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GAMETOCYTE DYNAMICS AND THE EFFECTS OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *Lophira Lanceolata* IN MICE INFECTED WITH *Plasmodium bergei*

Ezea, O. C[1]., Nwachukwu, M. O[1]., Nnodim, N. N[1]., Nwachukwu, C. N[1]., Ozioko, K. U[2]., Azorji, J. N[3].

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Abstract

Resistance of human malaria parasites to existing chemotherapeutic drugs remains a global challenge to malaria control. This study was an evaluation of the gametocyte dynamics and the potential roles of aqueous and ethanolic leaf extracts of *Lophira lanceolata* in the transmission of *Plasmodium* parasites using mice experimentally infected with *P.berghei* as a model. A total of 192 experimental mice divided into 15 groups in a randomized block design and replicated four times with four mice in each group were used for this study. Thirty-six (36) mice were used for acute toxicity test, 32 mice each for curative antiplasmodial assay, prophylactic assay and gametocyte evaluation, and analyses. Experimental mice were inoculated intraperitoneally with standard inoculum of 1 x 10^7*P.berghei* parasitized red blood cells on first day (DO). Body weights of mice were taken using sensitive digital weighing balance. Blood sample was collected by cardiac puncture using sterile needle and 5ml syringe and preserved in ethylene diaminetetraacetic acid (EDTA) bottle. Haematological components were determined using an autohaematology analyzer SYSMEX KX21. Data analysis was done using student's t-test and one way analysis of variance with multiple comparison tests. Phytochemical screening revealed the presence of phenols, terpenoids, tannins, saponins, alkaloids, flavonoids, steroids, oxalates and cardiac glycosides. The flavonoids had the largest concentrations of 388.24mg/ml, and 350.00mg/ml in ethanolic and aqueous extracts respectively. LD_{50} was found to be $\geq$ 5000mg/kg body weight in both extracts. At $\geq$ 100mg/kg body weight, infected treated mice experienced body weight increases while infected untreated mice experienced decreases in mean body weight from 23.33g to 20.08g (mean 10% weight loss). Aqueous and ethanolic leaf extracts produced variations in number of gametocyte from mice infected with *P.berghei*. On days 7, 14, 21, and 28, the mean gametocytes of the parasite in the control group were 18, 21, 22, and 20 respectively which were significantly ($p < 0.05$) higher compared with the mean gametocytes in the groups of mice treated with aqueous extract, ethanolic extract and chloroquine drugs. The dose level of 10mg/kg body weight of aqueous extract produced the mean gametocyte counts of 10, 10, 8 and 6 on days 7, 14, 21 and 28 respectively which were significantly ($p < 0.05$) lower than the mean gametocyte counts of the control

INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by eukaryotic protists of the genus *Plasmodium*. This genus *Plasmodium* infects mammals, birds, and lizards and is transmitted through the bites of female mosquitoes (*Anopheles* species in mammals or *Culex* species in birds and lizards) [1]. The signs and symptoms of malaria typically begin 8–25 days

following infection. However, symptoms of malaria may occur later in those who have taken antimalarial medications as prevention [2]. Initial manifestations of malaria are similar to flu-like symptoms [3] and can resemble other conditions such as sepsis, gastroenteritis, and viral diseases [4]. The presentation may include headache, fever, shivering, joint pain, vomiting, haemolytic anaemia, jaundice, haemoglobin in the urine, retinal damage, and convulsions [5].

In 2015, there were an estimated 438,000 malaria deaths worldwide. Most of these deaths occurred in the African Region (90%), followed by the South - East Asia Region (7%) and the Eastern Mediterranean Region (2%) [6]. Between 2000 and 2015, malaria incidence rates (new malaria cases) fell by 37% globally, and by 42% in Africa. During this same period, malaria mortality rates fell by 60% globally and by 66% in the African Region [6]. Other regions have achieved impressive reductions in their malaria burden. Since 2000, the malaria mortality rate declined by 72% in the Region of the Americas, by 65% in the Western Pacific Region, by 64% in the Mediterranean Region, and by 49% in the South-East Asia Regions [6]. Children under five are particularly susceptible to malaria illness, infection and death. In 2015, malaria killed an estimated 306,000 under five globally, including 292,000 children in the African Region [6]. Between 2000 and 2015, the mortality rate among children under five fell by 65% worldwide and by 71% in Africa [6] According to the latest estimates in 2018, there were an estimated 228 million cases of malaria worldwide; the estimated number of malaria deaths stood at 405 000 [7]. Children aged under 5 years are the most vulnerable group affected by malaria and they accounted for 67% (272 000) of all malaria deaths worldwide [7]. The WHO African Region carries a disproportionately high share of the global malaria burden. Total funding for malaria control and elimination reached an estimated US$ 2.7 billion in 2018 [8]. Contributions from governments of endemic countries amounted to US$ 900 million, representing 30% of total funding [7]. In Nigeria, the burden of malaria is well documented, and has been shown to be a big contributor to the economic burden of disease in communities where it is endemic and is responsible for annual economic loss of 132 billion naira [9]. It is estimated that 300,000 deaths occur each year, and 60% of outpatient visits and 30% hospitalizations are all attributed to malaria [10]. About 50% of the population has at least one episode of malaria annually resulting in high productivity losses [10] The disease is particularly virulent among pregnant women and children under 5 years of age due to their low levels of immunity [11]. The trend is rapidly increasing due to the current malaria resistance to first line antimalarial drugs [11] It is responsible for over 90% of reported cases of tropical disease

in Nigeria [11]. Five species of *Plasmodium* can infect humans. Severe disease is largely caused by *Plasmodium falciparum* while the disease caused by *Plasmodium ovale* and *Plasmodium malariae* are generally a mild disease and is rarely fatal [12]. *Plasmodium falciparum* is the most predominant parasite species accounting for about 98% of malaria cases [13]. In Nigeria, malaria transmission occurs all year round in the South, and is more seasonal in the North. The country accounts for a quarter of all malaria cases in the WHO African Region [6].

