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Antiplasmodial activity of Azanza Garckeana root bark extract and its effect on hematological indices in plasmoduim berghei infected mice

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Abstract

***Background & Aims*:** Malaria disease imposes a substantial global health burden, urging the search for effective treatments amid escalating drug resistance. This study investigates the antiplasmodial potential of Azanza garckeana root bark extract in Plasmodium berghei-infected mice and evaluates its impact on hematological indices.

Materials & Methods: In this experimental research, we studied on six groups of 30 mice (Groups A to F) comprising of five mice per group. Group A was only given food and water with no inoculation and treatment, B was inoculated with Plasmodium berghei but no treatment was given, C was infected and treated with artemether while D E F were infected and treated with 100mg/kg, 200mg/kg and 300mg/kg of Azanza garckeana root-bark extract respectively for 4 days. Parasitemia levels, chemo-suppressive effect and hematological parameters were assessed on four different days following the start of the treatment. The findings were expressed as mean ± SEM. One-way analysis of variance was used to find the differences between the four groups, and $p < 0.05$ was considered statistically significant.

Results: Phytochemical analysis revealed diverse bioactive compounds in the extract. The 4-day suppressive test demonstrated substantial antiplasmodial activity, with the 300 mg/kg dose achieving an 88.17% chemo-suppressive effect, comparable to Artemether's efficacy. Significant ($p < 0.05$) increases in hemoglobin, packed cell volume, red blood cells, white blood cells, and platelets were seen in hematological examinations in intervention groups, especially with the 300 mg/kg dosage.

Conclusions: Azanza garckeana root-bark extract exhibited potent antiplasmodial effects, possibly mediated by identified phytochemicals. The dose-dependent chemosuppression and modulation of hematological parameters underscore its potential as an alternative antimalarial therapy in mammals.

***Keywords*:** Antiplasmodial Activity, *Azanza Garckeana*, Hematological Indices, *Plasmodium Berghei*

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Introduction

Malaria represents a severe global health burden, particularly affecting vulnerable populations in sub-Saharan Africa, including children under the age of five and pregnant women (1). With a predicted 249 million cases in 85 malaria-endemic countries in 2022, the

number of malaria cases worldwide topped pre-COVID-19 levels, according to the WHO's 2023 World Malaria Report. The number of threats posed by climate change to global response is 55% more than anticipated for the 2025 Global Technical Strategy (2).

The growing risk of drug resistance imperils the effectiveness of existing antimalarial medications, including Artemisinin-based combination treatments (ACTs) (3). Drug-resistant parasite strains have emerged as a result of extended exposure to antimalarial medications; this phenomenon has been seen most prominently in South-East Asia, where ACT-resistant *P. falciparum* parasites impede the clearance of parasites (4). This growing resistance necessitates urgent efforts to develop novel antimalarial products.

Traditional medicinal plants, known for their safety and efficacy, have been a valuable source of drug discovery. About 94 plant species have yielded 122 drugs, emphasizing the importance of ethno botanical leads (5). *Azanza garckeana*, holds promise as a medicinal plant, grown exclusively in Tula village with the coordinates 9.83333, 11.46667, Gombe State of Nigeria. With a rich history of use in Northern Nigeria for over 20 human diseases, including cough, chest pains, infertility, and sexually transmitted infections, *A. garckeana* has been recognized for its diverse bioactive metabolites (6).

The purpose of this study was to assess the antimalarial efficacy of an aqueous extract of *A. garckeana's* root bark in mice harboring a *Plasmodium berghei* infection. Considering the high rates of morbidity and death in sub-Saharan Africa, particularly among vulnerable groups (7), the decision was motivated by the pressing need for innovative therapies for malaria. Traditional medicinal plants like *A. garckeana*, with their historical efficacy, offer a potential avenue for novel treatments (8). By exploring the antimalarial potential of *A. garckeana*'s root-bark extract, this study aims to contribute to the discovery of effective malaria treatments.

Despite the introduction of Artemisinin, mefloquine, sulphadoxine, and pyrimethamine in Nigeria, significant drug resistance against *Plasmodium falciparum* persists, highlighting the need for alternative treatments (11). The emergence of resistance in Artemisia, the latest plant used in malaria treatment, adds urgency to the situation (12). The declining efficacy of ACTs in regions with a history of antimalarial drug resistance, like the Thai-Cambodia border, underscores the global challenge posed by drug-resistant strains of *P. falciparum* (13). As drug-resistant parasites continue to spread, the development of effective control measures becomes imperative (14). In Nigeria, where accessibility to pharmaceutical drugs is often limited, traditional remedies, including *A. garckeana*, offer a potential alternative that is easily accessible (15). This study aims to investigate the antiplasmodial activities of *A. garckeana* and its effect on hematological parameters, contributing to the development of affordable and accessible antimalarial agents in Nigeria.

