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Full Length Research Paper

Antiplasmodial *in vitro* and *in vivo* activities of crude extracts from *Ozoroa obovata* (Oliv) R.Fern. &A. Fern collected in Burkina Faso

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Abstract

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Malaria remains the main health concern in sub-Saharan Africa. This study aimed to evaluate the *in vitro* and *in vivo* antiplasmodial activities of *Ozoroa obovata* extracts, a plant traditionally used to treat malaria in Burkina Faso. The macerate extracts were freeze-dried, and TLC analysis was used to analyze the phytochemical compounds. The *in vitro* antiplasmodial activity was evaluated using the *Plasmodium* lactate dehydrogenase (pLDH) method. The *in vivo* antimalarial activity of the extract was evaluated in mice infected with *Plasmodium berghei* using the Peters' 4-day suppressive test. Thin-layer chromatography (TLC) analysis revealed the presence of flavonoids, sterols, triterpenes and tannins in both the aqueous and hydroethanolic extracts. The IC_{50} of the aqueous extracts were 58.59 ± 9.05µg/ml against the 3D7 and 56.14 ± 7.84 µg/ml against NF54 strains. The hydro-ethanolic extracts shown the IC_{50} of 161.10 ± 12.51µg/ml against the 3D7 and 76.28 ± 3.20µg/ml against NF54 strains. The percentages of reduction in parasitemia with the aqueous extract were 2.5%, 54.00%, and 92.00% at 100mg/kg, 250mg/kg, and 500mg/kg body weight, respectively. The hydroethanolic extract showed reductions of 7.00, 65.00, and 83.75% for the respective doses 100mg/kg, 250mg/kg and 500mg/kg body weight. These activities shown could justify the using of *O.obovata* in traditional medicine to treat malaria.

Keywords: *Ozoroa obovata (Oliv) R.Fern. &A. Fern*, Malaria, *Antiplasmodial activity,* Burkina Faso, *In vitro, in vivo*

1. Introduction

Malaria is a parasitic disease that continues to pose a significant threat to global health, particularly in endemic regions where resources for treatment and prevention are limited. According to the World Health Organization's 2024 report, there were 263 million recorded cases of malaria worldwide, resulting in 597,000 deaths, the majority of which occurred in Africa [1]. In Burkina Faso, more than 11 million cases and over 4,000 deaths were

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recorded in the same year, including 2,925 children under the age of five [2]. Efforts to combat the disease include improving access to effective treatments, promoting preventive measures such as insecticide-treated nets, and advancing vaccine development strategies. Artemisinin-based combination therapy (ACT), recommended by the WHO for the treatment of uncomplicated malaria, has been effective in combating this disease; however, the incidence of the disease remains high [3, 4] . Unfortunately, ACT treatment failures have been reported in some countries [5–8]. Recent studies highlight the urgent need for innovative approaches to tackle the rising drug resistance in malaria, which complicates treatment efforts and necessitates the development of new therapeutic agents. New research emphasizes the potential of plant-based extracts as alternative antimalarial agents, particularly in regions where traditional treatments are becoming less effective. In light of these alarming statistics, exploring traditional medicinal plants for their antiplasmodial properties could provide valuable insights into new treatment options for malaria [9, 10]. Ongoing research into new treatments is crucial to combating this disease, particularly given the emergence of resistance to existing treatments. In this context, there is an urgent need to develop innovative, practical strategies that provide accessible treatments for vulnerable populations. Medicinal plants are a rich source of new drug discoveries. Many plants used in West Africa and in Burkina Faso to treat malaria have demonstrated antiplasmodial properties *in vitro* or *in vivo* tests. These include *Cochlospermum tinctorium* (Cochlospermaceae), *Azadirachta indica* (Meliaceae), *Vernonia colorata* (Asteraceae), *Vernonia cinerea* Less (Asteraceae) and *Combretum micranthum* (Combretaceae) [11–17]. One such plant is *Ozoroa obovata* (Oliv.) R. Fern. & A. Fern, which belongs to the Anacardiaceae family and the *Ozoroa genus*, is attracting growing interest due to its medicinal properties, and has been included in the list of therapeutic plants by the Burkina Faso Research Institute of Health Sciences (IRSS)"[18]. Previous phytochemical studies of *Ozoroa* species have identified bioactive substances, including flavonoids, triterpenoids and anacardic acids. These compounds have significant potential in the fight against malaria due to their antimalarial properties and ability to inhibit parasite growth [19–22]. *Ozoroa obovata* (Oliv.) R. Fern. & A. Fern plant is also traditionally used to treat various diseases, including dysentery, coughs, and stomachaches [20]. Other studies indicate that the plant exhibits antiproliferative activity against HeLa cells and antiplasmodial activity *in vitro* against 3D7 strains [23]. These properties make *Ozoroa obovata* a promising candidate for the development of new antimalarial treatments. However, there is minimal data available regarding the antiplasmodial activity of this plant. The aim of this study is, therefore, to investigate the *in vitro* and *in vivo* antiplasmodial activities of extracts from the leaves of *Ozoroa obovata* (Oliv.) R. Fern. & A. Fern. used in traditional medicine to treat malaria.

