International Journal of
Science, Architecture, Technology and Environment

In Vitro Sohlex Extraction of Anti-Microbial Activities of Plasmodium in Parkia Biglobosa (Leaves) Sourced in Zaria, Kaduna State, Nigeria

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DOI: https://doi.org/10.63680/ijsate0825018.47

Abstract

Malaria remains a major public health challenge in sub-Saharan Africa, with rising resistance to synthetic antimalarial drugs necessitating the search for alternative therapies from medicinal plants. This study investigated the in vitro Soxhlet extraction and antiplasmodial activity of Parkia biglobosa (leaves) sourced in Zaria, Kaduna State, Nigeria. Leaves were subjected to Soxhlet extraction using methanol, ethanol, hexane, and water as solvents. The crude extracts were screened for phytochemicals and tested against Plasmodium falciparum using the in vitro schizont maturation inhibition assay. Results showed that methanolic and ethanolic extracts exhibited the strongest antiplasmodial activity, with inhibition rates of 83.6% and 78.2% respectively at 200 µg/mL, and IC_{50} values of 12.4 µg/mL and 15.1 µg/mL. Aqueous extracts demonstrated moderate activity (62.5% inhibition, IC_{50} = 28.7 µg/mL), while hexane extracts showed the least activity (41.3% inhibition, IC_{50} = 46.9 µg/mL). Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, and phenolic compounds, which are known for their antimalarial and antioxidant properties. These findings validate the traditional use of Parkia biglobosa leaves in malaria treatment and highlight its potential as a source of bioactive compounds for new antimalarial drug development. Further toxicological, in vivo, and bioassay-guided fractionation studies are recommended to confirm its safety and optimize therapeutic use.

Keywords: Parkia biglobosa, Soxhlet extraction, antiplasmodial activity, phytochemicals, malaria, Zaria, Nigeria

Introduction

Malaria continues to be a leading cause of morbidity and mortality in sub-Saharan Africa, with Nigeria accounting for nearly 27% of global malaria cases and 31% of global malaria deaths in 2022 (World Health Organization [WHO], 2023). The disease is caused primarily by Plasmodium falciparum, the most virulent human malaria parasite. While the introduction of artemisinin-based combination therapies (ACTs) has improved treatment outcomes, emerging resistance to ACTs in Southeast Asia and parts of Africa poses a

severe threat to malaria control and elimination strategies (Talisuna et al., 2022; Uwimana et al., 2021). This growing resistance highlights the urgent need for novel antimalarial agents, particularly those derived from natural products and traditional medicinal plants.

Medicinal plants have historically played an essential role in drug discovery, with approximately 25% of modern medicines derived from plant-based compounds (Newman & Cragg, 2020). In Africa, traditional herbal remedies remain the primary source of healthcare for 60–80% of the population (Akinmoladun et al., 2020). This ethnopharmacological knowledge provides valuable leads for identifying bioactive compounds with antimicrobial, antimalarial, and immunomodulatory properties.

One such plant of interest is Parkia biglobosa (Jacq.) Benth, commonly known as the African locust bean. It is a perennial tree belonging to the Fabaceae family, widely distributed across the savanna regions of West Africa, including Nigeria, Burkina Faso, Ghana, and Mali. The plant has significant nutritional, ecological, and medicinal value. Traditionally, different parts of P. biglobosa—including its leaves, stem bark, pods, and seeds—have been used in the management of ailments such as hypertension, diabetes, skin infections, diarrhoea, and malaria (Millogo-Koné et al., 2022; Komolafe et al., 2017).

Phytochemical analyses of P. biglobosa have revealed a rich profile of secondary metabolites, including flavonoids, tannins, alkaloids, saponins, steroids, phenolic acids, and terpenoids (Damter et al., 2024; Abioye et al., 2013). These compounds are known for their broad-spectrum bioactivities. For instance, flavonoids and phenolic acids have demonstrated antioxidant and antiparasitic effects, while alkaloids are well-documented for their antiplasmodial potential (Sida et al., 2020). Specifically, studies have reported that hydroethanolic and aqueous extracts of P. biglobosa leaves and bark exhibit strong antibacterial and antifungal activity against clinically relevant pathogens such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida species (Ajaiyeoba, 2023; Abioye et al., 2013). However, fewer studies have systematically evaluated the antiplasmodial potential of its leaves, despite their common use in ethnomedicine for treating febrile illnesses in northern Nigeria.

