Direct Research Journal of Biology and Biotechnology

Direct RESEARCH JOURNALS

Vol. 11(1), Pp. 34-42, October 2025,

https://journals.directresearchpublisher.org/index.php/drjbb; https://www.ajol.info/index.php/djbb

Research Article
ISSN: 2734-2158

Evaluation of Antimalarial Efficacy and Safety of *Boswellia dalzielii* Leaf Extract in *Plasmodium berghei* (NK-65)-Infected Mice

Mary M. Mankilik[1]*, Yunana Nehemiah Parah[1], Joel Paul[1], Peace Ori Abaya[1], Daniel H. Mhya[2], Akinbobola P. Otitoju[3] and Ishaya Y. Longdet[1]

[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Jos, Plateau State, Nigeria.
[2]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Abubakar Tafawa Balewa University, Bauchi. Bauchi State, Nigeria.
[3]National Biotechnology Development Agency (NABDA), FCT, Abuja, Nigeria.
Corresponding author email: mmankilik@gmail.com, mankilikma@unijos.edu.ng

ABSTRACT

Due to the malaria parasite's resistance to drugs, the use of medicinal plants in malaria treatment in Africa remains the most alternative option. The plant 'Boswellia dalzielii' was reported with antimalarial potential. However, little is known about the antimalarial properties of its root leaf. The study aimed to assess antimalarial effectiveness and safety of Boswellia dalzielli leaf extract in mice infected with Plasmodium berghei (NK-65). Fresh Boswellia dalzielii leaves were washed, air-dried, powdered, and macerated in distilled water for 48 hours. The concentrated butanol extract was used for phytochemical screening, toxicity testing, and in vivo antimalarial evaluation. Eighteen Swiss albino mice were divided into six groups of 3 rats each: P. berghei-infected treated with a standard drug, P. berghei-infected untreated, P. berghei-infected treated with 100 mg/kg extract, P. berghei-infected treated with 200 mg/kg extract, P. berghei-infected treated with 400 mg/kg extract, and uninfected untreated control. Parasitemia, packed cell volume, and mean survival time were measured to assess antimalarial activity. The phytochemical study revealed the presence of flavonoids, tannins, saponin, balsam, carbohydrates, and phenol. Acute toxicity testing confirmed the extract's safety. In vivo investigations showed a significant increase in mice weight, decrease in parasite levels, and restoration of PCV in a dose-dependent manner. Boswellia dalzielii extract at 400 mg/kg increased the average survival time to 18 days surpassing the standard drug's 17 days' duration and showing an antimalarial effect comparable to the standard drug (chloroquine phosphate 25 mg/kg). The study found that Boswellia dalzielii leaf extract is safe and contains chemicals with antimalarial activity.

Keywords: *Antiplasmodial, Boswellia dalzielii, Plasmodium berghei, Phytochemicals, Leaf extract*

Article information
Received 3 August 2025
Accepted 28 September 2025
Published 20 October 2025
https://doi.org/10.26765/DRJBB84818179

Citation Mankilik, M. M., Parah,Y. N., Paul, J., Abaya, P. O., Mhya, D. H., Otitoju, A. P., Longdet, I. Y. (2025). Evaluation of Antimalarial Efficacy and Safety of *Boswellia dalzielii* Leaf Extract in *Plasmodium berghei* (NK-65)-Infected Mice. *Direct Research Journal of Biology and Biotechnology*. Vol. 11(1), Pp. 34-42.

INTRODUCTION

Till date, malaria continues to be a significant public health concern Worldwide. In 2023, according to the World Malaria report by the world health organization (WHO) (2024), there were approximately 263 million malaria cases worldwide in 2023, an increase of 11 million from the previous year. The number case rose to 60.4 up from 58.6 in 2022. In a *recent study, increase in malaria cases have been observed with* Nigeria having the highest number of malaria cases globally *(Friedman-Klabanoff et al., 2024).* There are numerous over-the-counter antimalarial drugs, but many antimalarial agents, including artemisinin-based combination therapies (ACTs), currently used as first- and second-line treatments for malaria, have been reported to show reduced efficacy due to resistance in parasite strains (Tse et al.,2019; Blanshard and Hine, 2021). Poor efficacy noticed by the antimalarial drugs particularly artemisinin is reported to be a result of Plasmodium mutation (Straimer et al., 2022).

