Asian Journal of Biology

Volume 22, Issue 7, Page 1-10, 2026; Article no.AJOB.161977
ISSN: 2456-7124

Non-Hemolytic Effect and Antiplasmodial Activity of *Cassia sieberiana* Root Bark on *Plasmodium falciparum* Isolates

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Authors' contributions

This work was carried out in collaboration among all authors. Author KYY conceptualized the study, wrote and prepared the original draft of the manuscript, wrote, reviewed and edited the manuscript. KPMC harvested the roots of the plant material Cassia sieberiana. Authors KYKF and GK reading and correcting the manuscript. Author YHF supervised the work. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajob/2026/v22i7672

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/161977

Original Research Article

Received: 03/05/2026
Accepted: 09/07/2026
Published: 15/07/2026

Abstract

Introduction: Malaria remains endemic in Côte d'Ivoire, and *Plasmodium falciparum* is the predominant species associated with malaria attacks.
Aims: This study evaluated the phytochemical composition, haemolytic effect, and antiplasmodial activity of an aqueous extract of *Cassia sieberiana* root bark against *P. falciparum* isolates.
Methods: The aqueous extract was prepared by decoction, and selected secondary metabolites were identified using colorimetric methods. Haemolytic activity was assessed using uninfected O+ red blood cells, with

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Cite as: *Yves, K. Y., François, K. Y. K., Konan, G., Chantal, K. P. M., & Félix, Y. H. (2026). Non-Hemolytic Effect and Antiplasmodial Activity of Cassia sieberiana Root Bark on Plasmodium falciparum Isolates. Asian Journal of Biology, 22(7), 1–10. https://doi.org/10.9734/ajob/2026/v22i7672*

phosphate-buffered saline and Triton X-100 serving as the negative and positive controls, respectively. Antiplasmodial activity was evaluated against 10 *P. falciparum* isolates, with quinine used as the reference antimalarial molecule.

Results: The extract contained alkaloids, saponins, polyphenols, flavonoids, and catechic tannins, whereas gallic tannins, sterols and terpenes, and quinones were not detected. The measured haemolysis percentage was 0.619%. Antiplasmodial activity increased across the tested concentrations. At the highest tested concentration, the minimum and maximum IC50 values were 1.11 ± 0.00 µg/mL and 4.33 ± 0.00 µg/mL, respectively.

Conclusion: Under the reported *in vitro* conditions, the aqueous root-bark extract showed low haemolytic activity and inhibited *P. falciparum* isolates. These findings provide preliminary evidence that water-extractable constituents of *C. sieberiana* root bark may contribute to the observed antiplasmodial activity. Further standardised studies are required to confirm the dose-response relationship, cytotoxicity, selectivity, active constituents, and possible mechanisms.

Keywords: Malaria; Plasmodium falciparum; Cassia sieberiana; root bark; aqueous extract; antiplasmodial activity; haemolysis; phytochemical screening; IC50; quinine.

1. Introduction

Malaria is a disease caused by parasites of the genus *Plasmodium*. It is transmitted to humans through the bites of female mosquitoes of the genus *Anopheles*. The African region bears a substantial proportion of the global malaria burden (World Health Organization, 2024). In Côte d'Ivoire, malaria is endemic, and children under 5 years of age and pregnant women are particularly affected (U.S. President's Malaria Initiative, 2024). Parasites that were once sensitive to antimalarial treatments are developing resistance, while mosquitoes are becoming progressively less sensitive to insecticides (Kipre et al., 2015). Recent reviews have continued to identify medicinal plants and their secondary metabolites as relevant sources for antimalarial screening and subsequent compound-focused investigation (Irungu et al., 2023; Ribeiro et al., 2023). In this context, *Cassia sieberiana* (Fabaceae), a plant widely used in Africa for its medicinal properties, was selected. A recent phytochemical and acute-toxicity investigation of *C. sieberiana* leaves documented multiple constituent classes, providing species-specific context for examining alternative plant parts and extraction preparations (Enenche et al., 2024). Similarly, aqueous leaf extracts of other medicinal plants have shown antiplasmodial effects in a *P. berghei* mouse model, supporting the continued evaluation of aqueous preparations while recognising that findings from animal parasites are not directly equivalent to activity against *P. falciparum* isolates (Obia et al., 2023). An investigation of another Fabaceae species assessed root, stem-bark, and leaf extracts against laboratory strains and fresh *P. falciparum* field isolates, together with acute toxicity, illustrating the relevance of evaluating antiplasmodial activity and safety in parallel (Ochora et al., 2024). However, the available literature does not clearly establish the antiplasmodial activity and haemolytic profile of an aqueous *C. sieberiana* root-bark extract against *P. falciparum* isolates under the conditions examined in this study. This study therefore addresses the need for concurrent characterisation of extract constituents, red-blood-cell compatibility, and parasite inhibition. The general objective of this study was to promote traditional medicine by developing an improved traditional antimalarial. The specific objectives were to identify the secondary metabolites present in the aqueous extract of *Cassia sieberiana* root bark (Fabaceae), determine the haemolysis percentage of the aqueous extract of *Cassia sieberiana* root bark, and determine the concentrations that inhibit 50% of *Plasmodium falciparum*.