Malaria transmission can be reduced by preventing mosquito bites by distribution of mosquito nets and insect repellants, or by mosquito-control measures such as spraying insecticides and draining standing water (where mosquitoes breed). The challenge of producing a widely available vaccine that provides a high level of protection for a sustained period is still to be met, although several vaccines are under development [14]. Presently, vector control is the mainstay to prevention and reduction of malaria transmission. Two forms of vector control are effective in a wide range of circumstances.They are insecticide-treated mosquito nets (ITNs) and indoors residual spraying (IRS). Over the last 15years, there has been a major increase in coverage of ITNs in Sub-Sahara Africa. By 2014, more than half (56%) of the population had access to an ITN, compared to less than 2% in 2000 [6]. In 2014, 166 million people globally were protected by indoor residual spraying (IRS), including 50 million people in Africa. An estimated 663 million cases of malaria have been averted in Sub-Saharan Africa since 2001 as a direct result of the scale-u p of three (3) key interventions: Insecticide - treated mosquito nets (ITNs), artemisinin-based combination therapy (ACTs) and indoors residual spraying (IRS) (Bhatt *et al*., 2015). It is estimated that 69% of the 663 million fewer malaria cases attributable to interventions were due to the use of mosquito nets, 21% due to ACTs and 10% due to indoor spraying [6]. Across Africa, the prevention of new cases of malaria attributable to malaria control activities saved an estimated US $900 million in case management costs between 2001 and 2014 [6]. Despite substantial costs savings, malaria has placed a heavy economic burden on health systems in Africa. Since 2000, the average annual cost of case management alone is estimated at nearly US $300 million [6]. The efficacy of the conventional drugs against malaria parasite has been reported with variable successes [15]. The toxic effects of these chemicals on humans, the development of resistance to it by target parasites and the high cost of drugs have paved way for herbal remedies as reasonable alternative [16].

The continuous spread of *P. falciparum* resistance to antimalarial drugs possess serious threat to malaria control programmes. In Nigeria, a nationwide surveillance data on drugs efficacy showed that Chloroquine (CQ) and Sulphadoxine-pyrimethamine (SP) are no longer viable therapeutic options for the effective treatment of human malaria [17]. Although vaccines could be the best long term control option, they are still undergoing clinical trials. This in addition to the increased number of drug- resistant parasites, makes the development of novel antimalarial urgent. The high cost of malaria treatment has left the poor masses of Nigeria heavily reliant on traditional practitioners and medicinal plants for the treatment of the disease [8].

Numerous plants indigenous to Nigeria have been found with amazing antimalarial properties. It is therefore highly essential that indigenous plants used by the people to treat malaria be scientifically investigated to prove their ethno-therapeutic use [18]. Plants have always been considered to be a possible alternative and rich source of new drugs. The search for malaria remedies in plants and improved interest in plant drugs by many communities staying in endemic area led to the choice using *Lophira lanceolata* plant in establishing the scientific basis for the treatment of malaria. Phytochemical screening of *Lophira lanceolata* leaves and seeds revealed the presence of compounds such as flavonoids, anthraquinones, phenols, saponins and tannins [19]. *Lophira lanceolata* has a range of pharmacological effects. The plant has been found to possess antioxidant, antimalarial, anti-hypertensive effect, antibacterial, antiviral and sexual enhancement properties [20]. Besides the efficacy of herbal remedies, there are always serious concerns for their safety. Some researchers have earlier reported the safety of an aqueous stem bark extract of *Lophira lanceolata* in Sprague dawley rats [21].

MATERIALS AND METHODS

Plant Collection and Authentication

The fresh leaves of *Lophira lanceolata* were collected in July 2017 at Obinze, Owerri West Local Government Area, Imo State, Nigeria. The plant materials were transported in polythene bags to the Research Laboratory of Department of Biology, Federal University of Technology Owerri, Imo State for identification. The specimen was identified and authenticated by Dr. C. M. Duru, a

plant taxonomist in the Department of Biology, Federal University of Technology Owerri, Imo State, Nigeria.. Voucher specimen number was left in the Departmental herbarium **Study Area**

Preparation and administration of leaf extracts

Fresh leaves of the plant were sorted to remove any dead matter and other unwanted particles. The plant leaves were washed in tap water and air-dried for two (2) weeks [22]. The crude extracts were prepared by cold maceration technique according to [21].

Ethanol extraction

One hundred grams (100g) of the air-dried powdered leaf were weighed and mixed with ethanol 95% (500 ml) using a rotary shaker at 200 rpm for 24 hours at room temperature (25 $\pm$ 3^0C). The filtrates were pooled and filtered two times on cotton wool and once on Whatman (No 1) filter paper. The ethanol was evaporated at 50^0C using a rotary evaporator [2]. The prepared extract was weighed, labeled and stored at 4^0C in air tight bottles until ready for use.

Aqueous extraction

One hundred grams (100g) of leaf powder was dissolved in 500ml of distilled water. The solvents were evaporated using a rotary evaporator at 100 rpm. The prepared extract was weighed, labeled and stored at 4^0C in air tight bottles until ready for use. The yields of the extract obtained were calculated by the formula as shown below:

$$\text{Extraction yield} = \frac{\text{Weight of concentrated extract}}{\text{Weight of plant dried powder}} \times \frac{100}{1}$$

The weighed extracts were administered orally to animals using a metal orpharyngeal cannula.

Phytochemical screening

The aqueous and ethanolic leaf extracts were screened for bioactive ingredients such as terpenoids, flavonoids, steroids, cardiac glycosides, alkaloids, tannins, phenols, oxalate, and saponins using standard procedures [20]. The change in coloration determined the presence or absence of a particular bioactive parameters investigated.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of aqueous and ethanolic extracts of *Lophira lanceolata* was conducted following the standard procedures as described by [19].

Test for Alkaloids (Wagner's reagent)

Added 4ml of extract was treated with 4 drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100ml of water] and observed for the formation of reddish brown precipitate (or colouration).

Test for Cardiac glycosides (Keller Kelliani's test)

Added 5ml of leaf extract to 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar. A violet ring appeared below the ring while in the acetic acid layer, a greenish ring formed.

Test for Flavonoids (Alkaline reagent test)

Added 2ml of leaf extract to 4 drops of 20% sodium hydroxide solution in a test tube. Formation of intense yellow colour, which became colourless on addition of dilute hydrochloric acid, indicated the presence of flavonoids.

Test for Phenols (Ferric chloride test)

Added 2ml of the extract to aqueous 5% ferric chloride in a test tube and observed for formation of deep blue or black colour.

Test for Saponins (Foam test)

To 2mls of extract was added to 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for Steroids (Liebermann-Burchard test)

To 1ml of extract in a test tube was added 3 drops of chloroform, acetic anhydride and concentrated sulphuric acid (H_2SO_4) and observed for the formation of dark pink or red colour.

Test for Tannins (Braymer's test)

To 2mls of extract in a test tube was added 4 drops of 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Test for Terpenoids (Salkowki's test)

To 1ml of chloroform in a test tube was added to 2ml of each extract followed by 3 drops of concentrated sulphuric acid and observed for formation of a reddish brown precipitate.

Test for Oxalate

To 3ml of extract in a test tube was added 3 drops of ethanoic acid glacial and observed for formation of a greenish black colouration.

Quantitative phytochemical analysis

The phytochemicals which were present in the ethanol and aqueous extracts of *Lophira lanceolata* were determined and quantified by standard procedures [19].

