



Antiplasmodial Activity of Ethanolic Leaf Extract of *Cymbopogon citratus* (DC) Stapf in Swiss Albino Mice Infected with *Plasmodium berghei* NK 65

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Authors' contributions

This work was carried out in collaboration between both authors. Author EOD designed the study, wrote the protocol and managed the analyses of the study. Author ROA anchored the field study, gathered the initial data and performed the preliminary data analysis. Both authors managed the literature searches, produced the initial draft and approved the final manuscript.

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ABSTRACT

The study assessed the antiplasmodial activity of the ethanolic leaf extract of *Cymbopogon citratus* on chloroquine sensitive *Plasmodium berghei* in mice. Standard methods were used to determine the bioactive components of the leaf extract, acute toxicity test and antiplasmodial activity. Mice obtained (of body weight 20-25 g) were housed and acclimatized for seven days at room temperature before the commencement of the experiment. A total of 16 albino mice were randomized into four groups of four mice each for acute toxicity while 35 were grouped into five groups of seven mice each for antiplasmodial activity. All the groups 1-5 were infected with *P. berghei* and were treated for six consecutive days with leaf extract dosage of 200, 400 and 800 mg/kg, standard antimalarial drug (chloroquine) as positive control and normal saline as negative control respectively.

Phytochemical screening/ bioactive compounds of the leaf extract reveals the presence of saponins (10.3 mg/g), tannins (2.38 mg/g), flavonoids (1.87 mg/g), terpenoids (19.12 mg/g), steroids (6.21 mg/g) and glycosides (19.9 mg/g) as secondary metabolites. The leaf extract

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revealed decrease in body weight of the infected mice and did not show any toxicity at all dosage levels used.

The antiplasmodial investigation revealed a decrease in percentage parasitaemia level in mice of extract treated groups compared with mice infected and not treated. The parasitaemia reduction was higher in 800 mg/kg than 200 mg/kg and 400 mg/kg. This significant decrease ($P < 0.05$) in percentage parasitaemia level in the study was dose and time-dependent. The extract showed significant ($p < 0.05$) antiplasmodial activity and could serve as possible candidates for the development of new effective drugs for the treatment of malaria.

Keywords: *Cymbopogon citrates*; *in vivo*; *Plasmodium berghei*; *bioactive compounds*; *citral*; *lemon grass*.

1. INTRODUCTION

Malaria is a vector-borne life threatening disease and one of the deadliest infectious disease that have a significant burden on economic stability. According to the latest World malaria report, there were 229 million cases of malaria in 2019 compared to 228 million cases in 2018. The estimated number of malaria deaths stood at 409 000 in 2019, compared with 411 000 deaths in 2018 [1]. There are five parasite species that cause malaria in humans, and two of these species *P. falciparum* and *P. vivax* – pose the greatest threat. In 2018, *P. falciparum* accounted for 99.7% of estimated malaria cases in the WHO African Region, 50% of cases in the WHO South-East Asia Region, 71% of cases in the Eastern Mediterranean and 65% in the Western Pacific. *P. vivax* is the predominant parasite in the WHO Region of the Americas, representing 75% of malaria cases [1]. *P. falciparum* is found in most tropical and sub-tropical regions of the world, such as sub-Saharan Africa, and is the most dangerous of the five in terms of mortality and morbidity, whereas, *P. malariae* and *P. ovale* are rare and account for less than 1% of all confirmed malaria cases [2]. The fifth species (*P. knowlesi*) which causes malaria in macaque monkey, has recently been reported to infect humans in Southeast Asia. It infects monkey primarily and occurs rarely in human, the infection does occur when an *Anopheles* mosquito infected from monkey bites human [1]. The current efforts to reduce the global burden of malaria are threatened by the rapid emergence and spread of *P. falciparum* resistance to antimalarial drugs [3].

P. berghei, a rodent malaria parasite, is commonly used to assay antimalarial activity of medicinal plant extracts as well as conventional antimalarial drugs. The common strains of *P. berghei* are ANKA, K173, NK65, SP11, and LUKA. *P. berghei* provides a well-established

experimental model of malaria infection, producing pathological symptoms which closely mimic those of human malaria [4].

C. citratus belongs to the family Poaceae and commonly called lemongrass/ citronella which is also known as *eweti* or *kooko-oba* in Yoruba, *eti* in Edo, *Ikoneti* by the Eriks, *chiawasami* or *tsauri* in Hausa, and *acharaehi* in Igbo [5]. According to [6], *Cymbopogon* is a genus of about 55 species derived from the Greek words "kymbe" (boat) and pogon (beard), referring to the flower spike arrangement and citratus (Latin) means lemon-scented leaves. Lemon grass can grow up to 1 meter with numerous stiff leafy stems arising from short rhizomatous roots and has been cultivated over many years for medicinal purposes in different regions of the world. [7].

