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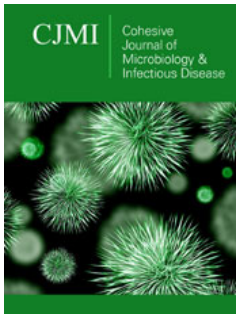

Potent *in Vitro* Antiplasmodial Effect of *Lawsonia inermis* (Henna) Extract Against *Plasmodium* Species

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Abstract

Malaria, a life-threatening disease caused by *Plasmodium* species, remains a major global health burden, particularly in tropical and subtropical regions. The increasing resistance of parasites to conventional antimalarial drugs highlights the urgent need for alternative therapeutic agents derived from natural sources. *Lawsonia inermis* (Henna) has been traditionally used in herbal medicine and is known for its diverse biological properties, including antimicrobial and antiparasitic effects. This study aimed to evaluate the *in vitro* antiplasmodial potential of *L. inermis* leaf extract against human malaria parasites. Fresh blood samples from malaria-infected patients were cultured and treated with different concentrations of the extract. Parasite development was monitored microscopically to assess the inhibitory effects. The results revealed a complete inhibition of parasite growth in all Henna-treated samples, whereas control cultures showed normal maturation of schizonts and gametocytes. These findings indicate that *Lawsonia inermis* possesses potent antimalarial activity, likely through interference with parasite nuclear division and support its further exploration as a promising source of plant-based antimalarial compounds.

Keywords: *Lawsonia inermis*; Henna; Antiplasmodial activity; *Plasmodium* species; *In vitro* study

Introduction

Malaria is a parasitic disease caused by *Plasmodium* species that replicate within human red blood cells, leading to significant morbidity and mortality worldwide [1]. Despite major advances in diagnosis and treatment, the emergence of drug-resistant *Plasmodium* strains continues to challenge malaria control and elimination efforts [2]. Given this challenge, natural products derived from medicinal plants have attracted increasing attention as potential sources of new antimalarial agents [3]. Among these plants, *Lawsonia inermis* (commonly known as Henna) has been traditionally used in various cultures for its medicinal, cosmetic and antimicrobial properties [4]. Several studies have reported that *L. inermis* exhibits a wide range of biological activities, including antioxidant, antibacterial and antiparasitic effects [5-7]. Phytochemical analyses have shown that *L. inermis* leaves contain phenolic compounds, flavonoids and naphthoquinones-particularly lawsone-that are believed to contribute to its pharmacological properties [6,7]. Recent *in vitro* and *in silico* studies have demonstrated the antiplasmodial potential of these bioactive constituents, suggesting that they may inhibit parasite heme polymerization, mitochondrial function and essential enzyme systems [8,9]. In particular, Afolayan FID et al. [9] identified potential molecular interactions between *L. inermis* compounds and key *Plasmodium* enzymes, providing a mechanistic explanation for its

inhibitory effect [9]. These findings support the scientific rationale for further evaluating *Lawsonia inermis* extracts as promising candidates for antimalarial drug development.

Materials and Methods

Preparation of *lawsonia inermis* (henna) extract

Fresh leaves of *Lawsonia inermis* were collected, thoroughly washed with distilled water and air-dried under shade in a dust-free environment for two weeks. The dried leaves were finely powdered using a clean mortar and pestle. A stock aqueous extract was prepared by dissolving 3.5g of the powdered leaves in 100mL of distilled water. The mixture was incubated in a water bath at 70 °C for 30 minutes with intermittent shaking. The extract was then filtered multiple times through sterile muslin cloth to obtain a clear solution. Serial dilutions were prepared from the stock solution to obtain six different concentrations for experimental use [6].

Collection and Processing of Blood Samples

Malaria-infected blood

Fresh blood samples from malaria-positive patients were collected in heparinized tubes. Samples were washed three times with RPMI 1640 medium to remove plasma and white blood cells [8].

Human serum

Venous blood from an O^+ healthy donor was collected in a plain tube, allowed to clot at room temperature for 45 minutes and centrifuged at 2000rpm for 10 minutes. The separated serum was heat-inactivated at 56 °C for 30 minutes with gentle mixing every 10 minutes to destroy complement activity [10].

Preparation of culture medium

A sodium bicarbonate solution was prepared by dissolving 84mg of sodium bicarbonate in 1mL of distilled water. The complete culture medium was prepared by mixing 9.5mL of RPMI 1640 with 0.2mL of human serum, 0.2mL of Gentamicin and 0.2mL of the sodium bicarbonate solution. The medium was equilibrated at 37 °C before use [11].

Preparation of red blood cells and hematocrit adjustment

The hematocrit of the culture mixture was adjusted to 3% by combining 0.3mL of infected red blood cells with 0.27mL of uninfected cells. When a matching blood type was unavailable, O-type red blood cells were used as substitutes [8].

