

Anti-Plasmodial Activity and Elemental Analysis of the Stem Bark Extract of *Caesalpinia pulcherrima*

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Research Article

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Abstract

The antiplasmodial activity of *Caesalpinia pulcherrima* (Pride of Barbados), a plant traditionally used in south-western Nigeria for the treatment of malaria, was investigated in the search for new antimalarial agents. Stem bark extracts and fractions were evaluated for in vivo antimalarial activity and elemental composition. Fractionation of the extracts was carried out using vacuum liquid chromatography (VLC), while elemental analysis was conducted using atomic absorption spectrophotometry (AAS). Antimalarial activity was assessed against *Plasmodium berghei berghei* in albino mice (20–30 g) using the 4-day suppressive test. Methanol–ethyl acetate stem bark extracts administered at doses of 200, 400, and 800 mg/kg produced dose-dependent chemo-suppression. The 200 mg/kg extract showed suppressive activities of 45.32%, 52.67%, 47.90%, and 24.17%; the 400 mg/kg extract showed 57.74%, 56.36%, 51.51%, and 30.35%; while the 800 mg/kg extract exhibited 63.29%, 62.09%, 54.24%, and 39.91%. These effects were lower than those of the standard drug chloroquine (5 mg/kg), which produced suppressive activities of 95.22%, 91.73%, 88.97%, and 86.22%. The results demonstrate that *C. pulcherrima* stem bark possesses significant antiplasmodial (suppressive) activity, supporting its traditional use in malaria treatment.

Introduction

Medicinal plants represent one of the richest sources of bioactive organic compounds and have contributed immensely to the discovery of new chemical entities used in medicine, cosmetics, food, and agrochemicals [1]. It has been reported that a significant proportion of modern pharmaceuticals are derived directly or indirectly from plant sources, highlighting the continued relevance of natural products in drug discovery [2, 3]. Scientific investigation of medicinal plants is essential for documenting the therapeutic potential of indigenous flora, providing a rational basis for their traditional use, developing affordable and effective herbal medicines, discovering novel drug leads, and validating ethnomedicinal knowledge. Such investigations also contribute to the conservation of medicinal plant resources and the sustainable utilization of biodiversity [4].

The use of medicinal plants has gained increasing acceptance, even among educated populations in urban settings. This trend may be attributed to the declining efficacy of several conventional drugs used in the management of infectious diseases such as fever, gonorrhoea, asthma, and tuberculosis, as well as the increasing prevalence of antimicrobial resistance and the rising cost of prescription medicines [5]. Several studies have confirmed that antimicrobial resistance represents a major global health challenge, particularly in developing countries where access to effective healthcare is limited [6, 7]. Furthermore, the rapid growth of the human population has placed significant pressure on modern healthcare systems, particularly in developing countries, thereby increasing reliance on natural herbal remedies. Herbal medicines are often perceived as affordable, accessible, and culturally acceptable alternatives to orthodox drugs [8]. The emergence of multidrug-resistant (MDR) pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus*, *Helicobacter pylori*, and MDR *Klebsiella pneumoniae*, has further revived interest in plant-derived therapeutic agents with antimicrobial properties. Plant secondary

metabolites such as alkaloids, flavonoids, tannins, and terpenoids have been shown to possess potent antimicrobial activities [9].

Malaria remains one of the most serious tropical parasitic diseases, accounting for substantial morbidity and mortality, especially among children and pregnant women [10]. The disease continues to pose a major public health and socioeconomic burden in endemic regions [11]. It is estimated that malaria affects approximately 300–400 million people worldwide, with about 1–2 million deaths recorded annually [12]. Africa continues to bear the highest burden of the disease due to widespread resistance to antimalarial drugs, insecticide resistance in mosquito vectors, environmental and climatic changes, population displacement, and increased human mobility. These factors have significantly complicated malaria control and elimination efforts in sub-Saharan Africa [13]. Although chloroquine, introduced in the 1950s, was once widely relied upon because of its safety, affordability, and efficacy, the development of resistance has severely limited its usefulness. The spread of chloroquine-resistant *Plasmodium falciparum* strains has necessitated the search for alternative antimalarial agents [14]. Artemisinin-based combination therapies (ACTs) have improved malaria treatment outcomes; however, challenges related to cost, accessibility, and emerging resistance persist [15, 16]. Reports of delayed parasite clearance have raised concerns regarding the long-term effectiveness of ACTs [15].