According to Christopher et al. [8] therapeutic potential of medicinal herbs could be associated to the presence of secondary metabolites. The biological effects ascribed to *C. citratus* have been attributed to its primary bioactive constituents derived from its leaves, stem and roots. Secondary metabolites such as citral (3, 7-dimethyl-2, 6-octadienal), myrcene and citronellal have been isolated from lemon grass and were characterized as antimalarial compounds. These isolated compounds show pronounced activity against *Plasmodium* species [9]. Bioactive constituents such as ketones, alcohols, phenols, terpenes, flavonoids, saponins, steroids, tannins, alkaloids, geraniol, terpenoids, polyphenols, esters, aldehyde and fatty acids have been isolated and analysed [10]. The most essential compounds in *C. citratus* according to literatures are essential oil and flavonoids, which contributed to the pronounced therapeutic and pharmacological activities of the plant [11]. The present study is aimed at determining the antiplasmodial effect of ethanolic leaf extracts of *C. citratus* in Swiss albino mice infected with *Plasmodium berghei*.

2. MATERIALS AND METHODS

2.1 Plant Leaf Collection

Fresh leaves of *C. citratus* were collected from a farmland within Seebi, Ilesha-Owo Express way, Akure, Ondo State, Nigeria. Identification and authentication were carried out and the voucher specimen number of the plant Bio/ FUTA/ 99 was left in the herbarium of the Department of Crop Soil and Pest Management, School of Agricultural Technology, Federal University of Technology, Akure, Ondo State, Nigeria.

2.2 Extraction of Plant Material

The leaves were washed, cut into smaller pieces and air dried for 3 weeks at room temperature ($28\pm 3^{\circ}\text{C}$) and were pulverized into fine powder by a high-speed blender. A mass of five hundred grams (500g) of the grounded powder was soaked into 3000mls of 75% ethanol for 72 hours and then filtered using a millipore filter (pore size 0.7 μm). The extracts was concentrated using rotary evaporator at a temperature of 40°C . It was further heated over a water bath to obtain a solvent-free extract and was thereafter stored in the refrigerator at 4°C [12].

2.3 Phytochemical Screening of *C. citratus*

The phytochemical analysis to determine the bioactive ingredients (Qualitative and Quantitative) present in *C. citratus* was carried out using standard procedures as described by Trace, [13], and employed by Arome [4].

2.3.1 Test for cardiac glycosides

About 2 ml of the extract solution was diluted with 1 ml of glacial acetic acid followed by six drops of 10% ferric chloride solution and six drops of concentrated sulfuric acid. The formation of green-blue color indicates the presence of cardiac glycosides.

2.3.2 Test for saponins

The extract was diluted with 20 ml of distilled water and then the test tube was shaken for about 10 min. The formation of lather or foam on top indicated the presence of saponins.

2.3.3 Test for tannins

The extract solution was dissolved into 4 ml of chloroform and 1 ml of acetic anhydride. About 1

ml of sulfuric acid was added to it along the wall sides of the test tube. The formation of green coloration showed the presence of tannins.

2.3.4 Test for steroids

The extract was dissolved in 10 ml of chloroform, and 1 ml of concentrated sulfuric acid was added into the test tube. The formation of red color in the upper layer and yellow color in the sulfuric acid layer showed the presence of steroids.

2.3.5 Test for carbohydrates

About 2 ml of the extract solution was diluted with two drops of Molisch's test reagent and mixed thoroughly. Then, 4 ml of concentrated sulfuric was added. The formation of purple color indicates the presence of carbohydrates.

2.3.6 Test for flavonoids

A few drops of diluted sodium hydroxide were added to the extract solution. The formation of intense yellow color which becomes colorless upon the addition of a few drops of diluted sulfuric acid showed the presence of flavonoids.

2.4 Collection of parasite and Experimental Mice

Chloroquine sensitive strain of malaria parasite (*Plasmodium berghei* NK 65) in a donor mouse and 55 healthy mice of weighs between 20-25g was obtained from Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Oyo State, Nigeria. They were transferred to Microbiology laboratory, at Federal University of Technology, Akure (FUTA). Mice were housed in plastic cages with wood saw dust beddings. They were fed with pellets (Supreme Pet food) and water ad libitum and acclimatized for 7 days at room temperature ($29-30^{\circ}\text{C}$) before the commencement of the experiment.

2.5 Acute Toxicity Test

The toxicological test for *C. citratus* was carried out according to Organization for Economic Cooperation and Development (OECD) guidelines [14] with slight modification as employed by Dada and Muhammed [12]. A total of 16 healthy mice were randomized into four groups of four mice per group. Each mouse in groups were treated with 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight of the extract,

respectively. The control group received normal saline. Before commencing the treatment, the body weight of mice was recorded. The mice were observed visually daily for 14 days throughout the experiment for signs of toxicity and behavioral.

2.6 Measurement of Weight

Body weights of each mouse in all group were measured before and after acute toxicity test at different doses and throughout the study using digital weighing balance for 5 days.