2. Materials and Methods

2.1 Plant collection

Leaves of Ozoroa obovata were harvested on 10 January 2024 in the rural commune of Saponé (latitude: 12°10'14.553"N; longitude: 1°33'16.747"W), Burkina Faso and then dried and pulverized at the Institut de Recherche en Sciences de la Santé (IRSS). Its description and other taxonomic data can be found on World Flora Online, id: wfo-0001050305. An aqueous maceration and a hydroethanolic maceration (10/90) were prepared from the pulverized material, and the extracts were freeze-dried at the Centre National de Recherche et de Formation sur le Paludisme (CNRFP). The different parts of plant are shown in the Figure 1.

2.2 Preparation of plant extracts

2.2.1 Aqueous maceration:

Two hundred grams (200 g) of the plant material was macerated in 1 L of distilled water. The resulting water-plant drug suspension was thoroughly homogenized and left covered at room temperature for 24 hours. After 24 hours, the solution was filtered using nylon fabric. One hundred milli-liters (100 mL) of distilled water was added to the plant residue on the filter paper until it was completely exhausted. The resulting filtrate was then centrifuged at 2000 rpm for ten minutes. The resulting supernatant is the aqueous macerate, which was freeze-dried.

2.2.2 Hydro-ethanolic maceration

200 g of the plant material was macerated in 1 L of 90% ethanol. The alcohol-plant drug solution obtained was thoroughly homogenized, left to stand for 24 hours and then filtered using filter paper. Then, 100 mL of 90% ethanol was added to the plant residue on the filter paper until it was completely exhausted. The resulting filtrate was evaporated in a rotary evaporator and then freeze-dried.

2.2.3. Extract Yield Determination

Extract yields were determined using the prepared extracts. For each extract, 3 mL of extract was collected in three 1 mL doses in three watch glasses. The extract contained in each watch glass was then dried in an oven at 105°C for three hours and weighed to determine the dry mass. Extraction yields were then evaluated by calculating the ratio of the mass of the extract obtained to the mass of the plant material used.

Figure 1: *Ozoroa obovata* (A): fruits (B), leaves (C) and packaged powder (D).

Yield extracts (%) = (mass of the dry extract obtained)/(mass of the plant material) x100

2.2.4 Chromatographic analysis of extracts

Phytochemical screening of the extracts was performed using thin-layer chromatography (TLC) on chromatographic plates, following the methods described by Wagner and Bladt in 1996 [24]. The metabolites sought were saponins, sterols, triterpenes, alkaloids, flavonoids and tannins. TLC involves the absorption and partitioning of compounds according to their affinity for a solid phase (silica gel) and a mobile phase (migrating solvent), thereby separating them. Five glass plates were prepared for phytochemical screening by cutting and tracing the deposit line and the migration limit line. Twenty (20 mg) milligrams of hydro-ethanolic extract and forty milligrams of aqueous extract were weighed and dissolved in one millilitre of distilled water and one millilitre of methanol, respectively. A deposit of 5 µL of each extract solution and reference substance was made at the deposit line. The spots were then eluted over a distance of 7 cm in a glass tank containing a solvent system whose composition depended on the chemical group being sought. After elution, the chromatographic plates were removed and dried first at room temperature (25 °C) and then in a ventilated oven at 40 °C for five minutes.

