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In-vitro Antimalarial Activity of *Plicosepalus acaciae* Extracts Against *Plasmodium falciparum*

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Abstract

The emergence of drug-resistant *Plasmodium* strains necessitates the continuous search for novel antimalarial agents. *Plicosepalus acaciae* (*P. acaciae*), a semi-parasitic mistletoe, is used in traditional medicine, but its antimalarial potential is underexplored. This study aimed to evaluate the *in vitro* antimalarial activity of *P. acaciae* leaf and stem extracts. Methanol and chloroform extracts of *P. acaciae* leaves and stems were prepared. Their antimalarial activity against the chloroquine-resistant K1 strain of *Plasmodium falciparum* (*P. falciparum*) was assessed using a SYBR Green I-based assay at concentrations of 500, 250, and 125 µg/mL. Artemisinin (51.20 nM/L) was used as a standard drug. The 50% inhibitory concentration (IC_{50}) was determined for active extracts. The methanolic stem extract exhibited the highest antimalarial activity with an IC_{50} of 3.26±0.10 µg/mL and achieved 91% parasite growth inhibition at 500 µg/mL, comparable to the 90% inhibition observed with artemisinin. The methanolic leaf extract and chloroform extracts showed lower activity, with IC_{50} values of 12.57 ± 0.03 µg/mL and 13.93 ± 0.07 µg/mL (leaves), and 7.61 ± 0.01 µg/mL (stems), respectively. *Plicosepalus acaciae* extracts, particularly the methanolic stem extract, possess promising *in vitro* antimalarial activity against *P. falciparum*. These findings justify further investigation to isolate the active compounds and evaluate their efficacy *in vivo*.

Keywords: *Plicosepalus Acaciae*, Antimalarial, *Plasmodium Falciparum*, Artemisinin, *In Vitro* Assay.

INTRODUCTION

Malaria remains a significant global health concern, particularly in sub-Saharan Africa, where it is a leading cause of morbidity and mortality (Kolawole et al., 2023; WHO, 2022). The emergence of drug-resistant strains of the Plasmodium parasite has heightened the need for the development of new antimalarial therapies (Belete, 2020; Shibeshi et al., 2020).

In this context, the exploration of traditional medicinal plants as potential sources of novel antimalarial compounds has become an area of increasing interest (Ceravolo et al., 2021). The exploration of medicinal plants as potential sources of antimalarial agents has gained significant momentum in recent years, driven by their rich chemical diversity and historical validation in traditional healing systems (Cragg & Newman, 2013). Numerous plant-derived compounds, including artemisinin

from *Artemisia annua*, have served as foundational templates for developing effective antimalarial drugs (Tu, 2016).

In Africa and various other regions, plant-based remedies continue to be widely utilized for the treatment of malaria and other health conditions, with 80% of the African population depending on traditional medicine for their primary health care needs (D'Almeida et al., 2024). *Plicosepalus acaciae* (Loranthaceae) is a hemiparasitic plant native to Sudan and other parts of Africa and the Middle East, traditionally used to treat various ailments, including infectious diseases (Abajue & Wogu, 2024; Kacholi, 2024). The Loranthaceae family, to which it belongs, is known to contain various bioactive metabolites, such as flavonoids, terpenoids, and phenolic compounds, which have demonstrated promising pharmacological activities against multiple pathogens (Zhang et al., 2022). Supporting this, previous phytochemical studies on *P. acaciae* itself have identified the presence of specific compounds like quercetin, tannins, and terpenoids, which are likely contributors to its biological activities (Eltamany et al., 2022). While previous research has primarily focused on the antidiabetic properties of *P. acaciae* (Kotb El-Sayed et al., 2020), a systematic evaluation of its antimalarial potential remains largely unexplored and represents a significant gap in the literature.

Therefore, this study was designed to conduct a systematic *in vitro* evaluation of the antimalarial activity of methanol and chloroform extracts from the leaves and stems of *P. acaciae* against the chloroquine-resistant *Plasmodium falciparum* K1 strain. The findings aim to provide a scientific basis for its traditional use and contribute to the discovery of new antimalarial leads.

