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Phytochemical Analysis, Antimicrobial Properties, and Antiplasmodial Activity of *Bauhinia rufescens* Root Extract

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Abstract

The increasing resistance to existing antimalarial drugs has elevated malaria to a critical global health concern, underscoring the urgent need for novel, safe, and effective therapeutic solutions. This study evaluated the phytochemical composition, antiplasmodial activity, and antibacterial properties of **Bauhinia rufescens** ***root extract. The roots were extracted using 85% methanol and screened for phytochemicals using standard methods. Antimicrobial activity was assessed using the Agar Well Diffusion Method, while the antiplasmodial efficacy was tested in*** **Plasmodium berghei-***infected male Swiss albino mice at doses of 150, 300, and 600 mg/kg. Phytochemical screening identified alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phlobatannins, and anthraquinones. The extract exhibited dose-dependent antiplasmodial activity, significantly reducing parasitaemia levels and extending the survival of infected mice. Additionally, antimicrobial assays demonstrated moderate inhibitory effects against* **Staphylococcus aureus** ***and*** **Escherichia coli**. ***These findings support the traditional use of*** **Bauhinia rufescens** ***in managing malaria and associated infections, highlighting its potential as a promising source for developing multi-target therapies.***

Keywords: Antiplasmodial activity, Antimicrobial properties, and Phytochemical analysis

INTRODUCTION

Malaria remains a significant public health challenge in developing nations, contributing substantially to illness and mortality despite global efforts to implement effective control measures, including enhanced vector management systems (Aliyu, 2022). In 2020, there were an estimated 241 million malaria cases and 627,000 deaths across 85 endemic countries, with sub-Saharan Africa (SSA) bearing the greatest burden (WHO, 2021). Nigeria, in particular, accounts for 39% of global malaria deaths among children under five years of age, with an estimated 55 million cases and nearly 90,000 deaths annually (Shekarau et al., 2024). This has highlighted the critical need for improved malaria management strategies.

The 2021 World Malaria Report revealed that the global impact of malaria is more severe than previously estimated and that malaria-related deaths have been underestimated (Mahamat & Kenyatta, 2021). This persistent burden underscores the urgent need for innovative malaria control and treatment solutions, particularly as resistance to widely used antimalarial drugs becomes more prevalent.

The emergence of chloroquine (CQ)-resistant strains of *Plasmodium falciparum* is a major contributor to the global malaria crisis, driven by the extensive use of CQ for prevention and treatment. The Resistance against chloroquine and other antimalarial drugs significantly complicates disease management in endemic areas (Peters, 1982). The World Health Organization (WHO) recommends artemisinin-based combination therapies (ACTs) such as artemether-lumefantrine (AL) and artesunate-amodiaquine (AS-AQ) as the first-line treatments for uncomplicated *Plasmodium falciparum* malaria (Ringwald et al., 2005). However, resistant strains of the parasite continue to emerge and spread rapidly (Ross & Fidock, 2019; Takala-Harrison & Laufer, 2015; Wellems & Plowe, 2001). Given the growing prevalence of resistance, medicinal plants are gaining attention as potential sources of novel antimalarial drugs. The effectiveness of quinine and its derivatives, including artemisinin, has spurred interest in plant-based compounds for treating resistant malaria (Olasehinde *et al.*, 2014). As drug resistance to

affordable and accessible antimalarials spreads globally, innovative malaria control and *Bauhinia species* (Fabaceae) are frequently referred to as "cow's hoofs." Due to the shape of its leaves. Most tropical nations, particularly those in Africa, Asia, and South America, are home to the species. Typically, bauhinia trees grow 6–12 m tall, with branches that extend 3–6 m from the trunk. The lobed leaves typically measure 10-15 cm in width (Aminu, 2013). In folk medicine, the plant is used for the treatment of gout, gingivitis, diarrhea, dysentery, diabetes, leprosy, and malaria (Compaoré *et al.*, 2011). The extract of Bauhinia was reported to show significant activity against *Salmonella spp* (Alamin *et al.*, 2021).

This study aims to evaluate the phytochemical composition, antiplasmodial, and antimicrobial effects of Bauhinia rufescens root extract against *Plasmodium berghei* in a model. The primary goal is to assess the extract's ability to reduce parasitemia and improve survival rates in infected mice. The findings from this study could provide scientific validation for the traditional use of *B. rufescens* and contribute to the search for new, plant-based antimalarial agents.

Methods

Collection and preparation of plant materials

Fresh roots of *Bauhinia rufescens* were collected from the Hadejia-Nguru Wetland in Jigawa State, Nigeria. The plant was authenticated at the Herbarium of Ahmadu Bello University, Zaria, Nigeria, where reference specimens (ABU0900230) were stored for future use. The collected plant materials were washed with deionized water to remove impurities and air-dried for 21 days. Subsequently, the dried materials were pulverized using a mortar and pestle. The pulverized plant bark was then soaked in 85% methanol and filtered using Whatman No. 1 filter paper. The resulting filtrate was dried under reduced pressure at 40°C.