2.7 Preparation Inoculation

The method described by Dada and Oloruntola [14] was used for the inoculation of malaria parasites. Chloroquine sensitive strain of malaria parasite (*Plasmodium berghei* NK 65) in a donor mouse was obtained. By cardiac puncture, parasitized blood were withdrawn from the infected mouse with a syringe, transferred into a screw cap sterile plastic tube containing 0.9% normal saline to obtain 1×10^7 *P. berghei* infected erythrocytes. Thirty - five (35) mice were randomized into five groups of seven mice each. Mice in Group 1, 2, 3, 4 and 5 were infected intravenously with 0.2 mls of 1×10^7 standard inoculum of chloroquine-sensitive *P. berghei* after the parasitemia level of the infected mouse had been ascertained to be high.

2.8 Drugs and Administration

The leaf extracts and chloroquine were administered orally to the test groups, negative group, and the positive group, respectively. A stainless metallic feeding cannula attached to a syringe was used during oral administration to deliver the compound into the stomach [12]. Extract concentrations were prepared by dissolving known weight of the concentration of ethanol extracts (200 mg/kg) in known volume of distilled water to give 200 mg/ml, 400 mg/ml and 800mg/ml of the crude extract. 250 mg/ml of the standard antibacterial agent (tetracycline) was similarly constituted.

2.9 In vivo Antiplasmodial Activity of the Extracts

The method of Ogundolie et al. [15] was used to administer the extract and drug. Three (3) hours after infections, 0.2 ml of 200, 400 and 800 mg/kg body weight of leaf extract were

administered orally to group 1, 2 and 3 respectively as treatment dose once daily, group 4 (positive control) were treated with 0.2 ml of 5 mg/kg body weight of chloroquine, and group 5 (negative control) were given 0.2 ml of normal saline for six consecutive days.

2.10 Determination of Parasitemia

The method described by Bankole [16] was used to determine the parasitemia level of the mice. It was determined on the sixth day by collecting 2 drops of blood on a microscopic slides from each mouse by venesection of the tail. Thick and thin blood smear was made and allowed to air-dry at room temperature. The films were then fixed with 75% ethanol for 2-3 minutes, after which they were stained with 10% Giemsa stain for 15 minutes. The blood smear samples were rinsed with buffer distilled water pH 7.2. The slides were examined and counted under light microscope at X100 magnification (oil immersion). The parasitemia was determined by counting minimum of three fields per slides with 100 RBC per field [15].

$$\text{Parasitemia} = (\text{Number of parasitized RBC} / \text{Total Number of RBC examined}) \times 100$$

2.11 Statistical Analysis

Analysed data were expressed as Mean \pm standard deviation. Statistical significant was determined by one-way analysis of variance. The analysis was performed using SPSS version 25. $P < 0.05$ was considered a significant difference between means (Duncan's multiple range test).

3. RESULTS

The qualitative screening of ethanol leaf extract of *C. citratus* revealed the presence of saponins, tannins, glycosides, terpenoids, flavonoids and steroids (Table 1) while in quantitative screening glycosides was higher with 19.92 mg/g, followed by terpenoids with 19.12 mg/g, saponins (10.3 mg/g), steroids (6.21 mg/g), tannins (2.38 mg/g) and the least flavonoids with 1.87mg/g (Fig. 1).

Toxicological test of *C.citratus* leaf extract in mice (Table 2) showed no noticeable sign of toxicity in the mice after 14 days and no death or mortality recorded for all the doses tested (250, 500 and 1000 mg/kg). This was a clear indication that doses used were safe. No physical and

behavioral signs of overtotoxicity such as decreased motor activity, decreased body/limb tone, writhing, respiration, and death amongst others were observed. The result obtained from Fig. 2 showed decreased in body weight of mice after administration at concentration tested 500 mg/ml (17-12g) and 1000 mg/ml (18- 20g) while there was no decrease at concentration 250 mg/ml.

Body weight of mice of groups 1 (200 mg/kg ethanol extract) is shown in Fig. 3 revealed an average decrease in body weight from day 1 to 5 after treatment, however, the mice treated with 400 mg/kg ethanol extract (Groups 2) showed increase body weight for all days except day 4 where there was little decreased in weight. 800mg/kg ethanol (group 3) showed average decreased in body weight in all the days of the treatment. Mice of positive control (Group 4) also showed decrease body weight after 4 days of treatment, but not as that of negative control mice (group 5) that showed increase body weight for day 1 to 3, and decreased body weight in day 4.

