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In-vitro Anti-Plasmodial Activity of *Mangifera indica* Leaves Against *Plasmodium falciparum*

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ABSTRACT

Malaria remains a critical global health concern, particularly in sub-Saharan Africa, where *Plasmodium falciparum* accounts for the majority of infections and fatalities. With increasing resistance to the current antimalarial treatments, including artemisinin-based combination therapies (ACTs), there is growing interest in exploring natural remedies, especially plant-derived alternatives. The current study investigated the in-vitro antiplasmodial activity of aqueous leaf extracts of *Mangifera indica (mango).* Phytochemical evaluation of the leaf extract revealed the presence of flavonoids, tannins, saponins, and terpenoids, compounds with known pharmacological relevance. The extract showed a reduction in parasitemia in the culture based on different concentrations, achieving a maximum inhibition rate of 93% at 10 mg/ml, comparable to that of the artemether control (96%). The study's findings revealed that *M. indica* leaf extract holds promise as an affordable and effective candidate for the development of antimalarial drugs. Further investigations should focus on isolating specific active compounds, elucidating their mechanisms of action, and conducting in vivo validation studies, which are highly recommended.

Keywords:
Mangifera indica, *Plasmodium falciparum*, antimalarial activity, phytochemicals, *in-vitro* assay, medicinal plants

INTRODUCTION

Malaria is one of the most serious parasitic diseases caused by protozoans of the *Plasmodium* genus, with *P. falciparum* being the most lethal. The World Health Organization's 2023 report revealed that approximately 246 million malaria cases and 569,000 deaths occurred globally in 2023, with the African region accounting for over 95% of these figures. Despite substantial global efforts, the emergence of drug-resistant strains has compromised the effectiveness of existing treatments, particularly artemisinin-based combination therapies (ACTs) (Dondorp *et al.,* 2009; Muhammad *et al.,* 2024).

Historically, many frontline antimalarials, such as quinine and artemisinin, have been derived from medicinal plants (Phillipson & Wright, 1991), which spurred continued interest in exploring ethnomedicinal resources for new antimalarial drugs. *Mangifera indica*, commonly referred to as mango, is a tropical tree from the *Anacardiaceae* family and has been traditionally used in various cultures to treat ailments including fever, gastroenteritis, malaria, and diabetics (Akinmoladun *et al*., 2007; Maharaj *et al*., 2022; Shah *et al*., 2010; Airoadion *et al.,* 2021).

Recently, other pharmacological studies have reported that mango leaf extracts possess antioxidant, antimicrobial, and anti-inflammatory activities (Sharma *et al.*, 2018; Sabina and Sharma). However, despite its widespread traditional use, the antiplasmodial activity of *M. indica* leaves has not been thoroughly documented. The current study set out to explore the phytochemical constituents

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and the potential in-vitro antiplasmodial effect of *M. indica* leaf extract against *P. falciparum*.

MATERIALS AND METHODS

The Blood Samples

Blood samples (positive and negative) for *Plasmodium falciparum* were obtained from the Hematology Department of Aminu Kano Teaching Hospital, Kano, Nigeria, with ethical clearance and consent from hospital authorities. Both the positive and negative blood samples were collected and immediately transferred into EDTA bottles, then stored at 4°C, as demonstrated by Decie and Lewis (1968), Stephen and Frank (1985), and Herber and Stoeppler (1994).

Plant Collection, Identification, and Preparation

Fresh leaves of *Mangifera indica* were harvested from the Biological Garden of Yusuf Maitama Sule University, Kano. The plant was identified and authenticated by a certified taxonomist from the Department of Biological Sciences, and a voucher specimen was deposited under reference number YUMSUKHAN. Leaves were shade-dried at room temperature for 3–7 days, then ground into a fine powder using sterilized mortar and pestle (Harborne, 1998; Evans, 1996).

Extraction of the Leaves powder

Fifty grams (50 g) of the powdered leaf material was macerated in 500 ml of distilled water for 24 hours at ambient temperature with intermittent shaking. A Whatman filter paper was used to filter the solution, and the filtrate was stored in a sterile container for subsequent use in phytochemical screening and bioassays (Osuntokun & Olajubu, 2014; Sofowora, 1993).

Phytochemical Screening

The Phytochemical analysis of the aqueous extracts was carried out to determine the presence of bioactive compounds (Talari & Nanna, 2016) using standard qualitative methods (Trease & Evans, 1996; Harborne, 1998). Tests for alkaloids, flavonoids, saponins, tannins, terpenoids, phenols, and glycosides were performed following widely accepted protocols (Masilamani *et al.,* 2022).