2. Materials and Methods

Plant Material: The plant material consisted of *Cassia sieberiana* root bark (Fig. 1) collected in Korhogo, Côte d'Ivoire. The National Floristic Centre of Félix HOUPHOUËT-BOIGNY University identified the plant under number UCJ 009184.

Biological material: The biological material (Fig. 2) consisted of malaria isolates (*Plasmodium falciparum*) obtained from the Research and Malaria Control Centre of the National Institute of Public Health in Abidjan, Côte d'Ivoire. Ethical matters were addressed in advance by the Research and Malaria Control Centre.

The Research and Malaria Control Centre of the National Institute of Public Health in Abidjan, Côte d'Ivoire, has a blood bank containing *Plasmodium falciparum*. This study involved testing the aqueous extract of *Cassia sieberiana* roots against *Plasmodium falciparum*.

Fig. 1. *Cassia sieberiana* tree

Fig. 2. Malaria isolates

Aqueous Extract Preparation: Before phytochemical screening, *Cassia sieberiana* root bark collected in Korhogo (Côte d'Ivoire) was dried away from sunlight for 7 weeks. The dried *Cassia sieberiana* root bark was ground, and the resulting powder was used to prepare the aqueous extract (Willcox et al., 2011). The *Cassia sieberiana* root bark collected in Korhogo (Côte d'Ivoire) was dried away from light for two months and ground into powder using an electric grinder. One hundred (100) grams of *Cassia sieberiana* root-bark powder was then added to a saucepan containing one litre of boiling distilled water, and the mixture was boiled for 20 minutes. After cooling, the mixture was first filtered through a sieve, and the resulting filtrate was filtered twice through hydrophilic cotton. The filtrate obtained after the final two filtrations was placed in a crystallising dish and dried completely in an oven at 40 °C. Finally, the dry material at the bottom of the crystallising dish was scraped and ground into a fine powder, which constituted the aqueous extract of *Cassia sieberiana* root bark.

Search for Secondary Metabolites: Selected secondary metabolites in the aqueous extract of *Cassia sieberiana* root bark were investigated using the colorimetric method (Evans, 2009).

Test for Flavonoids: Two (2) mL of plant extract was evaporated in a capsule without carbonising the residue. After cooling, the residue was reconstituted in 5 mL of hydrochloric alcohol (prepared by mixing 10 mL of 96° ethanol, 10 mL of distilled water, and 10 mL of concentrated hydrochloric acid), diluted twofold in a test tube. Two to three magnesium shavings were added, producing an exothermic reaction. A pink-orange or purple

colour developed. The addition of three drops of isoamyl alcohol intensified the pink-orange or purple colour, indicating the presence of flavonoids. The control was prepared using an alcoholic solution of quercetin.

Test for Saponins: Ten (10) mL of plant extract was placed in a test tube measuring 160 mm in height and 16 mm in diameter. The test tube was shaken vigorously for 10 seconds. The foam height was measured after 10 minutes of rest. A foam height greater than 1 cm indicated the presence of saponins. The presence of saponins could also be demonstrated by the persistence of the foam.

Test for Phenols: One drop of a 2% alcoholic ferric chloride solution was added to 2 mL of plant extract. The development of a blackish-blue or dark or light green colour indicated the presence of phenolic compounds.