Natural products had been known to play essential role in drug discovery and the treatment of various ailments. Traditionally, medicinal plants have been used for controlling multiple disorders only to their fewer adverse effect, affordability and easy accessibility. Natural products such as quinine and artemisinin and their derivatives and analogues, have saved millions of lives (Kingston and Cassera, 2022). *Plasmodium falciparum*, the most dangerous malaria parasite has become resistant to quinine and most of its derivatives and is becoming resistant to artemisinin and its derivatives (Kingston and Cassera, 2022).

Boswellia dalzielii is plant species that's belongs to the family *Burseraceae*. It is commonly known as frankincense. The tree species is commonly found in tropical region on Africa particularly in Cameroon. Nigeria and Sudan (Burkill, 1985; Owolabi *et al*., 2020; Mshelia *et al*., 2023). In Nigeria it is abundantly found in Northern Nigeria and called "Hano" or "Harrabi" in Hausa and very popular among the locals as rich source of ethnomedicine (Etuk *et al*., 2009).

The plant has been extensively studied for its pharmacological properties and use in the treatment of diseases such as inflammation and osteoarthritis (Ammon *et al*., 2010). These properties have been attributed to the presence of active phytoconstituents triterpenoids which include beta-boswellic acid (Adegboyi *et al*., 2019; Awada *et al*., 2020). Recent reports on *B. dalzielii* by various authors has explored its bioactive compounds for potential antimalarial properties (Zakariya *et al*., 2021; Dogara *et al*., 2021), antinflammatory (Salihu, 2018), antimicrobial (Sherifi *et al*., 2020), arthritis ((Salihu *et al*., 2018), fever, skin disease, gastrointestinal disorder (Adjarolium *et al*., 1996; Salihu *et al*., 2018) *B. dalzielii* root-bark has been traditionally used in Africa to treat various disease Ogbiuchi *et al*., 2012). Despite reports on the antimalarial potential of *B. dalzielii* root and bark, the antimalarial properties of its leaf remain largely underexplored, justifying the present study's focus on evaluating the leaf extract for potential therapeutic efficacy.

MATERIALS AND METHODS

Plant Sample Collection

Boswellia dalzielii leaves were collected from *Pilkong, Abwor-dyis* village Lankang district of Pankshin Local Government Area of Plateau State in the month of January, 2023. The leaves were validated at the herbarium of the Department of Botany Federal College of Forestry, Jos, Plateau State for future reference, a voucher specimen was deposited (Voucher number FHJ2023). The leaves were cleared from all dirt by washing under running tap water, and air-dried at room temperature (22-25^{o}C). After drying it was then pulverized into coarse powder using wooden mortar and pestle.

Preparation of Plant Extract

Boswelia dalzielli leaves powder (400g) was weighed using weighing scale (JJ200 G&G Deutschland) and soaked in butanol (2.25L). It was then stirred vigorously and kept at room temperature for 48hrs. Thereafter, the mixture was filtered using a whatman (No. 1 filter paper) and then concentrated by rotary evaporation at 50^{0}C. The extract obtained was kept in a clean air-tired sterile bottle for further analysis.

Ethical Clearance

All experimental procedures involving animals were conducted in accordance with the university's ethical committee guidelines for the care and use of laboratory animals, following the National Institutes of Health (NIH) Publication No. 8023, revised 1978. Approval for the research protocol was granted by the University Research and Ethics Committee in collaboration with the Office of Laboratory Animal Welfare (OLAW) under reference number F17-00379.

Animal Handling

Eighteen Swiss albino mice of both sexes, aged seven to eight weeks and weighing between 18.4 g and 31.5 g, were purchased from the Animal House, Department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos. The mice were housed in well-ventilated cages under standard environmental conditions and allowed free access to feed (Vital Feed, ECWA Feed, Jos) and clean water *ad libitum* throughout the experimental period.

Phytochemical Screening

Phytochemical screening of the extracts was performed for

the presence of secondary metabolites using standard methods as described by Trease and Evans (2002). The extract was screened for the presence of alkaloids, flavonoids, phenolics, saponins, taninins etc.