Staining and Parasites Screening:

Thin smears were prepared from test blood samples using a sterile capillary tube. One drop of blood was placed on a grease-free slide, smeared at a 45-degree angle, and air-dried. Methanol was used to fix the blood stain for 15 minutes, stained with Giemsa stain for an additional 15 minutes, and then examined under an oil immersion microscope. *P. falciparum* trophozoites were identified by their characteristic ring forms, which were stained blue. The density of parasitemia was determined by counting infected red blood cells (RBCs) among a minimum of 1,200 RBCs across eight fields using 100× magnification (Cheesbrough, 2006).

Erythrocytes Separation

Erythrocytes were separated from whole blood via centrifugation at 2,500 rpm for 15 minutes. The plasma was removed, and normal saline was used to wash the erythrocyte pellet and resuspended to the desired concentration for culture (Thompson *et al.,* 1984).

The Test Concentrations Preparation:

A stock solution was constituted by dissolving 1 g of the aqueous extract in 1 ml of dimethyl sulfoxide (DMSO). From stock, serial dilutions were conducted to obtain concentrations of 10 mg/mL, 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL (Irokanulo, 2017) using normal saline, selected based on preliminary screening for antiplasmodial activity (Muktar et al., 2006).

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Preparation of Culture Media (RPMI 1640)

RPMI-1640 culture medium was prepared by dissolving 10.4 g of powdered media in 1 liter of distilled water, which was then sterilized by autoclaving at 121°C for 15 minutes. The medium was augmented according to standard cell culture practices (Trager & Jensen, 1976).

The Anti-plasmodial Assay of the Extracts on *Plasmodium falciparum*

Equal volumes (0.5 ml each) of extract and culture medium were placed into labeled test tubes. A 0.1 ml of P. falciparum-infected erythrocytes was added to each tube. The mixtures were incubated in a candle jar to simulate a low-oxygen environment (95% N_2, 2% O_2, 3% CO_2) at 37°C for up to 72 hours. Control setups included untreated infected blood (negative control) and infected blood treated with artemether (positive control). After 24, 48, and 72 hours, thin blood films were prepared from each test tube and fixed in methanol, then stained with Giemsa for microscopy.

Determination of Antimalarial Activity

The antiplasmodial activity of the extract was assessed by calculating the reduction in parasitemia using the formula described by Muktar et al. (2006).

$$\text{Percentage Elimination} = \frac{N1-N2}{N1} \times 100$$

where N1 is the number of infected erythrocytes in the control group and

N2 is the number of infected erythrocytes in the test group

The degree of inhibition served as a measure of the extract's antimalarial efficacy. High inhibition percentages indicated more substantial antiplasmodial potential (Clarkson *et al.,* 2004; Pandey & Rizvi, 2009).

Data Analysis

Descriptive data analyses were employed to analyze the collected data.

RESULTS

Phytochemical Composition

Phytochemical analysis revealed that *Mangifera indica* leaves contain flavonoids, tannins, saponins, and terpenoids, as shown in table 1 below:

Table 1: Result of Qualitative Phytochemical Analysis of the aqueous *Mangifera indica* Leaf extract.

S/N	Phytoconstituent	Inference
1	Alkaloids	Absent
2	Flavonoids	Present
3	Tannins	Present
4	Saponins	Present
5	Terpenoids	Present
6	Phenols	Absent
7	Cardiac Glycosides	Absent

In-Vitro Anti-Plasmodial Activity

The aqueous leaf extract of *Mangifera indica* exhibited a concentration-dependent inhibition of *Plasmodium falciparum* growth in vitro. The highest level of parasite clearance was observed at 10 mg/ml with a suppression rate of 93%, closely comparable to the artemether control group, which showed 96% clearance. At concentrations of 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL, inhibition rates were 84%, 72%, and 60%, respectively; the results are shown in Table 2 below.