3.1 Percentage Parasitaemia Count

Mice in group 1 (200 mg/kg) shown in Fig. 4 revealed remarkable significant ($P < 0.05$)

decreased parasitaemia from day 1 to 6. Similar observations (decreased parasitaemia) occurred for those of group 2 (400mg/kg) in (Fig. 5) but significantly displayed higher decreased in percentage parasitaemia than group 1 in day 5 and 6. Mice of groups 3 (800 mg/kg) recorded lower percentage parasitaemia compared with groups 1 (200 mg/kg and 400 g/kg). The mice treated with chloroquine (Fig. 7) revealed lower percentage parasitaemia (more activities) compared with mice treated with the highest dose of the ethanol extract (800 mg/kg). Mice of negative control revealed significant increase ($P < 0.05$) percentage parasitaemia from day 1 to day 6, compared to other groups.

Table 1. Phytochemical screening of *C. citraus*

Phytochemicals	Ethanol
Saponins	+
Tannis	+
Alkaloids	+
Anthraquinones	-
Flavonoids	+
Glycosides	+
Steroids	+
Terpernoids	+
Phlobatannin	-

Key: + Present and - Absent

Table 2. Acute toxicity test

Groups	Number of mice	Toxic symptoms	Dosages	Mortality	% mortality
1	4	None	250	0/4	0
2	4	None	500	0/4	0
3	4	None	1000	0/4	0
4	4	None	Control	0/4	0

Key: Group 1=250 mg/kg of ethanol leaf Extract, Group Group 2=500 mg/kg of ethanol leaf Extract, Group 3=1000 mg/kg of ethanol leaf Extract, Group 4= Control

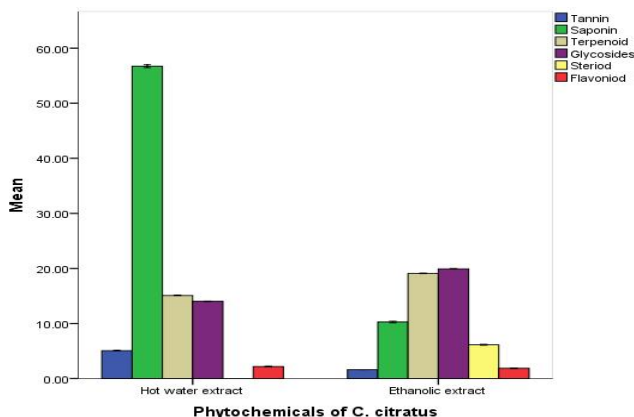


Fig. 1. Quantitative constituents of *C. citraus*
Bars are presented as Mean \pm S. D of replicates (n=3)