MATERIALS AND METHODS

Drugs and Reagent

Artemisinin, RPMI-1640 (Gibco-Brl, Life Technology), penicillin (100 U/ml), streptomycin, dimethyl sulfoxide (DMSO) (Merck Chemicals, Darmstadt, Germany), methanol, chloroform (Techno pharmchem, India), HEPES, phosphate buffer saline (PBS) (British Drug House, England), AB+ human serum, O-type human red blood cells, normal saline, and Giemsa stain (RICCA Chemical Company).

Ethical Approval:

Not applicable.

Plant materials

In Central Sudan, *P. acaciae* leaves and stems (Figure 1), were collected between January and February 2014. Taxonomists at the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) in Khartoum, Sudan identified and verified the plant species.

Preparation of crude extracts

The extraction of leaves and stems from *P. acaciae* was performed using overnight maceration methods, as described by Harbone (1984). Approximately 50 g of powdered material was soaked in 250 mL of methanol and chloroform for three hours at room temperature. The solution was intermittently shaken over a 24-hour period at room temperature, and the supernatant was discarded. The supernatant was filtered under reduced pressure using a rotary evaporator at 55°C. Each residue was weighed, and the yield percentage was determined (Table 1). The samples were stored at 4°C in tightly sealed glass vials and ready for use.

$$\text{Yield percentage} = \text{Weight of extract/weight of sample X } 100$$

Figure (1). Plant of *Plicosepalus acaciae.*

In vitro Antimalarial properties of crude extracts:

Preparation of crude extracts working solution:

Each extract was precisely weighed to 5 mg using a sensitive balance and then placed into Eppendorf tubes. Subsequently, 50 µL of DMSO was added to each sample, and distilled water was used to bring the total volume to 1 mL, resulting in a concentration of 5 mg/mL. The solution was then vortexed and stirred using a magnetic stirrer to ensure thorough mixing.

Parasite culture and *in vitro* bioassays for antimalarial testing:

The *P. falciparum* parasite strain was cultivated using the candle jar technique described by Trager and Jensen (1975). For the *in vitro* assessment of the antimalarial properties of the *P. acaciae* (leaves and stem) extracts selected for this study, the parasitemia levels in the cultures were maintained between 3% and 5%. The initial screening of the antimalarial effects of methanol and chloroform extracts from *P. acaciae* was conducted in 96-well microtiter plates using a SYBR Green I-based assay (Bennett et al., 2004), with all tests performed at 5% parasitemia and 7% hematocrit. Each extract was initially tested for antimalarial activity against the K1 strain of *P. falciparum* at a concentration of 50 µg/ml. Extracts that resulted in less than 50% parasite survival were further analyzed to determine their IC_{50} values. Plant extracts were used at a concentration of 100 µL/mL. Artemisinin (51.20 nM/L) was used as the standard antimalarial drug. Parasitemia was evaluated by analyzing ten fields on each slide. The percentage of inhibition were calculated using the following formula:

$$\text{Inhibition (\%)} = (1 - B) \times 100$$

Where: B = Number of infected erythrocytes in (samples/control).

Statistical analysis:

Data are presented as mean ± SD. Microsoft Excel (2016) was utilized to perform statistical analyses on all assay results. IC_{50} values were determined from dose-response curves.

RESULTS

Extraction yields:

The extraction yields are summarized in Table 1. The highest yield was obtained from the stem using methanol (32.2%), followed by the leaf methanol extract (25.0%). Chloroform extraction resulted in lower yields.

Table (1). Extraction yields of *P. acaciae* leaf and stem extracts:

Scientific name	Family name	Parts used	Solvents used	Yields (g)	Yields (%)
P. accaciae	Loranthaceae	Leaves	Methanol	12.5	25.0
			Chloroform	05.0	10.0
		Stems	Methanol	16.1	32.2
			Chloroform	06.3	12.6

Key: (g): gram; **(%):** percent.