Materials & Methods

Plant Material:

A fresh root bark of *A. garckeana* was collected from Gombe State and placed in a clean plastic bag. The leaves were collected for identification by a taxonomist in the Department of Plant Sciences, Modibbo Adama University, Yola.

Preparation of the Plant Material:

The root bark of *A. garckeana* was cleaned with water and rinsed with distilled water to remove mud and dirt. After that, it was gently spread out on a spotless surface and left for two weeks to air dry at room temperature. The dried root bark was then ground into a homogeneous powder using an electric mill. In preparation for a subsequent aqueous extraction, the powder was kept in sachets in a dry, ventilated cabinet out of direct sunlight.

Extraction of Plant Material:

The maceration process, as outlined by Bouratoua *et al.* (16), was used for extracting the plant material. In summary, one litter of distilled water was used to soak 100 g of *A. garckeana* root-bark powder, which was

periodically agitated and remained at room temperature for 72 hours. The weight of the powder was accurately determined using an analytical balance. Next, a Whatman filter paper was used to filter the mixture. After 48 hours in a tray, the filtrate was turned into a dry powder. Weighed and placed into airtight containers, the powdered extract was sealed and stored at 40 °C until it was analyzed. The extract's percentage yield was evaluated using the weight-by-weight (%w/w) method.

Phytochemical Screening of Root-bark Extracts of *Azanza garckeana:*

The conventional qualitative techniques used by Trease and Evans (17) were utilized to identify the phytochemical elements of root bark *A. garckeana*.

Laboratory Animals:

The study included thirty (30) mice weighing between 20 and 30 grams at 4 weeks of age, which were obtained from the National Veterinary Research Institute located in Vom, Plateau State, Nigeria. They received water and rodent pellets to eat. Before being employed, two weeks were allowed for acclimation to the experimental room temperature of 25°C and twelve hours of daylight. When dealing with the animals, all ethical protocols and recommendations were followed (18).

Parasite:

Plasmodium berghei, a strain susceptible to Artemether, was acquired from the National Veterinary Research Institute in Lagos State.

Parasite Inoculation:

Through cardiac plexus puncture, parasitized erythrocytes were removed from donor animals and diluted with trisodium citrate. On day 0, mice received an intraperitoneal injection of 0.2 ml blood solution containing 10^6–10^7 parasitized erythrocytes.

Experimental Design and Animal Grouping:

Thirty (30) mice were separated into six equal groups (Groups A through E) at random, each with five mice, the mice were then given the following treatment.

Group	Descriptions	Treatment
A	Naïve	No inoculation, no treatment
B	Negative control	Inoculation with *P. berghei*, no treatment
C	standard control (Artemether)	Inoculation with *P. berghei* + 10mg/kg of standard antimalarial drug (Artemether)
D	Treatment 1	Inoculation with *P. berghei* + 100mg/kg aqueous extract of *A. garckeana* root bark
E	Treatment 2	Inoculation with *P. berghei* + 200mg/kg aqueous extract of *A. garckeana* root bark
F	Treatment 3	Inoculation with *P. berghei* + 300mg/kg aqueous extract of *A. garckeana* root bark.

Hematological Analysis:

Hematological indices, using a hematology analyzer called HumaReader Plus (HUMAN Diagnostic Worldwide, Wiesbaden, Germany), the levels of packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBC), and white blood cells (WBC) were measured.

Determination of Parasitaemia Level and Chemo-suppression activity:

Parasitaemia levels were assessed on four different days following the start of the treatment. To determine this, a small sample of blood from each animal's tail was placed on glass slides and left to dry at air. The slides were then treated with methanol, stained using Giemsa,

and examined using a 100x objective lens under a microscope. The count of red blood cells infected with parasites observed on each slide was recorded. This count was used in the formula below to calculate the parasitaemia level.

$$\% \text{ Parasitaemia} = \frac{\text{Total number of PRBC X 100}}{\text{Total number of RBC}}$$

Where; **RBC**= Red Blood Cells and **PRBC**= Parasitized Red Blood Cells.

