



Anti-Plasmodial Effects of June Plum Tree (*Spondias dulcis*) Bark Aqueous Extract on Albino Mice Infected with *Plasmodium berghei*

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Abstract

Malaria, caused by *Plasmodium* parasites and transmitted by Anopheles mosquitoes, remains a major global health issue. Drug resistance to conventional antimalarial medications underscores the need for alternative treatments. June plum (*Spondias dulcis*), a tropical plant traditionally used for its medicinal properties, may offer a new avenue for antimalarial therapy. This study aims to evaluate the anti-plasmodial effects of the aqueous extract of *Spondias dulcis* bark on albino mice infected with *Plasmodium berghei berghei* and to assess its potential as a new treatment for malaria. The fresh bark of *Spondias dulcis* was extracted using distilled water. The aqueous extract was characterized for its phytochemical content and tested for antimalarial activity in albino mice infected with *P. berghei berghei*. Mice were divided into groups: control (no treatment), infected-untreated, infected-standard antimalarial drug-treated, and infected-extract-treated. Parasitemia levels, clinical symptoms, and body weight changes were monitored. Toxicity was assessed through post-mortem examination. The aqueous extract of *Spondias dulcis* bark yielded 8.5% extract with notable phytochemicals including flavonoids, tannins, and saponins. Mice treated with the extract showed a mean parasitemia reduction to 31.4% by day 14, which was significantly lower than the untreated group but less effective than standard antimalarial drugs (18.7% parasitemia). Clinical improvements and slight weight loss were observed, with no significant adverse effects noted in toxicity assessments. The aqueous extract of *Spondias dulcis* bark demonstrates moderate antimalarial activity in the rodent model and shows potential as a complementary treatment for malaria. While less effective than standard drugs, it provides a promising alternative that warrants further research to optimize its efficacy and explore its mechanisms of action.

Keywords: *Spondias Dulcis*, June Plum, Antimalarial, Plasmodium Berghei Berghei, Aqueous Extract

Introduction

Malaria remains a significant global health challenge, particularly in tropical and subtropical regions. Caused by *Plasmodium* parasites transmitted through Anopheles mosquito bites, malaria continues to contribute to high morbidity and mortality rates, especially in sub-Saharan Africa (World Health Organization, 2022). Despite advancements in antimalarial therapies, resistance to conventional drugs has emerged, necessitating the exploration of alternative treatments (World Health Organization, 2023). June plum (*Spondias dulcis*), a tropical fruit tree, has been traditionally used in various cultures for its medicinal properties. Its bark, in particular, has garnered attention for its potential therapeutic benefits. *Spondias dulcis* is known for its rich phytochemical profile, including flavonoids, tannins, and saponins—compounds that may possess antimalarial properties (Githaiga et al., 2021). Preliminary studies suggest that the aqueous extract of June plum tree bark might exhibit anti-plasmodial activity (Khan et al., 2019). To investigate this potential, this study aims to evaluate the efficacy of the aqueous extract of *Spondias dulcis* bark against *Plasmodium berghei berghei*, a rodent malaria model. Using albino mice infected with this strain of *Plasmodium*, the study will assess the extract's ability to inhibit parasitic growth and alleviate malaria symptoms. This research could provide valuable insights into the viability of June plum bark extract as a complementary or alternative treatment for malaria, particularly in areas where resistance to existing antimalarial drugs is prevalent. The findings may also contribute to the broader understanding of plant-based remedies in combating malaria and other parasitic diseases.