Tannins Determination by titration

The Folling Dennis titrating method as described by Pearson (1976) was used. To 20g of the crushed sample in a conical flask was added 100 ml of petroleum ether and covered for 24hrs.The sample was then filtered and allowed to stand for 15 minutes for petroleum ether to evaporate. It was then re-extracted by soaking in 100ml of 10% acetic acid in ethanol for 4 hours. The sample was then filtered. 25ml of ammonium hydroxide (NH_4OH) was added to the filterate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH_4OH still in solution. The remaining volume was measured and 5ml of this was added to 20ml of ethanol. It was titrated with 0.1 M soium hydroxide (NaOH) using phenolphthalein as indicator until pink end point was reached. Tannin content was then calculated in percentage molarity of sample analyzed.

Determination of saponins

Added 5g of the sample to 4 drops of 20% acetic acid a test tube and allowed to stand in a water bath at 50^0C for 24 hours. This was filtered and the extract was concentrated to one-quarter of the

volume, using a water bath. Concentrated NH_4OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage of sample analyzed.

Determination of alkaloids

The alkaloids content of the extract was determined using standard procedures as described by [23]. Five grams (5g) of the sample was weighed into a 250 ml beaker and 200ml of 20 % acetic acids in ethanol was added, covered and allowed to stand for 4hours at 25^0C.This was filtered with filter paper No 42 and the filtrate was concentrated using a water bath (Mammert), to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was collected and washed with dilute NH_4OH (1% ammonia solution). Then the solution was filtered with pre-weighed filter paper. The residue on the filter paper was the alkaloid which was dried in the precision oven at 800 ^{0}C.The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed.

2. 5. 2d Determination of steroids

Added 1ml of steroid solution into 10 ml volumetric flasks. Sulphuric acid (4 N, 2ml) and iron (iii) chloride (0.05% W/N, 2ml) were added followed by potassium hexacyanoferrat(iii) solution (0.5% W/N, 0.05ml) at 70 $\pm$ 20^0C for 30 minutes with occasional shaking and diluted to 8ml mark with distilled water. The absorbance was measured at 780nm against the reagent blank.

$$\text{Steroids} = \frac{\text{Concentration of Standard x Absorbance of sample}}{\text{Absorbance of standard x Sample weight (g)}}$$

Determination of cardiac glycosides

To 1ml of extract in a test tube was added 1ml of 2% solution of 3.5-DNS (Dinitrosallcyclic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 20 minutes (until brick - red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50^0C

till dryness and the weight of the filter paper with residue was noted. The càrdiac glycoside was calculated in percentage.

Determination of flavonoids

Total flavonoid content was determined by aluminium chloride method using a catechin as a standard. Added 1ml of test sample and 4 ml of water to a volumetric flask (10ml). After 5 minutes, 0.3ml of 5% sodium nitrate and 0.3ml of 10% aluminum chloride were added. After 6 minutes incubation at room temperature, 2ml of 1ml of NaOH was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically.

Determination of terpenoid

The total terpenoid content in the aqueous and ethanolic extract of *lophira lanceolata* were determined by the method as described by Ferguson (1956). Pippete1g of the plant extract into a conical flask and soaked in ethyl alcohol (70%) (10ml) for one day. Then it was filtered and the filtrate was extracted with petroleum ether. The ether extract was taken as the measure of total terpenoid.

Determination of oxalate by titration method

This was determined according to [24].This determination involved three major steps: digestion, oxalate precipitation and permanganate titration.

Digestion:

a) Suspended 2g of sample was in 100ml of distilled water in a 250 ml volumetric flask.

b) Added 10 ml of 0.1M HCl to the suspension and digested at 100^0C for 1hour.

c) Cooled the filtrate and then made up to 250ml mark with distilled water before titration.

Oxalate Precipitation:

Duplicated portions of 125ml of the filtrate were measured into beakers and four drops of methyl red indicators added. This was followed by the addition of NH_4OH solution (dropwise) until the test solution changed from pink colour to faint yellow colour (pH 4-4.5). Each portion was then heated 90^0C, cooled and filtered to remove precipitate containing ferrous ions.The filtrate was again heated to 90^0C and 10ml of 5% Calcium Chloride ($CaCl_2$) solution was added while being stirred constantly. After heating, it was cooled and left overnight at 25^0C. The solution was then centrifuged at 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H_2SO_4 solution.

Permanganate Titration:

At this point, the total filtration resulting from digestion of 2g of sample was made up to 300ml aliquots of 125ml of the filtrate was heated until near boiling and then titrated against 0.05M standard KMnO4 solution to faint pink colour which persisted for 30 seconds. The calcium oxalate content was calculated using the formular:

$$\text{Oxalate content} = \frac{T \times (Vme) \times (Df)}{(ME \times Mf)} \times (Mg/100g)$$

where T was the titre of KMnO4 (ml), Vme was the volume mass equivalent (ie 1ml of 0.05M solution solution is equivalent to 0.00225g anhydrous oxalic acid).Df is the dilution factor (Vt/A), Vt the total volume of titrate(300ml) and A is the aliquot used (250ml), ME is the molar equivalent of KMnO4 in oxalate and MF is the Mass of sample used.

Determination of phenol

The quantity of phenols was determined using the spectrophotometer method. Boiled 2g of the plant sample with 50 ml ethanol for 50 minutes. Pippetted 5 ml of the boiled sample into 50 volumetric flask and 10 ml of distilled water was added. After the addition of distilled water, 2ml of NH_4OH solution and 5 ml of concentrated pentanol was added to the mixture. The sample was made up to the 25ml mark with distilled water and left for 30 minutes to react for colour development and measured at 505nm wavelength using a spectrophotometer.

$$\text{Phenol Content} = \frac{\text{Concentration of Standard x Absorbance of sample}}{}$$

Absorbance of standard x Sample weight (g)

Parasite collection

A strain of *Plasmodium berghei* that is chloroquine sensitive was obtained from donor-infected mice maintained at Animal Facility Centre, Faculty of Veterinary Medicine, University of Nigeria, Nsukka

Experimental animals

Male Swiss albino mice were obtained from the Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria. The mice were allowed to acclimatize to the laboratory environment under ambient temperature of 26 - 32^{o}C and humidity for at least three days before being subjected to the experiments (Peter and Anatoli, 1998). The animals were housed in cages and fed with growers mash (Vital feeds) at the Department of Biology, Federal University of Technology, Owerri. All the mice were given free access to food and water ad libitum throughout the experimental period. Also, good hygiene was maintained by regular cleaning and removal of feaces and spilled feed from cages occasionally.

Inoculum preparation

The donor mice were monitored for signs of infection such as anorexia, ruffled appearance, shivering, heat-seeking behaviour and lethargy. Blood samples were taken from the second day to confirm the extent of parasitaemia in the donor mice. The mice were anaesthetized in a glass jar containing cotton wool soaked in chloroform. Blood were collected from the donor mice by cardiac puncture using 5ml sterile syringes and needles..The blood samples were diluted with Trisodium citrate (TC) medium so that each 0.2ml contain approximately $1.0x10^{7}$ infected red cells [20].