2.3 Evaluation of the *in vitro* antiplasmodial activity of *Ozoroa obovata* (Oliv.) R. Fern. & A. Fern

2.3.1 *Plasmodium falciparum* strains

The biological material consisted of the *P. falciparum* strain, which is responsible for the majority of malaria cases in Africa. *P. falciparum* NF54 and 3D7 strains were supplied by BEI Resources, National Institute of Allergy and Infectious Diseases (NIAID, NIH, USA). These strains were maintained in continuous culture in human blood at the Pharmacognosy Laboratory of the National Centre for Research and Training on Malaria (CNRFP) in Ouagadougou.

2.3.2 Continuous *in vitro* culture of parasites

This technique ensures the growth of *Plasmodium falciparum* parasites in an artificial environment [25]. The parasites were thawed from liquid nitrogen, washed, and cultured in flasks containing complete culture medium. Parasites were maintained in culture at 5% hematocrit (human type O-positive red blood cells for 3D7). RPMI 1640 medium containing 24 mM sodium bicarbonate (EuroClone; Celbio) was used with the addition of 0.01% hypoxanthine, 20 mM HEPES, and 2 mM glutamine at 37°C in a standard gas mixture consisting of 1% O2, 5% CO2, and 94% N2. Culture medium was supplemented with 1% Albumax II (lipid-rich bovine serum albumin). Parasitemia was monitored using Giemsa-stained smears. For a normal *in vitro* growth of the parasite, maintained between 1% and 5% (the number of infected RBCs relative to the total number counted).

2.3.3 *In vitro* evaluation of the antiplasmodial activity of *Ozoroa obovata* (Oliv.) R. Fern. & A. Fern against *Plasmodium falciparum*

The pLDH technique was used to evaluate the antiplasmodial activity of *O. obovata* extracts [26]. For this, extracts were first dissolved in dimethyl sulfoxide (DMSO) to create stock solutions of 10 mg/mL. A cascade dilution was performed, starting with an initial

extract concentration of 500 µg/mL to obtain doses of 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, and so on. Extracts were diluted with medium to achieve the required concentrations, ensuring a final DMSO concentration ≤1%, which is not toxic to the parasites. Chloroquine (CQ) was used as a positive control. Extracts were placed in 96-well plates (EuroClone) and serial dilutions were performed in a final volume of 100 µL/well. One hundred micro-liters of asexual parasite cultures with parasitemia of 1-1.5% were distributed into each well to achieve a final volume of 200 µL and final hematocrit of 1%. The plates were placed in an incubating chamber and inflated with the proper gas mixture for 2 minutes before being inserted into the incubator at 37 °C for 72 hours. The growth of parasites was determined spectrophotometrically by measuring the activity of the parasite lactate dehydrogenase (pLDH), according to a modified version of Makler's method. Briefly, the drug-treated culture was re-suspended and 20 µL/well were transferred into a new plate containing 100 µL of Malstat reagent (0.11% [vol/vol] Triton-100, 115.7mM lithium L-lactate, 30.27mM Tris, 0.62mM 3-acetylpyridine adenine dinucleotide [APAD] [Sigma-Aldrich], adjusted to pH 9 with 1M HCl) and 25 µL of PES/NBT (1.96mM nitroblue tetrazolium chloride and 0.24mM phenazine ethosulfate). The plate was incubated in the dark at room temperature (20 °C) for 15 min and then read at a wavelength of 650 nm using a microplate reader Synergy (BioTek). The results of the chemosensitivity assays were provided as the percent viability compared to the untreated controls, calculated with the following formula:

% Viability= ((OD product-OD Negative control)/(OD positive control))x100

Results were analyzed and expressed as the 50% inhibitory concentration (IC50) relative to control wells using Gen5 software. The IC_{50} of the obtained extracts were analyzed according to Deharo's criteria [27].

2.4 *In vivo* evaluation of the antiplasmodial activity of *Ozoroa obovata* (Oliv.) R. Fern. & A. Fern against *Plasmodium berghei* in a murine model

2.4.1 *Plasmodium berghei* strains

Plasmodium berghei (ANKA strain) was gradually obtained from the American Type Culture Collection (MR4) and maintained continuously at the Parasitology Laboratory of the National Centre for Research and Training on Malaria (CNRFP) through weekly cyclical passage from infected to healthy mice by injection of parasitized blood.