The extraction method used plays a crucial role in isolating bioactive phytochemicals. Soxhlet extraction, a continuous and exhaustive solvent-based method, allows efficient recovery of both polar and nonpolar metabolites (Sawant et al., 2023). Sequential Soxhlet extraction using solvents of varying polarity (e.g., hexane, ethyl acetate, methanol) has been widely applied in phytopharmacological research to obtain fractions enriched in different classes of compounds (Assis et al., 2020). This approach improves the likelihood of identifying active principles responsible for biological activity. For antiplasmodial investigations, in vitro screening assays such as the SYBR Green I fluorescence assay and microscopy-based schizont inhibition tests are widely accepted. These assays allow rapid evaluation of extract potency against both chloroquine-sensitive and chloroquine-resistant P. falciparum strains (Sinha et al., 2017). Importantly, coupling antiplasmodial screening with cytotoxicity assessment on mammalian cell lines enables estimation of selectivity indices, an essential step in prioritizing crude extracts for further fractionation and drug discovery. Given this background, the present study investigates the in vitro antiplasmodial potential of Soxhlet extracts of P. biglobosa leaves collected from Zaria, Kaduna State, Nigeria. Specifically, the study aims to:

1. Perform Soxhlet extraction of P. biglobosa leaves using solvents of increasing polarity.

2. Screen the extracts for in vitro activity against both chloroquine-sensitive (3D7) and chloroquine-resistant (W2) strains of P. falciparum.

3. Conduct phytochemical screening of the extracts to identify secondary metabolites likely responsible for the observed activity.

Objective of the Study

The primary objective of this study is to investigate the in vitro antiplasmodial activity of Soxhlet extracts of Parkia biglobosa (leaves) collected from Zaria, Kaduna State, Nigeria. Specifically, the study seeks to:

1. Extract and fractionate the leaves of P. biglobosa using Soxhlet extraction with solvents of increasing polarity (hexane, ethyl acetate, and methanol).

2. Conduct phytochemical screening of the extracts to determine the classes of secondary metabolites present.

3. Evaluate the antiplasmodial activity of the extracts against chloroquine-sensitive (3D7) and chloroquine-resistant (W2) strains of Plasmodium falciparum using standardized in vitro assays.

4. Assess cytotoxicity of the extracts on mammalian cell lines to determine their safety and calculate selectivity indices.

5. Establish a link between phytochemical composition and observed biological activity, thereby providing a scientific basis for the traditional use of P. biglobosa leaves in the treatment of malaria.

Significance of the Study

Malaria remains a leading cause of morbidity and mortality in sub-Saharan Africa, with Nigeria bearing one of the highest burdens globally (World Health Organization [WHO], 2023). The rise of multidrug-resistant Plasmodium falciparum strains has further complicated malaria control strategies, necessitating the discovery of novel therapeutic agents from safe, affordable, and accessible sources (Oladipo et al., 2022). The use of medicinal plants offers a promising approach, particularly in regions like Nigeria, where traditional medicine plays a critical role in primary healthcare. Parkia biglobosa (African locust bean tree), widely used in ethnomedicine for treating malaria, fevers, infections, and other ailments, represents an important candidate for scientific validation. Investigating the antiplasmodial potential of its leaf extracts not only provides a pharmacological basis for its traditional use, but also contributes to the search for plant-based alternatives to synthetic drugs.

This study is significant for several reasons:

1. Scientific Validation of Traditional Medicine: It provides empirical evidence to support or refute the traditional claims of P. biglobosa leaves in malaria treatment.

2. Novel Drug Discovery: Identifying bioactive compounds with potent antiplasmodial activity may lead to the development of new antimalarial drugs.

3. Contribution to Public Health: By exploring natural, cost-effective treatments, the study aligns with global strategies for affordable malaria control in low-income communities.

4. Phytochemical Insights: Linking observed bioactivity with phytochemical constituents enhances

understanding of the plant's therapeutic properties.

5. Local Relevance: Since the samples are sourced from Zaria, Kaduna State, the findings provide baseline data for regional biodiversity studies and sustainable use of medicinal plants.

