtar *et al*., 2006).

Presentation and analysis of data

The obtained results 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

Phytochemical Constituents of the Leaf and Stem bark Extracts of Ficus asperifolia and Ficus vallis-choudae

The preliminary phytochemical analysis of *Ficus asperifolia* methanol leaf extract (FaMLE), *Ficus vallis-choudae* methanol leaf extract (FvMLE), *Ficus asperifolia* methanol stem bark extract (FaMSE) and *Ficus vallis-choudae* methanol stem bark extract (FvMSE) revealed the presence of various phytochemicals such as cardiac glycosides, tannins, flavonoids, alkaloids, saponins, steroids and terpenoids. The plant extracts showed similarity in the content of secondary metabolites found in them. The qualitative test however, revealed a negative result for the presence of anthraquinones in all the extracts (Table 1).

Table 1: Phytochemical Constituents of Methanol Leaf and Stem bark Extracts of *Ficus asperifolia* and *Ficus vallis-choudae*

Chemical constituents	FaMLE	FvMLE	FaMSE	FvMSE
Alkaloids	+	+	+	+
Anthraquinone	-	-	-	-
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+

Key: + =present, **-** = absent, FaMLE = *Ficus asperifolia* methanol leaf extract, FvMLE =*Ficus vallis* methanol leaf extract, FaMSE = *Ficus asperifolia* methanol stem bark extract and FvMSE = *Ficus vallis* methanol stem bark extrac.

Median Lethal Dose (LD_{50})

The oral median lethal dose (LD_{50}) of FaMLE, FaMSE, FvMLE and FvMSE in mice was above 5000 mg/kg (Table 2).

Table 2: Oral Median Lethal Dose (LD_{50}) of FaMLE, FaMSE, FvMLE and FvMSE

Extract	Animal Specie	Value (mg/kg)
FaMLE	Mice	>5000
FaMSE	Mice	>5000
FvMLE	Mice	>5000
FvMSE	Mice	>5000

Key: FaMLE = *Ficus asperifolia* methanol leaf extract, FaMSE = *Ficus asperifolia* methanol stem bark extract, FvMLE = *Ficus vallis-choudae* methanol leaf extract, FvMSE = *Ficus vallis-choudae* methanol stem bark extract.

In vitro* Antiplasmodial Studies of the Methanol Leaf and Stem bark Extract of *Ficus asperifolia* and *Ficus vallis-choudae.

The FaMLE, FvMLE, FaMSE and FvMSE showed significant ($p<0.05$) parasite reduction when compared to the negative control as was observed with Chloroquine and Artesunate (10 µg/ml). However, the extracts in a concentration dependent manner (6.25, 12.5, 25.0, 50.0 and 100 µg/ml) exhibited the reduction with varying percentage of parasite clearance. The FaMLE had 37.96, 55.02, 66.89, 75.92 and 80.94% respectively while the FvMLE revealed 24.04, 45.06, 57.96, 63.06 and 75.96 as percentage parasite clearance respectively. The percentage parasite clearance for FaMSE and FvMSE was found to be 20.49, 35.07, 52.09, 60.06 68.04 and 33.93, 56.12, 62.97, 70.96, 78.95 respectively. Therefore, it was observed that FaMLE and FvMSE showed better *invitro* parasite clearing activity and they were selected for the *invivo* studies using laboratory animals (Table 3, 4, 5 and 6).

Table 3: *In vitro Plasmodium falciparum* Clearing Activity of the Methanol Leaf Extract of *Ficus asperifolia* in Parasite Infected RBCs

Treatment	Conc (µg/ml)	Mean no. of Parasitised RBC	% Clearance
NControl	-	59.8±1.03	-
FaMLE	6.25	37.1±0.26	37.96
FaMLE	12.50	26.9±0.08*	55.02
FaMLE	25.00	19.8±0.34*	66.89
FaMLE	50.00	14.4±0.11*	75.92
FaMLE	100.00	11.4±0.07*	80.94
CQ	10.00	6.3±0.55*	89.46
ATS	10.00	1.2±0.01*	97.99

Key: * significantly different from negative control at p<0.05 analysed using one-way ANOVA followed by Dunnett's post hoc test: NControl = Negative contol, FaMLE = *Ficus asperifolia* methanol leaf extract, CQ = Chloroquine, ATS = Artesunate, RBC = Red blood cells.

