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Phytochemical profiling of *Cassia sieberiana* parts utilized in indigenous Sierra Leonean malaria therapy

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ABSTRACT

Objective: To qualitatively analyse the phytochemical composition of the extracts of the different *Cassia sieberiana* (Gbangba) plant parts and to evaluate the anti-plasmodial activity of extracts of the different parts against *Plasmodium* parasites. To compare the anti-plasmodial activity of the different plant extracts against a control standard.

Methodology and Results: Phytochemical screening and thin-layer chromatography (TLC) were employed to identify and quantify the bioactive compounds present. A diverse range, including alkaloids, flavonoids, tannins, saponins, anthraquinones, and terpenoids, were observed, with varying concentrations across the different plant parts. However, only alkaloids and saponins were seen consistently across the roots, stems, and leaves. The stems were found to be particularly rich in almost all the phytochemicals, followed by the roots. Leaves, however, only showed trace amounts. An anti-plasmodial assay demonstrated concentration-dependent activity over 24, 48, and 72 hours, with roots showing the highest activity, followed by stems.

Conclusion and Application of findings: Cassia sieberiana demonstrates a rich phytochemical composition, with alkaloids, flavonoids, saponins, and anthraquinones contributing to its notable antimalarial efficacy. The stem and root extracts showed strong anti-plasmodial activity, eliminating parasites within 24 hours at 100% concentration, with the root extract being effective even at lower concentrations. In contrast, the leaf extract, with fewer metabolites, displayed minimal activity. These findings highlight the therapeutic potential of *C. sieberiana*, particularly its stem and root extracts, as promising sources of bioactive compounds for antimalarial drug development. To advance these results toward practical application, further work should focus on identifying and quantifying individual phytochemicals using high-performance liquid chromatography (HPLC), clarifying dose-response relationships through controlled experiments, and determining whether activity arises from synergistic interactions or single compounds. Clinical validation and mechanistic studies will be essential to translate laboratory findings into effective therapies, positioning *C. sieberiana* as a candidate for integration into malaria control programs.

Keywords: Malaria, *Cassia sieberiana,* Extract, Anti-plasmodial

INTRODUCTION

Malaria continues to pose a serious global health threat, with nearly half of the world's population at risk across almost 100 countries and territories (World Health Organization [WHO], 2023). The disease remains most prevalent among vulnerable populations such as children and pregnant women in tropical and subtropical regions, particularly in sub-Saharan Africa, which accounts for more than 90% of cases (Namusisi, 2025). In 2022 alone, WHO reported 249 million malaria episodes worldwide, 94% of which occurred in Africa, resulting in over 600,000 deaths (WHO, 2023). Malaria, caused by *Plasmodium* parasites transmitted through infected *Anopheles* mosquitoes, significantly impacts public health, healthcare systems, and economic productivity in endemic countries such as Sierra Leone (Turay *et al*., 2025). Despite progress with insecticide-treated nets and antimalarial drugs, the emergence of drug resistance, particularly in *Plasmodium falciparum*, remains a major challenge (González Sanz *et al*., 2023). Given these challenges, increasing interest has turned to traditional herbal medicine (THM), which has long been used across civilizations such as Greece, Egypt, China, and India (Mwendwa, 2025). Many modern pharmaceuticals derive from THM, with notable examples including curcumin from turmeric, St. John's Wort, garlic, ginger, and most significantly, artemisinin from *Artemisia annua*, discovered by Tu Youyou in 1972 as a breakthrough against chloroquine-resistant malaria (Geoffrey, 2025). Herbal medicine remains essential in Africa, where up to 80% of populations in some nations rely on traditional practitioners due to affordability and accessibility (Namusisi, 2025). However, concerns persist regarding assumptions of efficacy, placebo effects, and lack of standardized dosage or characterization of active compounds (Geoffrey, 2025). *Cassia sieberiana*, locally known in Sierra Leone as 'gbangba', is widely used in traditional medicine for fever, constipation, skin infections, and malaria (Kébé *et al*., 2024; Turay *et al*., 2025). Phytochemical studies reveal bioactive compounds such as alkaloids, flavonoids, glycosides, terpenoids, saponins, and anthraquinones, supporting its use as an antibacterial, anti-inflammatory, and analgesic agent (Turay *et al*., 2025). Despite its widespread traditional use, limited scientific evidence exists to confirm its efficacy and safety, particularly in Sierra Leone. Previous studies have examined samples from Nigeria or Senegal (Kébé *et al*., 2024) or explored traditional uses without detailed phytochemical analysis, leaving a gap in evidence-based validation of Sierra Leonean sources. This study, therefore, aimed to conduct a comprehensive phytochemical analysis of *C. sieberiana* leaves, stems, and roots sourced from Sierra Leone, with specific objectives to qualitatively analyse phytochemical composition, evaluate anti-plasmodial activity against *Plasmodium falciparum*, and compare the activity of different plant extracts against a control standard. This research sought to validate traditional use, contribute to evidence-based healthcare practices, and support integration into national malaria control programs.