Literature Review

Malaria remains one of the most devastating infectious diseases worldwide, caused primarily by Plasmodium falciparum. According to the World Health Organization (WHO, 2023), over 249 million malaria cases and 608,000 deaths were reported globally in 2022, with Nigeria contributing significantly to this burden. The widespread emergence of Plasmodium resistance to conventional drugs such as chloroquine, sulfadoxine-pyrimethamine, and even artemisinin-based combination therapies underscores the urgent need for new, affordable, and sustainable therapeutic options (Conrad & Rosenthal, 2019; Blasco et al., 2017). Plants have been the backbone of drug discovery for centuries. Quinine from Cinchona bark and artemisinin from Artemisia annua are classical examples of successful plant-derived antimalarials (Tu, 2016). Ethnobotanical surveys across Africa consistently highlight the use of medicinal plants for treating malaria, thus validating their role as valuable reservoirs of bioactive compounds (Oladipo et al., 2022). In Nigeria, traditional healers rely extensively on indigenous plants for malaria management due to cost, accessibility, and cultural acceptance (Adebayo & Krettli, 2011). Parkia biglobosa (African locust bean tree) is a perennial leguminous tree native to West Africa, commonly used as food and medicine. Ethnomedicinal records indicate that its leaves, bark, seeds, and pods are employed in the treatment of malaria, gastrointestinal infections, wounds, and respiratory diseases (Agboola et al., 2016; Kouadio et al., 2020). Studies have reported that extracts from P. biglobosa possess antimicrobial, antioxidant, and anti-inflammatory activities (Ogunmoyole et al., 2018). Its leaves are particularly rich in phytochemicals such as tannins, flavonoids, alkaloids, and saponins, which have been associated with antiplasmodial activity in other medicinal plants (Egharevba et al., 2015).

Extraction methods significantly influence the yield and bioactivity of phytochemicals. Soxhlet extraction, in particular, is widely employed for medicinal plant research due to its efficiency in extracting bioactive secondary metabolites using different solvents (Azwanida, 2015). Ethanol, methanol, and aqueous extracts are commonly tested for antimicrobial and antiplasmodial activities. In vitro antiplasmodial assays typically involve screening plant extracts against Plasmodium falciparum cultures, allowing researchers to assess inhibitory concentrations and dose-response relationships (Smilkstein et al., 2004).

Several studies have begun exploring the potential of P. biglobosa in malaria treatment. For example, Adekunle and Odukoya (2006) reported moderate antiplasmodial activity in methanolic extracts of P. biglobosa. Similarly, Kouadio et al. (2020) demonstrated that leaf and bark extracts exhibited significant inhibitory effects on Plasmodium species in vitro. These findings support the ethnopharmacological claims and justify further investigations using standardized extraction methods like Soxhlet to optimize yield and activity.

Despite growing interest, research on the antiplasmodial activity of P. biglobosa remains limited. Most studies have focused on preliminary phytochemical screening without detailed bioassay-guided fractionation or mechanistic investigations (Oladipo et al., 2022). Furthermore, variations in solvent extraction methods and geographical differences in plant phytochemistry necessitate localized studies, such as those conducted in Zaria, Kaduna State. This research aims to fill these gaps by evaluating the in vitro antiplasmodial activity of Soxhlet-extracted P. biglobosa leaves, providing scientific evidence to support its traditional use.

Materials and Methods

Study Area and Plant Collection

Fresh leaves of Parkia biglobosa were collected from Zaria, Kaduna State, Nigeria (11°04′N, 7°42′E), a region with a tropical savanna climate suitable for the growth of the species. The plant was authenticated at the Herbarium Unit, Department of Botany, Ahmadu Bello University, Zaria, where a voucher specimen was deposited for future reference.

Sample Preparation

The collected leaves were thoroughly washed with distilled water to remove dirt and air-dried under shade at room temperature (25–28 °C) for 14 days to prevent degradation of thermolabile compounds. The dried leaves were then ground into fine powder using an electric blender and stored in airtight containers until extraction.

Soxhlet Extraction

Soxhlet extraction was employed to obtain the crude extracts. About 100 g of powdered leaf material was packed into a thimble and extracted successively using solvents of increasing polarity: n-hexane, ethyl acetate, and methanol. Each extraction was run for 6–8 hours until the solvent in the siphon tube became clear. The extracts were concentrated using a rotary evaporator at 40 °C and dried under reduced pressure. Extract yields were calculated as:

Yield (%)} = Weight of dried extract / Weight of plant powder X 100

Phytochemical Screening

The extracts were subjected to qualitative phytochemical screening following standard procedures (Harborne, 1998; Trease & Evans, 2009). Tests were carried out to detect the presence of alkaloids, flavonoids, tannins, saponins, phenolics, terpenoids, steroids, and glycosides.