Antimalarial activity:

All tested extracts demonstrated concentration-dependent antimalarial activity (Figure 2). The methanolic stem extract was the most potent, with an IC_{50} of 3.26 ± 0.10 µg/mL and 91% inhibition at 500 µg/mL. The chloroform stem extract and methanolic leaf extract showed moderate activity, while the chloroform leaf extract was the least active (Table 2). The positive control, artemisinin, showed 90% inhibition at 51.20 nM/L.

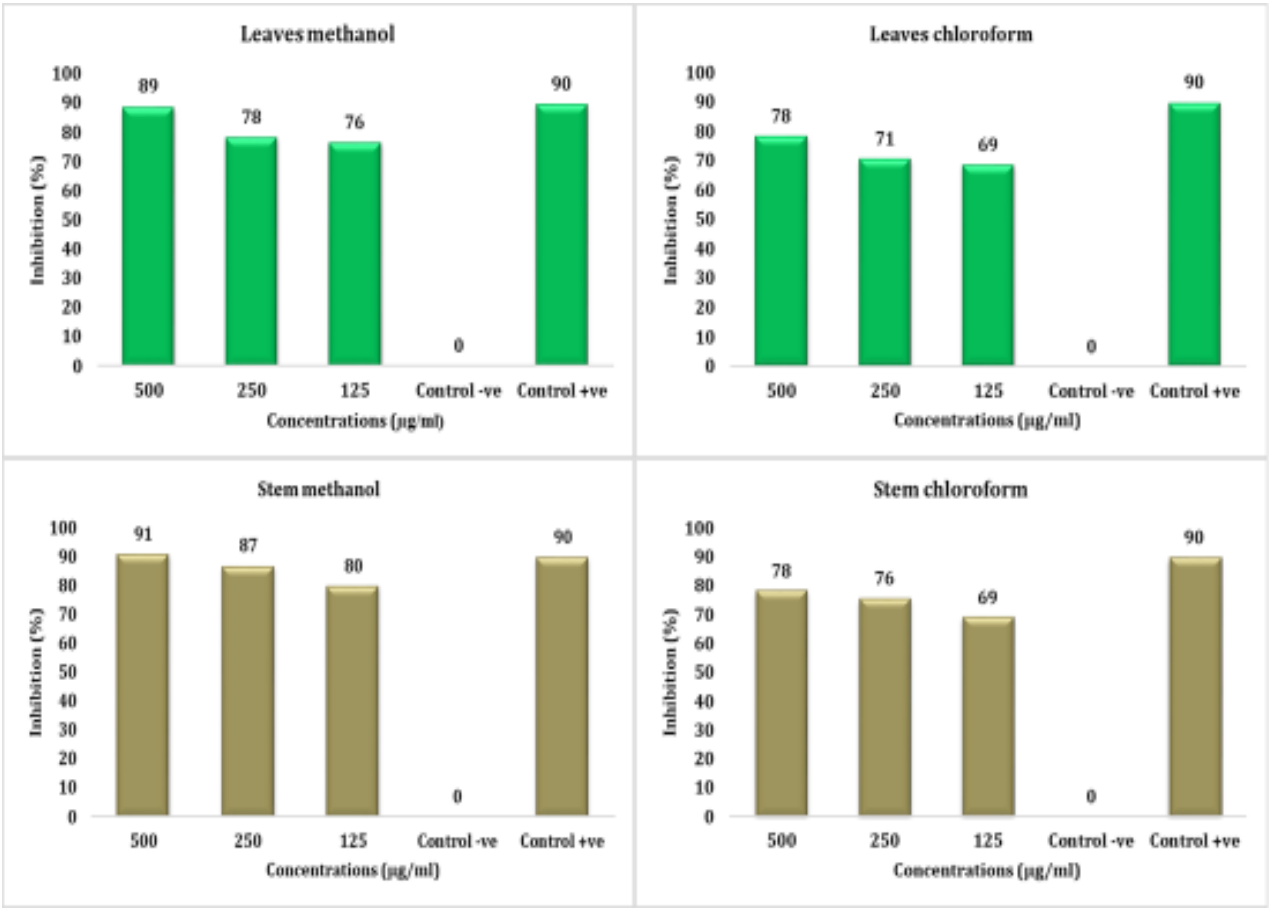

Figure (2). *In vitro* antimalarial activity of *P. acaciae* extracts against *P. falciparum*.