In vitro culture and henna treatment

Parasite cultures were established in 24-well culture plates, with each sample inoculated into two wells-one for treatment and one as a control. Each well contained 0.97mL of complete medium mixed with 0.3mL of red blood cell suspension. Cultures were maintained in a CO_2-rich atmosphere using the candle jar method and incubated at 37 °C for 24 hours. On the second day, parasite growth was assessed microscopically and the culture medium was replaced with fresh medium. Henna extract was then added to the treatment wells at the predetermined concentrations, while the control wells remained untreated. The culture medium was refreshed daily and parasite development was monitored for up to four days (72 hours post-seeding and 48 hours after extract addition) [12].

Microscopic examination

For microscopic evaluation, 100µL of the culture was withdrawn after gently removing most of the supernatant. Thin and thick blood smears were prepared on clean glass slides. Thin smears were fixed by gentle flame heating, while both thin and thick smears were stained with Giemsa stain (10%)-thin films for 20 minutes and thick films for 10 minutes. Slides were examined under a light microscope at ×1000 magnification to assess parasite developmental stages and parasitemia levels [13].

Results

Microscopic examination of the cultured samples revealed different developmental stages of *Plasmodium* species, including *P. vivax* and *P. falciparum*, in the control wells. Parasite growth was characterized by the presence of trophozoites, schizonts and gametocytes under light microscopy. In contrast, cultures treated with *Lawsonia inermis* (Henna) extract demonstrated a marked reduction in parasite development across all concentrations. Complete inhibition of parasite growth was observed in all treated wells compared with the untreated controls. Morphological alterations, such as chromatin condensation and distorted nuclear structures, were evident in parasites exposed to Henna extract, suggesting interference with nuclear division and cellular replication. Sample 3, which was identified as non-fresh, failed to show parasite development even in the control well, highlighting the importance of using fresh and viable blood samples for successful *in vitro* culture. The comparative results of parasite growth in treated and control wells are summarized in Table 1.

Table 1: Effect of Lawsonia inermis (Henna) extract on parasite growth.

Sample	Parasite Species	Growth in Control	Growth in Treated Wells	Observation
1	*Plasmodium* vivax	Normal schizonts observed	No growth	Complete inhibition
2	*Plasmodium* falciparum	Gametocytes observed	No growth	Complete inhibition
3	-	No growth	No growth	Non-viable sample (not fresh)

Discussion

The findings of the present study clearly demonstrate that *Lawsonia inermis* (Henna) extract possesses strong *in vitro* antiplasmodial activity against *Plasmodium falciparum* and *P. vivax*. The complete inhibition of parasite growth observed in the treated cultures suggests that the extract contains bioactive constituents capable of interfering with key metabolic and reproductive pathways of the parasite. This aligns with previous reports that highlighted the antimalarial potential of plant-derived compounds as alternative therapeutic agents [3,5]. Several studies have supported the current findings. Sharma et al. reported that ethanolic extracts of *L. inermis* effectively inhibited *P. falciparum* schizont maturation *in vitro* [5]. Similarly, Kumar et al. attributed the potent antiplasmodial activity of *L. inermis* to its rich content of phenolic compounds, flavonoids and naphthoquinones, which may disrupt the parasite's oxidative metabolism and inhibit heme polymerization [6]. These phytochemical constituents, particularly lawsone, have been recognized as the major bioactive molecules contributing to the observed inhibition of parasite development [4-7]. Microscopic observations in this study revealed chromatin condensation and nuclear deformation in parasites treated with Henna extract, suggesting possible interference with DNA synthesis or mitochondrial function. Comparable morphological changes were described by Singh N et al. [7], who associated similar alterations with mitochondrial inhibition and Reactive Oxygen Species (ROS) generation in *Plasmodium* species [7]. Furthermore, *in silico* molecular docking analyses conducted by Afolayan FID et al. [9] demonstrated that major constituents of *L. inermis* have a high binding affinity to Plasmodium Dihydrofolate Reductase (PfDHFR) and cytochrome bc1 complex, two essential enzymes in parasite metabolism and survival [9]. These findings strongly support the mechanistic hypothesis that *L. inermis* may exert its antiplasmodial effects through inhibition of enzymatic pathways vital for parasite energy production and replication. Taken together, the results of the present study, along with supporting literature, validate the traditional ethnopharmacological use of Henna as an antimalarial remedy in various regions. The observed *in vitro* activity highlights the potential of *Lawsonia inermis* as a promising natural source for the development of novel plant-based antimalarial drugs. However, further studies involving compound isolation, structural characterization and *in vivo* evaluation are essential to confirm its efficacy, determine optimal dosages and assess toxicity and pharmacokinetics before potential clinical application [6,8].

Conclusion

Lawsonia inermis (Henna) extract showed a strong *in vitro* antiplasmodial effect, completely inhibiting the growth of *Plasmodium falciparum* and *P. vivax*. This confirms its potential as a natural source of antimalarial compounds. The use of fresh blood samples proved essential for successful parasite culture. Further studies are recommended to isolate and characterize the active constituents of *L. inermis*, clarify their mechanisms of action and evaluate their safety and efficacy through *in vivo* and clinical investigations.

Consent

The patient's written consent has been collected.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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