Comparing the extracts to the untreated control, the percentage of parasitaemia suppression was calculated. This computation was made using a particular formula that Dikasso *et al*. (19) detailed. This formula was used to measure the level of parasitaemia reduction that the extracts were able to accomplish.

$$\% \text{ chemosuppression} = \frac{\text{Mean parasitemia of untreated control} - \text{mean parasitemia of treated group}}{\text{Mean parasitemia of untreated control}} X\ 100$$

Data Analysis:

The data was presented as mean ± standard deviation. With SPSS Version 27, analysis for the *in vivo* task was accomplished. ANOVA, a one-way test of statistical significance, and Tukey's Honest Significant Difference post-hoc test was used to determine the difference in survival time and parasitaemia decrease within each class.

Results

Phytochemical Composition of the Root-bark Extract of *Azanza garckeana:*

Table 1 displays the phytochemical makeup of the *A. garckeana* root-bark extract. There were phytochemicals such as tannins, flavonoids, alkaloids, phenols, glycosides, and steroids but not terpenoids.

Table 1. Phytochemical constituent of *Azanza garckeana* root-bark extract.

Phytochemical	*Azanza garckeana*
Saponins	+
Tannins	+
Flavonoids	+
Alkaloids	+
Glycosides	+
Phenols	+
Steroids	+
Terpenoids	–

Key: Absent (–); Present (+).

Parasitaemia and Chemosuppression Level of Treated *P. berghei* Infected Mice

Figure 1 shows how the 4-day suppressive test with *A. garckeana* root-bark extract affected the parasitaemia levels in *P. berghei*-infected mice. The root-bark extract of *A. garckeana* showed a dosage-dependent reduction in parasitaemia levels that was both quick and significant, with the dose of 300 mg/kg showing the greatest drop.

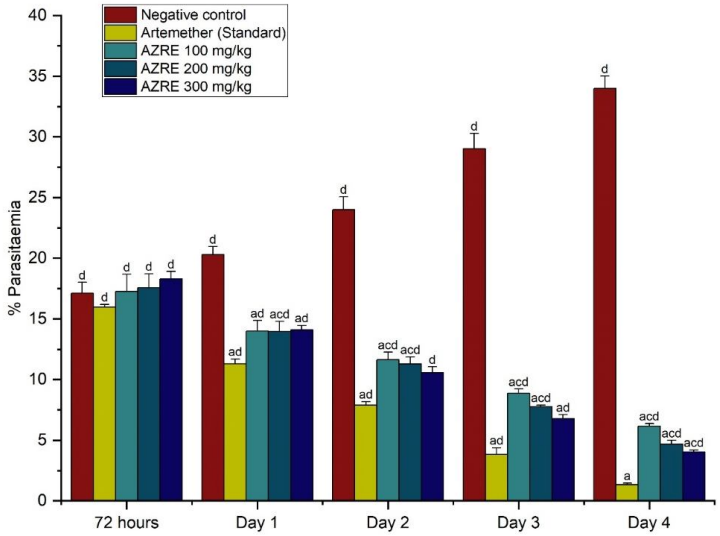

Fig. 1. Effects of *Azanza garckeana* root-bark extract on parasitaemia level in *Plasmodium berghei*-infected mice
[a]$P<0.05$ lower compared to negative control, [b]$P<0.05$ lower compared to Artemether, [c]$P<0.05$ higher compared to Artemether, [d]$P<0.05$ higher compared to normal control. *AZRE*: *Azanza garckeana* root-bark extract.

Figure 2 illustrates the impact of *A. garckeana* root-bark extract on the chemosuppression levels in *P. berghei*-infected mice during the 4-day suppressive test. Values represent Mean ± S.E.M for each group on Days 1 to 4. Treatment groups at different concentrations show varying degrees of chemosuppression. The 300 mg/kg dose shows the highest chemosuppresion on day-4 but below the standard control (Artemether).