MATERIALS AND METHODS

Study Setting and Design: This experimental laboratory study was conducted at the Biological Sciences Laboratory, Fourah Bay College; the Sierra Leone Pharmacy Board Laboratory; the Department of Pharmaceutical Sciences Laboratory, College of Medicine and Allied Health Sciences; and the Connaught Hospital Laboratory, University of Sierra Leone Teaching Hospital Complex. The study focused on assessing the phytochemical

composition of *Cassia sieberiana* leaves, stems, and roots and evaluating their anti-plasmodial properties.

Figure 1: *Cassia sieberiana* tree (Tropical The Ferns, accessed 16 May 2026)

Sample Collection and Identification: Plant samples (leaves, stems, and roots) of *C. sieberiana* were collected from the Gloucester Community, Western Area, Sierra Leone. The samples were verified as pest- and disease-free and authenticated at the Biological Sciences Laboratory, Fourah Bay College.

Preparation of Extracts: Leaves, stems, and roots were separately washed, sterilized with ethanol, and air-dried for 28 days at 27°C. Each dried sample was ground into powder, stored in sterilized Ziploc bags, and labelled accordingly. For extraction, 60 g of powdered material was soaked in 500 ml of 95% ethanol in a 1000 ml conical flask for seven days with intermittent swirling to ensure thorough mixing of the contents. The mixtures were separately filtered using linen cloths and funnels, and the filtrates were allowed to sediment before drying in a desiccator. Both dried and liquid extracts were stored in sterilized containers.

Qualitative Phytochemical Analysis: Phytochemical profiling was performed using thin-layer chromatography (TLC) to detect alkaloids, anthraquinones, flavonoids, saponins, and terpenoids. Silica gel plates were spotted with extracts and developed in solvent systems specific to each compound class (e.g., methanol–ammonium hydroxide for alkaloids, chloroform–methanol for anthraquinones, chloroform–ethanol for flavonoids). Plates were exposed to iodine vapour, visualized under UV light, and retention factor (Rf) values calculated to compare compounds. Further phytochemical screening was conducted using standard methods described by Fulton (1932), Harper (1975), Baibaa (1986), Wall *et al.* (1954), and Trease and Evans (1989), with modifications. Tests included Dragendorff's reagent for alkaloids, Borntrager's test for anthraquinones, Molisch's, Fehling's, and Benedict's tests for carbohydrates, acid–base reactions for flavonoids, frothing tests for saponins, and ferric chloride/vanillin hydrochloric acid tests for tannins.

Test Samples and test organism: Blood samples averaging 3 ml were obtained from five patients (ages 8 months, 3, 7, 18, and 32 years) diagnosed positive for malaria via the Rapid Diagnostic Test (RDT) kits. The samples exhibited high parasitaemia of *Plasmodium falciparum* (10,000–32,000 parasites/µl).

Anti-plasmodial Assay: Stock solutions of extracts were prepared at concentrations of 100%, 10%, and 1% using serial dilution. Thick blood smears were prepared, fixed in methanol, and stained with Giemsa for microscopic examination. For anti-plasmodial testing, 0.5 ml of infected blood was mixed with 50 µl of extract in test tubes, thoroughly mixed on a roller mixer, and incubated for 24, 48, and 72 hours. Parasite counts were observed under a light microscope at 100x magnification with immersion oil.

Parasite load was calculated using the formula:

$$P = \frac{\text{No. of parasites left} \times 8000}{200}$$

RESULTS

Determination of constituents from thin-layer chromatography: The results obtained from thin-layer chromatography on the silica gel plates are shown below in Figures 1- 5.