Acute toxicity tests

The aqueous and ethanolic extracts of *Lophira lanceolata* leaves were evaluated for their toxicity in *P. berghei* non-infected male Swiss albino mice using modified Lorke's method [25] method

of determining toxicity level of extract in mice.The study for each leaf extract was carried out in two phases. In phase one of the study, nine mice were randomized into three groups of three mice each and were given 10, 100, and 1000mg/kg body weight of the extract orally.The mice were observed for signs of toxicity which included but not limited to salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the first four hours and subsequently daily for seven days. In the second phase of the study another fresh set of nine mice were randomized into three groups of three mice each and were given 1600, 2900 and 5000 mg/kg body weight of the extract orally based on the result of the first phase. These were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for seven days.The LD_{50} were calculated as the square root of the product of the lowest lethal dose and highest non-lethal dose i.e.,the geometric mean of the consecutive doses for which 0 and100% survival rates were recorded in the second phase. The oral median lethal dose was calculated using the formula [26].

$$LD_{50} = \sqrt{(\text{Minimum toxic dose} \times \text{maximum tolerated dose})}$$

Determination of Parasitaemia

Thin film from tail blood sample was prepared by placing a drop of blood on a microscope slide and thereafter used a second slide held at an angle to spread the blood and pulled it across the slide, forming a “feathered edge” consisting of a single layer of cells at the end of the smear [26]. The thin blood film was allowed to air dry at room temperature before staining with 10% Giemsa at pH 7.2 for 10 minutes. The blood film was rinsed in distilled water (and back of the slide carefully cleaned) and air dried to remove particles that might obstruct the film examination. Slides were viewed using a compound microscope with oil immersion (x100 magnification). The percentage parasitaemia was obtained by counting the number of parasitized red blood cells out of 500 erythrocytes in random fields of the microscope Percentage parasitaemia and the average chemosuppression were calculated using the formula below:

$$\% \text{ parasitemia} = \frac{\text{No of parasitised RBC}}{\text{Total No of RBC counted}} \times \frac{100}{1}$$

Average percentage chemosuppression was calculatd as $\frac{A - B}{A} \times \frac{100}{1}$

Were A is the average percentage parasitaemia in negative control group and B average percentage parasitaemia in the test group.

Curative antiplasmodium assay

To assess the schizonticidal activity of either aqueous or ethanolic extracts, 32 mice were inoculated intraperitoneally with standard inoculum of $1x10^7$ *Plasmodium berghei* parasitized red blood cells on the first day (D_0). 72 hours later, the mice were randomized into 8 groups of 4 animals per group. Group 1 received 10ml/kg/day of distilled water served as a negative control group. Groups 2, 3, and 4 animals were administered with 10, 100 and 200mg/kg/day of the aqueous extract orally respectively. Groups 5, 6, and 7 animals were administered with 10, 100 and 200mg/kg/day of the ethanolic extract orally respectively. Group 8 animals were administered with 5mg/kg/day of chloroquine as a positive control group. All the drugs were administered to the animals once daily for 5 days.

Evaluation of gametocyte dynamics

To evaluate the dynamics of gametocyte production of infected mice treated with either aqueous or ethanolic extracts, 32 mice were inoculated intraperitoneally with standard inoculum of $1x10^7$ *Plasmodium berghei* parasitized red blood cells on the first day (D_0). 72 hours later, the mice were randomized into 8 groups of 4 animals per group. Group 1 received 10ml/kg/day of distilled water as a negative control group.. Groups 2, 3, and 4 animals were administered with 10, 100 and 200mg/kg/day of the aqueous extract orally respectively. Groups 5, 6, and 7 animals were administered with 10, 100 and 200mg/kg/day of the ethanolic extract orally respectively.Group 8 animals were administered with 5mg/kg/day of chloroquine served as the positive control group.. All the drugs were administered to the animals once daily for 28 days. Thin films from tail blood samples obtained on Day 7, Day 14, Day 21 and Day 28 were examined microscopically to monitor gametocytaemia level. On 29th Day, thin film from tail blood sample of each mouse was prepared. The percentage gametocytaemia was obtained by counting the number of red blood cells (RBC) having gametocytes in every 500 erythrocytes in random fields of the microscope

Percentage gametocytaemia was calculated using the formula below:

$$\% \text{ gametocytaemia} = \frac{\text{No of RBC infected with gametocytes}}{\text{Total No of RBC counted}} \times \frac{100}{1}$$

Data Analysis

The data was analysed using the Statistical Package for Social Sciences (SPSS) version 17. T-test and and one way analysis of variance (ANOVA) with multiple comparison tests were used to compare parameters.

RESULTS

Extract Yield

The aqueous and ethanolic leaf extracts of *L. lanceolata* yielded 17.43g (17.43%) and 36.17g (36.17%) respectively (**Table 1**).

Phytochemical Screening

The results of the qualitative phytochemical analysis of aqueous and ethanolic leaf extracts of *L. lanceolata* showed the presence of tannins, saponins, alkaloids, flavonoids, phenols, steroids, terpenoids, oxalate, and cardiac glycosides (**Table 2**).

Table 1: Yield of aqueous and ethanolic crude leaf extract of *L. lanceolata*

Solvent	Plant powder (g)	Extraction solvent (ml)	Yield (g)	Yield (%)
Aqueous	100	500	36.17	36.17*
Ethanolic	100	500	17.43	17.43*

Legend: ml:milliliter; g:gram; %:percentage; *****:there was significant difference between these values ($p < 0.05$).

Table 2: Qualitative analysis of ethanolic and aqueous extracts of *Lophira lanceolata*

S/N	Parameters	Ethanolic extract	Aqueous extract
1	Tannins	Present	Present
2	Saponins	Present	Present
3	Alkaloids	Present	Present
4	Flavonoids	Present	Present
5	Cardiac glycosides	Present	Present
6	Phenols	Present	Present
7	Steroids	Present	Present
8	Terpenoids	Present	Present
9	Oxalate	Present	Present

The results of quantitative phytochemical analysis showed that the flavonoid content had the highest value in both ethanolic leaf extract (388,240 mg/ml) and aqueous leaf extract (350.00 mg/ml). Of all the phytochemical constituents analysed, steroids was the lowest in both aqueous and ethanolic leaf extract of *L. lanceolata* (**Table 3**).