Test for alkaloids: Alkaloids were detected using Dragendorff's and Bouchardat's reagents. Six millilitres of plant extract was evaporated on a sand bath. The extract residue was reconstituted in 6 mL of 60° alcohol, and the resulting alcoholic solution was divided between two test tubes. Two drops of Dragendorff's reagent were added to the first tube. The formation of a precipitate or an orange colour indicated the presence of alkaloids. Two drops of Bouchardat's reagent were added to the second tube. The development of a reddish-brown colour indicated their presence.

Test for Tannins: Five (5) mL of extract was evaporated. Fifteen millilitres of Stiasny reagent was added to the dry residue. The mixture was maintained in a water bath at 80 °C for 30 minutes and then cooled under running water. The formation of a large, flaky precipitate indicated the presence of catechic tannins. The solution containing the flakes was filtered, and the collected filtrate was saturated with sodium acetate. Three drops of 2% ferric chloride were added to the mixture. The development of an intense blue-black colour indicated the presence of gallic tannins.

Test for Sterols and Polyterpenes: These compounds were detected using the Liebermann reaction. Five (5) mL of plant extract was evaporated on a sand bath. The residue was dissolved in 1 mL of hot acetic anhydride, and 0.5 mL of concentrated sulphuric acid was added. The appearance of a purple ring at the interface, which changed from blue to green, indicated a positive reaction.

Test for Quinones: Quinones were identified using Borntraeger's reagent, which enables the detection of quinone substances. Two millilitres of plant extract was evaporated to dryness in a capsule. The residue was mixed with 5 mL of hydrochloric acid diluted 1:5. The solution was placed in a boiling water bath for 30 minutes in a test tube, cooled under running cold water, and extracted with 20 mL of chloroform. The chloroform phase was collected in a test tube and mixed twice with 0.5 mL of dilute ammonia. The development of a red-to-purple colour indicated the presence of quinones.

Evaluation of the Haemolytic Effect of *Cassia sieberiana* Root Bark: Haemolytic activity was assessed by determining the percentage of haemolysis (Malagoli, 2007). For this purpose, O+ blood not infected with *Plasmodium falciparum* was used. Ten (10) µL of the aqueous extract of *Cassia sieberiana* was placed in an Eppendorf microtube and mixed with 190 µL of a 10% red-blood-cell suspension. Positive and negative control solutions were prepared. The negative control consisted of 10 µL of PBS and 190 µL of a 10% red-blood-cell suspension, while the positive control consisted of 10 µL of 20% Triton X-100 and 190 µL of a 10% red-blood-cell suspension. The sample and control solutions were incubated for 1 hour at room temperature with gentle agitation. The tubes were then centrifuged for 5 minutes at 2,200 rpm, and 150 µL of each supernatant was transferred to a 96-well culture plate. Absorbance was measured at 550 nm using a plate reader (Multiskan FC, Thermo Scientific). The formula below was used to calculate the percentage of haemolysis:

$$\% \text{ Hemolysis} = \frac{\text{Absorbance of } \textit{Cassia sieberiana} - \text{Absorbance of negative control}}{\text{Absorbance of positive control} - \text{Absorbance of negative control}} \text{ x } 100$$

Determination of the Antiplasmodial Effect of *Cassia sieberiana* Root Bark: Ten milligrams (10 mg) of the dry aqueous extract of *Cassia sieberiana* root bark was weighed and dissolved in 10 mL. The mixture was vortexed for 10 minutes to dissolve the extract completely. The resulting aqueous solution was filtered through a 0.22 µm Millipore filter. The newly filtered aqueous solution was used to evaluate the antiplasmodial effect of *Cassia sieberiana* root bark.

Reference Antimalarial Molecule: Quinine was used as the reference antimalarial molecule.

Obtaining the Inoculum: Ten (10) malaria isolates were used to evaluate the antimalarial effect of the aqueous root-bark extract of *Cassia sieberiana*. A total of four (04) culture plates were used for these ten *Plasmodium falciparum* isolates. Each malaria isolate was centrifuged at 3,000 rpm for 5 minutes. After centrifugation, the serum was removed from each tube using a sterile Pasteur pipette, leaving a red-blood-cell pellet at the bottom of the tube. RPMI-160 wash medium was added to the pellet in each tube, and the tubes were sealed and centrifuged at 3,000 rpm for 5 minutes. After centrifugation, the supernatant was removed from each tube using a sterile Pasteur pipette. Three washing steps were performed on the cell pellet (Trager & Jensen, 1976).