Figure 2: Alkaloid thin chromatography plates for *C. sieberiana* extract (root, stem and leaf)

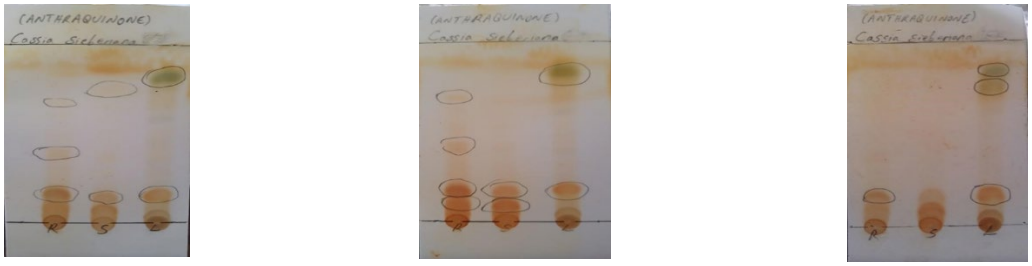

Figure 3: Anthraquinone thin-layer chromatography plates for *C. sieberiana* extracts (root, stem and leaf)

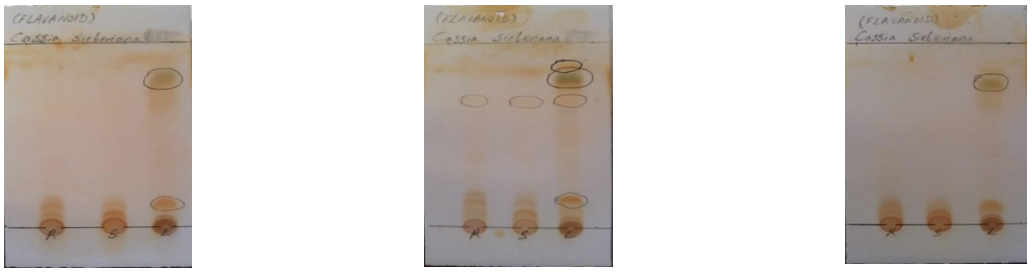

Figure 4: Flavonoid thin chromatography plates for *C. sieberiana* extracts (root, stem and leaf)

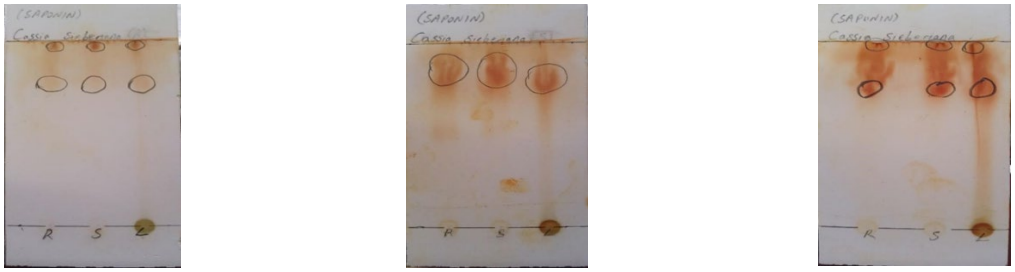

Figure 5: Saponin thin-layer chromatography plates for *C. sieberiana* extracts (root, stem and leaf)

Figure 6: Terpenoid thin layer chromatography plates for *C. sieberiana* extracts (root, stem and leaf)

From Figure 1, alkaloids were detected in all three extracts. Figures 2 and 3 indicate that the leaf extract contained higher levels of anthraquinones and flavonoids compared to the root and stem. According to Figure 4, saponins were present in all three extracts, while Figure 5 shows that terpenoids were also detected across all extracts, with the leaf exhibiting the highest.

