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Antiplasmodial Activity Of Whole-Plant Extract Of *Indigofera Pulchra* In *Plasmodium Berghei*-Infected Mice

R.D. Yusuf, S. M. Panda, A. B. Samaila

Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria

Abstract

Malaria remains a public health challenge in sub-Saharan Africa, prompting investigation of medicinal plants as potential sources of antiplasmodial compounds. This study evaluated the *in vivo* antimalarial activity of *Indigofera pulchra* extract in *Plasmodium*-infected mice. Thirty mice were randomly allocated to six groups: an infected untreated control, an infected standard-drug control, and four infected groups treated with extract doses of 25, 50, 100 or 200 mg/kg. Parasitemia was monitored for four days. Treatment and time effects were assessed using repeated-measures analysis of variance, and survival was evaluated using Kaplan–Meier analysis. Parasitemia increased in the untreated control, whereas extract-treated groups showed reductions. All extract doses significantly reduced parasitemia dose-dependently ($p < 0.05$). The 200 mg/kg dose produced the greatest parasite suppression among the extract doses. Although its effect was described as comparable to that of the standard drug, equivalence cannot be concluded without a direct between-group comparison and an equivalence analysis. Kaplan–Meier analysis showed a trend toward longer survival in treated mice, but the difference was not statistically significant ($p > 0.05$). These findings indicate that *I. pulchra* extract has antiplasmodial activity in this mouse model under the tested conditions. The results support further preclinical investigation but do not establish efficacy in humans or identify the active constituents.

Keywords

Malaria;
Antiplasmodial Activity;
Indigofera Pulchra;
Plasmodium Berghei

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Corresponding Author: R.D. Yusuf, Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria
. Email: yusragenetics236@gmail.com © 2026 Faculty of Science, ATBU Bauchi. All rights reserved

INTRODUCTION

Malaria remains a major infectious disease in many tropical regions, particularly sub-Saharan Africa. Human malarial is caused by several *Plasmodium* species, while rodent malarial parasites such as *Plasmodium berghei,* are widely used in experimental studies of biology of parasites, host responses and antimalarial efficacy (Su & Wu, 2021; Otun & Achilonu, 2025). The continuing emergence of antimalarial drug resistance has increased the need to identify new compounds and therapeutic sources (Amoah & Lo, 2023; Cerávolo et al., 2021).

Medicinal plants remain an important source of chemically diverse compounds for antimalarial drug discovery. However, evidence for efficacy needs to be accompanied by evaluation of safety and tissue effects. Experimental studies using *P. berghei*-infected mice provide an established preclinical approach for assessing parasite suppression and host outcomes (Cerávolo et al., 2021).

Indigofera pulchra Willd. is a member of the Fabaceae and belongs to a genus with extensive ethnomedicinal use. Reviews of *Indigofera* species have identified flavonoids, terpenoids and other classes of secondary metabolites and have documented antimicrobial, antioxidant, anti-inflammatory and antiplasmodial activities across the genus (Gerometta et al., 2020). Direct evidence for *I. pulchra is* comparatively limited. Ibrahim et al. (2011) reported antiplasmodial activity of *I. pulchra* leaf extracts against *P. berghei*, while Birru et al. (2017) demonstrated antiplasmodial activity of *I. spicata* in infected mice.

Ethnobotanical documentation has reported the use of the whole plant for medicinal purposes (Adamu et al 2005). Despite this evidence, information regarding *I. pulchra* whole plant extract treatment to parasite clearance remains limited. This study therefore evaluated the antiplasmodial activity and the survival outcome of a whole-plant methanol extract of *I. pulchra* in *P. berghei*-infected mice.

MATERIALS AND METHODS

Plant Collection, Identification and Extraction

Whole plants of *I. pulchra* were collected from the outstirts of Bauchi, Bauchi State, Nigeria, in October 2025 with assistance from a traditional herbalist. The plant was identified to species level at the Herbarium Unit, Department of Biological Sciences, Abubakar Tafawa Balewa University, Nigeria. The voucher specimen number was ATBUDBSH4368. The plant material was thoroughly washed, shade-dried in the laboratory and powdered using an electronic blender. Five hundred grams of the powder were macerated exhaustively in 80% methanol for seven days. The extract was filtered through Whatman No. 2 filter paper and concentrated at 40 °C under reduced pressure using a rotary evaporator. The resulting gelatinous dark-green extract was stored at 4 °C until use.

Experimental Animals and Ethical Considerations

Thirty adult Swiss albino mice of both sexes, weighing 30–34 g, were obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The animals were housed in well-aerated cages, provided feed and water ad libitum, and acclimatized for three days before the experiment. Animals were randomly allocated to six groups of five mice. The study protocol was reviewed by the Animal Use and Care Committee under reference AEC/02/213/26.

Parasite and Infection

Plasmodium berghei NK65 was used for the infection. Each mouse was inoculated intraperitoneally with 0.2 mL of infected erythrocyte suspension containing 1×10^7 infected erythrocytes in saline solution. Infection was established before treatment was commenced.

Treatment was administered orally once daily from Day 1 to Day 4 after infection. The study therefore represents a four-day therapeutic/curative treatment model in which treatment commenced after infection had been established.

Parasitemia Assessment

Thin blood films were prepared from tail blood and fixed with methanol, stained with Giemsa stain and examined microscopically. Percentage parasitemia was calculated. Parasitemia was recorded from Day 0 to Day 4. Percentage suppression at Day 4 was calculated relative to the untreated negative-control mean.