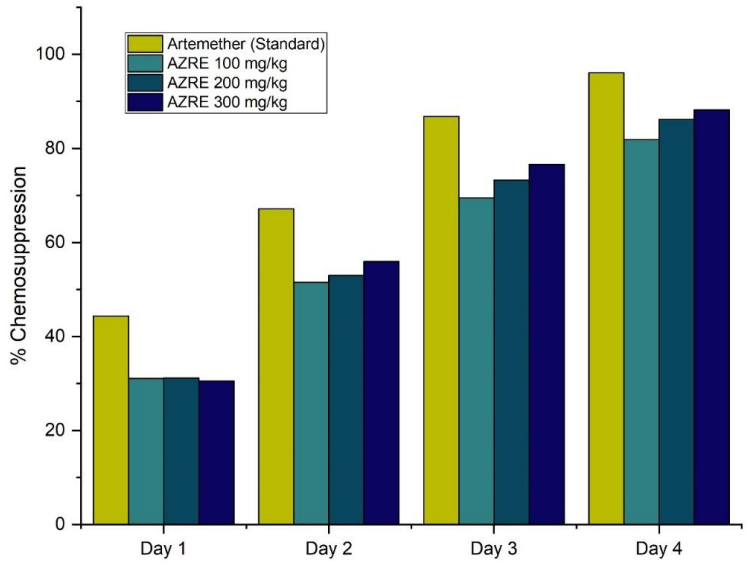

Fig. 2. Effects of *Azanza garckeana* root-bark extract on chemosuppression level of *Plasmodium berghei* infected mice

Hematological Level:

Table 2 summarizes the effects of *A. garckeana* root-bark extract on the hematological parameters in *P. berghei* infected mice after the 4-day suppressive test. It shows that *A. garckeana* root-bark extract has a modulatory effect on hematological parameters in *P. berghei* infected mice, with the 300 mg/kg dose demonstrating more pronounced positive effects.

Table 2. Effects of *A. garckeana* root-bark extract on the hematological level of *P. berghei* infected mice after the 4-day suppressive test

Parameter	WBC ($x10^9$/L)	RBC ($x10^{12}$/L)	PLT ($x10^9$/L)	PCV (%)	HGB (g/dL)	MCV (fl)	MHC (%)	LYM ($x10^9$/L)	NEU ($x10^9$/L)	BAS (%)	MCHC (%)
Naïve	11.70 ± 0.19	7.14 ± 0.15	1070.75 ± 24.36	38.28 ± 0.86	12.65 ± 0.38	54.73 ± 0.57	18.38 ± 0.59	87.63 ± 0.53	15.75 ± 0.63	1.03 ± 0.17	31.98 ± 0.89
Negative Control	9.15 ± 0.22^{d}	3.97 ± 0.49^{d}	590.50 ± 24.55^{d}	21.83 ± 1.13^{d}	7.40 ± 0.69^{d}	51.00 ± 0.96	17.65 ± 0.64	80.48 ± 1.86^{d}	6.95 ± 0.62^{d}	0.48 ± 0.15	39.60 ± 1.66^{b}
Artemether	16.93 ± 0.39^{ab}	7.24 ± 0.38^{a}	1090.75 ± 23.73^{a}	33.85 ± 0.29^{ad}	11.15 ± 1.22^{a}	54.30 ± 1.04	17.28 ± 0.49	79.10 ± 0.47^{d}	11.83 ± 0.89^{ad}	1.45 ± 0.30^{a}	31.83 ± 0.74^{c}
AZRE 100 mg/kg	9.70 ± 0.68^{df}	3.88 ± 0.24^{df}	823.75 ± 36.46^{adf}	22.05 ± 0.70^{df}	7.33 ± 0.50^{df}	50.75 ± 0.77	19.20 ± 0.28^{e}	71.23 ± 1.55^{cdf}	8.68 ± 0.29^{df}	2.83 ± 0.27^{abe}	33.98 ± 0.36^{c}
AZRE 200 mg/kg	13.90 ± 0.08^{abf}	5.34 ± 0.25^{adf}	928.50 ± 15.92^{adf}	29.28 ± 1.62^{adf}	10.08 ± 0.24^{ad}	53.13 ± 1.32	18.00 ± 0.83	72.13 ± 1.54^{cdf}	11.58 ± 0.77^{ad}	2.38 ± 0.19^{abe}	33.93 ± 0.31^{c}
AZRE 300 mg/kg	17.08 ± 1.33^{ab}	6.82 ± 0.13^{a}	1037.50 ± 30.31^{a}	34.08 ± 1.25^{ad}	10.53 ± 0.40^{ad}	57.98 ± 3.36^{a}	18.08 ± 0.11	79.85 ± 1.23^{d}	13.53 ± 0.80^{ad}	2.1 ± 0.14^{abe}	31.03 ± 0.23^{c}

Values are expressed as Mean ± S.E.M, n=5; $^{a}P < 0.05$ higher compared to negative control; $^{b}P < 0.05$ higher compared to normal control; $^{c}P < 0.05$ lower compared to negative control; $^{d}P < 0.05$ lower compared to normal control; $^{e}P < 0.05$ higher compared to Artemether; $^{f}P < 0.05$ lower compared to Artemether.