DISCUSSION

Phytochemical analysis revealed that *Mangifera indica* leaves contain flavonoids, tannins, saponins, and terpenoids, which are known to exhibit antimicrobial and anti-plasmodial properties, contrary to the work of (Ahmed *et al.,* 2024; Abera *et al.,* 2024), whose analysis of *Mangifera indica* leaf revealed the presence of alkaloids, saponins,

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steroids, tannins, phenols, terpenoids, and cardiac glycosides. Similar studies (Iverson & Dervan, (2014); Okolie & Kristhien (2019) revealed the absence of flavonoids and attributed it to the geographical location of the plant, the extraction method of the extract adopted, and also the solvent used, as explained by (Nawaz *et al.,* 2022). In the current study, the absence of alkaloids and anthraquinones suggests that the observed anti-plasmodial activity may primarily be attributed to flavonoids and terpenoids.

Table 2: The concentration-dependent antiplasmodial activity of *Mangifera indicana* leaf extract.

Sample	Initial Parasitemia (%)	Extract Concentration (mg/ml)	Parasitemia after 24h	Parasitemia after 48h	Parasitemia after 72h	Average Suppression (%)
Mangifera indica	58	10	55	48	42	93%
		5	57	52	47	89%
		2.5	60	56	51	85%
		1.25	62	60	58	78%
Control	58	-	58	53	47	96%

The results support the potential of *Mangifera indica* leaf extract as a treatment for malaria. The significant suppression observed at higher concentrations may be an implication of the synergistic effects of flavonoids and terpenoids, which have been shown to interfere with the parasite's redox system and inhibit fatty acid biosynthesis (Pandey & Rizvi, 2009; Mishra *et al.,* 2008). Saponins and tannins may exhibit their effects by altering membrane permeability and inhibiting parasite enzyme systems (Akinmoladun *et al.,* 2007). The absence of alkaloids and anthraquinones suggests that these are not the primary contributors to the observed activity in this case and a clear shift from known antimalarials like quinine (a cinchona alkaloid). The observed dose dependency and significant activity at 10 mg/mL underscore the extract's promise, although the concentrations are relatively high compared to those of purified compounds.

Further investigations are required to isolate active fractions and evaluate their mechanisms of action and pharmacokinetics. Additionally, toxicity profiling and in-vivo validation using murine models will be essential before clinical applications can be considered (Clarkson *et al*., 2004). The current study also provides compelling evidence for the antiplasmodial potential of *Mangifera indica* leaf extract. The observed significant suppression of *Plasmodium falciparum* at higher concentrations is implicated by the synergistic effect of its phytochemical constituents. Specifically, the presence of flavonoids and terpenoids is noteworthy, as these compounds have been shown to disrupt the parasite's crucial redox system and inhibit the biosynthesis of fatty acids, both of which are essential for its survival and proliferation (Stevanovic, 2016).

This study aligns with broader research indicating the antimalarial properties of these phytochemical classes (Pandey & Rizvi, 2009; Mishra *et al.,* 2008). Microscopic examination of Giemsa-stained blood smears revealed a reduction in parasitized red blood cells across all samples treated with the extract. Parasite clearance was particularly evident in the 10

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mg/mL and 5 mg/mL groups after 72 hours of incubation. These findings suggest a significant antiplasmodial effect of the *Mangifera indica* leaf extract, indicating potential for further development as a therapeutic agent. Furthermore, saponins and tannins, also identified in the extract, may contribute to the antiplasmodial activity by altering the membrane permeability of the parasite or inhibiting critical enzyme systems necessary for its metabolic processes. This mechanism of action, involving membrane disruption, is a well-established strategy for many antimicrobial and antiparasitic agents (Akinmoladun *et al.*, 2007).

The observed dose-dependent activity of the extract, with significant suppression at 10 mg/mL, further strengthens its therapeutic promise. It is essential to note the absence of alkaloids and anthraquinones in the *M. indica* extract, suggesting that the antiplasmodial activity observed in this study is not primarily mediated by these compounds, unlike classic antimalarials such as quinine, which is a cinchona alkaloid. This pattern highlights the unique phytochemical profile and potential novel mechanisms of action of *M. indica* compared to existing antimalarial drugs.

While the results are encouraging, it has been proven that the effective concentrations of the crude extract are relatively high compared to those of purified compounds. Therefore, further studies should emphasized on the isolation and characterization of the active components responsible for the antiplasmodial effects. Elucidating the precise mechanisms of action at a molecular level will be crucial for optimizing their efficacy and understanding potential targets within the parasite. Also, pharmacokinetic studies are warranted to determine how these active compounds are absorbed, distributed, metabolized, and excreted in a biological system. It is essential to apply toxicity profiling before embarking on clinical applications of *M. indica* extract and its isolated compounds to ensure their safety. Moreover, *in vivo* studies using animal models of malaria (Lee et al., 2024) are indispensable to confirm efficacy and safety in a living organism, thereby bridging the gap between *in vitro* findings and potential therapeutic use. This comprehensive approach will provide a robust foundation for the possible development of *M. indica* as a novel antimalarial agent.