Acute Oral Toxicity

To assess the toxic effect of the butanol root extract from *B. dalzelii*, the Lorke's (1983) method was employed. Briefly, 3 groups of mice containing 3 mice each were fasted for 12hr prior to test, they were administered with exact at different doses; 1000, 2000, and 5000mg/kg body weight respectively, mice were observed over a period of 24hrs for signs of morbidity and mortality.

Inoculation of Parasite in Mice

From the donor mice infected with *P. berghei* (NK-65) obtained, about 10mls of the infected blood was collected in a beaker containing 30mls of normal saliva. The naïve mice were then injected with 0.5ml of *P. berghei* (suspension) intraperitoneally.

Experimental Groupings

Twenty-one (21) Swiss albino mice of both sexes were used for the experiment and grouped as follows;

a. Group A (Positive control)– Mice infected with $1x10^{-7}$ *Plasmodium (P. berghei* NK-65) strain and treated with chloroquine (25kg/b.wt)
b. Group B (Negative control) - Mice infected with $1x10^{-7}$ *Plasmodium (P. berghei* NK-65) strain and is untreated
c. Group C (Normal control) - Mice were uninfected and untreated (Norma control group) – they were given only feed and water ad libitum.
d. Group D (Extract treated at lower dose) - Mice infected with $1x10^{-7}$ *Plasmodium (P. berghei* NK-65) strain and treated with 100mg/kg b.wt of *B. dalzielii* leaf butanol extract.
e. Group E (Extract treated at median dose) - Mice infected with $1x10^{-7}$ *Plasmodium (P. berghei* NK-65) strain and treated with 200mg/kg b.wt of *B. dalzielii* leaf butanol extract.
f. Group F (Extract treated at higher dose) - Mice infected with $1x10^{-7}$ *Plasmodium (P. berghei* NK-65) strain and treated with 400mg/kg b.wt of *B. dalzielii* leaf butanol extract.

Extracts were given once daily from day 3 to day 8. The parasitemia were examined microscopically by counting the parasites red blood cells. The mice were observed for 30 days and the Mean Survival Time of each group recorded.

Antimalarial Activity (Curative Test)

Mice in groups (A-E) were infected intraperitoneally on day one (D1) with $1x10^{-7}$ parasitized red blood cells contained in 200µL and then treated on D4, D5, D6 and D7 at 24hr interval with the butanol extract (AE). Mice in group (C, D, and E) received the extract at a dose of 100, 200, and 400mg/kg respectively. Group B (negative control) untreated and group A (Positive control) received chloroquine (CQ) at a dose of 25mg/kg. Parasitemia level was determined by May Grunwald-Giemsa (10%) strain. The mean survival rate of mice infected with *Plasmodium berghei* NK-65 following treatment were determined by calculating the average survival time in days from the day of infestation until the 30th day (D1-D30) as described by Sidiki et al. (2012). The activity of the extract on the parasites was expressed as a function of the reduction in parasitemia of treated mice compared to mice in the negative control group (Singh et al., 2015). Percentage inhibition and parasitemia were each determined.

$$\%\ parasetima = number\ of\ infected\ RBC/total\ number\ of\ RBC\ ^{x}\ 100$$

PCV Determination

The PCV, packed cell volume was determined using a modified Wintrobe's method. Blood samples were obtained from the tails of mice using heparinized micro-hematocrit tubes. These tubes were filled to three quarters of their capacity and sealed with crystoseal. The sealed tubes were then centrifuged at 12,000 rpm for five minutes in a micro-hematocrit centrifuge, with the sealed end facing outwards. Following centrifugation, PCV values were determined using a hematocrit reader. Baseline PCV measurements were taken prior to parasite inoculation.

$$PCV = Volume\ of\ erythrocytes\ in\ a\ given\ volume\ of\ blood/Total\ blood\ volume$$

Mice Body Temperature Measurement

The rectal temperature of each mouse was recorded using a digital thermometer one hour prior to infection to assess the potential impact of the extract on temperature regulation during a 5-day curative test.

Mice Body Weight Measurement

Mice's body weight (in grams) was assessed using a sensitive weighing balance 3 hours before infection and during the curative treatment. To get the mean body weight of a group of mice, divide the total weight by the total number of mice in the group.

Data Analysis

The data obtained were presented as mean ± standard error of the mean (SEM) for each analysis. Results were presented using bar charts and SEM indicated.