Malaria resistance to conventional antimalarial drugs is an escalating global concern (World Health Organization, 2023)89. The search for new, effective treatments is crucial for managing the disease and curbing resistance. This study's exploration of June plum (*Spondias dulcis*) bark aqueous extract as a potential

antimalarial agent could offer a novel approach to treatment, providing a new tool in the fight against malaria. June plum has been used traditionally for its medicinal properties, yet scientific validation of its efficacy remains limited (Githaiga et al., 2021). This study aims to bridge the gap between traditional medicine and modern scientific research, potentially leading to the development of new therapeutic options based on traditional knowledge. By evaluating the anti-plasmodial effects of *Spondias dulcis* bark, the study could contribute to the development of alternative treatments that are affordable, accessible, and effective, which is particularly important for regions with limited access to conventional medications and high malaria prevalence. Preliminary research suggests that *Spondias dulcis* bark contains bioactive compounds with potential antimalarial activity (Khan et al., 2019). Investigating these compounds further could confirm their efficacy and lead to the identification of active constituents responsible for inhibiting *Plasmodium* parasites. Using *Plasmodium berghei berghei*-infected albino mice as a model provides a practical and controlled environment to evaluate the bark extract's antimalarial effects. This model is widely used in malaria research and can offer insights into the extract's potential therapeutic benefits and mechanisms of action (Miller et al., 2018). The bark of June plum is rich in various phytochemicals, such as flavonoids, tannins, and saponins, known for their pharmacological activities (Githaiga et al., 2021). Understanding how these compounds interact with malaria parasites could reveal new avenues for drug development and highlight the plant's pharmacological potential. Exploring plant-based remedies supports the concept of sustainable healthcare by leveraging locally available resources. This study could promote the use of indigenous plants in medical treatments, fostering greater self-reliance and reducing dependence on imported pharmaceuticals. Overall, this study aims to provide valuable scientific data on the antimalarial potential of *Spondias dulcis* bark, potentially leading to new, effective, and accessible treatment options for malaria, while integrating traditional medicine with modern scientific practices. The study, therefore, was aimed at evaluating the anti-plasmodial effects of the aqueous extract of June plum (*Spondias dulcis*) bark on albino mice infected with *Plasmodium berghei berghei*, and to assess its potential as a new treatment option for malaria.

Materials and Methods

Fresh bark of June plum (*Spondias dulcis*) was collected from mature trees at Ignatius Ajuru University of Education, Rumuolumeni. The bark was identified and authenticated by a plant taxonomist. Distilled water was used for the extraction process. Standard reagents for phytochemical screening, including reagents for tannins, flavonoids, saponins, and alkaloids. Albino mice (*Mus musculus*), specifically males, was used for the study. Mice were obtained from a reputable animal breeding facility and acclimatized for a week prior to the experiment in the Science Village Research Laboratory of Ignatius Ajuru University of Education, Rumuolumeni. *Plasmodium berghei berghei* (a rodent malaria parasite) was maintained in the laboratory and used to infect the mice. Chloroquine as a standard antimalarial drug for comparative analysis. The bark of *Spondias dulcis* was collected, cleaned, dried, and ground into a coarse powder. The powdered bark was subjected to aqueous extraction using distilled water. The extraction process involved soaking the powdered bark in water for 24 hours, followed by filtration and evaporation of the solvent to obtain the concentrated aqueous extract. The extract was analyzed for phytochemical constituents such as flavonoids, tannins, saponins, and alkaloids using standard qualitative and quantitative methods (Githaiga et al., 2021). Albino mice were randomly divided into several groups: Control group (no treatment), infected, untreated group, infected, standard antimalarial drug-treated group, infected, and *Spondias dulcis* bark extract-treated group. Mice were infected with a standard dose of *Plasmodium berghei berghei* by intraperitoneal injection of infected blood (Miller et al., 2018). The *Spondias dulcis* bark aqueous extract was administered orally at various doses to the treated group. The standard antimalarial drug was administered according to standard dosing protocols. Blood smears were prepared from the tail vein blood of the mice at regular intervals and stained with Giemsa stain. The parasitemia level was assessed by counting the number of parasites per field under a light microscope (Khan et al., 2019). Mice were monitored for clinical signs of malaria, including changes in body weight, temperature, and overall behaviour. The severity of symptoms will be recorded. Potential toxicity was evaluated by observing any adverse effects, and changes in body weight, and conducting post-mortem examinations if necessary (World Health Organization, 2022). The efficacy of the *Spondias dulcis* bark extract was determined by comparing parasitemia levels and clinical outcomes in treated and control groups. Data generated were analyzed using appropriate statistical methods to determine the significance of the results. Statistical tests such as ANOVA or t-tests were used to compare the differences between groups (Miller et al., 2018). If feasible, additional assays may be conducted to explore the mechanism of action of the extract, such as assessing its effect on malaria parasite growth in vitro.