Table 3: Quantitative analysis of ethanolic and aqueous extracts of *Lophira lanceolata*

S/N	Parameters	Ethanolic extract (mg/ml)	Aqeous extract (mg/ml)
1	Tannins	$35.50^{e} \pm 2.05$	$22.70^{e} \pm 1.72$
2	Saponins	$0.038^{g} \pm 0.02$	$0.030^{h} \pm 0.01$
3	Alkaloids	$124.00^{d} \pm 12.03$	$69.00^{c} \pm 4.11$
4	Flavonoids	$388.24^{a} \pm 19.14$	$350.00^{a} \pm 17.16$
5	Cardiac glycosides	$188.00^{c} \pm 14.11$	$97.00^{b} \pm 8.17$
6	Phenols	$0.04^{g} \pm 0.02$	$0.11^{g} \pm 0.07$
7	Steroids	$0.02^{h} \pm 0.00$	$0.01^{i} \pm 0.00$
8	Terpenoids	$0.40^{f} \pm 0.10$	$0.73^{f} \pm 0.30$
9	Oxalate	$294.75^{b} \pm 16.23$	$29.25^{d} \pm 1.96$

Values are mean $\pm$ S. D.Mean values having different superscripts along the same column are significantly different ($P<0.05$)

Acute Toxicity Test

The result of the acute toxicity study indicated that none of the different doses of extract (10 – 5000mg/kg body weight of the extract)in all the groups and phases caused mortality of mice for over ten days (**Table 4**). The behavioural signs of toxicity observed in mice given 100mg/kg body weight and above included stretching, salivation, and reduced activity. The median lethal dose (LD_{50}) was found to be ≥ 5000mg/kg body weight.

Table 4: LD_{50} determination of aqueous and ethanolic leaf extract of *L. lanceolata*

Phases	Aqueous Number of mice (ratio)	Ethanolic Number of mice (ratio)
Phase one		
Dosed (mg/kg)		
10	$3(^0/_3)$	$3(^0/_3)$
100	$3(^0/_3)$	$3(^0/_3)$
1000	$3(^0/_3)$	$3(^0/_3)$
Phase two		
Dosed (mg/kg)		
1600	$3(^0/_3)$	$3(^0/_3)$
2900	$3(^0/_3)$	$3(^0/_3)$
5000	$3(^0/_3)$	$3(^0/_3)$

Effects of Aqueous and Ethanolic Leaf Extracts of *Lophira lanceolata* on Gametocyte Production

The oral administration of aqueous and ethanolic leaf extract of *Lophira lanceolata* on mice infected with *Plasmodium berghei* produced variations in number of the gametocyte production (**Table 5**). On day 7, 14, 21, and 28, the mean gametocytes of the parasite in the control group were 18, 21, 22, and 20 respectively which were significantly ($p< 0.05$) higher compared with the mean gametocytes in the groups of mice treated with aqueous extract, ethanolic extract and chloroquine drugs. The dose level of 10mg/kg body weight of aqueous extract produced the mean gametocyte counts of 10, 10, 8 and 6 on Day 7, Day 14, Day 21 and Day 28 respectively which were significantly ($p < 0.05$) lower than the mean gametocyte counts of the control. The mice treated with chloroquine at 5mg/kg body weight showed a significant decrease in gametocyte count ($p < 0.05$) on Day 7, Day 14, Day 21 and Day 28 compared with the three different dose levels of aqueous and ethanolic extracts. There were significant differences ($p < 0.05$) in mean gamatocytes counts at dose levels of 10mg/kg body weight , 100mg/kg body weight, 200mg/kg body weight of aqueous extract. However, there were no significant difference at dose levels of 10mg/kg body weight, 100mg/kg body weight and 200mg/kg body weight of ethanolic extract.

The percentage gametocytaemia of *P. berghei* of mice in the negative control group were 3.60%, 4.10%, 4.35%, and 4.05% on Day 7, 14, 21 and 28 respectively (**Figure 1**). The dose level of 100mg/kg body weight of aqueous extract of *L. lanceolata* recorded low percentage gametocytaemia of 0.70% on Day 28. The lowest percentage gametocytaemia (0.10%) was recorded in the positive control group of mice on Day 7 while the highest percentage gametocytaemia (4.35%) was recorded in the negative control group of mice on Day 21.

Table 5: Effects of aqueous and ethanolic leaf extracts of *Lophira lanceolata* and chloroquine on gametocyte production of *P. berghei* in mice

Treatments (mg / kg)	Mean Gametocyte Count in Mice in Days			
	Day 7	Day 14	Day 21	Day 28
Distilled water treated 10	$18^{a} \pm 1.53$	$21^{a} \pm 1.57$	$22^{a} \pm 1.59$	$20^{a} \pm 1.56$
Aqueous extract treated				
10	$10^{de} \pm 0.40$	$10^{d} \pm 0.40$	$8^{c} \pm 0.35$	$6^{c} \pm 0.31$
100	$11^{d} \pm 0.41$	$12^{d} \pm 0.43$	$7^{c} \pm 0.33$	$4^{d} \pm 0.25$
200	$15^{c} \pm 1.48$	$16^{c} \pm 1.50$	$12^{b} \pm 0.43$	$8^{b} \pm 0.35$
Ethanolic extract treated				
10	$16^{bc} \pm 1.50$	$16^{c} \pm 1.50$	$10^{bc} \pm 0.40$	$6^{c} \pm 0.31$
100	$17^{b} \pm 1.52$	$19^{b} \pm 1.54$	$11^{b} \pm 0.41$	$6^{c} \pm 0.31$
200	$12^{d} \pm 0.43$	$15^{c} \pm 1.48$	$3^{d} \pm 0.22$	$4^{c} \pm 0.25$
Chloroquine treated				
5	$9^{e} \pm 0.38$	$7^{e} \pm 0.33$	$1^{d} \pm 0.13$	$1^{e} \pm 0.13$

Values are mean $\pm$ S. D n = 4. Mean values having different superscripts along the same column are significantly different ($P<0.05$)