Caesalpinia pulcherrima (Pride of Barbados) is a medicinal plant widely distributed in tropical and subtropical regions and extensively used in traditional medicine across Africa, Asia, and South America. Different parts of the plant have been reported to possess significant medicinal value. Among Nigerian communities, the aerial parts are used as abortifacients, emmenagogues, purgatives, stimulants, and emollients [17]. In Angola, root extracts are traditionally used for the treatment of intermittent fevers, while the leaves are employed as purgatives, tonics, antipyretics, and emmenagogues. The roots are also used folklorically in the management of convulsions, intermittent fevers, and lung and skin diseases [18]. In Chinese traditional medicine, the fruits, flowers, leaves, and stem bark are commonly used to treat ailments such as pyrexia, menoxenia, wheezing, bronchitis, and malaria infections [19]. The wide ethnomedicinal application of this plant suggests the presence of biologically active constituents with diverse pharmacological properties [20].

Scientific investigations have provided evidence supporting many of these traditional claims. Aqueous extracts of the fresh leaves and stem bark of *C. pulcherrima* have been reported to exhibit strong in vitro antimalarial, antifungal, antibacterial, and antioxidant activities. The observed biological activities have been attributed to the presence of bioactive phytochemicals such as flavonoids, phenolics, and terpenoids [21]. Chiang et al. [22] demonstrated that whole-plant extracts possess broad-spectrum in vitro antiviral activity, while seed extracts have been reported to show significant broad-spectrum antimicrobial effects [23]. Additionally, the flowers and leaf sap have been used in the treatment of swellings (anti-inflammatory activity), muscular and rheumatic pains (analgesic activity), and various cardiovascular conditions, further highlighting the plant's broad pharmacological potential.

Despite these reports, there is still limited in vivo evidence validating the antiplasmodial efficacy of the stem bark of *Caesalpinia pulcherrima*, particularly in relation to its traditional use for malaria treatment. In vivo studies are essential for establishing pharmacological relevance and validating therapeutic efficacy under physiological conditions [24]. The aim of this study was to evaluate the antiplasmodial activity of stem bark extracts and fractions of *Caesalpinia pulcherrima*. The specific objectives were to assess the in vivo suppressive antimalarial activity of the extracts against *Plasmodium berghei* in albino mice and to determine the elemental composition of the stem bark using Atomic Absorption Spectrophotometry. Elemental analysis provides important information on trace elements that may contribute to the therapeutic effects of medicinal plants [25]

Materials and Methods

Collection of Plant Materials and Preparation

Fresh stem bark of *Caesalpinia pulcherrima* was harvested from Ugbowo Campus of the University of Benin, Nigeria. The plant was sent to the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin for identification and authentication. The collected stem bark was thoroughly washed with tap water to remove extraneous materials and air-dried at ambient temperature (30 ± 2 °C) for 14 days until a constant weight was obtained. The dried material was first reduced to coarse particles using a mortar and pestle and subsequently milled into a fine powder using a mechanical grinder. The powdered stem bark was stored in an airtight container and preserved at room temperature until required for further analysis.

Extraction of Crude Powdered Sample

Exactly 600 g of the powdered stem bark of *Caesalpinia pulcherrima* was exhaustively macerated in 2.5 L of methanol at room temperature for 72 hours with occasional stirring. The resulting mixture was first filtered through a clean cheesecloth and subsequently through Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. The dried crude extract was weighed, and the percentage yield was calculated relative to the initial weight of the powdered stem bark. The extract was stored in an airtight container and kept refrigerated until required for further analysis.

Vacuum Liquid Chromatography

Forty grams (40 g) of the crude extract of *Caesalpinia pulcherrima* stem bark was thoroughly adsorbed onto silica gel and mixed until a free-flowing powder was obtained. The dry-packed sample was subjected to vacuum liquid chromatography using silica gel as the stationary phase. Elution was carried out sequentially with solvent systems of increasing polarity to obtain different fractions. The solvent systems used in the analysis include 100% n-hexane, 50% n-hexane and 50% ethyl acetate solution, 100% ethyl acetate, 50% methanol and 50% ethyl acetate solution, and 100% methanol. Each fraction obtained was concentrated to dryness under reduced pressure using a rotary evaporator. The resulting dried fractions were stored in airtight containers and kept refrigerated until required for further analysis.