A quantity of RPMI-1640 washing medium was added to the red-blood-cell pellet, and the mixture was homogenised using a vortex mixer for 5 minutes. The supernatant (malaria-infected blood cleared of serum and white blood cells) was collected to prepare the inoculum. The inoculum for one 96-well culture plate was prepared by mixing 0.6 mL of the supernatant with 11.4 mL of complete medium (RPMI-160 medium enriched with 0.5% Albumax) (Trager & Jensen, 1976).

Arrangement of the Aqueous Extract of *Cassia sieberiana* and Quinine Solutions on the Culture Plate: The culture plate consisted of 96 wells arranged in a matrix of 8 rows (A to H) and 12 columns (1 to 12). The aqueous extract of *Cassia sieberiana* root bark and quinine were cultured in duplicate on the plate in an 8-row (A to H) by 6-column (1 to 6) matrix. Fig. 3 shows duplicate cultures of the aqueous extract of *Cassia sieberiana* root bark and quinine in the presence of *Plasmodium falciparum* (*P. falciparum*) on the culture plate. The mass concentrations of the aqueous extract solution in rows A, B, C, D, E, F, G, and H were 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0 g/mL, respectively, while the corresponding mass concentrations of quinine were 2.496, 1.248, 0.624, 0.312, 0.156, 0.078, 0.039, and 0 g/mL, respectively. Each culture plate contained three different isolates (I).

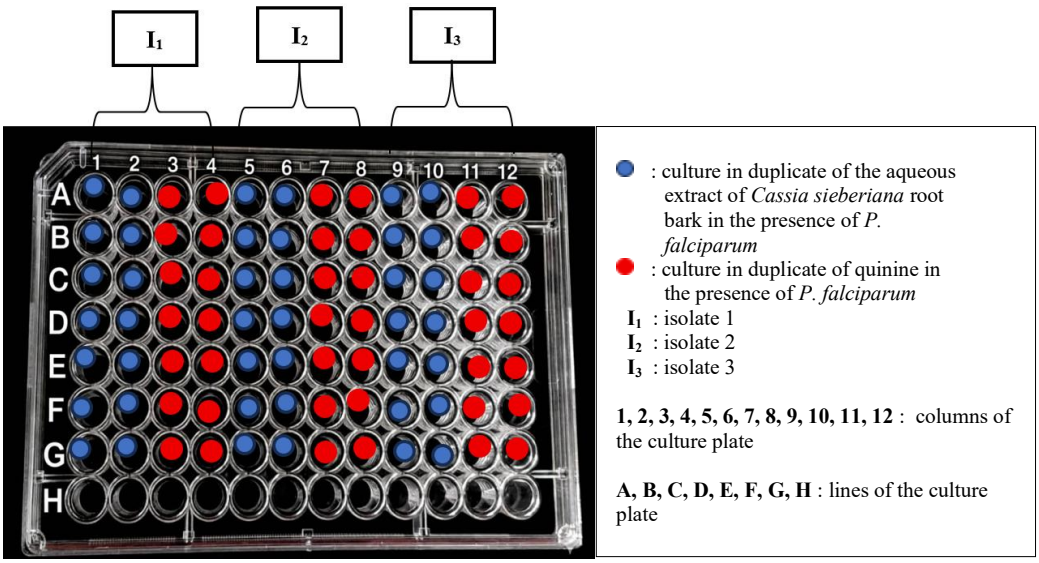

Fig. 3. Duplicate culture of the aqueous extract of *Cassia sieberiana* root bark and quinine in the presence of *Plasmodium falciparum*

Incubation of culture plates: The plates containing the cultures were placed in a bell jar containing a sheet of moistened blotting paper, two lit candles measuring 2 cm in height, and two capsules filled with water. The bell jar was closed, and once the candles had been extinguished, the culture plates were placed in an incubator at 37 °C for 72 hours (Trager & Jensen, 1976).