Table (2): IC_{50} values of *P. acaciae* extracts against *P. falciparum*:

Scientific Name	Family name	Parts Used	Solvents	IC_{50} ± SD (mg/ml)
P. accaciae	Loranthaceae	leaves	Methanol	12.57±0.03
			Chloroform	13.93±0.07
		Stems	Methanol	3.26±0.10
			Chloroform	7.61±0.01

Key: IC_{50}: 50% inhibitory concentration; **SD:** Standard Deviation.

DISCUSSION

The escalating challenge of antimalarial drug resistance, particularly in *Plasmodium falciparum*, underscores the critical need for continuous discovery and development of novel therapeutic agents (Organization, 2022). This study provides the first comprehensive *in vitro* evaluation of the antimalarial potential of *Plicosepalus acaciae* extracts against the chloroquine-resistant K1 strain of *P. falciparum*. Our findings demonstrate that crude extracts from *P. acaciae*, particularly the methanolic stem extract, possess significant antiplasmodial activity.

The most promising result was observed with the methanolic stem extract, which exhibited an IC_{50} value of 3.26±0.10 µg/mL. According to established criteria for antimalarial activity of crude plant extracts, an IC_{50} value below 5 µg/mL is considered highly active, while values between 5 and 15 µg/mL indicate moderate activity (Ogbeide et al., 2018). Therefore, the methanolic stem extract of *P. acaciae* demonstrates high antimalarial potential. Its remarkable inhibition rate of 91% at 500 µg/mL further reinforces its potency, showing comparable efficacy to artemisinin, which achieved 90% inhibition at 51.20 nM/L under identical experimental conditions. This finding is particularly significant given the global challenge of artemisinin resistance (Balikagala et al., 2021).

The superior efficacy of methanol extracts over chloroform extracts from both plant parts suggests that the active antimalarial constituents in *P. acaciae* are relatively polar compounds. This observation aligns with previous phytochemical investigations of medicinal plants where methanol has proven to be a more effective solvent for extracting antimalarial compounds such as flavonoids, phenolic acids, and other polar terpenoids (Kaur et al., 2009). The enhanced extraction efficiency of methanol can be attributed to its ability to dissolve a wider range of medium-polarity to high-polarity compounds compared to the non-polar chloroform (Uchôa et al., 2010). This finding is consistent with research by Vishakha et al. (2020), who also reported superior antimicrobial activity in methanolic extracts compared to chloroform extracts from various medicinal plants.

The significant variation in antimalarial activity between different plant parts (stem versus leaf) highlights the importance of specifying the plant organ used for extraction. The stem extracts consistently demonstrated higher activity than their leaf counterparts, regardless of the solvent used. This differential bioactivity likely reflects variations in the biosynthesis, accumulation, and distribution of secondary metabolites within different plant tissues (Yang et al., 2018). Plants often compartmentalize defense compounds in specific organs as an adaptive strategy against pathogens and herbivores (Mithöfer & Boland, 2012). Our results suggest that the stem of *P. acaciae* may accumulate higher concentrations of the active antimalarial principles or contain unique compounds not present in the leaves.

While *P. acaciae* has been previously investigated primarily for its antidiabetic properties (Kotb El-Sayed et al., 2020), this study reveals a previously undocumented pharmacological property of considerable importance. The traditional use of *P. acaciae* for treating febrile illnesses in some com-

munities (Abajue & Wogu, 2024) may indirectly support our findings, as malaria typically presents with fever. The potent antimalarial activity of the stem extract warrants its prioritization in future phytochemical investigations aimed at isolating the specific bioactive compounds.