Table 4: *In vitro Plasmodium falciparum* Clearing Activity of the Methanol Leaf Extract of *Ficus vallis-choudae* in Parasite Infected RBCs

Treatment	Conc (µg/ml)	Mean no. of Parasitised RBC	% Clearance
NControl	-	62.8±1.14	-
FvMLE	6.25	47.7±0.72	24.04
FvMLE	12.50	34.5±0.40*	45.06
FvMLE	25.00	26.4±0.16*	57.96
FvMLE	50.00	23.2±0.06*	63.06
FvMLE	100.00	15.1±0.12*	75.96
CQ	10.00	5.0±0.04*	92.04
ATS	10.00	3.1±0.06*	95.06

Key: * significantly different from control at p<0.05 analysed using one-way ANOVA followed by Dunnett's post hoc test: NControl = Negative control, FvMLE = *Ficus vallis-choudae* methanol leaf extract, CQ = Chloroquine, ATS = Artesunate, RBC = Red blood cells.

Table 5: *In vitro Plasmodium falciparum* Clearing Activity of the Methanol Stem bark Extract of *Ficus asperifolia* in Parasite Infected RBCs

Treatment	Conc (µg/ml)	Mean no. of Parasitised RBC	% Clearance
NControl	-	57.6±1.02	-
FaMSE	6.25	45.8±1.15	20.49
FaMSE	12.50	37.4±1.04	35.07
FaMSE	25.00	27.6±0.84*	52.09
FaMSE	50.00	23.0±0.04*	60.06
FaMSE	100.00	18.4±0.32*	68.04
CQ	10.00	6.3±0.36*	89.07
ATS	10.00	4.0±0.22*	93.05

Key: * significantly different from control at p<0.05 analysed using one-way ANOVA followed by Dunnett's post hoc test: NControl = Negative control, FaMSE = *Ficus asperifolia* methanol stem bark extract, CQ = Chloroquine, ATS = Artesunate, RBC = Red blood cells.

Table 6: *In vitro Plasmodium falciparum* Clearing Activity of the Methanol Stem bark Extract of *Ficus vallis-choudae* in Parasite Infected RBCs

Treatment	Conc (µg/ml)	Mean no. of Parasitised RBC	% Clearance
NControl	-	61.3±1.15	-
FvMSE	6.25	40.5±1.08	33.93
FvMSE	12.50	26.9±0.72*	56.12
FvMSE	25.00	22.7±0.81*	62.97
FvMSE	50.00	17.8±0.32*	70.96
FvMSE	100.00	12.9±0.08*	78.95
CQ	10.00	5.5±0.17*	91.03
ATS	10.00	1.8±0.09*	97.06

Key: * significantly different from control at p<0.05 analysed using one-way ANOVA followed by Dunnett's post hoc test: NControl = Negative control, FvMSE = *Ficus vallis-choudae* methanol stem bark extract, CQ = Chloroquine, ATS = Artesunate, RBC = Red blood cells.

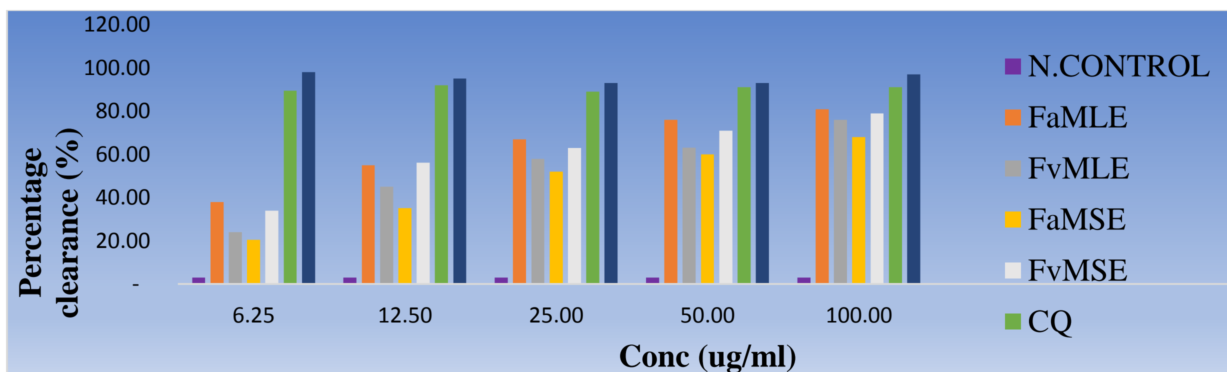

Figure 1: *Invitro* Antiplasmodial Studies of the Leaf and Stem bark Extracts of *Ficus asperifolia* and *Ficus vallis-choudae.* Ncontrol = Distilled water, FaMLE = *Ficus asperifolia* Methanol Leaf Extract, FvMLE =*Ficus vallis-choudae* Methanol Leaf Extract, FaMSE = *Ficus asperifolia* Methanol Stem bark Extract and FvMSE = *Ficus vallis-choudae* Methanol Stem bark extract.