Plate-reading Phase: After 72 hours of incubation, the culture plates were removed from the incubator and then from the desiccator. For each culture plate, a new empty culture plate was prepared to receive the cultures in the same arrangement as the previous plate. The new culture plate was closed, and the isolate numbers and the date and time at which filling was completed were recorded on the lid. The new culture plate was then wrapped in aluminium foil and placed in the dark for 1 hour at 37 °C. After incubation in the dark, the new culture plates were removed, and the aluminium foil was taken off. The lid of each new culture plate was removed, and the

plate was placed in a BioTek multimode reader to determine the inhibitory concentrations in each well at 535 nm (Trager & Jensen, 1976).

Classification of Antiplasmodial Activity: The antiplasmodial effects of the aqueous extract of *Cassia sieberiana* root bark and the reference antimalarial molecule, quinine, were classified according to the concentrations that inhibited 50% (IC50) of *Plasmodium falciparum* (Table 1) (Bero & Quetin-Leclercq, 2011).

Table 1. Classification of the antiplasmodial effect of plant extracts and pure antimalarial molecules (Bero & Quetin-Leclercq, 2011)

IC50 of plant extracts	Antiplasmodial effect	IC50 of pure antimalarial molecules	Antiplasmodial effect
IC50 > 50 µg/mL	Inactive	IC50 > 50 µM	Inactive
15 < IC50 ≤ 50 µg/mL	Moderate	11 < IC50 ≤ 50 µM	Moderate
5 < IC50 ≤ 15 µg/mL	Promising	2 < IC50 ≤ 11 µM	Active
IC50 ≤ 5 µg/mL	Very active	IC50 ≤ 1 µM	Very active

3. Results

3.1 Secondary Metabolites

Phytochemical analyses revealed the presence of secondary metabolites, including alkaloids, saponins, polyphenols, flavonoids, and catechic tannins, in the aqueous extract of *Cassia sieberiana* root bark. However, sterols and terpenes, gallic tannins, and quinones were absent from the extract (Table 2).

Table 2. Secondary metabolites in the aqueous extract of *Cassia sieberiana* roots bark

Secondary metabolites	Aqueous extract of *Cassia sieberiana* root bark
Saponins	+
Polyphenols	+
Flavonoids	+
Catechic Tannins	+
Gallic Tannins	-
sterols and terpenes	-
Quinones	-
Alkaloids	+

Presence : +
Absence : -

3.2 Haemolysis Percentage

The absorbance of the negative control, comprising phosphate-buffered saline (PBS) and 10% red blood cells, was zero, while that of the positive control, comprising Triton X-100 and 10% red blood cells, was 0.49700. The absorbance of the sample, comprising the aqueous extract of *Cassia sieberiana* root bark and 10% red blood cells, was 0.00308. These data yielded a haemolysis percentage of 0.619%.

3.3 Antiplasmodial Effect

At mass concentrations of 0.3125, 0.625, 1.25, and 2.5 g/mL, the aqueous root-bark extract of *Cassia sieberiana* proved inactive against the ten (10) *Plasmodium falciparum* isolates distributed across four (04) culture plates, as the obtained IC50 values were greater than 50 µg/mL (IC50 > 50 µg/mL).

At a mass concentration of 5 g/mL, the extract had a moderate effect on the ten (10) *Plasmodium falciparum* isolates distributed across four (04) culture plates because the obtained IC50 values ranged from 15 to 50 µg/mL (15 < IC50 ≤ 50 µg/mL). At this concentration, the minimum IC50 was 17.08 ± 0.00 µg/mL, and the maximum IC50 was 43.00 ± 0.00 µg/mL.

At a mass concentration of 10 g/mL, the aqueous extract of *Cassia sieberiana* root bark showed a promising effect on the ten *Plasmodium falciparum* malaria isolates, as the obtained IC50 values ranged from 5 to 15 µg/mL (5 < IC50 ≤ 15 µg/mL). At this concentration, the minimum IC50 was 6.09 ± 0.05 µg/mL, and the maximum IC50 was 14.11 ± 0.00 µg/mL.

At a concentration of 20 g/mL, the aqueous extract of *Cassia sieberiana* root bark had a strong effect on the ten (10) *Plasmodium falciparum* isolates distributed across our (04) culture plates because the obtained IC50 values were below 5 µg/mL (IC50 ≤ 5 µg/mL). At this concentration, the minimum IC50 was 1.11 ± 0.00 µg/mL, and the maximum IC50 was 4.33 ± 0.00 µg/mL.