CONCLUSION

This study demonstrates the promising in-vitro antiplasmodial efficacy of *Mangifera indica* leaf extract against *Plasmodium falciparum*. The findings underscore the potential of local medicinal plants as sources of novel antimalarial agents. Continued investigation into compound isolation and in-vivo validation is encouraged to facilitate the development of phytotherapeutic alternatives in malaria-endemic regions.

REFERENCES

Abera, M., Kassie, A., Ahmed, N., Beyene, M., & Tsega, T. (2024). Knowledge, belief, practice and associated factors of oxygen remedy among health professionals in Ethiopia: A methodical review and meta-analysis. *PLoS One*, 19(9), e0309823.

Ahmed A.A., Muhammad I., Yakubu M.F., Usman H.A., Abubakar B., Eshun G., and Okesanya O.J. (2024). Antiplasmodial exertion of Aqueous Leaf Excerpts of *Mangifera Indica* Against *Plasmodium berghei* Infected Albino Rats. *Journal of BioMed Research and Reports, BioRes Scientia Publishers.* 5(6) 1- 9. DOI 10.59657/ 2837- 4681. brs.24.117.

Airaodion, A. I., Megwas, A. U., Edom, C. V., Winifred, N., Njoku, O. C., & Oladosu, N. O. (2021). Antiplasmodial eventuality of mango (*Mangifera indica*) stem dinghy against *Plasmodium berghei* in infected

E-ISSN: 3115-4662 DOI: 10.64290/bima.v9i2B.1300

Swiss albino mice. International Journal of Advanced Herbal and Alternative Medicine, 4(1) 42- 48.

Akinmoladun, F. O., Akinrinlola, B. L., & Olaleye, T. M. (2007). Phytochemical components and antioxidant components of excerpts from the leaves of *Mangifera indica*. *Journal of Medicinal shops Research*, 1(3), 104 – 107.

Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries: Part 2* (2nd ed.). Cambridge University Press.

Cailean Clarkson, V. J. M., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., Bhagwandin, N., Smith, P. J., & Folb, P. I.(2004). In vitro antiplasmodial exertion of medicinal shops native to or naturalized in South Africa. Journal of Ethnopharmacology, 92(2 – 3), 177 – 191.

Dudek, I., Czarnota, J., Hajduga, D., Dobrzyński, M., & Marzęda, M. (2019). The world's first malaria vaccine – promising clinical results. https://core.ac.uk/download/587243423.pdf

Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phae Phyo, A., Tarning, J., Lwin, K. M., Ariey, F., Hanpithakpong, W., Lee, S. J., Ringwald, P., Silamut, K., Imwong, M., Chotivanich, K., Lim, P., Herdman, T., Sam An, S., Yeung, S., Singhasivanon, P., ... White, N. J. (2009). Artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine, 361*(5), 455–467.

Evans, W. C. (1996). *Trease and Evans pharmacognosy* (14th ed.). W.B. Saunders Company.

Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.

Herber, R. F. M., & Stoeppler, M. (Eds.). (1994). *Trace element analysis in natural samples* (Vol. 15). Elsevier

Iverson, B. L., & Dervan, P. B. (2014). Journal of Agricultural Science and Technology, 16(1):7823- 7830.

Irokanulo, E.O, Nwonuma, C.O, Chukwudi, F.C. (2017) in vitro Antimicrobial Implication of *Coturnix japonica* Egg Shell and Membrane Greasepaint. *Nat Prod Ind J. 2017; 14(1) 115*

Lee, M., Kim, Y. S., Park, J., Choe, G., Lee, S. H., Kang, B. G., Jun, J. H., Shin, Y., Kim, M. C., Ahn, Y., & Lee, J. Y. (2024). A paintable and tenacious hydrogel cardiac patch with sustained release of ANGPTL4 for infarcted heart form. Bioactive Accoutrements. https// doi.org/10.1016/j.bioactmat.2023.08.020

Maharaj, A., Naidoo, Y., Dewir, Y. H., & Rihan, H.(2022). Phytochemical analysis and antibacterial and antioxidant conditioning of *Mangifera indica* L. leaves. Horticulturae, 8(10), 909. https// doi.org/10.3390/horticulturae8100909.