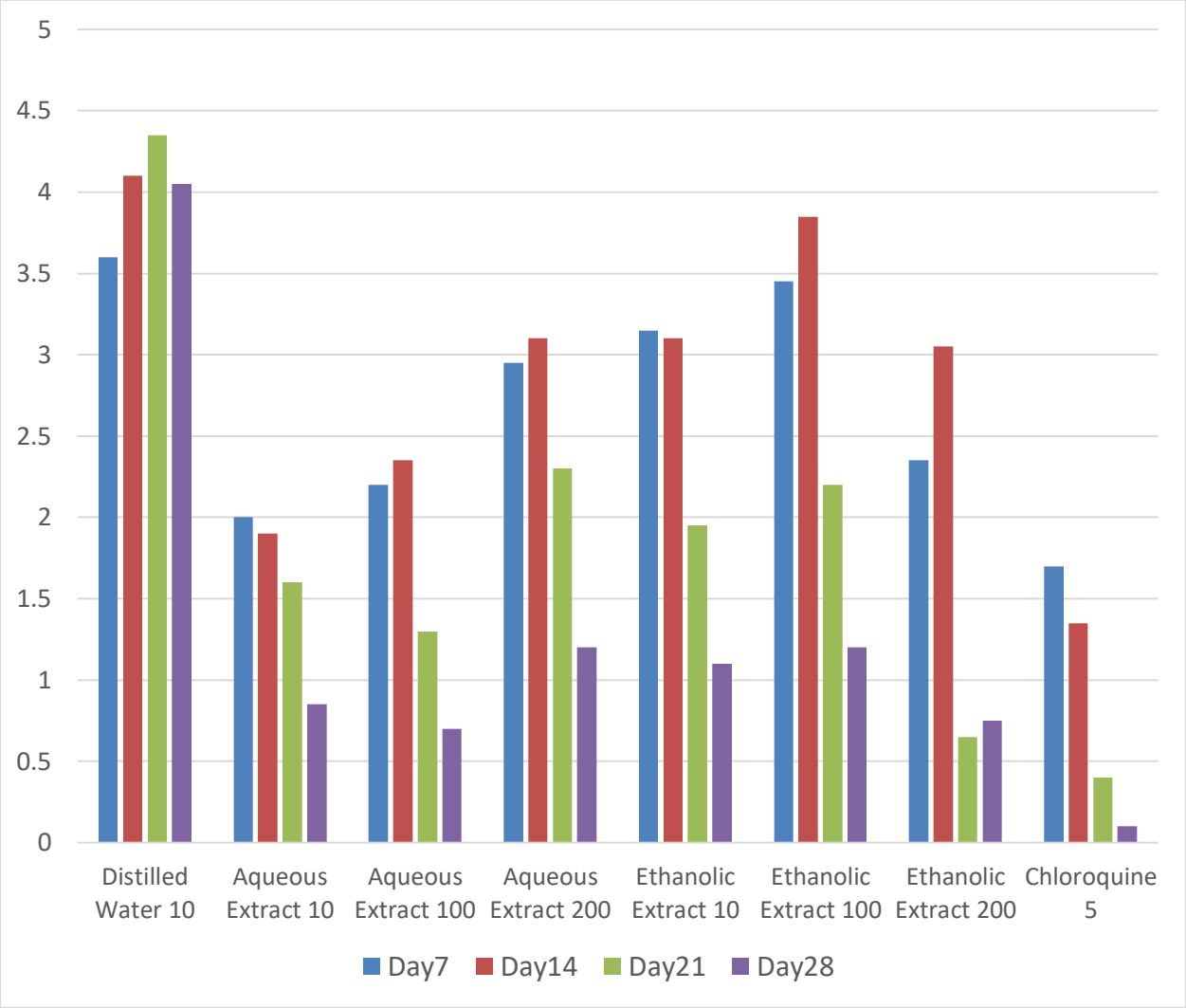

Figure 1: **Percentage gametocytaemia of *P. berghei* of mice in days**

Prophylactic Effect of Aqueous and Ethanolic Leaf Extract of *Lophira lanceolata* and chloroquine against *P. berghei* infection in mice

The aqueous leaf extract of *Lophira lanceolata* exerted significant (<0.05) non – dose - dependent reduction in level of parasitaemia with chemosuppressions of 48.79%, 24.26%, 29.38% and 80.32% at 10mg/kg, 100mg/kg, 200mg/kg body weight and the chloroquine treated groups respectively (**Table 6**). Also the ethanolic leaf extract of *Lophira lanceolata* exhibited significant ($p < 0.05$) non – dose - dependent reduction in levels of parasitaemia with chemosuppressions of 43.94%, 35.74%, 22.10% and 80.32% at 10mg/kg, 100mg/kg, 200mg/kg and 5mg/kg the chloroquine treated groups respectively.

Table 6: Prophylactic Effects of aqueous and ethanolic leaf extracts of *L. lanceolata and* chloroquine against *P. berghei in mice*

Test substances	Dose mg/kg	% parasitaemia	% inhibition
Distilled water treated	10	18.55^{a} ± 0.29	0.00 ±0.00
Aqueous extracts treated	10	9.50^{d} ± 0.19	48.79 ±1.58
	100	14.05^{b} ± 0.23	24.26±24.26
	200	13.05^{c} ± 0.22	29.38 ±0.91
Ethanolic extract treated	10	10.40^{d} ± 0.15	43.94 ±1.41
	100	12.25^{c} ± 0.18	35.74 ±1.02
	200	14.45^{b} ± 0.26	22.10 ±0.67
Chloroquine treated	5	3.65^{e}±0.09	80.32 ±2.00

(P<0.05) Each result is a mean of 4 mice ± controls

Curative effect of aqueous and ethanolic leaf extract *Lophira lanceolata* and chloroquine against *P. berghei* infection in mice

The aqueous and ethanolic leaf extract of *Lophira lanceolata* showed significant dose dependent antiplasmodial activity at the various concentrations. The dose level of 10mg/kg, 100mg/kg, 200mg/kg body weight of aqueous extract and the 5mg/kg chloroquine showed chemosuppressions of 12.09%, 20.60%, 60.44% and 89.56% respectively (**Table 7**).

Moreso, the dose level of 10mg/kg, 100mg/kg, 200mg/kg body weight of the ethanolic extract and 5mg/kg of the chloroquine drug showed chemosuppression of 12.64%, 17.31%, 41.21% and 89.56% respectively.

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Table 7: Curative Effects of aqueous and ethanolic leaf extracts of *L. lanceolata* and chloroquine against *P. berghei in mice*

Test substances	Dose mg/kg	% parasitaemia	% inhibition
Distilled water treated	10	18.20^{a}± 0.53	0.00 ±0.00
Aqueous extracts treated	10	16.00^{b} ± 0.50	12.00 ±0.49
	100	14.45^{c} ± 0.48	20.60 ±0.61
	200	7.20^{e} ± 0.34	60.44 ±1.78
Ethanolic extract treated	10	15.90^{b} ± 0.49	12.64 ±0.43
	100	15.05^{c} ± 0.48	17.31 ±0.4
	200	10.70^{d} ± 0.41	41.21 ±1.38
Chloroquine treated	5	1.90^{f} ± 0.17	89.56 ±2.11

(P<0.05) Each result is a mean ot 4 mice ± controls

Mean body weight of mice

The mean body weight of the mice in the negative and positive control groups were 22.53g and 22.60g respectively at the commencement of the study (**Table 8**). The mean body weight for the negative and positive control groups increased to 23.33g and 23.63g at the end of the experimental period, indicating a weight gain of 3.55% and 4.56% respectively. The infected untreated mice had a decrease in mean body weight from 23.33g to 20.08g, showing a 10.08% weight loss. The infected mice treated with aqueous extract at the dose levels of 10mg/kg body weight, 100mg/kg body weight and 200mg/kg body weight had the mean weight of between 23.68 -24.53g at the beginning of the experiment to between 24.28 -24.93g at the end of the treatment indicating 2.53%, 0.61% and 2.17% weight gains at the three dose levels respectively. The uninfected mice treated with aqueous extract at the dose level of 200mg/kg body weight had Mean weight of 26.63g at the beginning of the experiment and 26.88 at the end of the experimental period resulting in 0.95% weight gain.