2.4.2 Laboratory animals

Forty-two albino mice NMRI (Naval Medical Research Institute), aged 4–7 weeks and with an average weight 21.69 g, were obtained from the Institute for Health Sciences Research (IRSS). Mice were maintained under regulated laboratory conditions (Temperature 23°C ± 2°C, relative humidity 60%–70%, and a 12-h light-dark cycle) at the National Centre for Malaria Research and Training (NCMRT), acclimated to the working environment for two weeks before the start of the experiment. They were fed a 29% fattening feed supplied by the Livestock Department of the Bobo-Dioulasso Regional Livestock Directorate, and given access to drinking water. The experiments were conducted in accordance with the Organization for Economic Co-operation and Development's (OECD) principles for the use of laboratory animals [28–33]. Consent for the research was obtained from the Institutional Ethics Committee of the IRSS [34].

2.4.3 Preparation of the inoculum

Parasitized blood from donor mice, which are constantly maintained in the laboratory, is used to infect the test mice. The parasitaemia of each donor mouse is assessed to determine the amount of parasitized blood required to obtain 1x 10^7 parasitized red blood cells for injection into the test mice. Blood smears were made from blood taken from the tails of the donor mice. The smears were fixed with methanol, stained with 10% Giemsa, and examined under an optical microscope at 100x magnification. Parasitaemia was determined by examining 10 fields, each containing 100 red blood cells [35]. Donor mice were euthanized through 2 minutes exposure to petroleum ether for blood collection by cardiac puncture.

2.4.4 Infection of mice

Three doses of 100, 250 and 500 mg/kg body weight were prepared using aqueous and hydroethanolic extracts. Before the infection, the mice were weighed and grouped into batches of six. Blood was collected from euthanized donor mice 3–4 days post-infection via cardiac puncture using a sterile syringe and heparinized tubes. Parasitized blood was then diluted in physiological water (1% PBS), taking into consideration the parasitaemia and red blood cell count to reach 1 x 10^7 infected red blood cells in 200 µL for each mouse infection.

2.4.5 Peter's 4-day suppression test

The *in vivo* antiplasmodial activity of the extracts was assessed in mice as described by Peters (1975) [36]. Briefly, three hours post-infection and post-fasting, each of the six batches received orally either the aqueous or hydroethanolic extract of *Ozoroa obovata* at doses of 100, 250, and 500 mg/kg body weight. The remaining batch received water (negative control). Treatment administration was performed daily from D0 to D3 at the same time. On day 4 (D4), blood smears were made with

blood collected from the tails of the mice. The slides were stained with 15% Giemsa for 15 minutes after fixation with methanol, then examined under an optical microscope at 100x magnification. Parasitemia was measured by counting three fields on each slide. The animals were observed for a further 24 hours and then sacrificed. Parasitemia and the percentage suppression of parasitemia were calculated using the following formulas:

Parasitaemia (%)=((Number of infected RBCs)/(Total number of RBCs))x100

Suppression (%)=((% parasitaemia of Negative control-% parasitaemia of treated group)/(% parasitaemia of Negative control)).

2.5 Beta-haematin formation inhibition test

The semi-quantitative *in vitro* antimalarial activity screening test was performed according to the method described by Baelmans et *al*. (2000)[37], with some modifications. Ferriprotoporphyrin IX (FPIX) is a toxic residue resulting from the oxidation of haem. It is transformed into hemozoin (malaria pigment) during a biomineralization process commonly referred to as 'crystallization' in the digestive vacuole. Antimalarial drugs inhibit the formation of hemozoin (beta-haematin), a unique phenomenon that occurs only in the parasite at a pH range of 5.2 to 5.4. Under acidic conditions, haem spontaneously polymerizes in a simple test tube without external input. These observations have led to the development of various techniques for synthesizing hemozoin. These techniques all involve incubating hemin in a more or less acidic environment with or without parasitic residues for 18 to 24 hours at 37°C. The hemozoin formed can be detected using spectrophotometry or radiolabelling[38].