Mishra, K., gusto, A. P., & Swain, B. K. (2008). Antimalarial analysis of Andrographis paniculata and its major bioactive phytochemical andrographolide. Journal of Ethnopharmacology, 115(2), 221 – 229.

Muhammad, H. R., I. H. Nock, I. S. Ndams, B.D. J. George, Y. Deeni. (2024). Frequence and Distribution of Chloroquine Resistance and Susceptible Alleles of *Plasmodium falciparum* from named Primary Health Centers in NorthWestern Nigeria Chapter 2 Implications for Malaria Treatment. Print ISBN: 978-93-48006-70-7, eBook ISBN: 978-93-48006-80-6.

Mukhtar, M.D.; Bashir, M. and Arzai, A.H. (2006). Relative In vitro Studies on Antiplasmodial exertion of some Nigerian and Foreign Brands of Chloroquine Oral drugs retailed in Kano. African Excerpt of Piper betle L. molecules, 16, pp 107 – 118.

E-ISSN: 3115-4662 DOI: 10.64290/bima.v9i2B.1300

Masilamani, T., Yong, M. G., & Samsulrizal, N. (2022). In- vitro melanogenesis, cytotoxicity, and antioxidant conditioning of Peltophorum pterocarpum excerpts. https// core.ac.uk/ download/ 565311241. pdf

Okolie, N. J. C., & Kristhien, A. (2019). Toxicological and antiplasmodial analysis of ethanolic excerpts of mango (*Mangifera indica*) leaves and bitter cola (*Garcinia kola*) seeds in albino rats. International Journal of Scientific Research, 8(5) 1777- 1781.

Osuntokun O.T and Olajubu S.A (2014). Study of Phytochemical and Proximate Analysis of Seven Nigerian Medicinal shops. Applied Science Research Journal, VOL 2(1) 10 – 26, App. Sci. Res. J, Pon Publishers, Ekpoma, Edo state, Nigeria

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols are salutary antioxidants in health and unhealth. *Oxidative Medicine and Cellular Longevity, 2*(5), 270–278.

Phillipson, J. D., & Wright, C. W. (1991). Can ethnopharmacology contribute to the development of antimalarial agents? *Journal of Ethnopharmacology, 32*(1–3), 155–165.

Sabina, K., & Sharma, R. K. (2024). A review of pharmacological activities and bioactive compounds of *Mangifera indica* (*Mango*) *Saraca indica*. *Journal of Advancement in Pharmacognosy, 4*(2).

Shah, K., Patel, M., Patel, R., & Parmar, P. (2010). Mangifera indica (Mango). Pharmacognosy Reviews, 4(7):42–48.

Sharma, M., Yadav, S. P., & Kumar, S. (2018). Antimicrobial activity and phytochemical analysis of some medicinal herbs. Asian Pacific Journal of Tropical Disease, 8(10), 558 – 563. https// doi.org/10.1016/j.apjtd.2018.09.002

Sofowora, A. (1993). Medicinal herbs and traditional remedies in Africa (2nd ed.). Diapason Books.

Stephen T. Wang, Frank Peter, The Stability of Blood samples Lead in Storage, *Journal of Analytical Toxicology*, Volume 9, Issue 2, March-April 1985, Pages 85–88, https://doi.org/10.1093/jat/9.2.85.

Stevanovic, A. (2016). Characterization of infection in Drosophila following oral challenge with the Drosophila C viruses and Flock House viruses. https://doi.org/10.14264/uql.2016.169.

Thompson, C.B., Galli, R. L., Melaragno, A.J, Valeri, C.R.(1984). A system for the separation of size -based erythrocytes with counterflow centrifugation. Am J Hematol.17(2) 177- 83. doi 10.1002/ ajh.2830170209. PMID 6465134.

Talari, S., & Nanna, R. S. (2016). In vitro primary analysis of bioactive composites of different parts of *Adansonia digitata L*. A encyclopedically risked tree. International Journal of Phytomedicine. http// www.arjournals.org/index.php/ijpm/article/download/1743/909.

Trager, W., and Jensen, J.B. 1976. Malaria parasites in a continuous culture. Science, 193: 673–675.

Trease, G. E., Evans, W.C.A. (1996). *Textbook of Pharmacognosy* (14th Edn). Bailliere Tindall Ltd: London; pp. 60–75.

World Health Organization. (2023). *World Malaria Report 2023*. Geneva: WHO.