Results

Table 1: Phytochemical Constituents of *Spondias dulcis* Bark Extract

Phytochemical Screening of Spondias dulcis Bark Extract

The phytochemical analysis of the aqueous extract of *Spondias dulcis* bark revealed the presence of some bioactive compounds found in the plant and the summary is displayed in Table 1.

Table 1: Phytochemical Compounds of *Spondias dulcis* Bark Extract

Phytochemical Result

Phenols	Present
Flavonoids	Present
Tannins	Present
Alkaloids	Present
Saponins	Present
Terpenoids	Present
Glycosides	Present

Proximate Composition of Spondias dulcis Bark

The proximate composition of the bark of *Spondias dulcis* is presented in Table 2.

Table 2: Proximate Composition of *Spondias dulcis* Bark

<u>Component</u>	<u>Composition (%)</u>
Moisture content	8.5
Ash content	6.7
Crude fiber	14.2
Protein	12.5
Lipids	4.1
Carbohydrates	54.0

Anti-malarial Activity

The anti-malarial activity of *Spondias dulcis* bark extract was evaluated through suppressive and curative tests. The results of these tests are summarized below.

Suppressive Activity on Early Infection (5-Day Test)

The suppressive activity of *Spondias dulcis* bark extract against *Plasmodium berghei* is presented in Table 3.

Table 3: Suppressive Activity of *Spondias dulcis* Bark Extract on *Plasmodium berghei* Infection

<u>Group</u>	<u>Treatment</u>	<u>Average % Parasitemia</u>	<u>Average % Inhibition</u>
Group 1	Distilled water (Uninoculated)	0.0	0.0
Group 2	Inoculated, untreated	34.5	0.0
Group 3	Chloroquine (10 mg/kg)	5.2	84.9
Group 4	<i>Spondias dulcis</i> bark extract (200 mg/kg)	12.3	64.3
Group 5	<i>Spondias dulcis</i> bark extract (1000 mg/kg)	8.1	76.5

The results indicated that both doses of *Spondias dulcis* bark extract exhibited significant anti-malarial activity, with the 1000 mg/kg dose showing greater suppression of parasitemia than the 200 mg/kg dose. The curative potential of *Spondias dulcis* bark extract was assessed by monitoring the parasitemia levels on days 1, 3, and 5 post-treatment. The results are shown in Table 4.

Table 4: Curative Activity of *Spondias dulcis* Bark Extract on *Plasmodium berghei* Infection

Group	Treatment	Day 1 Parasitemia	% Day 3 Parasitemia	% Day 5 Parasitemia	%
Group 1	Distilled water (Uninoculated)	0.0	0.0	0.0	
Group 2	Inoculated, untreated	35.0	40.2	47.8	
Group 3	Chloroquine (10 mg/kg)	34.5	15.6	4.3	
Group 4	<i>Spondias dulcis</i> bark extract (200 mg/kg)	35.3	22.8	12.5	
Group 5	<i>Spondias dulcis</i> bark extract (1000 mg/kg)	34.8	18.4	6.9	

The curative test results demonstrated that *Spondias dulcis* bark extract significantly reduced parasitemia levels in a dose-dependent manner, with the higher dose (1000 mg/kg) showing a more pronounced reduction in parasitemia compared to the lower dose (200 mg/kg). Data analysis showed that at a 5% significant level, there was a significant difference between the treated group and the untreated group. Also, the suppressive and curative tests indicate that the *Spondias dulcis* bark extract had a $p < 0.05$.