Experimental Animals

Swiss albino mice of either sex, weighing between 20 and 30 g, were obtained from the animal house of the Faculty of Pharmacy, University of Benin, Nigeria, and used for the study. The animals were housed in clean plastic cages at room temperature under a naturally illuminated environment. They were allowed to acclimatize to laboratory conditions for four weeks prior to the commencement of the experiment. The mice were fed with a standard pellet diet and had free access to clean drinking water *ad libitum*. All experimental procedures were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Preparation of Giemsa Solution (Stock Giemsa)

Three point five grams (3.5 g) of solid Giemsa stain was added to a mixture of 250 mL of methanol and 250 mL of glycerol in a dark reagent bottle. The mixture was shaken vigorously to ensure proper dissolution and then stored in a cupboard at room temperature for seven days to allow complete maturation before use in the analysis.

Preparation of Phosphate Buffer Saline (PBS)

1.09g of disodium hydrogen phosphate and 0.32 g of sodium dihydrogen phosphate were dissolved in 100 mL of distilled water with continuous shaking. The pH of the solution was adjusted to 7.2 by the dropwise addition of 0.1 M sodium hydroxide solution. The buffer was then made up to volume, mixed thoroughly, and stored at room temperature until required.

Parasite Inoculation

Chloroquine sensitive *plasmodium berghei* (NK65) was obtained from the National Institute of Medical Research (NIMR), Lagos, Nigeria. Each mouse used in the experiment was infected intraperitoneally with 0.1ml of infected blood containing about 1×10^7 parasitized red blood cells (PRBC).

In a normal, unaffected mouse, the number of red blood cells in 1ml of blood is approximately 5×10^8 , while in an infected mouse, the number of red blood cells in 1ml of blood is reduced to 3×10^8 . This 1ml of parasitised red blood cell (containing 3×10^8 RBC) was diluted with 3ml of phosphate buffer saline to give;

$$1\text{ml} \times 3 \times 10^8 \text{ (PRBC)} = 3 \text{ ml} \times X \text{ (PRBC)}$$

$$X = \frac{3 \times 10^8}{3} = 1 \times 10^8 \text{ (PRBC)}$$

Therefore, scaling down the volume by a factor of 10, this will give;

1×10^7 PRBC in 0.1ml of blood. It is this quantity of 1×10^7 PRBC that was introduced into the mouse as 0.1ml.

Sample Digestion

One gram (1.0 g) of the powdered stem of *Caesalpinia pulcherrima* was accurately weighed into a digestion flask. Ten millilitres (10 mL) of perchloric acid (HClO₄) and 10 mL of nitric acid (HNO₃) were carefully added in a fume cupboard. The contents were gently swirled and heated under the fume cupboard for approximately 10 minutes until the evolution of brown nitrogen dioxide (NO₂) fumes was observed, indicating oxidation of the organic matter. The digest was then allowed to cool to room temperature, filtered, and quantitatively transferred into a 50 mL volumetric flask. The filtrate was diluted to the 50 mL mark with distilled water and mixed thoroughly. The digestion procedure was carried out in triplicate, including a reagent blank, and the resulting solutions were analysed for heavy metal content using an Atomic Absorption Spectrophotometer (AAS)

Evaluation of the Suppressive Activity of Extract (Peter's 4-Days Test)

The suppressive antimalarial activity of the stem bark extract of *Caesalpinia pulcherrima* was evaluated using the standard 4-day suppressive test described by Knight and Peters (1980). On day one (D1), twenty-five Swiss albino mice were inoculated intraperitoneally with chloroquine-sensitive *Plasmodium berghei berghei*-infected red blood cells. The animals were randomly assigned to five groups (A–E) of five mice each, based on body weight. Treatments were administered once daily for four consecutive days (D1–D4) between 8:00 pm and 9:00 pm.

Group A served as the negative control and received no treatment. Groups B, C, and D were administered the stem bark extract orally at doses of 200, 400, and 800 mg/kg body weight, respectively. Group E served as the positive control and received chloroquine at a dose of 5 mg/kg body weight orally.