The mechanism of action of *P. acaciae* extracts against *P. falciparum* remains to be elucidated. However, based on the known bioactive constituents of related species in the Loranthaceae family, the activity may be attributed to various secondary metabolites. Plants in this family are known to produce flavonoids, terpenoids, and alkaloids, many of which have demonstrated antimalarial properties through various mechanisms, including inhibition of hemozoin formation, interference with parasite metabolic pathways, and induction of oxidative stress (Kacholi, 2024; Muthaura et al., 2007). Future studies should focus on identifying the specific compounds responsible for the observed activity and their molecular targets.

It is noteworthy that the methanolic stem extract demonstrated potent activity against the chloroquine-resistant K1 strain, suggesting that its mechanism of action may differ from that of chloroquine. This is particularly important in the context of multidrug-resistant malaria, where compounds with novel mechanisms are urgently needed (Belete, 2020). The potential for overcoming existing resistance mechanisms makes *P. acaciae* an interesting candidate for further development.

Despite these promising findings, this study has several limitations. First, the absence of *in vivo* validation means we cannot yet ascertain the efficacy, bioavailability, or safety profile of these extracts in a whole-organism system. Future research should include *in vivo* studies using appropriate animal models of malaria to confirm the therapeutic potential. Second, the crude nature of the extracts means that the observed activity could result from single compounds or synergistic interactions among multiple constituents. Bioassay-guided fractionation is necessary to isolate and characterize the active principles.

CONCLUSION

In conclusion, this study provides scientific validation for the traditional use of *P. acaciae* and identifies it as a promising source of antimalarial agents. The potent activity of the methanolic stem extract against a drug-resistant strain of *P. falciparum* highlights its potential for development into a novel antimalarial therapy. Further investigation is warranted to isolate the active compounds, elucidate their mechanism of action, and evaluate their efficacy and safety in *in vivo* models.

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Duality of interest:

The authors declare that they have no duality of interest.

Author contributions:

Conceptualization, A.S.K., A.M.A, and H.A.F., proposed and designed compounds, A.S.A., and A.M.A., conducting experiments, ASK., A.M.A., and H.A.F., writing-original draft preparation, A.S.K, A.M.A., and H.A.F., writing-review and editing, A.S.K., and A.M.A., all authors reviewing and editing this work.

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REFERENCES

Abajue, M. C., & Wogu, M. N. (2024). Medicinal Plants in the Tropics Used in the Treatment and Management of Parasitic Diseases Transmitted by Mosquitoes: Administration, Challenges, and Strategic Options for Management. In *Herbal Medicine Phytochemistry: Applications and Trends* (pp. 417-450). Springer.

Balikagala, B., Fukuda, N., Ikeda, M., Katuro, O. T., Tachibana, S.-I., Yamauchi, M., Opio, W., Emoto, S., Anywar, D. A., & Kimura, E. (2021). Evidence of artemisinin-resistant malaria in Africa. *New England Journal of Medicine, 385*(13), 1163-1171.

Belete, T. M. (2020). Recent progress in the development of new antimalarial drugs with novel targets. *Drug design, development and therapy*, 3875-3889.

Ceravolo, I. P., Aguiar, A. C., Adebayo, J. O., & Krettli, A. U. (2021). Studies on activities and chemical characterization of medicinal plants in search for new Antimalarials: a ten year review on Ethnopharmacology. *Frontiers in Pharmacology, 12*, 734263.

Cragg, G. M., & Newman, D. J. (2013). Natural products: a continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1830*(6), 3670-3695.

D'Almeida, S. A., Gbomor, S. E., Osaio-Kamara, B., Olagunju, M. T., Abodunrin, O. R., & Foláyan, M. n. O. (2024). A scoping review of the use of traditional medicine for the management of ailments in West Africa. *PloS one, 19*(7), e0306594.