Table 8: Mean body weight of mice before and after treatment with *L. lanceolata* aqueous leaf extract

Treatments groups (mg / kg)	Mean weight (g)		
	Day 0	Day 28	% change
Negative control	22.53^{c}± 0.59	23.33^{b} ± 0.60	3.55 ±0.27
10			
Infected, untreated			
0	22.33^{c} ± 0.58	20.08^{c} ± 0.56	-10.08 ±0.05
Infected, aqueous			
extract treated			
10	23.68^{c} ± 0.61	24.28^{b} ± 0.62	2.53 ±0.14
100	24.53^{b} ± 0.63	24.68^{b} ± 0.64	0.61 ±0.07
200	24.40^{b} ± 0.62	24.93^{b} ± 0.65	2.17 ±0.10
Uninfected, aqueous			
extract treated	25.48^{a} ±0.63		
10	26.43 ^{a}±0.64	26.43^{a} ± 0.67	3.73 ±0.23
100	26.63^{a} ± 0.65	27.05^{a} ± 0.69	
200		26.88^{a} ± 0.68	2.35 ±0.12
Infected chloroquine			0.95 ±0.04
treated	22.60^{c} ± 0.60		
5		23.63 ^{b}± 0.61	4.56 ±0.38

Values are mean ± S. D n = 4. Mean values having different superscripts along the same column are significantly different ($P < 0.05$).

The oral administration of ethanolic leaf extract of *L. lanceolata* caused some changes in the weight of the individual mice subjected to the experiment (**Table 9**). The dose level of 200mg/kg body weight of the ethanolic extract in infected mice caused an increase in body weight of the mice from 26.35g at the beginning of the experiment to 27.13g at the end of the experiment resulting in 2.96% weight gain. The mean weight of the uninfected mice treated with ethanolic extract at the dose levels of 10mg/kg body weight,100mg/kg body weight and 200mg/kg body weight caused 6.44%, 3.62% and 3.81% weight gains respectively.

Table 9: Mean body weight of mice before and after treatment with *L. lanceolata* ethenolic leaf extract

Treatments groups (mg / kg)	**Mean weight** (g)		
	Day 0	**Day** 28	% **change**
Negative control	22.53^{e} ± 0.59	23.33^{d} ± 0.60	3.55 ±0.27
10			
Infected, untreated			
0	22.33^{e} ± 0.58	20.08^{e} ± 0.56	-10.08 ±0.05
Infected, ethanolic extract treated			
10	27.25^{a} ± 0.71	27.75^{a} ± 0.73	1.83 ±0.08
100	24.30^{d} ± 0.62	24.93^{c} ± 0.64	2.59 ±0.16
200	26.35^{b} ± 0.64	27.13^{a} ± 0.68	2.96 ±0.15
Uninfected, ethanolic extract treated			
10	23.75^{d} ± 0.61		[
100	25.38^{c} ± 0.63	25.28^{c} ± 0.65	6.44 ±0.52
200	23.60^{d} ± 0.61	26.30^{b} ±0.69	
Infected, chloroquine		24.50^{c} ± 0.62	3.62 ±0.31
treated			3.81 ±0.43
5	22.60^{e} ± 0.60		
		23.63^{d} ± 0.61	
			4.56 ±0.46

Values are mean ± S. D n = 4. Mean values having different superscripts along the same column are significantly different (P<0.05).

Mean weights of mice organs

The organs of mice harvested for weight assessment were kidneys, livers, hearts and lungs. There was a significant decrease (p<0.05) in weight of kidneys and livers of infected untreated mice compared to the control (**Table 10**). However, there was no significant difference in weight of hearts and lungs of infected untreated mice compared to control.

The dose level of 10mg/kg body weight of aqueous extract in infected mice caused a significant decrease (p<0.05) in kidney and heart weight of the mice compared with the control. There was no significant difference (p>0.05) in weight of liver and lung of the infected mice treated with 10mg/kg body weight of the aqueous extract compared to the control. There was no significant difference (p >0.05) in the weight of kidney, heart and lung of the infected mice treated with 10mg/kg body weight of the aqueous extract compared with the infected mice treated with 100mg/kg body weight of the aqueous extract (**Table 10**). The dose level of 200mg/kg body weight of aqueous extract caused a significant decrease (p<0.05) in weight of kidney and liver organs of uninfected treated mice compared with the control.

Table 10: Mean weight(g) of mice organs after treatment with *L. lanceolata* aqueous leaf extract

Treatments Groups (mg / kg)	Organs in mice(g) Kidney	Liver	Heart	Lung
Negative control 10	0.46^{a} ± 0.08	1.41^{a} ± 0.15	0.11^{b} ± 0.04	0.15^{a} ± 0.05
Infected, untreated 0	0.39^{b} ± 0.07	1.04^{d} ± 0.13	0.11^{b} ± 0.04	0.14^{a} ± 0.05
Infected , aqueous extract treated 10	0.35^{c} ± 0.07	1.41^{a} ± 0.15	0.13^{a} ± 0.05	0.14^{a} ± 0.05
100	0.34^{c} ± 0.07	1.36^{b} ± 0.15	0.13^{a} ± 0.05	0.15^{a} ± 0.05
200	0.41^{b} ± 0.08	1.37^{b} ± 0.15	0.11^{b}±0.04	0.15^{a} ± 0.05
uninfected , aqueous extract treated 10	0.35^{c} ± 0.07	1.42 ^{a} ± 0.15	0.13^{a} ± 0.05	0.13^{a} ± 0.05
100	0.36^{c} ± 0.07	1.20^{c}± 0.14	0.11^{b} ± 0.04	0.14^{a} ± 0.05
200	0.40^{b} ± 0.08	1.29^{bc} ± 0.14	0.11^{b} ± 0.04	0.16^{a} ± 0.05
Infected ,chloroquine treated 5	0.27^{d} ± 0.06	0.79^{e} ± 0.11	0.06^{c} ± 0.03	[0.14^{a} ± 0.05

Values are mean ± S. D n = 4. Mean values having different superscripts along the same column are significantly different (P<0.05).