RESULTS

Extract yield and phytochemicals analysis

The yield of crude butanolic extract of *Boswellia dalzielii* leaf was 152g, which accounted for 38%. The phytochemical examination of *Boswellia dalzielii* leaf extract revealed the presence of a number of bioactive components, including alkaloids, flavonoids, tannins etc. These chemicals are renowned for their therapeutic qualities and may add to the plant's medicinal potential. The phytochemical identified are presented in (Table 1).

Table 1: Phytochemical composition of *Boswellia dalzielii* leaf butanol extract.

Test	Result
Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	-
Steroids	-
Cardiac glycoside	-
Balsam	+
Carbohydrates	-
Phenols	+
Resin	-

NB: + signifies present and – indicate absent

Effect of *Boswellia dalzielii* Leaf Extract on Parasitemia Levels of Mice Infected with *P. berghei* Nk-65

Figure 1 depicts how parasitemia levels varied throughout the groups during the investigation. Mice infected with *Plasmodium berghei* Nk-65 untreated (negative control group) show a significant rise in parasitemia from 19.3% to 26%. Mice infected with *Plasmodium berghei* Nk-65 but treated with normal medicines or extracts showed an increase in parasitemia on day three, followed by a reduction on day six, which lasted until day 18. The decrease in parasitemia in mice infected with *Plasmodium berghei* Nk-65 but treated with chloroquine (standard drug) drops from 4% to 0%. In the mice infected with *Plasmodium berghei* Nk-65 groups and treated with extract at varied doses, the decrease in parasitemia was noticed in a dose-dependent manner where the extract dose at 400 mg/kg had a reduction of parasitemia level from 3.3 to 0.3% and is the highest compared with the doses at 200 mg/kg body weight and 100 mg/kg body weight. The butanol extract of *Boswellia dalzielii* leaf showed curative action in vivo, with a decrease rate of > 90% at the higher dose (400 mg/kg) and ≥ 80% at the lower dose (100 mg/kg).

Effect of *Boswellia dalzielii* Leaf Extract on Survival Rate of Mice Infected with *P. berghei* Nk-65

In comparison to the negative control group, administering a butanol extract of *Boswellia dalzielii* leaf to infected mice resulted in a longer survival time, with mean survival times ranging up to 18 days. Figure 2 shows a significant increase in survival time ($P < 0.05$) with a higher dose of extract (400 mg/kg body weight). A mean survival duration was observed with an increasing extract dose in this order: 18 days (400 mg/kg), 16 days (200 mg/kg), and 15 days (100 mg/kg body weight). These findings suggest that *Boswellia dalzielii* leaf may possess potent therapeutic properties that could enhance survival in infected hosts. Notably, the chloroquine-treated group exhibited a good mean survival time, reaching 17 days. The extract appears to have increased the survival time more than the conventional medicine. These findings imply that administering leaf extract of *Boswellia dalzielii* may protect mice from malaria progression.

Effect of *Boswellia dalzielii* Leaf Extract on PCV of Mice Infected with *P. berghei* Nk-65

Figure 3 depicted the effects of *Boswellia dalzielii* leaf extract on mice's PCV. *Boswellia dalzielii* leaf extract demonstrated PCV protection in a time-dependent way; low (100 mg/kg), medium (200 mg/kg), and high doses (400 mg/kg) were found to have a protective effect against PCV. At the outset, PCV in all groups rose but gradually decrease as the trial progressed. However, the standard drug and *Boswellia dalzielii* leaf extract dramatically improved PCV by decreasing it compared to the negative control treatment.

Effect of *Boswellia dalzielii* Leaf Extract on Rectal Temperature of Mice Infected with *P. berghei* Nk-65

As shown in Figure 4, treatment with extract or standard medication effectively prevented a drop in rectal temperature in *P. berghei*-infected mice. When compared to *P. berghei*-infected mice untreated, a decrease in temperature is noticed throughout the study duration. There is a little decrease in body temperature from the *P. berghei*-infected mice treated with chloroquine, whereas the extract –treated at varied doses: 100, 200, and 400 mg/kg body weight showed a decrease from all the groups as the study prolonged. However, the decrease in temperature by the extract at 200 and 400 mg/kg body weight seem to be averted compared to the extract at 100 mg/kg body weight and the negative control groups.