Eltamany, E. E., Goda, M. S., Nafie, M. S., Abu-Elsaoud, A. M., Hareeri, R. H., Aldurdunji, M. M., Elhady, S. S., Badr, J. M., & Eltahawy, N. A. (2022). Comparative assessment of the antioxidant and anticancer activities of Plicosepalus acacia and Plicosepalus curviflorus: metabolomic profiling and in silico studies. *Antioxidants, 11*(7), 1249.

Harbone, B. (1984). Phytochemical methods. 2nd. *New York, Champan Hall, 4*, 4-7.

Kacholi, D. S. (2024). A comprehensive review of antimalarial medicinal plants used by Tanzanians. *Pharmaceutical Biology, 62*(1), 133-152.

Kaur, K., Jain, M., Kaur, T., & Jain, R. (2009). Antimalarials from nature. *Bioorganic & medicinal chemistry, 17*(9), 3229-3256.

Kolawole, E. O., Ayeni, E. T., Abolade, S. A., Ugwu, S. E., Awoyinka, T. B., Ofeh, A. S., & Okolo, B. O. (2023). Malaria endemicity in Sub-Saharan Africa: Past and present issues in public health. *Microbes and Infectious Diseases, 4*(1), 242-251.

Kotb El-Sayed, M.-I., Al-Massarani, S., El Gamal, A., El-Shaibany, A., & Al-Mahbashi, H. M. (2020). Mechanism of antidiabetic effects of Plicosepalus Acaciae flower in streptozotocin-induced type 2 diabetic rats, as complementary and alternative therapy. *BMC complementary medicine and therapies, 20*(1), 290.

Mithöfer, A., & Boland, W. (2012). Plant defense against herbivores: chemical aspects. *Annual Review of Plant Biology, 63*(1), 431-450.

Muthaura, C., Rukunga, G., Chhabra, S., Omar, S., Guantai, A., Gathirwa, J., Tolo, F., Mwitari, P., Keter, L., & Kirira, P. (2007). Antimalarial activity of some plants traditionally used in treatment of malaria in Kwale district of Kenya. *Journal of Ethnopharmacology, 112*(3), 545-551.

Ogbeide, O. K., Dickson, V. O., Jebba, R. D., Owhiroro, D. A., Olaoluwa, M. O., Imieje, V. O., Erharuyi, O., Owolabi, B. J., Fasinu, P., & Falodun, A. (2018). Antiplasmodial and acute toxicity studies of fractions and cassane-type diterpenoids from the stem bark of Caesalpinia pulcherrima (L.) Sw. *Trop J Nat Prod Res, 2*(4), 179-184.

Organization, W. H. (2022). *WHO Malaria Policy Advisory Group (MPAG) meeting, October 2022*. World Health Organization.

Shibeshi, M. A., Kifle, Z. D., & Atnafie, S. A. (2020). Antimalarial drug resistance and novel targets for antimalarial drug discovery. *Infection and drug resistance*, 4047-4060.

Tu, Y. (2016). Artemisinin-A gift from traditional chinese medicine to the world (Nobel Lecture). *Angewandte Chemie International Edition, 55*(35).

Uchôa, V. T., de Paula, R. C., Krettli, L. G., Santana, A. E. G., & Krettli, A. U. (2010). Antimalarial activity of compounds and mixed fractions of Cecropia pachystachya. *Drug Development Research, 71*(1), 82-91.

Vishakha, K., Das, S., Banerjee, S., Mondal, S., & Ganguli, A. (2020). Allelochemical catechol comprehensively impedes bacterial blight of rice caused by Xanthomonas oryzae pv. oryzae. *Microbial Pathogenesis, 149*, 104559.

WHO. (2022). *World malaria report 2022*. World Health Organization.

Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. *Molecules, 23*(4), 762.

Zhang, Y., Cai, P., Cheng, G., & Zhang, Y. (2022). A brief review of phenolic compounds identified from plants: Their extraction, analysis, and biological activity. *Natural Product Communications, 17*(1), 1934578X211069721.