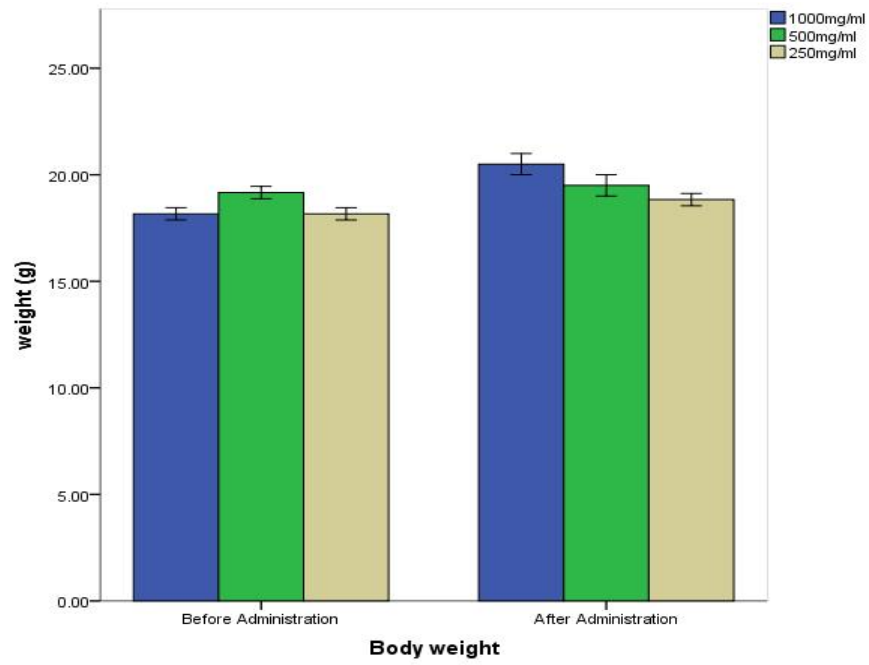


Fig. 2. Body weight of mice before and after acute toxicity
 Error bars ± 1 SE

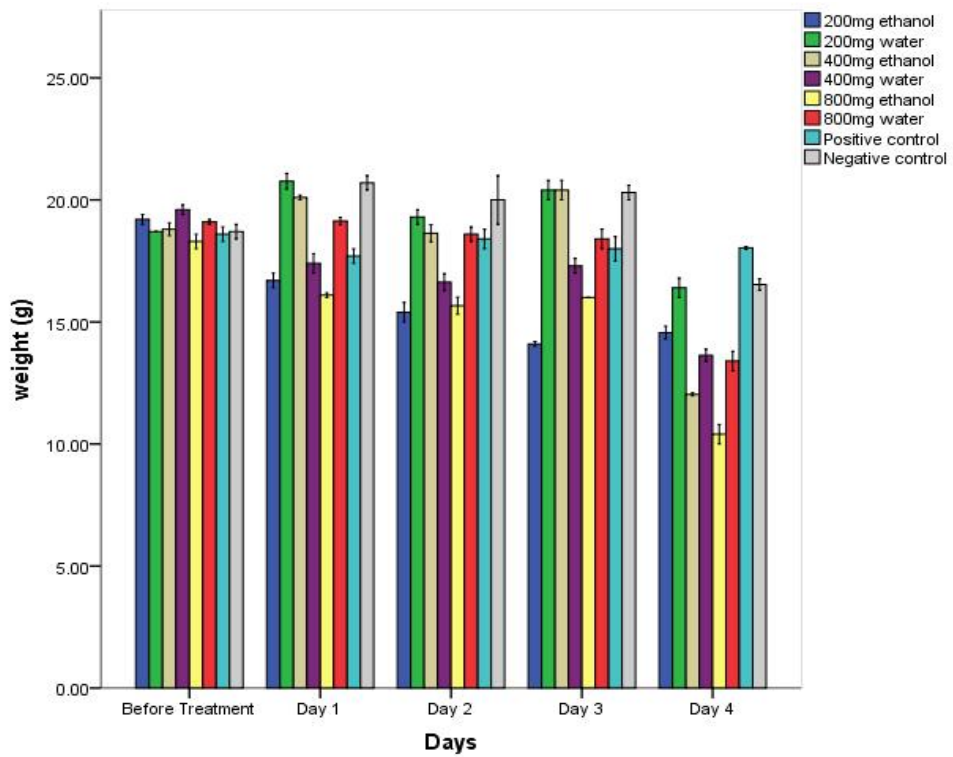


Fig. 3. Weight of mice before and after treatment
 Error bars ± 1 SE, Bars are presented as Mean \pm S. D of replicates (n=3)

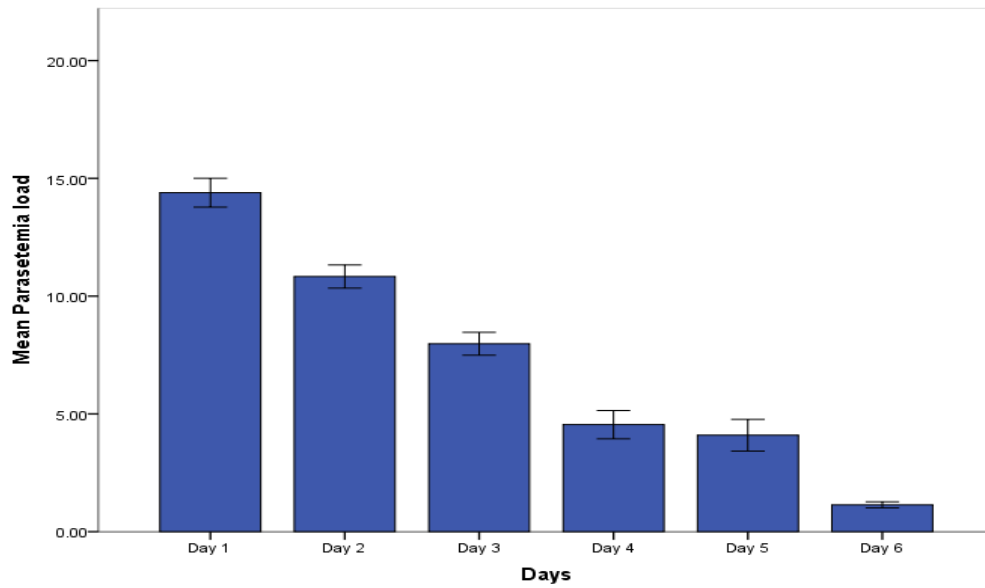


Fig. 4. % Parasitemia for group 1

Keys: Group 1: *P. berghei* + 0.2 ml 200 mg/kg ethanol extract; Bars are presented as Mean \pm S. D of replicates (n=3)