RBC: Red blood cells, WBC: White blood cells, PLT: Platelets, PCV: Packed cell volume, HGB: Hemoglobin MCV: Mean corpuscular volume, MHC: Mean hemoglobin concentration, LYM: Lymphocytes, NEU: Neutrophils, BAS: Basophils, MCHC: Mean corpuscular hemoglobin concentration; AZRE: *Azanza garckeana* root-bark extract.

Discussion

Phytochemical Composition of the Root-bark Extract of *Azanza garckeana:*

The phytochemical screening of the aqueous extract of *A. garckeana* root bark revealed the presence of various bioactive compounds, aligning with previous studies on different parts of the plant (8,9,10). The identified phytochemicals, including saponins, tannins, flavonoids, alkaloids, glycosides, phenols, and steroids, provide a foundation for understanding the potential therapeutic effects of the root-bark extract against *P. berghei* in mice.

Parasitaemia and Chemosuppression Level of Treated *P. berghei* Infected Mice:

The 4-day suppressive test results on the effects of *A. garckeana* root-bark extract on the parasitaemia level of *P. berghei*-infected mice revealed promising outcomes, suggesting its significant antiplasmodial properties as a potential therapeutic agent for malaria treatment. In comparison to the negative control, which exhibited a steady increase in parasitaemia levels, and the artemether group, where parasitaemia levels decreased, the *A. garckeana* root-bark extract demonstrated a rapid and substantial significant reduction in parasitaemia levels but not surpassing the efficacy of artemether. The dose-dependent nature of the antiplasmodial activity was evident, with the 300 mg/kg dose exhibiting the most significant decrease in parasitaemia levels, aligning with previous studies highlighting the dose-dependent antiplasmodial effects of *A. garckeana* (20). These findings underscore the potential of *A garckeana* as a promising and dose-responsive alternative therapy for malaria (20).

The results of the 4-day suppressive test evaluating the effects of *A. garckeana* root-bark extract on the chemosuppression level in *P. berghei*-infected mice revealed a dose-dependent response. The negative control exhibited no chemosuppression, serving as a baseline for uncontrolled parasitemia growth. In comparison, the standard antimalarial drug, artemether, demonstrated a substantial increase in chemosuppression over the treatment period, reaching an impressive 96.09% on day 4. Notably, the *A. garckeana* root-bark extract groups at various concentrations (100 mg/kg, 200 mg/kg, and 300 mg/kg) exhibited dose-dependent chemosuppressive effects. The highest dose, 300 mg/kg, demonstrated a significant chemosuppression level of 88.17% on day 4, approaching the efficacy of artemether. These findings show the ability of *A. garckeana* to impede the development of *P. berghei*, indicating its potential utility as an antimalarial agent. This is consistent with the findings of a study on the antimalarial activity of *Zingiber Officinale* and *Echinops Kebericho* (21). Similar results were obtained by another study when using *Salvadora persica* root extract and *Balanites rotundifolia* leaf extract to prevent *P. berghei* growth (22).

The aqueous root-bark extract of *A. garckeana* demonstrated appreciable and dose-dependent chemosuppressive effects against *P. berghei* infection in mice. The identified phytochemicals, including flavonoids, saponins and alkaloids, likely mediate antiparasitic effects through well-established mechanisms of cell membrane disruption, protein binding, and heme polymerization to exert parasite clearance (23, 24). These multimodal actions help circumvent current drug resistance issues. About 88% suppression at 300 mg/kg is therapeutically relevant, considering prior studies demonstrating >60% efficacy as a cut-off for antimalarial potential (24).

The observed reduction in malaria parasite growth by the *A. garckeana* root-bark extract may occur in different ways that we are not fully aware of. It could enhance the immune system indirectly or block certain pathways in the body. The plant contains phytosteroids,

phenolic compounds, and flavonoids, which are known for their potential to boost the immune system, reduce inflammation, and act as antioxidants. Additionally, these plant components might interact with the known factors involved in the development and life cycle of the malaria parasite, using a similar or unique method (26).