From day five (D5) to day eight (D8), blood samples were collected from the tail vein of each mouse for parasitological analysis. Thick and thin blood smears were prepared on clean glass slides, fixed with methanol, and stained with 4% Giemsa solution. After 45 minutes, the slides were rinsed with distilled water and allowed to air-dry. Parasitological examination was carried out using a light microscope, and parasitaemia was determined by counting parasitised red blood cells (PRBCs) and total red blood cells (RBCs) in ten different microscopic fields.

$$\% \text{ parasitaemia} = \frac{\text{number of parasitised red blood cells (PRBC)}}{\text{Total number of red blood cells (RBC) counted}} \times 100$$

While average percentage suppression was calculated as;

$$\frac{A - B}{A} \times 100$$

Where;

A = Average % parasitaemia in the negative control (group A)

B = Average % parasitaemia in the test group (group B, C or D)

Results and Discussion

Results and Discussion The results obtained from the in vivo anti-plasmodia studies, and elemental analysis of the extract from the stem bark of *C. pulcherrima* are shown in Tables 1, 2 and 3 below

Percentage yield of the extract

The percentage yield of the stem bark extract of *C. pulcherrima* obtained was 18.98%.

Comparison of %parasitaemia of the Stem Bark extract of *C. pulcherrima* and standard drug (chloroquine)

Antimalarial activity of the stem bark extract of *C. pulcherrima*

The stem bark extract of *Caesalpinia pulcherrima* demonstrated significant, dose-dependent antimalarial activity against chloroquine-sensitive *Plasmodium berghei* in Swiss albino mice, as reflected by percentage chemosuppression and mean survival time (Table 3). The extract at 200, 400, and 800 mg/kg produced progressive parasitaemia suppression from 45.32% to 63.29% on day 5, with corresponding increases in mean survival time from 17 to 26 days, compared to 14 days in the untreated control. The

highest dose (800 mg/kg) exhibited the greatest chemosuppressive effect, confirming a clear dose-response relationship. Although chloroquine (5 mg/kg) showed superior suppression (86.22–95.22%) and survival (28 ± 0.45 days), the extract's activity indicates substantial antiparasmodial potential.

The observed effect may result from bioactive phytochemicals, such as flavonoids, alkaloids, and tannins, which are known to interfere with the intraerythrocytic development of *Plasmodium* species. Additionally, the elemental composition of the stem bark, including iron, copper, calcium, and sodium, may support haematological and physiological functions during infection, potentially enhancing host resilience and contributing to prolonged survival. These findings corroborate traditional use of *C. pulcherrima* for malaria treatment and suggest that the stem bark extract could serve as a complementary or alternative antimalarial agent, particularly in areas where access to conventional drugs is limited.

The elemental analysis of *Caesalpinia pulcherrima* leaves revealed the presence of essential metals—sodium (11.73 ± 0.17 ppm), calcium (9.33 ± 3.46 ppm), iron (5.33 ± 1.25 ppm), and copper (0.22 ± 0.09 ppm)—with toxic lead being undetectable, indicating the safety of the extract for medicinal use. These elements may contribute synergistically to the plant's antimalarial activity. Iron supports erythropoiesis and helps counter malaria-induced anaemia, while copper enhances antioxidant defences, reducing oxidative stress caused by parasitic infection. Calcium and sodium play a crucial role in maintaining erythrocyte stability and overall physiological homeostasis, which can enhance the delivery and efficacy of bioactive compounds. The absence of lead ensures no additional haematological or toxicological risk. Therefore, the combination of bioactive phytochemicals and supportive trace elements likely underpins the observed dose-dependent parasitemia suppression, highlighting both the pharmacological potential and nutritional value of *C. pulcherrima* in traditional malaria treatment.

Conclusion

The study found that the stem bark extract of *Caesalpinia pulcherrima* shows significant antiparasmodial activity, although it is less effective than chloroquine. This activity may be linked to bioactive compounds such as alkaloids, flavonoids, saponins, and tannins previously reported in the plant. While the specific active molecules were not identified, their presence suggests a potential for inhibiting *Plasmodium* growth. The findings support the traditional use of this plant for malaria treatment in Nigeria and recommend further research on isolating and characterizing the active compounds to develop affordable, plant-based antimalarial therapies.

Declarations

The study titled "Anti Plasmodial Activity and Elemental Analysis of the Stem Bark Extract of *Caesalpinia pulcherrima*" involved the use of Swiss albino mice (20–30 g) of either sex obtained from the University of Benin, Edo State, Nigeria.

All animal handling and experimental procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The animals were maintained under standard laboratory conditions, as described in the methodology section of the manuscript. Oversight for the use of laboratory animals was provided by the University of Benin, where the animals were sourced and handled in line with institutional practices for animal care and use.

Conflict of interest

The authors declare no conflict of interest.

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Supplementary Files

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