Effect of *Boswellia dalzielii* Leaf Extract on Body Weight of Mice Infected with P berghei Nk-65

Figure 5 shows that *Boswellia dalzielli* leaf extract prevented body weight loss as compared to the negative control, but the effect varied by extract dose. The highest level of protection was achieved with 200 mg/kg of *Boswellia dalzielli* leaf extract, while the lowest level was at 400 mg/kg. In contrast to the *P. berghei*-infected mice who got the conventional medication, a rise in body weight

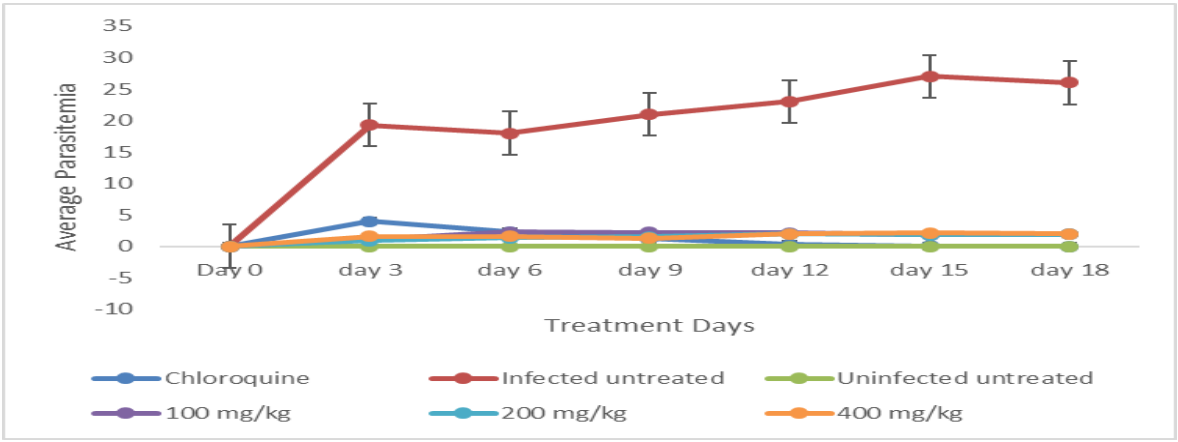

Figure 1: Effect of *Boswellia dalzielii* Leaf Extract on Parasitemia Levels of Mice Infected with Plasmodium berghei Nk-6

100 mg/kg = Infected mice treated with 100 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
200 mg/kg = Infected mice treated with 200 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
400 mg/kg = Infected mice treated with 1400 mg/kg b. wt. *Boswellia dalzielii* Leaf extract