Table 1: Thin layer chromatography of the different *Cassia sieberiana*

Phytochemical	Solvent System	Plant Part	Rf value	Vapor Reagent
Alkaloid	Methanol Ammonia Hydroxide (17:3)	Root	0.84, 0.82, 0.82	Iodine
		Stem	0.86, 0.81, 0.83	
		Leaf	0.86, 0.81, 0.81	
Flavonoid	Chloroform Ethanol (18:3)	Root	0.68, 0.81	Iodine
		Stem	0.68	
		Leaf	0.13, 0.79, 0.11, 0.77, 0.15, 0.81, 0.87	
Anthraquinone	Chloroform Methanol (18:2)	Root	0.42, 0.70, 0.33, 0.67	Iodine
		Stem	0.75	
		Leaf	0.61, 0.81, 0.59, 0.83, 0.73, 0.82	
Saponin		Root	0.74, 0.76	Iodine

	Chloroform Glacial acetic acid Methanol Water (6:2:1:1)	Stem	0.74, 0.74	
		Leaf	0.74, 0.74	
Terpenoid	Petroleum ether Ethyl acetate (1:1)	Root	0.85, 0.87, 0.26, 0.65	Iodine
		Stem	0.87	
		Leaf	0.59, 0.75, 0.83, 0.61, 0.78, 0.87, 0.59, 0.75	

The observed Rf values for alkaloids in the roots, stem, and leaves fall within the typical range, indicating that these values are consistent with known standards for alkaloids. The observed Rf values for flavonoids in the roots and stem align well with the standard range, although some values for leaves (0.11, 0.13, 0.15) fall slightly outside the typical range. This suggests a diverse presence of flavonoid compounds in the leaves. The Rf values for anthraquinones in the roots and stem fit well within the standard range. Some values for leaves (0.81, 0.83, 0.82) exceed the standard range, indicating higher mobility in the chosen solvent system. The observed Rf values for saponins in all plant parts (roots, stem, leaves) are higher than the typical range. This suggests a strong interaction between the saponins and the solvent system used. The Rf values for terpenoids in the roots, stem, and leaves are mostly consistent with the standard range, although some values (e.g., 0.26 for root) are on the lower end. The 0.87 value in the stem indicates a strong presence in this part. Phytochemical screening of the leaves, stem, and roots of *Cassia sieberiana,* with ethanol extracts.The screening of the aqueous extracts of *C. sieberiana,* shows the following phytochemical composition in the different plant parts. The tables below show the presence of phytochemical components in the leaves, stems, and the roots.

Table 2: Phytochemical Screening of the leaves, stem and roots of *C. sieberiana*

Components	**A**			**B**		
	L	**S**	**R**	**L**	**S**	**R**
Tannins	++	+++	--	++	--	++
Saponins	+	+++	+			
Flavonoids	+	+++	++			
Anthraquinones	+	++	+++			
Alkaloids	+	++	+			
Carbohydrates						
Molisch's	+	--	++			
Fehling's	+	++	+++			
Benedict's	+	+	++			

Key: A – *Cassia sieberiana test;* **B** – *Confirmatory test;* **L** – Leaves; **S** – Stem; **R** – Roots; +++ Abundance, ++ Moderate, + Trace and – Absent.

In the *C. sieberiana* analyses, tannins were most prevalent in the stem, moderate in the leaves, and absent in the roots; in contrast, the confirmatory test revealed moderate levels in the roots. The stem contains a lot of saponins, which are also found in the leaves and roots. The stem has a lot of flavonoids, the roots have modest amounts, and the leaves have trace amounts. There are minimal amounts of anthraquinones in leaves, considerable amounts in stems, but an abundance in roots. Alkaloids appeared in traces in leaves and roots, and are moderately present in stems. According to Molisch's test, there are trace amounts of carbohydrates in leaves, substantial amounts in roots, and none in the stems. Fehling's test indicates that in the roots carbohydrates are abundant, moderate in the stems, and show traces in the leaves. Benedict's test indicates that the roots had moderate carbohydrates and the leaves and stem had traces.

Anti-plasmodial tests results of the leaves, stem, and roots of *Cassia sieberiana,* ethanol extract within 24 hours: The anti-plasmodial testing of *C. sieberiana* ethanol extracts showed that the leaves, stem and root of *C. sieberiana* eradicate the parasite from the blood samples.