The oral administration of ethanolic leaf extract of *L. lanceolata* in mice induced some changes in weight of the mice organs. The dose level of 100mg/kg body weight of ethanolic extract had a significant decrease ($p<0.05$) in weight of kidney, liver, and heart of infected mice compared with the control (**Table 11**). There was no significant difference ($p > 0.05$) in the weight of kidney, liver, and lung of the infected mice treated with 100mg/kg body weight of ethanolic extract compared with the infected mice at the dose level of 200mg/kg body weight of the ethanolic extract.

The dose level of 200mg/kg body weight of ethanolic extract in uninfected mice caused a significant decrease ($p < 0.05$) in liver and kidney of mice compared with the control. However, there was no significant difference in weight of heart, and lung of uninfected mice treated with 200mg/kg body weight of the ethanolic extract compared with the control.

Table 11: Mean weight (g) of mice organs after treatment with *L. lanceolata* ethanolic leaf extract.

Treatments	Organs in mice(g)			
Groups (mg / kg)	Kidney	Liver	Heart	Lung
Negative control				
10	$0.46^{a} \pm 0.08$	$1.41^{a} \pm 0.15$	$0.11^{b} \pm 0.04$	$0.15^{a} \pm 0.05$
Infected, untreated				
0	$0.39^{b} \pm 0.08$	$1.04^{d} \pm 0.12$	$0.11^{b} \pm 0.04$	$0.14^{a} \pm 0.05$
Infected, ethanolic				
extract treated	$0.40^{b} \pm 0.08$	$1.29^{c} \pm 0.14$	[	
10			$0.09^{c} \pm 0.04$	$0.15^{a} \pm 0.05$
100	$0.34^{c} \pm 0.07$	$1.32^{c} \pm 0.14$	$0.13^{a} \pm 0.05$	$0.15^{a} \pm 0.05$
200	$0.35^{c} \pm 0.07$	$1.32^{c} \pm 0.14$	$0.09^{c} \pm 0.04$	$0.15^{a} \pm 0.05$
Uninfected, ethanolic extract treated	$0.37^{bc} \pm 0.08$	$1.36^{b} \pm 0.15$		
10			$0.13^{a} \pm 0.05$	$0.14^{a} \pm 0.05$
100	$0.39^{b} \pm 0.08$	$1.37^{b} \pm 0.15$	$0.10^{b} \pm 0.04$	$0.16^{a} \pm 0.05$
200	$0.35^{c} \pm 0.07$	$1.37^{bc} \pm 0.15$	$0.1a^{b} \pm 0.05$	$0.14^{a} \pm 0.05$
Infected, chloroquine treated	$0.27^{d} \pm 0.06$	$0.79^{e} \pm 0.11$		
5			$0.06^{c} \pm 0.03$	

	$0.14^{a} \pm 0.05$

Values are mean ± S. D n = 4. Mean values having different superscripts along the same column are significantly different (P<0.05).

DISCUSSION

The quality and quantity of phytochemicals extracted from the plant materials differed according to the solvent type used. Some reports show that ethanol extracts had more number and types of compounds in plant materials than other extraction solvents such as water [27]. The results of the investigation into the phytochemical compositions of aqueous and ethanolic leaf extracts of *L. lanceolata* employed in this study have revealed the presence of tannins, saponins, alkaloids, flavonoids, phenols, steroids, terpernoids, oxalate, and cardiac glycosides. The presence of these phytochemicals has also been reported in the leaves of *Anacardium occidentalis* [28]. The result is also in accordance with the reports of [29] whose phytochemical analysis of *Lophira lanceolata* revealed the presence of alkaloids, phenols, flavonoids, steroids, tannins and saponins. These constituents have been found in other plant products which exhibited antimalarial activity [30].

The result of the acute toxicity study indicated that none of the different doses (10 – 5000mg/kg) of aqueous and ethanolic leaf extracts in all the groups and phases caused mortality of mice for over ten days up to 5000mg/kg body weight. The behavioural signs of toxicity observed in mice given 100mg/kg body weight and above included stretching, salivation, and reduced activity. These signs were not seen in 10mg/kg body weight dose group but progressed and became increasingly pronounced as the dose increased towards 5000mg/kg body weight. The median lethal dose (LD_{50}) was found to be ≥ 5000mg/kg body weight of the aqueous and ethanolic extract. The LD_{50} being greater than 5000mg/ kg body weight is thought to be safe as suggested by [20]. The results of acute toxicity study in this research work could explain the routine use of the plant by the local people for traditional management of malaria. Furthermore, the findings of this work are in agreement with the previous results of the acute toxicity study of the *Lophira lanceolata* leaf extracts on mice [31]. Also, these results are in conformity with the previous results of the acute toxicity study of the *Lophira lanceolata* leaf extracts on rats [32-33].

The oral administration of aqueous and ethanolic leaf extracts of *Lophira lanceolata* caused increases in the weights of the mice subjected to the experiment. The infected untreated mice had a decrease in mean body weight from 23.33g to 20.08g showing a 10.08% weight loss. The increase in weight observed in the treated mice showed that the animals responded to the treatment given.

This implies that the extract may act through mechanisms which are similar to those through which chloroquine acts [34]. Chloroquine is lethal to malaria parasites by causing the accumulation of toxic heme in the parasite food vacuole [35]. Heme may be toxic to the parasite by its interference with the nucleic acid biosynthesis [36-37]. The aqueous and ethanolic leaf extract of *Lophira lanceolata* showed significant dose-dependent antiplasmodial activity at the various concentrations. The dose level of 10mg/kg, 100mg/kg, 200mg/kg body weight of aqueous extract and the 5mg/kg chloroquine showed chemosuppressions of 12.09%, 20.60%, 60.44% and 89.56% respectively. Moreso, the dose level of 10mg/kg, 100mg/kg, 200mg/kg body weight of the ethanolic extract and 5mg/kg of the chloroquine drug showed chemosuppression of 12.64%, 17.31%, 41.21% and 89.56% respectively.

4.0 Conclusion

The study was undertaken to evaluate gametocyte dynamics and the effects of aqueous and ethanolic leaf extracts of *Lophira lanceolata* in mice infected with *Plasmodium bergei*. Thus, gametocytes resulting from sequestration of parasites seen on Day 0 should appear in circulation after Day 7. Although the level of gametocytaemia does not perfectly correlate with infectivity because it is easier to measure, it may be a useful indicator of an antimalarial's probable impact on malaria transmission. These results on gametocyte studies support previous studies demonstrating that the addition of artemisinins to standard antimalarial regimens reduces post- treatment gametocytaemia as gametocyte carriage did significantly decrease by the end of the 28 - day follow- up.

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Affiliation of authors:

1. Department of Biology, Federal University of Technology, Owerri Imo State Nigeria
2. Department of Zoology and Environmental Biology, University of Nigeria Nsukka.
3. Department of Biological science, Hezekiah University, Umudi Imo State Nigeria

Correspondence Author's Email: nwachukwumichael025@gmail.com