Phytochemical screening

phytochemical tests were conducted on the extract to identify the bioactive constituents of the plant material, following the procedures outlined by Sofowora (1993) and Trease & Evans (1989).

Invitro Antimicrobial Assay of Crude Extracts

The antimicrobial properties of the extracts were evaluated using the agar well diffusion method, following the protocols described by Ara et al., (2012) and Artizzu et al., (1995). A sterile petri dish containing 0.1 ml of various organisms cultured in nutrient broth was filled with melted nutrient agar for bacteria and Sabouraud Dextrose Agar (SDA) for fungi and allowed to set. Using a sterile maize borer, wells were created in the solidified agar. Each well was filled with 0.1 ml of the extract solution at varying concentrations. The plates were left to pre-diffuse for 30 minutes before incubation. Dimethyl sulfoxide (DMSO) served as the negative control, while ketoconazole and Ciprofloxacin were the positive controls for antifungal and antibacterial activities. The bacterial plates were incubated at 37°C for 24 hours, and the fungal plates were incubated at 25°C for 27 hours. The inhibition zones, measured in millimetres, were recorded to assess the antimicrobial activity of each extract.

Antimalarial studies

Animals used for antimalarial studies

Thirty-six male swiss albino mice (6-8 weeks) were obtained from the Department of Pharmacology Bayero University Kano. They were randomly divided into six groups, each consisting of six mice. The mice were housed and fed according to the recommended standard (NIH 2007).

Parasite

The study utilized *plasmodium berghei* NK65 obtained from Aminu Kano teaching hospital, which is sensitive to chloroquine. Mice were infected with *plasmodium berghei* for the survey. All the mice used in the study received an intraperitoneal inoculation of 1+107 p. berghei parasitized erythrocytes on day 1. The parasitized erythrocytes were obtained by suitable dilution with 0.9% saline from a donor-infected mouse through cardiac puncture.

In vivo antiplasmodial study

With some slight modifications, the approach described by (Akuodor *et al.*, 2011; Peters *et al.*, 2002) was used to evaluate the efficacy of *Bauhinia rufescens* root extract in treating a condition. In this investigation, a blood suspension (0.2 ml) containing 1x107 *Plasmodium berghei* was intraperitoneally administered to thirty mice on the first day of the experiment. The experimental animals were split into six groups, with 6 mice in each cage, 72 hours after the inoculation. *Bauhinia rufescens* root extract was administered in varying quantities to groups 1-3 (180, 300, and 600 mg/kg), while groups 4 and 5 received attermeter (5 ml/kg) and chloroquine (10 ml/kg) as positive controls. Group 6 received 10 millilitres per kilogramme of distilled water as a negative control. There were four, five,

and six days of oral therapy. Each mouse's 16mm blood film was produced and dried on the seventh day. The dried films were fixed in methanol and dyed with Giemsa. Later, the parasite density was investigated under a microscope by counting the parasitised red blood cells in ten distinct fields. The average time of the mice in each group over a 30-day period was calculated to estimate each group's mean survival time (in days).

$$\text{parasitemia (\%)} = \left(\frac{\text{Number of parasitized red blood cell}}{\text{Total Number of red blood cell}}\right) \times 100$$

$$\text{Mean survival time} = \frac{\text{Total survival time in all mice in the group}}{\text{Total number of mice in the group}}$$

$$(\%)\text{inhibition} = \left(\frac{\text{Mean parasitemia of control group-Mean parasitemia of treatment group}}{\text{Total Number of mice in the group}}\right) \times 100$$

Statistical analysis

The data obtained were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 20. Differences between the means were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. Values of $p < 0.05$ were considered statistically significant, and the results were expressed as mean ± standard error of the mean (SEM).

RESULTS

Phytochemical composition of root bark *Bauhinia rufescen*

Phytochemical screening of the root bark of methanol extract of *Bauhinia rufucens* showed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phlobatanins, and anthraquinines

TABLE 1: Zone of inhibition (mm) of crude *Bauhinia rufucens* root extract at different concentrations (µg/ml) against clinical isolate

Organism/con. µg/ml	4000	3000	1000	500	Cipro	Keto
Staphylococcus aureus	14.00	11.00	9.00	8.00	27.00	-
Streptococcus pyogenes	11.00	10.00	8.00	0.00	24.00	-
Escherichia Coli	15.00	13.00	11.00	9.00	30.00	-
klebsiella pneumoniae	14.00	12.00	10.00	8.00	22.00	-
Candida albicans	13.00	12.00	10.00	8.00	-	20.00
aspergillus fumigatus	12.00	11.00	9.00	0.00	-	18.00

Table 2: Effect of Methanol Root Extract of Bauhinia rufucenson Parasitaemia Level of *Plasmodium berghei* Infected Mice in Rane's Test