In this study, it was found that a high dose of the plant extract was effective against the parasite. By nanoformulating the extract, the same therapeutic outcomes may be achieved with lower doses, minimizing toxicity and improving patient compliance. Nanoformulation involves encapsulating active compounds into nanoparticles to enhance their effectiveness and reduce dosage requirements (27). This enhances the extract's solubility, stability, and targeted delivery, leading to better bioavailability and therapeutic outcomes. Additionally, it allows for controlled release kinetics, optimizing treatment strategies (27). Overall, nanoformulation of medicinal plant extracts shows promise for improving antimalarial therapies and herbal medicine in general (28).

Hematological Level:

The hematological analysis following the 4-day suppressive test with *A. garckeana* root-bark extract on *P. berghei*-infected mice reveals notable impacts on various blood parameters. In comparison to the naive (non-infected) group, the negative control group, representing untreated infected mice, exhibited significant reductions in key parameters, including white blood cells (WBC), red blood cells (RBC), platelets (PLT), packed cell volume (PCV), and hemoglobin (HGB). These reductions align with the expected effects of *P. berghei* infection on blood components. Artemether, the standard antimalarial drug, showed some restoration of these parameters, indicating its efficacy in mitigating malaria-induced hematological alterations. Interestingly, *A. garckeana* at different doses demonstrated varying effects on the hematological parameters which concise other findings on *Ocimum gratissimum* (29) and *Jatropha tanjorensis* leaf (30). The 300 mg/kg dose of *A. garckeana* showed an increase in WBC, RBC, PLT, PCV, and HGB compared to the negative control, suggesting a potential protective effect against malaria-induced hematological changes. However, some parameters, such as mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and lymphocytes (LYM), showed mixed responses across *A. garckeana* groups. These findings suggest that *A. garckeana* root-bark extract may have a modulatory effect on hematological parameters in *P. berghei*-infected mice, with the 300 mg/kg dose demonstrating more pronounced positive effects.

The study revealed that administering an aqueous root-bark extract of *A. garckeana* to mice infected with *P. berghei* resulted in a significant increase in white blood cells (WBC) and neutrophil (NEU) counts when compared to the uninfected control group. With 100 and 200 mg/kg *A. garckeana* compared to naïve mice, basophil percentages were similarly increased.

White blood cells play an important role in the immune response against malarial infection. Specifically, neutrophils are involved in the initial innate response and help control parasite multiplication through phagocytosis and cytokine release (31). The observed increase in WBCs and neutrophils suggests that *A. garckeana* extract may stimulate immune cell production and activity, enhancing the clearance of parasitized erythrocytes. This immune-boosting effect could contribute to the antimalarial efficacy noted in prior *in vivo* studies testing extracts of this plant (26).

Elevations in circulating basophils were also seen with 100 and 200 mg/kg *A. garckeana* extract treatment. Though the function of basophils in malaria is less clear, some evidence suggests they may play a regulatory role through interactions with T-cells and the release of IL-4 (32). The higher percentages could reflect increased activation and recruitment related to the extract's influence on immune processes during infection.

No significant alterations were detected between groups for other hematological indices like red blood cells, hemoglobin, platelets, or red cell volume and morphology. The lack of major changes indicates the extract does not cause haematoxicity and is well-tolerated at the tested doses. Artemether similarly did no effect on these measures at its curative concentration.

Conclusion

The medicinal plant *A. garckeana*'s aqueous root-bark extract showed strong antiplasmodial action against *P. berghei* infection in mice. Significant chemosuppression was attained, and hematological parameters were modulated by it. The findings confirm the ethnomedical significance of *A. garckeana* and encourage more research into the phytochemical components of the plant to develop antimalarial medications. However, limited access to advanced analytical tools like gas chromatography and mass spectrometry hindered detailed characterization of the plant's active compounds. Also, the majority of previous studies that sought to confirm the antimalarial properties of plants employed methodologies akin to ours. Despite this, the plant's antimalarial potential warrants further exploration, particularly in isolating and characterizing its active compounds. Additionally, exploring nanoformulation of the plant extract could offer promising avenues to enhance its therapeutic benefits and reduce dosage requirements.

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Ethical Statement

The Animal Ethics Committee at the Department of Animal Science, Faculty of Agricultural Science, Modibbo Adama University, Yola, gave the clearance and authorization for the use of animals in this study (MAU/FAS/AEC/AS/2023/036).

Data availability

The supporting data of this study is always obtainable on request from the corresponding author.

Authors' contributions

Author MSJ designed the study, conducted the statistical analysis and wrote the first draft of the manuscript; author YU performed the experiments; author AUW managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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Conflict of interest

The authors have no conflict of interest in this study.

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