2.5.1 Preparation of sample solutions and chloroquine

The extracts were dissolved in double-distilled water or 10% DMSO and diluted to obtain initial concentrations of 4, 2, 1, 0.5, 0.25 and 0.125 mg/mL. Chloroquine diphosphate was prepared in successive half dilutions at initial concentrations of 3.125–100 µg/mL.

2.5.2 Methodology for the haemozine formation inhibition test

In an Eppendorf tube, 200 µL of haemoglobin solution (0.5 mg/mL in DMSO), 400 µL of sodium acetate buffer solution (0.5 M; pH 4.4) and 200 µL of the sample solution (or distilled water for the negative control) were successively added. The mixture was then incubated at 37 °C for 24 hours. After centrifugation for 10 minutes at 4000 x g at 25 °C, the supernatant was removed. The mixture was rinsed with 200 µL of DMSO to separate the free haemoglobin, then centrifuged and the resulting supernatant was discarded. The remaining β-haematin was then dissolved in 400 µL of 0.1 N NaOH to form an alkaline haematin, which can be measured by spectrophotometry [39, 40]. Absorbance was read at 405 nm. The results are expressed as the percentage inhibition (I%) of hemozoin formation using the following formula:

%I = (AB – AA)/(AB) x 100, where the letters mean:

AB = absorbance of the blank (negative control); AA = absorbance of the test sample.

2.6 Data analysis

Statistical analysis of the data was performed using Microsoft Excel 2013 to obtain the following standard curves, as well as the means and standard deviations of secondary metabolite content and parasitaemia. It was used to calculate the 50% inhibitory concentration of beta-haematin formation and the percentage suppression of parasitaemia. The percentage of viability was plotted as a function of drug concentrations. The curve fitting was obtained by nonlinear regression analysis using a four-parameter logistic method (software Gen5 1.10 provided with the Synergy plate reader [Biotek]). The IC_{50}, which is the dose capable of inducing 50% inhibition of parasite viability, was calculated from the sigmoidal dose-response curve. One-way ANOVA was also used to measure the statistical significance of the results using the GraphPad Prism Analysis software module version 10.4.0. The differences were statistically significant at a p-value of less than 0.05. TableCurve software was used to analyze the in vitro activity and determine the IC50 of extracts.

3. Results

3.1 Extraction yields of *Ozoroa Obovata* (Oliv) leaves R.Fern. &A.Fern.

The results of extraction yield are presented in the Table 1 below.

3.2. Chromatographic analysis

Phytochemical screening revealed the groups shown in Table 2. The presence of flavonoids, tannins, sterols and triterpenes and saponins in the extracts as well as the absence of alkaloids.

3.2. Beta-haematin formation inhibition test

The results of the test, expressed as the 50% inhibitory concentration (IC_{50}) are shown in the following Figure 2: The 50% inhibitory concentration (IC_{50}) of the hydroethanolic extract was higher than that of the aqueous extract.

Table 1. Results of extraction yields of aqueous and hydroethanolic extracts *Ozoroa Obovata* (Oliv) leaves R.Fern. & A.Fern.

Extracts	Yield (%)
Aqueous extracts	11.08± 0,24
Hydroethanolic extracts	23.41± 0,22

Table 2. Results of phytochemical screening by TLC.

Secondary metabolites	Aqueous extract	Hydro-ethanolic extract
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Alkaloids	-	-
Sterols and triterpenes	+	+

Key: (+) present; (-) negative result.

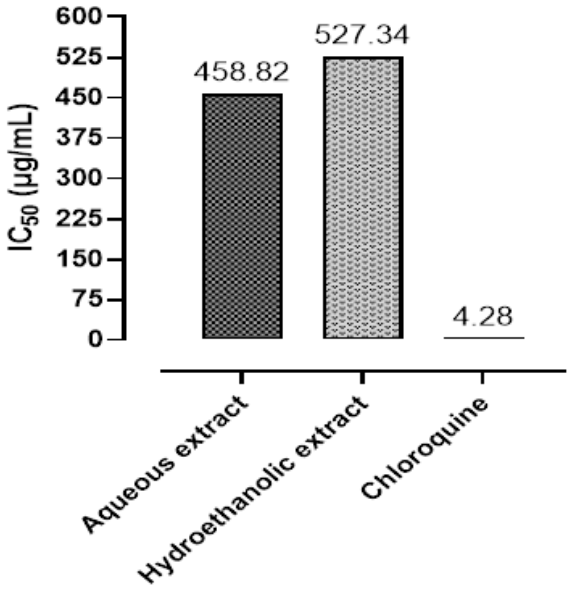

Figure 2: IC_{50} of the beta-haematin formation test.