Table 3: Anti-plasmodial tests results produced by ethanol extract of *C. sieberiana* within 24 hours

	Treatment group/Plant extract									
Concentration 100% (g/ml)	**Water p/*µl* (p count)**		**Artesunatep/*µl* (p count)**		**Leaf p/*µl* (p count)**		**Stem p/*µl* (p count)**		**Root p/*µl* (p count)**	
	A	B	A	B	A	B	A	B	A	B
1%	10,000 (250P)	10,000 (250P)	10,000 (250P)	1,000 (25P)	22,000 (550P)	22,000 (550P)	32,000 (800P)	32,000 (800P)	10,000 (250P)	8,000 (200P)
10%	10,000 (250P)	10,000 (250P)	10,000 (250P)	20 (5P)	22,000 (550P)	22,000 (550P)	32,000 (800P)	32,000 (800P)	10,000 (250P)	2,320 (58 P)
100%	10,000 (250P)	10,000 (250P)	10,000 (250P)	NMPS	22,000 (550P)	22,000 (550P)	32,000 (800P)	NMPS	10,000 (250P)	NMPS

Keys: **A**= Initial parasite count **B**= Final parasite count **NMPS**= No malaria parasite seen **P**= Parasite
(Water was a negative control used in this experiment with Artesunate as the positive control.)

From Table 3, the positive control is highly effective in reducing the parasite count, with higher concentrations resulting in near-total elimination of parasites. Leaf extracts show no reduction in parasite count across all concentrations, indicating low or no antimalarial activity. Stem extracts are also largely ineffective except at the highest concentration (100%), where they eliminate all measurable parasites. Root extracts exhibit a gradual reduction in parasite count with increasing concentration, with complete elimination at 100% concentration.

Anti-plasmodial test results of the leaves, stem, and roots of *Cassia sieberiana,* ethanol extract within 48 hours: The anti-plasmodial testing of *C. sieberiana* ethanol extracts showed that leaves, stem the and root of *C. sieberiana* eradicating the parasite from the blood sample.

Table 4: Antiplasmodial tests results produced by ethanol extract of *C. sieberiana* within 48 hours

	Treatment group/Plant extract									
Concentration 100% (g/ml)	**Water p/μl (p count)**		**Artesunate p/μl (p count)**		**Leaf p/μl (p count)**		**Stem p/μl (p count)**		**Root p/μl (p count)**	
	A	B	A	B	A	B	A	B	A	B
1%	10,000 (250P)	10,000 (250P)	1,000 (25P)	20 (5P)	22,000 (550P)	22,000 (550P)	32,000 (800P)	32,000 (800P)	8,000 (200P)	8,000 (200P)
10%	10,000 (250P)	10,000 (250P)	20 (5P)	NMPS	22,000 (550P)	22,000 (550P)	32,000 (800P)	32,000 (800P)	2,320 (58 P)	2,000 (50P)
100%	10,000 (250P)	10,000 (250P)	NMPS	NMPS	22,000 (550P)	16,600 (415P)	NMPS	NMPS	NMPS	NMPS

Keys: **A=** Initial parasite count **B=** Final parasite count **NMPS=** No malaria parasite seen **P**= Parasite
(Water was a negative control used in this experiment with, Artesunate as the positive control.)

From table 4, Artesunate was most effective at 10% and 100%, with complete elimination of the parasites at both percentages. The leaf extract had limited efficacy, only showing moderate activity at the highest concentration (100%). The stem had no activity at low (1%) and medium (10%) concentrations but highly effective at 100%, while the root extract shows moderate to high efficacy, with complete elimination at 100%.

Anti-plasmodial tests results of the leaves, stem, and roots of *Cassia sieberiana,* ethanol extract within 72 hours: The anti-plasmodial testing of *C. sieberiana* ethanol extracts showed that the leaves stem and root of *C. sieberiana* eradicating the parasite from the blood sample.

Table 5: Anti-plasmodial tests results produced by ethanol extract of Cassia sieberiana within 72 hours.

	Treatment group/Plant extract									
Concentration 100% (g/ml)	**Water p/μl (p count)**		**Artesunatep/μl (p count)**		**Leaf p/μl (p count)**		**Stem p/μl (p count)**		**Root p/μl (p count)**	
	A	B	A	B	A	B	A	B	A	B
1%	10,000 (250P)	10,000 (250P)	20 (5P)	NMPS	22,000 (550P)	18,000 (450P)	32,000 (800P)	30,000 (750P)	8,000 (200P)	8,000 (200P)
10%	10,000 (250P)	10,000 (250P)	NMPS	NMPS	22,000 (550P)	18,000 (450P)	32,000 (800P)	16,000 (400P)	2,000 (50 P)	800 (20 P)
100%	10,000 (250P)	10,000 (250P)	NMPS	NMPS	16,600 (415P)	3,040 (76 P)	NMPS	NMPS	NMPS	NMPS

Keys: A= Initial parasite count **B=** Final parasite count **NMPS=** No malaria parasite seen **P**=Parasite
(Water was a negative control used in this experiment with, Artesunate as the positive control.)