Discussion

The present study has demonstrated that the aqueous extract of *Spondias dulcis* bark possesses significant anti-malarial properties, as evidenced by the suppression of parasitemia and the curative effects observed in albino mice infected with *Plasmodium berghei*. The phytochemical screening of the extract revealed the presence of several bioactive compounds, including phenols, flavonoids, tannins, alkaloids, saponins, terpenoids, and glycosides. These compounds have been associated with anti-malarial activity in various plants, supporting the potential of *Spondias dulcis* as a source of anti-malarial agents. Several studies have investigated the anti-malarial potential of various plant extracts, and our findings align with these previous reports. For instance, Ajayi (2021) examined the anti-malarial properties of *Azadirachta indica* (neem) and observed a significant reduction in parasitemia levels in mice, which was attributed to the presence of bioactive compounds such as flavonoids and alkaloids. Similarly, our study identified flavonoids and alkaloids in *Spondias dulcis*, which may contribute to its observed anti-malarial effects. Additionally, Oduola (2010) evaluated the anti-malarial activity of *Carica papaya* leaf extract, finding that it effectively reduced parasitemia in infected mice. The study suggested that the phenolic compounds and saponins in *Carica papaya* were responsible for its anti-malarial properties. In our research, the detection of phenols and saponins in *Spondias dulcis* supports a comparable mechanism of action. Furthermore, Iwalokun (2008) investigated the anti-malarial properties of *Momordica charantia* (bitter melon) and reported significant suppression of parasitemia and increased survival rates in infected mice. The presence of terpenoids and glycosides in *Momordica charantia* was highlighted as a crucial factor in its anti-malarial activity. The identification of terpenoids and glycosides in *Spondias dulcis* in our study is consistent with these findings, suggesting that these compounds may play a significant role in the anti-malarial effects we observed. The bioactive compounds identified in *Spondias dulcis* bark extract likely contribute to its anti-malarial activity through various mechanisms. Phenolic compounds are known to have antioxidant properties that can mitigate oxidative stress caused by malaria infection. Flavonoids and alkaloids have been shown to interfere with the lifecycle of *Plasmodium* species, inhibiting their growth and replication. Saponins and terpenoids may disrupt the integrity of the parasite's cell membrane, leading to its death. Glycosides, particularly cardiac glycosides, have been reported to have immunomodulatory effects that enhance the host's immune response against the parasite. The statistical analysis using ANOVA indicated that the differences in parasitemia levels between the treated and untreated groups were statistically significant ($p < 0.05$). This suggests that the anti-malarial effects observed in the study are not due to random variation but are a direct result of the administration of *Spondias dulcis* bark extract.

Conclusion

The findings of this study provide strong evidence that the aqueous extract of *Spondias dulcis* bark has significant anti-malarial properties. The presence of various bioactive compounds such as phenols, flavonoids, tannins, alkaloids, saponins, terpenoids, and glycosides supports the potential of this plant as a source of novel anti-malarial agents. The suppression of parasitemia and the curative effects observed in the study indicate that *Spondias dulcis* bark extract could be an effective treatment for malaria. This study aligns with previous research on other plants with anti-malarial properties, highlighting the importance of exploring plant-based remedies for malaria treatment. Future research should focus on isolating and characterizing the specific compounds responsible for the anti-malarial effects of *Spondias dulcis*, as well as evaluating the safety and toxicity of the extract. Clinical trials will be necessary to determine the efficacy and safety of *Spondias dulcis* bark extract in human populations. *Spondias dulcis* bark extract represents a promising candidate for the development of new anti-malarial therapies. Its potential to provide an affordable and accessible treatment option could significantly contribute to the global fight against malaria, particularly in regions where the disease is endemic.

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