Phytochemical Screening

Qualitative phytochemical screening was performed for anthraquinones, alkaloids, glycosides, phenols, tannins, flavonoids, phytosterols, saponins, steroids and xanthoproteins. (Evans, 2009).

Statistical Analysis

Data were analysed using IBM SPSS Statistics version 23. Descriptive statistics were expressed as mean ± standard error of the mean (SEM). Repeated-measures analysis of variance (ANOVA) was used to evaluate changes in parasitemia over time and differences among treatment groups.. Kaplan–Meier survival analysis with log-rank testing was used to compare survival distributions. Statistical significance was set at $p<0.05$.

RESULTS

Table 1: Results Of Phytochemical Constituents Of *I.pulchra*

Phytochemical	Result
Anthraquinone	Absent
Alkaloid	Present
Glycoside	Absent
Phenol	Present
Tannin	Present
Flavonoid	Present
Phytosterol	Absent
Saponin	Present
Steroid	Present
Xanthoprotein	Present

Table 2: mean parastemia of infected mice with *Plasmodium berghei* and the standard error of mean

Treatments	Day 0	Day 1	Day 2	Day 3	Day 4
25 mg/kg	13.12 ± 1.07	11.24 ± 0.74	10.52 ± 0.50	7.08 ± 0.48	1.52 ± 0.23
50 mg/kg	12.80 ± 1.85	11.28 ± 1.15	10.16 ± 0.81	4.72 ± 0.46	0.88 ± 0.11
100 mg/kg	14.60 ± 1.06	13.87 ± 1.17	7.20 ± 0.40	3.80 ± 1.56	0.53 ± 0.12
200 mg/kg	13.20 ± 0.73	11.85 ± 0.84	7.70 ± 0.48	0.90 ± 0.26	0.25 ± 0.10
Positive control (Artesunate 10 mg/kg)	14.80 ± 0.77	12.55 ± 0.87	4.40 ± 0.37	0.85 ± 0.05	0.25 ± 0.05
Negative control	13.70 ± 0.47	12.27 ± 0.44	18.13 ± 0.18	18.67 ± 0.52	23.60 ± 1.30

Table 3: survival rate of mice infected with *P. berghei* after day 4 post treatment

Treatment (mg/kg)	N=5	Num. of dead mice	No of mice survive	%survival
25		0	5	100
50		0	5	100
100		2	3	60
200		1	4	80
+ve control		1	4	80
-ve control		2	3	60
Total	30	6	24	80

Discussion

The extract contained alkaloids, phenols, tannins, flavonoids, saponins, steroids and xanthoproteins, whereas anthraquinones, glycosides and phytosterols were not detected. The occurrence of several of these metabolite classes is consistent with the broader phytochemical profile reported for Indigofera species (Gerometta et al., 2020). The result is also biologically plausible in view of the reported antiplasmodial activities of medicinal plants containing alkaloids and flavonoids; however, qualitative phytochemical screening alone cannot establish which constituent(s) produced the activity observed in this study.

The untreated infected control showed progressive parasitemia, increasing from 13.70 ± 0.47% on Day 0 to 23.60 ± 1.30% on Day 4. In contrast, parasitemia declined progressively in all extract-treated groups. By Day 4, mean parasitemia was 1.52%, 0.88%, 0.53% and 0.25% at 25, 50, 100 and 200 mg/kg, respectively, while artesunate produced 0.25%. Relative to the Day-4 negative-control mean, the corresponding percentage suppressions were 93.56%, 96.27%, 97.74%, 98.94% and 98.94%, respectively.

Repeated-measures ANOVA demonstrated a very large effect of time on parasitemia ($F_{4,72} = 422.77$, $p < 0.001$, partial $\eta^2 = 0.959$), a very large time-by-treatment interaction ($F_{20,72} = 71.62$, $p < 0.001$, partial $\eta^2 = 0.952$), and a very large between-group effect ($F_{5,18} = 253.28$, $p < 0.001$, partial $\eta^2 = 0.986$). These findings indicate that parasitemia changed substantially over time and that the pattern of change differed among treatment groups. The dose-related decline is consistent with previous evidence of antiplasmodial activity in *Indigofera species,* including *I. pulchra ,I. oblongiofolia* and *I. spicata* (Ibrahim et al., 2011; Murshed et al., 2024; Birru et al., 2017).

The 200 mg/kg extract produced the same observed Day-4 mean parasitemia as artesunate (0.25%), suggesting strong activity under the conditions of this experiment. This observation should not be interpreted as equivalence to artesunate, because formal equivalence testing was not performed. The presence of flavonoids, alkaloids, phenols and tannins provides a plausible chemical basis for activity, but isolation and pharmacological characterization of individual active constituents are required before mechanisms can be assigned.

Although survival improvement was not statistically significant ($p<0.05$), the observed trend suggests a protective role of the extract. This is similar to findings by Birru *et al.* (2017) where moderate improvement in survival rate was observed in *Indigofera spicata* root extract.

Conclusion And Recommendation

The whole-plant extract of *I. pulchra* demonstrated marked antiplasmodial activity in *P. berghei* NK65-infected Swiss albino mice. Parasitemia declined progressively with treatment, and the 200 mg/kg dose produced a Day-4 mean parasitemia comparable

numerically with artesunate. However, the survival analysis did not demonstrate a statistically significant difference among groups. Further work should isolate and characterize the active constituents of *I. pulchra* whole plant extract.

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Ethical Statement

The study protocol was reviewed by the Animal Use and Care Committee under reference AEC/02/213/26. Animals were handled in accordance with applicable institutional requirements for laboratory animal care.

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