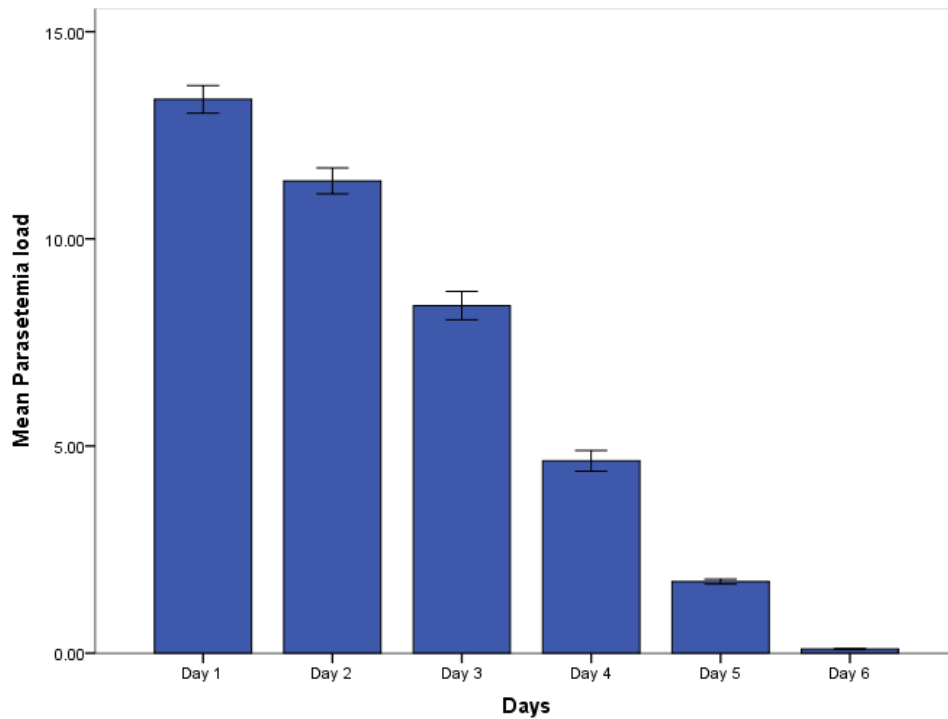


Fig. 5. % Parasitemia count for group 2

Keys; Group 2: *P. berghei* + 0.2 ml 400 mg/kg ethanol leaf extract of *C. citratus*; Bars are presented as Mean \pm S. D of replicates (n=3)

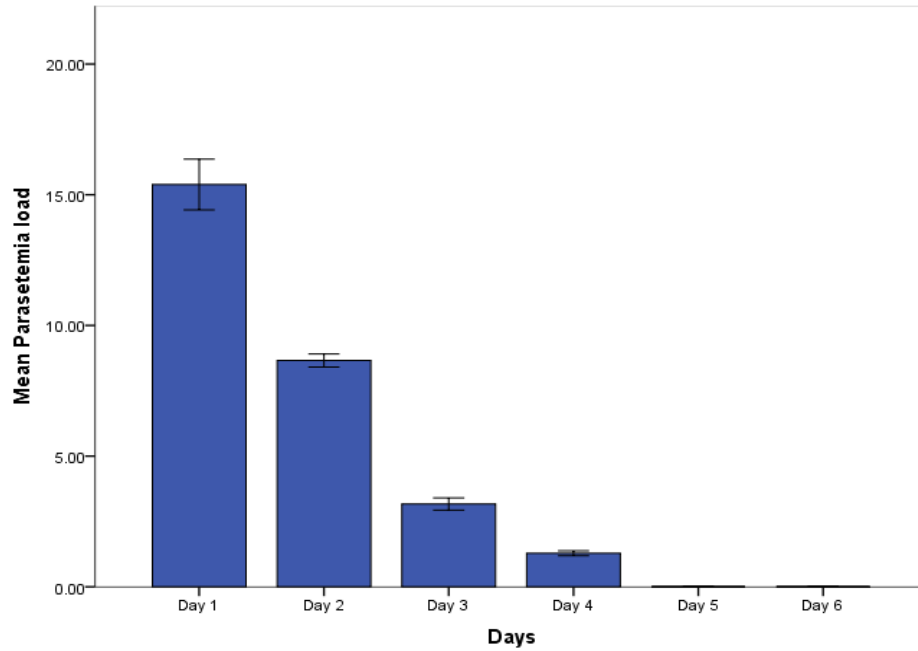


Fig. 6. % Parasitemia count for group 3
Bars are presented as Mean \pm S. D of replicates (n=3); Keys; Group 3: *P. berghei* + 0.2 ml 800 mg/kg body weight hot water leaf extract of *C. citratus*