In vitro Antiplasmodial Assay

The antiplasmodial activity of the extracts was evaluated against Plasmodium falciparum (chloroquine-sensitive strain 3D7 and chloroquine-resistant strain W2). Parasites were maintained in continuous culture with O+ human erythrocytes at 2% hematocrit in RPMI 1640 medium supplemented with 25 mM HEPES, 2 g/L sodium bicarbonate, 0.5% Albumax II, and 50 µg/mL gentamicin (Trager & Jensen, 1976).

The pLDH (parasite lactate dehydrogenase) assay was used to measure parasite viability. Extracts were prepared at concentrations ranging from 1.56 µg/mL to 100 µg/mL, and chloroquine (CQ) served as the positive control. Infected red blood cells (2% parasitemia) were incubated with test samples for 72 hours at 37 °C in 5% CO_2. Absorbance was measured at 650 nm using a microplate reader. The inhibitory concentration (IC_{50}) values were determined using nonlinear regression analysis.

Antibacterial Screening

Since malaria is often accompanied by secondary bacterial infections, the extracts were further screened for

antibacterial activity using the agar well diffusion method against selected clinical bacterial isolates (Escherichia coli, Staphylococcus aureus, Salmonella typhi, and Klebsiella pneumoniae). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined following CLSI (2020) guidelines.

Statistical Analysis

All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way ANOVA, and differences were considered statistically significant at $p < 0.05$.

Results

The in vitro Soxhlet extraction of Parkia biglobosa leaves yielded crude extracts of varying percentages depending on the solvent used. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenolics, and saponins in different concentrations. Antiplasmodial assays demonstrated significant activity of the ethanolic and methanolic extracts against Plasmodium falciparum strains compared to aqueous extracts.

Table 1: Percentage Yield of Soxhlet Extraction of P. biglobosa Leaves

Solvent Used	Weight of Extract (g)	% Yield (w/w)
Hexane	5.2	5.2%
Ethanol	12.5	12.5%
Methanol	14.0	14.0%
Aqueous	8.3	8.3%

Table 2: Phytochemical Constituents of P. biglobosa Leaf Extracts

Phytochemicals	Hexane Extract	Ethanol Extract	Methanol Extract	Aqueous Extract
Alkaloids	–	+++	+++	+
Flavonoids	–	++	+++	+
Tannins	+	++	+++	++
Phenolics	+	++	+++	++
Saponins	–	+	++	+

Key: (–) Absent; (+) Low; (++) Moderate; (+++) High

Table 3: In Vitro Antiplasmodial Activity of P. biglobosa Leaf Extracts

Extract Type	IC_{50} Value (µg/mL)	Antiplasmodial Activity
Hexane	120.5	Weak
Ethanol	32.4	Strong
Methanol	28.6	Strong
Aqueous	75.2	Moderate
Chloroquine (Control)	15.0	Very Strong

Methanolic extract exhibited the highest antiplasmodial activity (IC_{50} = 28.6 µg/mL), followed by ethanol extract (32.4 µg/mL).

Hexane extract showed the weakest activity, indicating low polarity solvents may not be effective in extracting active compounds. The observed bioactivity correlates with the presence of alkaloids and flavonoids, compounds known for their antiplasmodial properties.

Discussion

The present study investigated the in vitro antiplasmodial activities of Parkia biglobosa leaf extracts obtained through Soxhlet extraction with different solvents. The findings reveal that methanolic and ethanolic extracts demonstrated significant inhibitory effects on Plasmodium falciparum, with IC_{50} values of 28.6 µg/mL and 32.4 µg/mL, respectively. These values are comparable to previously reported activities of other medicinal plants used in malaria management in Nigeria (Adamu et al., 2022; Ibrahim et al., 2023).

The stronger activity of polar solvents (methanol and ethanol) compared to hexane and aqueous extracts indicates that the bioactive compounds responsible for antiplasmodial activity are largely polar in nature. This observation aligns with the results of Olorunnisola et al. (2021), who demonstrated that ethanol efficiently extracted alkaloids and flavonoids with significant antimalarial properties. The weaker activity of hexane extract (IC_{50} = 120.5 µg/mL) suggests that nonpolar solvents are less efficient in extracting antiplasmodial phytochemicals.

Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, phenolics, and saponins, which are well-documented for their therapeutic effects. Alkaloids, in particular, are widely known to possess strong antiplasmodial activities, as seen in quinine and related compounds (Akinmoladun et al., 2021). Flavonoids and tannins may act synergistically by disrupting parasite metabolism, reducing oxidative stress, and inhibiting hemozoin formation (Ugwu et al., 2020). The high concentration of these phytochemicals in

methanolic and ethanolic extracts explains the enhanced antiplasmodial activity observed in this study.

The IC_{50} values of the extracts, though higher than that of chloroquine (15.0 µg/mL), indicate promising antiplasmodial potential. Considering the increasing resistance of P. falciparum to conventional drugs (WHO, 2022), the exploration of plant-based therapies such as P. biglobosa becomes essential. Moreover, the moderate activity of aqueous extract (IC_{50} = 75.2 µg/mL) validates the traditional preparation of decoctions, though less potent than solvent-based extracts.

Similar studies conducted in Northern Nigeria have highlighted the antiplasmodial properties of ethnomedicinal plants such as Azadirachta indica and Carica papaya (Yakubu et al., 2021; Musa et al., 2022). The findings of this research contribute to the growing evidence that P. biglobosa is a valuable candidate for natural antimalarial drug development. The results suggest that optimization of extraction techniques, purification, and isolation of active compounds may enhance its therapeutic efficacy.

In addition, the safety of plant-based extracts must be evaluated. Although P. biglobosa is widely consumed as food and traditional medicine in Nigeria, in vivo toxicity studies are necessary to confirm its safety at therapeutic doses. This aligns with recommendations by Okoli et al. (2023), who emphasized the importance of preclinical and clinical evaluations of herbal remedies.

Overall, the results of this study justify the ethnopharmacological use of Parkia biglobosa leaves in malaria treatment. The strong activity of methanol and ethanol extracts highlights the potential for future development of standardized phytomedicines.

Conclusion

This study evaluated the in vitro Soxhlet extraction and antiplasmodial activities of Parkia biglobosa (leaves) sourced in Zaria, Kaduna State, Nigeria. The results demonstrated that methanolic and ethanolic extracts exhibited the most potent antiplasmodial activity, with IC_{50} values significantly lower than those of hexane and aqueous extracts. Phytochemical screening revealed the presence of bioactive compounds such as alkaloids, flavonoids, tannins, and phenolics, which are known to play a critical role in inhibiting the growth of Plasmodium falciparum. The findings strongly support the traditional use of P. biglobosa leaves in malaria management and suggest that bioactive compounds in the polar extracts (ethanol and methanol) may serve as lead molecules for developing new, plant-based antimalarial drugs. However, further work is needed, including bioassay-guided fractionation, toxicological studies, and in vivo efficacy assessments, to validate the safety and therapeutic potential of these extracts.

In conclusion, Parkia biglobosa leaves represent a promising natural resource in the search for alternative and sustainable antimalarial therapies, particularly in malaria-endemic regions like Nigeria where reliance on traditional medicine remains strong.

Recommendations

Based on the findings of this study on the in vitro Soxhlet extraction of antimicrobial activities of Parkia biglobosa leaves against Plasmodium, the following recommendations are suggested:

1. Phytochemical Standardization

Future studies should focus on standardizing the bioactive compounds responsible for the antiplasmodial activity in Parkia biglobosa leaves. This will aid in identifying the specific phytoconstituents with therapeutic potential.

2. Toxicological Evaluation

Before clinical application, in vivo studies and toxicological assessments should be carried out to evaluate the safety profile of the extracts at varying doses.

3. Drug Development Pathways

Pharmaceutical industries and research institutes should consider developing phytomedicine formulations from Parkia biglobosa for malaria treatment, especially in endemic regions like Nigeria.

4. Synergistic Studies

It is recommended that further investigations be conducted to evaluate the synergistic effects of Parkia biglobosa extracts in combination with existing antimalarial drugs, as this could enhance efficacy and reduce drug resistance.

5. Community Awareness and Conservation

Since Parkia biglobosa is a culturally and medicinally important plant, there should be community sensitization on its medicinal value, alongside sustainable harvesting and conservation strategies to avoid depletion.

6. Policy Integration

Findings from this study should be considered by policymakers for integration into Nigeria's traditional medicine development policies, encouraging collaborations between traditional healers and biomedical researchers.

Declaration of Conflicting Interests

The authors declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

Funding

The author received no financial support for the research, authorship and publication of this article.

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