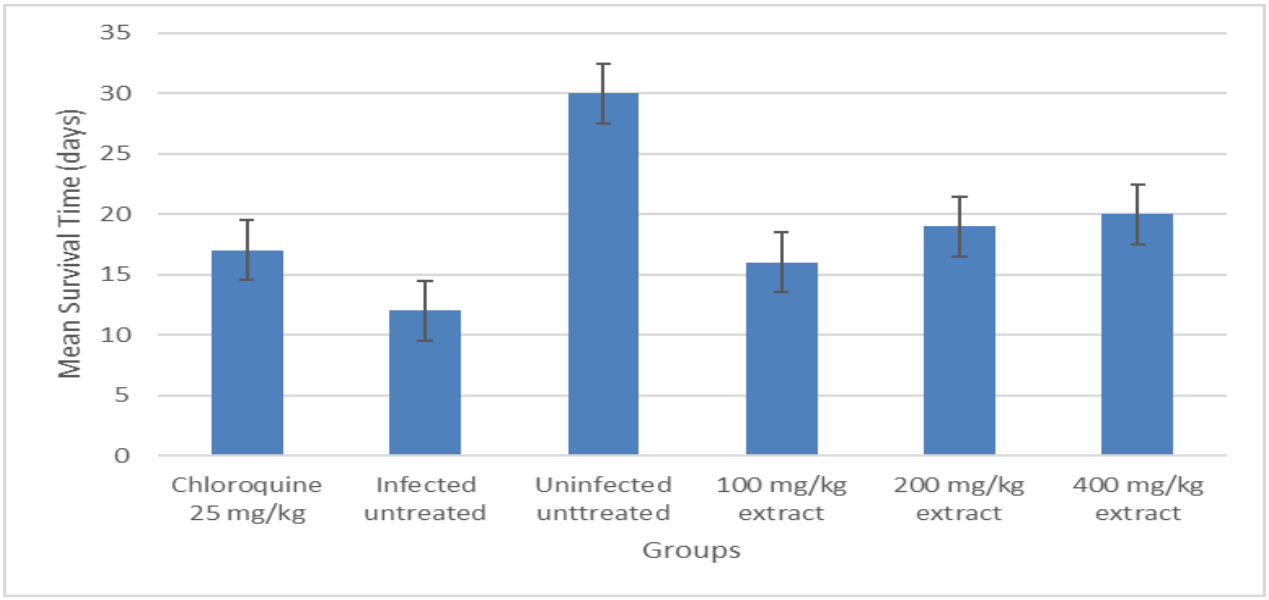

Figure 2: Effect of *Boswellia dalzielii* Leaf Extract on Survival Rate of Mice Infected with *Plasmodium berghei* Nk-65
Values were present as mean ± standard error of mean (SEM) of three determinants (n = 3).
100 mg/kg = Infected mice treated with 100 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
200 mg/kg = Infected mice treated with 200 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
400 mg/kg = Infected mice treated with 1400 mg/kg b. wt. *Boswellia dalzielii* Leaf extract

was observed, equivalent to normal control mice. Throughout the study period, *P. berghei*-infected mice untreated lost weight consistently.

DISCUSSION

Medicinal plants remain an important source of new therapeutic agents, particularly as resistance to existing antimalarial drugs continues to rise. This study demonstrated that *Boswellia dalzielii* leaf butanol extract possesses significant, dose-dependent antimalarial activity in *Plasmodium berghei* (NK-65)-infected mice. The extract reduced parasitemia, prolonged survival time, improved PCV, stabilized rectal temperature, and prevented body weight loss, indicating both antiparasitic and host-protective effects. The reduction in parasitemia supports earlier reports that *B. dalzielii* contains active compounds with antiplasmodial properties (Zakariya et al., 2021; Dogara et al., 2021). Its phytochemical constituents, particularly alkaloids, flavonoids, phenolics, and tannins,