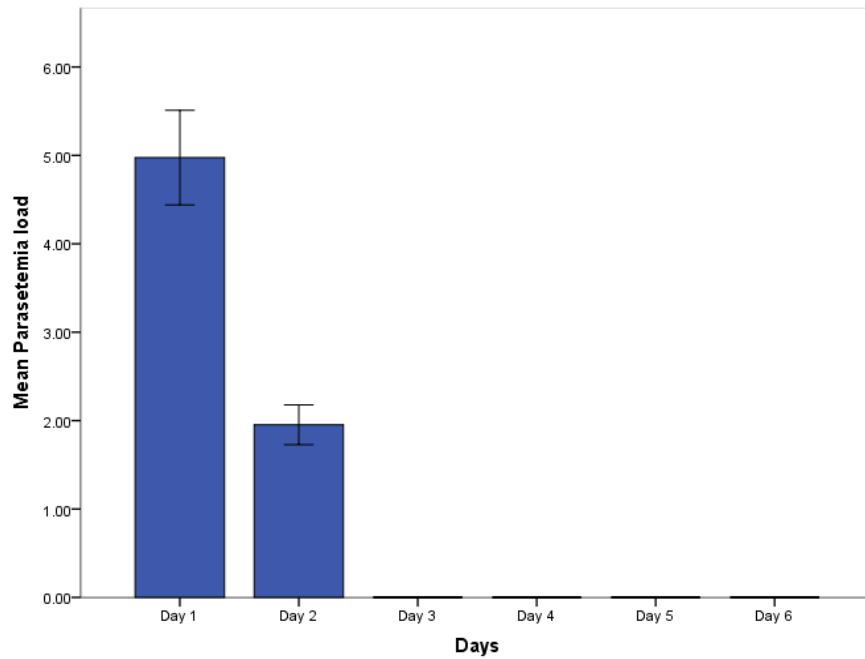


Fig. 7. % Parasitemia count for group 4
Bars are presented as Mean \pm S. D of replicates (n=3); Keys: Group 4: *P. berghei* + 0.2 ml 5 mg/kg body weight chloroquine

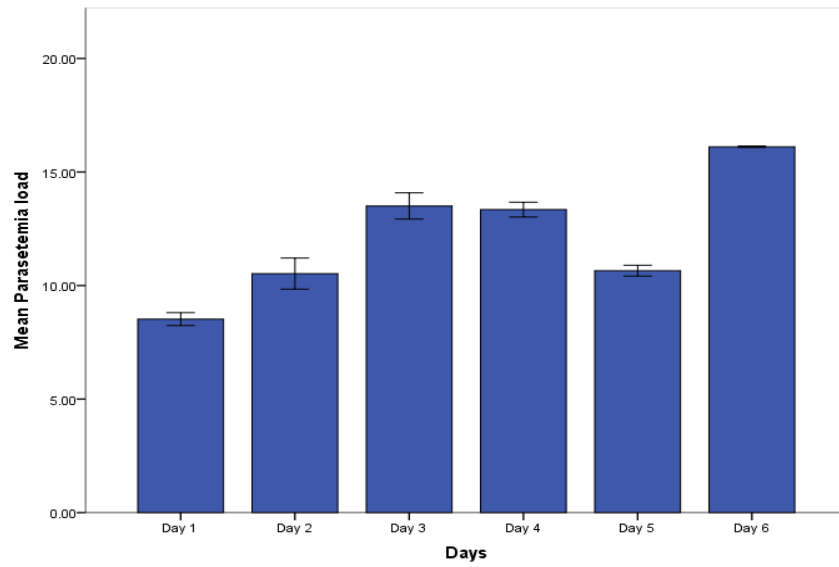


Fig. 8. % Parasitemia count for group 5

Bars are presented as Mean \pm S. D of replicates (n=3); Keys: *P. berghei* + 0.2 normal saline (Negative control)