Discussion

Medicinal plants have been recognized and used throughout human history. They have been found to be the number one source of biologically active compounds, and many documented medicinal plants have been scientifically proven to have therapeutic applications (Faustino *et al.* 2010). Medicinal plants, especially in developing countries, have been the subject of intense research due to their potentials as sources of commercial drugs or as lead compounds in drug development (Zhang, 2004). It is on record that about 80% of people living in the developing world, depend on traditional medicine for their primary health care needs (Schippmann *et al*., 2006; WHO, 2019). This is due to the perceived low cost, easy access and the belief that these medicines are devoid of adverse effects as well as blending readily into the socio cultural life of the people (Da- Nóbrega et *al*., 2008). Researches on medicinal plants have led to the discovery of novel lead compounds

for potential development as drugs (Adebayo *et al*., 2015; Seo *et al*., 2018). In malaria treatment notably quinine was isolated from Cinchona (Rubiaceae) and artemisinin from *Artemisia annua* (Asteraceae) (Rathore *et al*., 2005). There are several species of the Ficus genus, commonly known as figs, which have been studied for their medicinal properties, including their potential antiplasmodial activity (Muregi *et al*., 2001; Ajaiyeoba *et al*., 2013; Folade *et al*., 2014; Mukhtar *et al*., 2019). However, it is important to note that the effectiveness of different species may vary and hence the essence for this pharmacological activity studies.

Preliminary phytochemical screening provides basic information about the different classes of secondary metabolites present in a plant and the medicinal importance of such plant (Shabbir *et al*., 2013). The preliminary phytochemical screening of the leaf and stem bark extracts of *Ficus asperifolia* and *Ficus vallis-choudae* revealed the presence of saponins, flavonoids tannins, alkaloids, terpenoids, steroids and cardiac glycosides which might be responsible singly or in complement for the observed antiplasmodial activity of the extracts. This finding is in tandem with the reported work of Omaniwa and Luka, (2012) and Abdullahi *et al*., (2020) in the case of *Ficus asperifolia* and Lawan *et al*., (2008) in the case of *Ficus vallis-choudae*. Secondary metabolites such alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides and steroids are responsible for plants' biological activities (Edewor-Kuponiyi, 2013; Rungsung *et al*., 2015). Phytochemicals constitute an integral part of medicinal plants and are responsible for their numerous bioactivities. Numerous plants have a wide variety of phytochemicals as their bioactive principles and are reported to possess antiplasmodial activity (Alshawsh *et al*., 2007; Matur *et al*., 2009). Although some studies have tracked antiplasmodial activity of plants to their alkaloids, flavonoids and terpenoids contents (Akuodor *et al*., 2010; Philip, 2020; Tajjuddeen *et al*., 2021), there are reported studies indicating antiplasmodial activity with saponins (Akanbi *et al*., 2018; Nafiu *et al*., 2021) and glycosides (Yun *et al*., 2021).

Some of these secondary metabolites have been suggested to act as primary antioxidants or free radical scavengers that can mitigate the oxidative damages induced by the malaria parasites. Others, such as some polyphenols and flavonoids, may be pro-oxidants in high ingested amounts (Halliwell, 2007; Asanga *et al*., 2017) this therefore suggest that better activity does not always depend on higher contents of these secondary metabolites but optimality to function either as anti or pro oxidants depending on what is targeted at a given period. Antioxidant property of saponins have been reported by some studies towards preventing generation of free radicals and are able to block protein synthesis in the parasites' apicoplast ribosome through structure-related interaction (Abdullelah and Zainal 2007). This therefore suggests that the observed activity by the extract might be due to its ability to elicit antioxidant activities which inhibited parasites growth and multiplication, and consequently, a reduction in percentage parasitaemia. However, the dual nature of phenolic compounds enables them to function both as antioxidants and pro oxidants. Similarly, depending on the extent of their pro oxidant activity, ROS can either trigger pathogenesis, exacerbate existing conditions, compromise immunity (thus increasing vulnerability to certain diseases like malaria), or serve as potential agents for eradicating malignant cells and foodborne pathogens (Rajashekar, 2023). Free radicals also play important roles in cellular signaling and since iron is necessary for the survival and replication of microorganisms such as plasmodium parasites, the potency of an antioxidant in chelating iron needed by these parasites will adversely negate the survival of the parasite in the host system (Asanga *et al*., 2017).