3.3 *In vitro* evaluation of antiplasmodial activity

The Table 3 presents the 50% inhibitory concentrations of *O. obovata* plant extracts. The aqueous and hydroethanolic extracts were evaluated for *in vitro* activity against the 3D7 and NF54 strains**.**

* IC_{50} values are given as the mean ± SD from at least three independent dose-response experiments conducted in duplicate or triplicate wells. Chloroquine was used as a positive control.

Dose-response curves of plant extracts on parasites are shown in Figure 3 below.

3.4 Evaluation of antiplasmodial activity *in vivo*

The mean parasitaemia levels and the percentage of reduction on day 4 for the three groups of mice used in the antiplasmodial trial are shown in Table 4 below.

4. Discussion

In this study, the aqueous and hydroethanolic extracts were prepared. The results of phytochemical screening revealed the presence of saponins, flavonoids, tannins, triterpenes and sterols[41]. These various phytochemical groups indicate the local use of *Ozoroa obovata* in traditional medicine to treat microbial diseases [42–44].

Table 3. Summary of *in vitro* antiplasmodial activity of crude extracts obtained from *O.obovata*.

Extracts	IC_{50}	
	3D7	**NF54**
Aqueous extracts (µg/mL)	58.59 ± 9.05	56.14 ± 7.84
Hydro-ethanolic extracts (µg/mL)	161.10 ± 12.51	76.28 ± 3.20
Chloroquine (ng/mL)	13.87 ± 5.9	20.36 ± 1.7

*IC_{50} values are given as the mean ± SD from at least three independent dose-response experiments conducted in duplicate or triplicate wells. Chloroquine was used as a positive control.

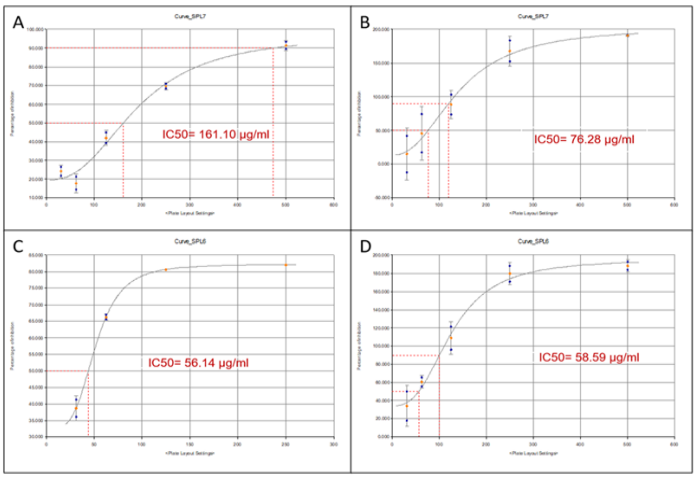

Figure 3.: Dose-response curves of extracts compared on parasites. (A) represents a curve plot of percentage of inhibition of hydroethanolic extract against *P.falciparum* 3D7; (B) represents a curve plot of percentage of inhibition of hydroethanolic extract against *P.falciparum* NF54. (C) represents the curve plot of percentage of inhibition of aqueous extract against *P.falciparum* NF54, and (D) represents the curve plot of percentage of inhibition of aqueous extract against *P.falciparum* 3D7.