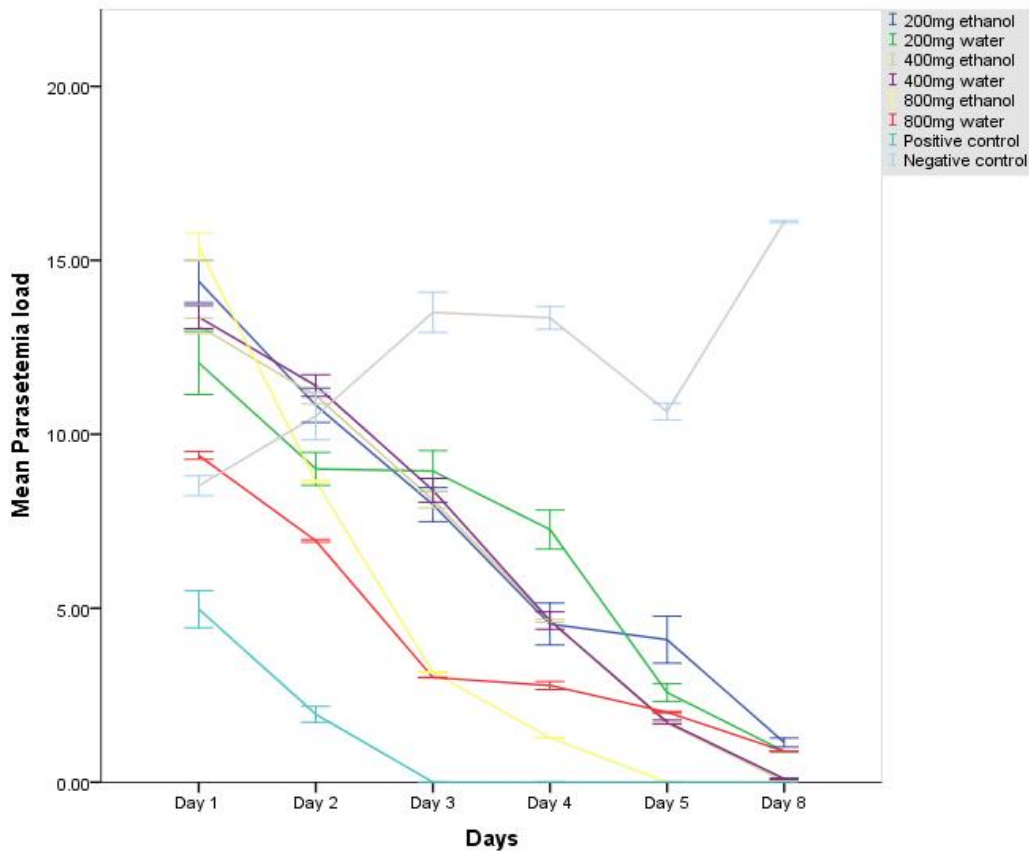


Fig. 9. Line graph showing thean parasitemia load of all group

4. DISCUSSION

The emergence of widespread resistance of *Plasmodium* species to most antimalarial drugs has led to a more vigorous and concerted research in traditional medicinal plants for the treatment of malaria.

The study clearly demonstrates the potential activity of *C. citratus* leaf extract against malaria. The phytochemicals screening of the ethanol leaf extract evaluated were found to contain steroids, tannins, flavonoids, saponins, alkaloids, terpenoids, except, phlobatannin and anthraquinones that were absent. Notably, our results was in conformity with Arome [4] who reported the presence of all the aforementioned phytochemicals in the in *C. citratus* extract. According to Kiliobas [17], secondary metabolites are one of the major classes of compounds possessing antimalarial activity, the presence of tannins, terpenoids, saponins in *C. citratus* extracts have contributed to antimalarial activities exhibited by the plant extracts. Also, flavonoids are compounds with a widespread occurrence in the plant kingdom which have also been detected in *Artemisia* species [18].

Change in body weight is a sensitive indices of toxicity evaluations after exposure to toxic compound. The body weight of mice in all groups significantly ($P < 0.05$) increased before and after toxicological test. This agrees with Gebremickael [19]. This is also an indicated positive health status of the mice or safety of both extracts for further studies.

The results of acute toxicity test implied that ethanolic extracts of *C. citratus* at the different dosages tested (250 mg/kg, 500 mg/kg and 1000 mg/kg body weight) in this study were not toxic to the mice. The extracts can therefore be considered safe, because no death and general sign of toxicity observe. The findings disagree with Ukpai and Amaechi, [20] who reported that acute toxicity of the ethanolic leaf extracts of *C. citratus* was considered slightly toxic. The acute toxicity test of the extract in mice, which revealed no death and a general sign of toxicity is expected. This is in line with the findings of Muhammad et al. [12], who indicated that herbal extracts with LD_{50} above 3000 mg/kg/oral may be considered safe and nontoxic.

Higher antiplasmodial activity was observed on the ethanol extract of *C. citratus* in all

concentrations tested. This finding could be due to reasons advanced by Umar et al. [18] that is as a result of high polarity and the ability of ethanol to extract more of the plant active components.

The reduced parasitaemia counts in mice of extracts treated groups (groups 1, 2, and 3) compared to group 5 (negative control) for all days is envisaged. The degree of parasitaemia counts decreased was high in group 3 Fig. 6 (800 mg/kg ethanolic extract). The pattern of reduction was dose and time dependent. This agrees with the work of Kiliobas, [17], who reported dose dependent antimalarial activities against *P. berghei* in fractions of *C. citratus*. The decreased percentage parasitaemia in ethanolic treated mice compared to negative control could be attached to the findings of Muhammed *et al.*, [21], that saponins and tannins in extracts could inhibit haem polymerisation and the unpolymerised haem is very toxic for intraerythrocytic *Plasmodia*. Comparatively, the parasitaemia load reductions exhibited by ethanolic treated group at difference concentrations for all days were not as effective as chloroquine (positive control). This conformed to the work of Ukpai and Amaechi, [20], who noted that the leaf extract of *C. citratus* exerted some antimalarial activity as was observed in the chemosuppression obtained but this was not as effective as the positive control group (chloroquine standard 5 mg/kg)

5. CONCLUSION

From the results of this preliminary work, it is concluded that ethanol extract of *Cymbopogon citratus* showed antiplasmodial activity and could be apply as an effective. The use of *C. citratus* could be a better choice or a substitute used as a supportive therapy for malaria treatment. However, further evaluation is needed before this plant is used to cure and manage human malaria.

ETHICAL APPROVAL

The whole experimental management, handling and care were approved by the Research and Ethics Committee of the Department of Microbiology, School of Sciences, The Federal University of Technology, Akure, Nigeria. Animal Ethic committee approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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