At mass concentrations of 0.039 and 0.312 g/mL, the reference antimalarial molecule, quinine, was inactive against the ten (10) *Plasmodium falciparum* isolates distributed across four (04) culture plates because the obtained IC50 values were greater than 50 µM (IC50 > 50 µM).

At a mass concentration of 0.624 g/mL, quinine was not very active against the ten (10) *Plasmodium falciparum* isolates distributed across four (04) culture plates because the IC50 values ranged from 11 to 50 µM (11 < IC50 ≤ 50 µM). At this concentration, the minimum IC50 was 13.29 ± 0.00 µM, and the maximum IC50 was 39.64 ± 0.00 µM.

At a mass concentration of 1.248 g/mL, quinine was active against all ten (10) *Plasmodium falciparum* isolates distributed across four (04) culture plates because the IC50 values ranged from 2 to 11 µM (2 < IC50 ≤ 11 µM). At this concentration, the minimum IC50 was 3.17 ± 0.00 µM, and the maximum IC50 was 10.76 ± 0.0 µM.

At a mass concentration of 2.496 mg/mL, quinine was very active against the ten (10) *Plasmodium falciparum* isolates distributed across four (04) culture plates because the IC50 values were less than or equal to 1 µM (IC50 ≤ 1 µM). At this concentration, the minimum IC50 was 0.71 ± 0.00 µM, and the maximum IC50 was 0.99 ± 0.00 µM.

Table 3 summarises the effects of the aqueous extract of *Cassia sieberiana* and quinine on *Plasmodium falciparum*.

Table 3. Effects of aqueous extract of *Cassia sieberiana* and quinine on *Plasmodium falciparum*

	Aqueous extract of Cassia sieberiana (0.3125, 0.625, 1.25, and 2.5 g/mL)	**Quinine (0.039 and 0.312 g/mL)**
Minimum IC50	Not determined because the aqueous extract of *Cassia sieberiana* was inactive against *Plasmodium falciparum*	Not determined because the aqueous extract of *Cassia sieberiana* was inactive against *Plasmodium falciparum*
Maximum IC50	Not determined because the aqueous extract of *Cassia sieberiana* was inactive against *Plasmodium falciparum*	Not determined because the aqueous extract of *Cassia sieberiana* was inactive against *Plasmodium falciparum*
	Aqueous extract of Cassia sieberiana (5 g/mL)	**Quinine (0.624 g/mL)**
Minimum IC50	17.08 ± 0.00 µg/mL	13.29 ± 0.00 µM
Maximum IC50	43.00 ± 0.00 µg/ mL	39.64 ± 0.00 µM
	Extrait aqueux de *Cassia sieberiana* (10 g/mL)	**Quinine (1.248 g/mL)**
Minimum IC50	6.09 ± 0.05 µg/mL	3.17 ± 0.00 µM
Maximum IC50	14.11 ± 0.00 µg/mL	10.76 ± 0.0 µM
	Extrait aqueux de *Cassia sieberiana* (20 g/mL)	**Quinine (2.496 g/mL)**
Minimum IC50	1.11 ± 0.00 µg/mL	0.71 ± 0.00 µM
Maximum IC50	4.33 ± 0.00 µg/mL	0.99 ± 0.00 µM

4. Discussion

This study began with the harvesting and drying of *Cassia sieberiana* roots away from sunlight. Drying the *Cassia sieberiana* roots was intended, in part, to stop enzymatic reactions within the plant-material cells (Marston & Hostettmann, 2006). Grinding the dried *Cassia sieberiana* root bark increased the surface area in contact with the solvent and facilitated solvent penetration during decoction at 100 °C for 20 minutes.

Phytochemical screening of the aqueous extract of *Cassia sieberiana* root bark revealed the presence of secondary metabolites, including alkaloids, saponins, polyphenols, flavonoids, and catechic tannins. The presence of secondary metabolites in the aqueous extract may have been facilitated by boiling at 100 °C for 20 minutes. Boiling caused cellular disruption, thereby facilitating water penetration and the dissolution of hydrophilic molecules (Miguel, 2010). The presence of secondary metabolites in the aqueous extract of *Cassia sieberiana* root bark may also be explained by the role of plant roots as sites for the synthesis and storage of many secondary metabolites (Wink, 2010). However, gallic tannins, sterols, and quinones were absent. The polyphenols, flavonoids, alkaloids, and catechic tannins identified in the aqueous extract of *Cassia sieberiana* roots were also reported in the aqueous extract of *Cassia sieberiana* leaves (Kouamé et al., 2025).