Table 4: *In vivo* antiplasmodial activity of *O. obovata*

Extract	Aqueous extract		Hydroethanolic extract	
Dose	**Parasitemia**	**% Reduction**	**Parasitemia**	**% Reduction**
Control	20.17 ± 6%		20.17 ± 6%	
100 mg/kg	20.5 ± 6%	-2,5	18.6 ± 5%	7
250 mg/kg	9.2 ± 1%	54	7 ± 1%	65
500 mg/kg	1.6 ± 2%	92	3.25 ± 2%	83.75

*Results are the average of the parasitemia of 6 mice ± SD

The *in vitro* assay of *O. obovata* was performed against two strains of *Plasmodium falciparum*. The data were obtained and presented in Table 3. The inhibitory activity of aqueous extract against *P.falciparum* 3D7 and NF54 shown IC_{50} =58.59 ± 9.05µg/mL and 56.14 ± 7.84µg/mL respectively. The inhibitory activity of hydroethanolic extract shown $IC_{50=}$ 161.10 ± 12.51µg/mL and 76.28 ± 3.20µg/mL, respectively against *P.falciparum* 3D7 and NF54.These results of *in vitro* evaluation against *Plasmodium falciparum* 3D7 (chloroquine-sensitive clone

clone) and NF54 (isolate clone) revealed that the aqueous and hydroethanolic extracts were active with moderate activity, according to Deharo et al.'s criteria. The IC_{50} of the aqueous extract can be compare to *Vismia guinensis* studies on 3D7 *P. falciparum* strains which show moderate activity [45]. The hydroethanolic extracts show the highest values of $IC_{50,}$ which can demonstrate low antimalarial activity. This low *in vitro* activity may be due to the plant acting through another mechanism of action. The findings from the 4-day suppressive tests indicated that the aqueous extract of *Ozoroa obovata* possesses *in vivo* antiplasmodial activity in NMRI mice infected with *Plasmodium berghei.* The measure of parasitemia is the most reliable indicator of antimalarial efficacy of the plant extract in an *in vivo* assay. The 500 mg/kg dose of the aqueous extract demonstrated a high plasmodial suppression effect (92%) in the 4-day suppressive test, which was higher than the 83.75% suppression observed with the same dose of the hydroethanolic extract. This parasitemia-suppressive effect was similar to that observed in investigations conducted on *Coriandrum sativum* Linn, *Eleucine indica* and *Echinops kebericho* [46–48]. These values confirm the antiplasmodial effect of the plant extracts, as classified by Rasoanaivo et al.[49]. The extracts can be considered to have low-to-moderate antiplasmodial activity, thus confirming their traditional medicinal use in the treatment of malaria. The inhibition of parasitaemia by the two extracts may be due to the presence of phytochemical compounds with antiplasmodial activity in the plant. This corroborates the 2021 findings of Tajuddeen et *al*[17, 23], who discovered that certain compounds isolated from *Ozoroa obovata*, such as amentoflavone and quercetin 3-O-arabinofuranoside, exhibited antiplasmodial activity against *Plasmodium* 3D7 strains *in vitro*. In addition, the antiplasmodial effect exerted by both extracts of *Ozoroa obovata* could be due to the abundant phytochemicals localised in the plant [43, 44, 50].

The inhibitory activity on beta-haematin formation showed that the extracts inhibited beta-haematin formation, with an IC_{50} ranging from 458.82 µg/mL for the aqueous extract to 527.34 µg/mL for the hydroethanolic extract. This is 100 times higher than the reference (chloroquine IC_{50}= 4.28 µg/mL). The high IC_{50} values for beta-haematin formation indicate low antiplasmodial activity, suggesting that the plant extracts may act through a different mechanism involving secondary metabolites. The findings from the phytochemical profiling indicated that the plant contained flavonoids, tannins, saponins, and terpenoids. The terpenoids and flavonoids contained in the plant are molecules endowed with demonstrated pharmacological activities. These host protective actions could be responsible for the low antiplasmodial activity observed with the extract[51]. Future research, such as isolating active compounds, elucidating mechanisms, or conducting further preclinical/clinical studies could be done.

5.Conclusion

Our study demonstrated the presence of flavonoids, steroids, triterpenes, tannins, and saponins in the plant extracts. This study also showed that the aqueous and hydroethanolic extracts of *Ozoroa obovata* possess antimalarial properties *in vitro* and *in vivo* (in NMRI mice), highlighting their potential as sources of bioactive compounds for the development of antimalarial treatments. Appropriately research studies could be done to identify and isolate the lead active compounds which are responsible for the antiplasmodial activity and the way to develop a new antimalarial drug based on plant.

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