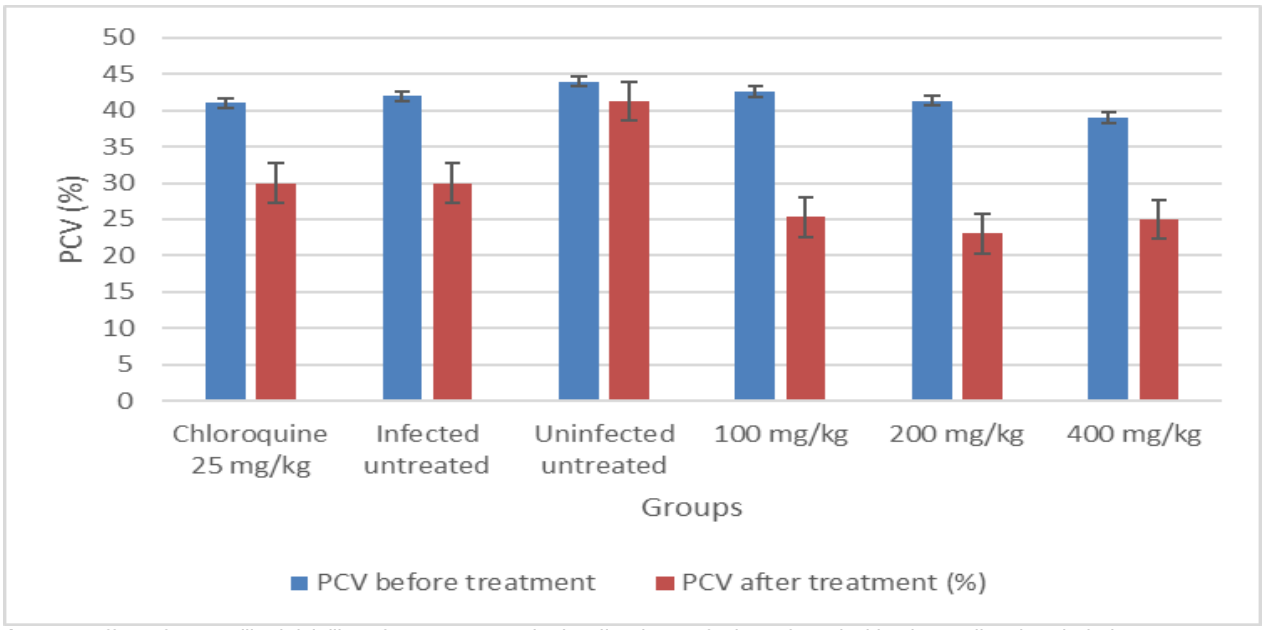

Figure 3. Effect of *Boswellia dalzielii* Leaf Extract on Packed Cell Volume of Mice Infected with *Plasmodium berghei* Nk-65
Values were present as mean ± standard error of mean (SEM) of three determinants (n = 3).
100 mg/kg = Infected mice treated with 100 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
200 mg/kg = Infected mice treated with 200 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
400 mg/kg = Infected mice treated with 1400 mg/kg b. wt. *Boswellia dalzielii* Leaf extract

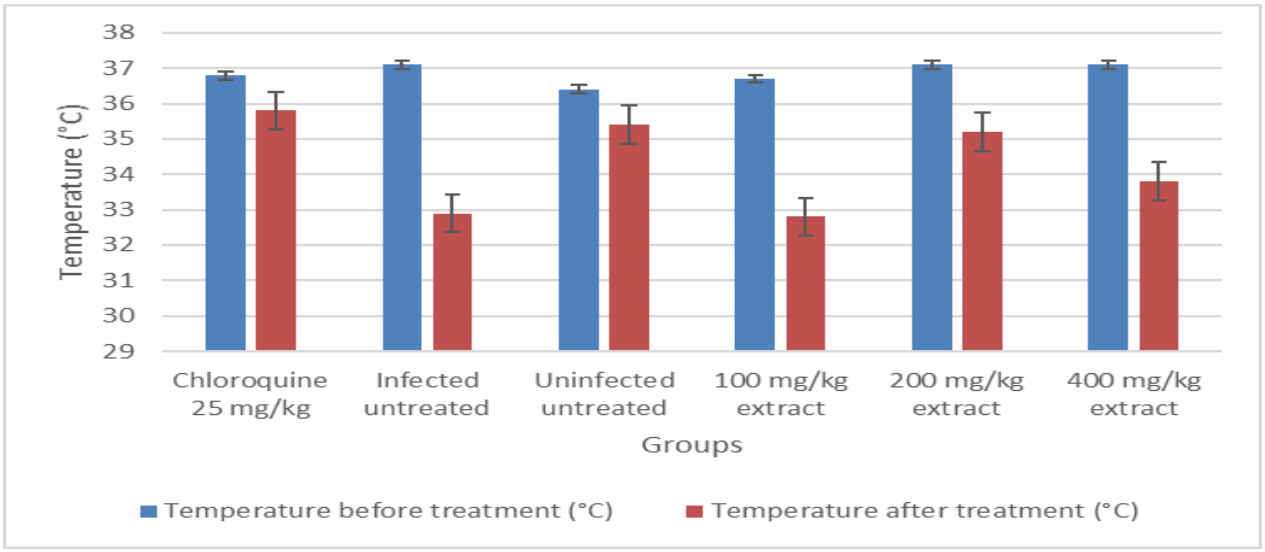

Figure 4. Effect of *Boswellia dalzielii* Leaf Extract on Rectal Temperature of Mice Infected with *Plasmodium berghei* Nk-65
Values were present as mean ± standard error of mean (SEM) of three determinants (n = 3).
100 mg/kg = Infected mice treated with 100 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
200 mg/kg = Infected mice treated with 200 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
400 mg/kg = Infected mice treated with 1400 mg/kg b. wt. *Boswellia dalzielii* Leaf extract

are known to interfere with parasite metabolism and oxidative balance (Atawodi et al., 2011; Dogara et al., 2022). Alkaloids such as quinine have long served as effective antimalarials (Gachelin et al., 2017), while flavonoids and phenolics inhibit parasite growth through redox modulation (Amin et al., 2023). These compounds likely account for the extract's antimalarial effects.