The haemolysis percentage of 0.619% indicates that the aqueous extract of *Cassia sieberiana* root bark was non-haemolytic because the value was below 5% (Koffi, 2017). This result indicates that the antiplasmodial test was not confounded by extract-induced red-blood-cell lysis. In the context of malaria, the haemolysis test assesses the ability of a plant extract to lyse red blood cells and is important for evaluating the preliminary safety of an extract with antimalarial potential. Such testing can support the traditional use of plants for treating malaria by assessing their compatibility with blood cells, particularly red blood cells (Koffi, 2017).

The antiplasmodial effect of the aqueous extract of *Cassia sieberiana* root bark may be associated with the secondary metabolites present in the extract, including alkaloids, polyphenols, flavonoids, tannins, and saponins. Part of this effect may be associated with alkaloids, as quinine, a reference antimalarial molecule, is an antimalarial alkaloid extracted from the root bark of *Cinchona officinalis* (Achan et al., 2011). Quinoline alkaloids act by inhibiting the degradation of haemoglobin by *Plasmodium falciparum*. The antiplasmodial activity of the aqueous extract may also be associated with tannins and flavonoids (Cushnie et al., 2014). Flavonoids are thought to inhibit the intra-erythrocytic growth of *Plasmodium falciparum*, while tannins may damage the parasite by chelating metal ions and reducing ferric iron (Fe3+) to ferrous iron (Fe2+), which is essential for parasite survival. Saponins have surfactant properties that may promote pore formation, thereby slowing the development of *Plasmodium falciparum* and damaging the parasite (Bero et al., 2009). At a mass concentration of 20 mg/mL, the aqueous extract of *Cassia sieberiana* root bark may have acted similarly to quinine because it inhibited the development of *Plasmodium falciparum* more effectively at this concentration.

5. Conclusion

This study showed that the aqueous extract of *Cassia sieberiana* root bark contained several classes of secondary metabolites, including alkaloids, saponins, polyphenols, flavonoids, and catechic tannins. Under the tested conditions, the extract showed low haemolytic activity, suggesting that it did not cause marked red-blood-cell lysis in the in vitro haemolysis assay. The extract also showed antiplasmodial activity against *Plasmodium falciparum* isolates, with stronger inhibition at higher tested concentrations. These findings suggest that *C. sieberiana* root bark contains water-extractable constituents that may contribute to antiplasmodial activity. However, the results should be interpreted only as preliminary in vitro evidence. Further studies are required to confirm the activity through standardised dose-response assays, isolate and identify the active compounds, evaluate cytotoxicity and selectivity, clarify possible mechanisms of action, and assess safety and efficacy in appropriate experimental models before any therapeutic use can be recommended.

6. Limitation

This study was limited to the *in vitro* evaluation of a single aqueous extract of *Cassia sieberiana* root bark against *Plasmodium falciparum* isolates. The findings therefore cannot be directly extrapolated to therapeutic efficacy or safety in humans. Cytotoxicity against non-erythrocytic cells, selectivity indices, and broader dose–response relationships were not assessed. Moreover, the active phytochemical constituents were not isolated or quantified, and their mechanisms of antiplasmodial action were not experimentally confirmed. Further standardised *in vitro* studies, compound characterisation, and appropriate *in vivo* investigations are required.

Ethical Approval and Consent

The Research and malaria Control Center of the National Institute of Public Health in Abidjan - Côte d'Ivoire has a bank of *Plasmodium falciparum* isolates. The written consent of malaria patients for blood sampling is handled beforehand with the research and malaria control center. Our aqueous extract from *Cassia sieberiana* roots has only been tested on these isolates.

Acknowledgement

The authors thank the Research and Malaria Control Center of the National Institute of Public Health of Côte d'Ivoire for allowing our aqueous extract of *Cassia sieberiana* root bark to be tested on *Plasmodium falciparum* malaria isolates that they had in their laboratory.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all authors, including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The authors acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, and reference formatting. These AI-assisted tools were not used as authors and did not replace the authors' intellectual contributions or scholarly judgement. All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the authors. The authors accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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