Treatment	Dose (mg/kg)	Parasitaemia level	% Inhibition
Distilled water	10 mL/kg	5.34±0.26	-
MEAR	150	3.63±0.19*	32.02
MEAR	300	2.53±0.16*	52.62
MEAR	600	1.96±0.19*	63.30
Chloroquine	10	0.37±0.12*	93.07
Artemether	5	0.51±0.11*	90.45

Values are presented as Mean ± S.E.M., * = $p<0.001$ as compared to distilled water group - One way ANOVA followed by Tukey's post hoc test, n = 6, MEAR = Methanol root extract of *Bauhinia rufucens*

Table 3: Effect of Methanol Root Extraction Survival Time of *Plasmodium berghei* Infected Mice in Rane's Test

Treatment	Dose (mg/kg)	Survival time (days)
Distilled water	10 mL/kg	13.83±3.33
MEAR	150	18.67±2.33
MEAR	300	18.00±3.00
MEAR	600	18.83±2.07
Chloroquine	10	16.17±3.19
Artemether	5	21.00±0.00

Values are presented as Mean ± S.E.M., No significant difference when compared to the distilled water group - One way ANOVA followed by Tukey's post hoc test, n = 6, MEAR = Methanol root extract of *Bauhinia rufucens*

DISCUSSION

The phytochemical screening of *Bauhinia rufescens* revealed a rich diversity of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, phlobatannins, and anthraquinones. These results align with those of Garbi *et al.*, (2015), who identified flavonoids, tannins, triterpenes, saponins, and alkaloids in the leaves of *Bauhinia rufescens*. Such secondary metabolites are well-documented for their medicinal properties, including antimicrobial, antimalarial, and other therapeutic activities (Hassan *et al.*, 2009; Hostettmann & Marston, 1995).

The antimicrobial activity of the root extract highlighted its therapeutic potential. The extract exhibited significant inhibition against *Staphylococcus aureus* (14 mm zone of inhibition at 4000 µg/ml), indicating its efficacy against Gram-positive bacteria commonly associated with skin and soft tissue infections. In contrast, *Streptococcus pyogenes* showed lower sensitivity, with no inhibition observed below 500 µg/ml. This variability may be attributed to differences in bacterial cell wall structures or efflux mechanisms. The extract also demonstrated activity against Gram-negative pathogens such as *Escherichia coli* (15-9 mm) and *Klebsiella pneumoniae* (14-8 mm), suggesting potential efficacy against multidrug-resistant infections. These findings corroborate the antimicrobial potential of *Bauhinia rufescens*, as reported by (Hassan *et al.*, 2009).

The antifungal activity of the extract, evident from its inhibition of *Candida albicans* (13-8 mm) and *Aspergillus fumigatus* (12 mm at 4000 µg/ml), further supports its broad-spectrum antimicrobial capabilities. This aligns with prior studies on the antimicrobial activity of *B. rufescens* (Issa *et al.*, 2021) and related species such as *B. Purpurea* (Negi et al., 2012), reinforcing its therapeutic promise.

Additionally, the methanol root extract (MEAR) exhibited significant antiplasmodial activity. In *Plasmodium berghei*-infected mice, MEAR showed dose-dependent parasitaemia reduction, achieving 63.30% inhibition at 600 mg/kg, although this was lower than chloroquine (93.07%) and artemether (90.45%). The antimalarial activities observed in *Bauhinia rufucens* extract may be attributed to various phytochemicals, including alkaloids, terpenes, flavonoids, xanthones, anthraquinones, and phenolic compounds, which are well-documented for their bioactivities (Batista *et al.*, 2009; Mazid *et al.*, 2011). However, these findings differ from previous reports, such as the study by Nadège Bonkian *et al.* (2017), which indicated that *Bauhinia rufescens Lam.* lacks significant antiplasmodial activity. In this study, MEAR-treated mice exhibited extended survival times, with a maximum mean survival time (MST) of 18.83 days at 600 mg/kg compared to 13.83 days in the control group. Although the increase was not statistically significant, the extract's potential to reduce parasitic load and mitigate malaria pathology is noteworthy. This prolonged MST aligns with findings by Chutoam & Klongthalay (2015), who reported significantly extended MST in *Bauhinia strychnifolia* treated mice compared to untreated controls. These findings highlight the extract's potential as a complementary therapeutic agent in malaria management.

CONCLUSION

In conclusion, *Bauhinia rufescens* root extract demonstrates significant potential as a natural therapeutic agent, offering both antiplasmodial and antimicrobial benefits. The dose-dependent reduction of parasitemia in *Plasmodium berghei*-infected mice and its ability to inhibit bacterial strains linked to secondary infections underscore the plant's dual bioactivity. The phytochemicals identified in the extract, including alkaloids, flavonoids, tannins, and saponins, likely contribute to its effectiveness. These findings support the traditional use of *Bauhinia rufescens* in malaria treatment and suggest that the plant could be a valuable source for developing novel, multi-target therapies against malaria, particularly in the face of growing resistance to conventional antimalarial drugs. Further studies on the isolation of bioactive compounds and their mechanisms of action are recommended to fully realize their therapeutic potential.

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