The determination of median lethal dose (LD_{50}) is a crucial aspect in acute toxicity testing, serving as a key entry point for pharmacological research (Abdullahi *et al*., 2020). This method allows for the rapid evaluation of potential hazards associated with a test substance after a single dose. LD_{50} testing is commonly employed in risk assessments for both synthetic and naturally occurring chemicals, providing valuable guidelines for subsequent clinical studies involving humans and non-target environmental organisms (Agrawal and Paridhavi, 2007). Acute toxicity studies are typically conducted to ascertain the dosage range that could be harmful to animals. Additionally, these studies enable the estimation of the therapeutic index (LD_{50}/ED_{50}) for drugs and xenobiotics (Maikai *et al*., 2008; Abdullahi *et al*., 2020). The oral LD_{50} values for both the methanol stem bark extract of *Ficus vallis-choudae* (FvMSE), methanol leaf extract of *Ficus asperifolia* (FaMLE) were found to be above 5000 mg/kg, indicating relative safety of these substances (which agrees the reported work of Lawan *et al*., 2008; Malami *et al*., 2010 and Abdullahi *et al*., 2020). It is important to note that while LD_{50} serves as a useful measure for assessing the safety margin of a substance, it should not be regarded as an absolute value or a comprehensive evaluation of its properties and may not fully capture the entire range of toxicity or hazards associated with a drug or chemical (Cassarette *et al*., 1996).

Plasmodium falciparum like other malaria parasites will develop within their host erythrocytes when they are incubated outside under appropriate conditions. This *in vitro* technique originally carried out by Trager, 1971 has developed into antimalarial drug screening model by exploiting the percentage parasite clearing ability of test compounds. The model has been slightly modified also to serve as antiplasmodial mechanism screening technique through the schizont inhibition test (Trager and Jensen, 1976). The *in vitro* percentage parasite clearing ability of the methanol leaf and stem back extract of *Ficus vallis-choudae*, together with that of the *Ficus asperifolia* revealed a concentration dependent results and showed different activity when compared. All the extracts exhibited better percentage clearance at higher concentrations. This is likely because the active components in the plant extracts are present in very low concentrations or forms part of *in vivo* complex mixtures with many other compounds within the extract (Batista *et al*., 2009).

In numerous investigations, many plants have demonstrated promising antiplasmodial activity in laboratory settings (*in vitro*). However, it has been observed in several studies that this activity tends to decrease when tested in living organisms (*in vivo*), with most plants exhibiting only moderate activity. In some cases, certain plants that initially displayed good activity *in vitro* became inactive *in vivo*, indicating a decrease in effectiveness (Gathirwa *et al*., 2008; Tajbakhsh *et al*., 2021). Nevertheless, there are few exceptions where the antiplasmodial activity of plants actually increased from *in vitro* to *in vivo* analysis. For instance, Ngbolua *et al*., (2011) demonstrated an increase

in activity of *Vernonia ambigua* when tested in living organisms. Other studies, such as those conducted by Muthaura *et al*., (2015) using *Boscia angustifolia*, Kweyamba *et al*., (2019) using *Commiphora africana*, and Ajaiyeoba *et al*., (2006) using *Annona senegalensis,* also reported similar findings. These instances suggest that certain plants may possess significant antimalarial activity *in vivo*, even if they did not exhibit it *in vitro*. It is worth noting that most researchers typically proceed to *in vivo* studies only when they observe substantial antiplasmodial activity *in vitro* (Tajbakhsh *et al*., 2021). It is therefore reasonable to recommend that the study should progressed to *in vivo* analysis after the earlier findings on the *in vitro* antiplasmodial activity of the methanol stem bark extract of *Ficus vallis-choudae* (FvMSE) methanol leaf extract of *Ficus asperifolia* (FaMLE) in order to conclude more authoritatively, on the antiplasmodial activity of the plants.

Conclusion

Based on the available data, the study concludes that the methanol leaf and stem bark extracts of *Ficus asperifolia* and *Ficus vallis-choudae* possess *in vitro* antiplasmodial activity. However, FaMLE and FvMSE showed better *in vitro* parasite clearing activity and were recommended for the *in vivo* studies using laboratory animals to further confirm their antiplasmodial potentials.

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Conflict of Interest

The authors state that no any conflict of interest is associated with this work or its publication.

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