From table 5, Artesunate demonstrates high efficacy at all concentrations, with complete parasite elimination at 100% concentration. The leaf extract shows limited to moderate antimalarial activity, with some reduction only at the highest concentration (100%). Stem extract exhibits some reduction of the parasite load at 1%, further reduction is seen at 10% and a total elimination of the parasites was maintained at the highest concentration being 100%. The root extract at 10% shows significant reduction at the 10% concentration and continues to maintain high efficacy in reduction at 100%.

DISCUSSION

The phytochemical screening of *Cassia sieberiana* revealed a diverse profile across roots, stems, and leaves, with alkaloids, flavonoids, anthraquinones, and saponins detected in varying abundance. The Rf values obtained for these compounds fall within typical ranges, confirming the reliability of the plant extracts for medicinal use. The prevalence of tannins in the stem agrees with earlier findings by Parekh and Chanda (2008), while the moderate presence in leaves and absence in roots reflects tissue-specific distribution. Alkaloids were detected in trace amounts in leaves and roots and moderately in stems, consistent with Bhambhani *et al*. (2021), who reported alkaloids as more concentrated in stems and roots than leaves. Carbohydrate detection showed some divergence: Molisch's test indicated absence in stems, contradicting Trease and Evans (2009), while Fehling's and Benedict's tests confirmed reducing sugars in roots and stems, aligning with Sofowora (1993) and Edeoga *et al*. (2005). These variations highlight the importance of using multiple confirmatory assays in phytochemical analysis. The anti-plasmodial assays demonstrated that stem and root extracts eliminated parasites at 100%

concentration within 24 hours, while the root extract also showed significant activity at 10%. The leaf extract, however, exhibited limited efficacy even at 100% concentration after 72 hours. This strong activity in stem and root extracts can be attributed to their high concentrations of flavonoids, saponins, and anthraquinones, compounds widely recognized for their antimalarial properties (Masi and Evidente, 2020). Similar findings have been reported by Kuete and Efferth (2010) and Ajaiyeoba *et al*. (2003), who demonstrated the anti-plasmodial potential of flavonoids and anthraquinones in other medicinal plants. Baba et al. (2015) also highlighted the therapeutic relevance of stem extracts rich in diverse metabolites. The results therefore place *C. sieberiana* within the broader context of African medicinal plants with promising antimalarial activity. The ability of root and stem extracts to eliminate parasites rapidly suggests potential for developing plant-based therapies that complement or enhance existing antimalarial drugs. Given the global challenge of drug resistance in malaria treatment, these findings underscore the relevance of *C. sieberiana* as a candidate for further pharmacological development.

CONCLUSION AND APPLICATION OF RESULTS

Cassia sieberiana demonstrates a rich and varied phytochemical composition, with alkaloids, flavonoids, saponins, and anthraquinones contributing to its notable antimalarial efficacy. The stem and root extracts showed strong anti-plasmodial activity, eliminating parasites within 24 hours at 100% concentration, while the root extract was effective even at lower concentrations. The leaf extract, with fewer metabolites, displayed minimal activity. These findings highlight the therapeutic potential of *C. sieberiana*, particularly its stem and root extracts, as sources of bioactive compounds for antimalarial drug development. To strengthen these findings and move toward practical application, further work is recommended. High-performance liquid chromatography (HPLC) should be used to identify and quantify individual phytochemicals. Studies should determine whether the anti-plasmodial activity of stem and root extracts results from single active compounds or from synergistic interactions among phytochemicals, requiring isolation and purification. Additional experiments at concentrations between 10% and 100% with statistical analysis will clarify dose-response relationships. Controlled trials with larger sample sizes are needed to validate reproducibility, while mechanistic studies should explore how *C. sieberiana* phytochemicals exert their anti-plasmodial effects. These steps will help translate laboratory findings into therapeutic applications, positioning *C. sieberiana* as a potential source of novel antimalarial agents.

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