The increase in survival time and improvement in PCV among treated mice are consistent with previous reports on plant extracts that enhanced hematological recovery and survival in malaria-infected models (*Myrica salicifolia*, *Dorstenia barnimiana*, and *Leonotis ocymifolia*) (Kifle et al., 2020; Derebe et al., 2021; Teklu et al., 2020). The stabilization of body temperature and prevention of weight

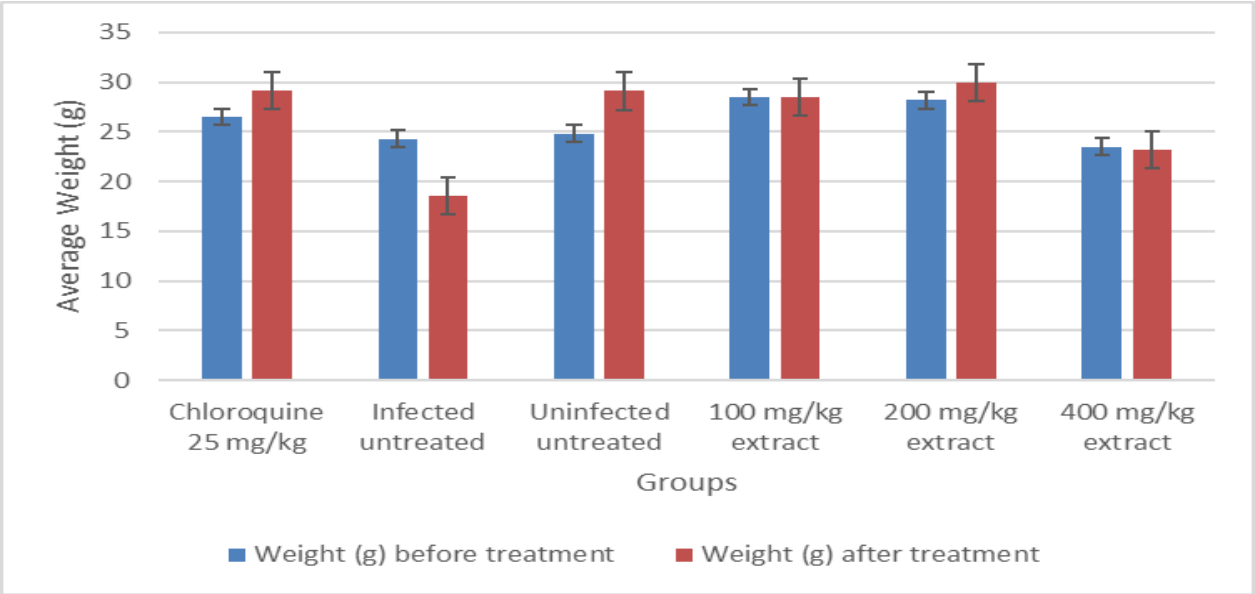

Figure 5. Effect of *Boswellia dalzielii* Leaf Extract on Body Weight of Mice Infected with Plasmodium *berghei* Nk-6
Values were present as mean ± standard error of mean (SEM) of three determinants (n = 3).
100 mg/kg = Infected mice treated with 100 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
200 mg/kg = Infected mice treated with 200 mg/kg b. wt. *Boswellia dalzielii* Leaf extract
400 mg/kg = Infected mice treated with 1400 mg/kg b. wt. *Boswellia dalzielii* Leaf extract

loss observed in this study align with findings that effective antimalarial agents mitigate malaria-induced metabolic disruptions (Chilombe et al., 2022; Mankilik et al., 2025). Collectively, the results corroborate previous evidence on the antimalarial potential of *B. dalzielii* stem bark and other ethnomedicinal plants used in traditional malaria therapy (Salihu et al., 2020; Jansen et al., 2010; Phuwajaroanpong et al., 2023). The extract's comparable efficacy to chloroquine further supports its promise as a potential natural antimalarial candidate.

Conclusion

The butanol extract of *Boswellia dalzielii* leaf exhibited notable antimalarial activity, improving parasitemia levels, survival time, PCV, and physiological stability in infected mice. Its effects were dose-dependent, with the 400 mg/kg dose producing outcomes comparable to chloroquine. These findings validate the traditional use of *B. dalzielii* in malaria treatment and suggest that its leaf extract could serve as a potential source of new antimalarial compounds warranting further isolation and characterization.

Conflict of Interest

All authors affirm that they have no conflicts of interest pertaining to this publication.

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