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Antimalarial activity of the aqueous extract and anthraquinones from the root of *Senna siamea* (LAM) H.S. Irwin & Barneby (Fabaceae).

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ABSTRACT

This study evaluated the antimalarial properties of the aqueous extract of the root of *S. siamea* in mice and identified two bioactive phytoconstituents from the antimalarial ethyl acetate fraction. The extract demonstrated interesting antimalarial activity with significant ($P < 0.5$) percentage parasitaemia suppression of 88.02±1.46 % and clearance of 80.96±3.25 % in *Plasmodium beighei*-infected mice. The ED_{50} values were 194.98 for the suppressive assay and 100 mg/kg for the curative assay. LD_{50} was above 2000 mg/kg. Two anthraquinones, Chrysophanol and Cassiamin A, with remarkable antimalarial activity were isolated. These compounds at 10 mg/kg body weight produced % parasitaemia clearance of 68.90 % by Chrysophanol and 70.25 % by Cassiamin A in Rane's curative assay. Mice treated with both compounds recorded a higher haem concentration relative to the untreated group, suggesting haem polymerization as a possible mechanism of antiplasmodial action. The aqueous root extract of *S. siamea* possesses antimalarial properties. Chrysophanol and Cassiamin A from the ethyl acetate fraction showed antimalarial activity. The compounds act by preventing the conversion of parasite-toxic haem into inactive haemozoin within parasitized erythrocytes. The antimalarial activity of the aqueous extract of *S. siamea* and Cassiamin A are reported for the first time. Our findings provide a rationale for the continued use of *S. siamea* root decoction in the traditional treatment of malaria in Ghana.

Introduction

Malaria remains one of the world's most debilitating diseases in terms of morbidity and mortality. This is particularly so in communities with inadequate health facilities [40]. According to a recent World Health Organization (WHO) report [41], a staggering 2.2 billion cases of malaria and 12.7 million deaths have been prevented worldwide since 2000. Despite this progress, malaria remains a noteworthy global health concern, with the WHO African Region bearing the brunt of the disease. In 2023, this region accounted for

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approximately 94 % of malaria cases and 95 % of malaria deaths globally, with children under 5 years old being disproportionately affected [41].

The World Health Organization recommends Artemisinin Combination Therapy (ACT) as the primary treatment for malaria. However, there are reported emergence of *P. falciparum* resistance to Artemisinin Combination Therapy (ACT) in Asia [4] and Africa [35]. This underscores the urgent need to discover novel antimalarial compounds or/and develop alternative antimalarial remedies, especially from botanicals which have, over the years, served as sources of many drugs including antimalarial agents such as quinine [14] and artemisinin [25].

In Ghana and other Sub-Saharan African countries, many people use medicinal plants to treat malaria. These plants may be employed solely or as adjuncts to orthodox drugs [10]. Many such plants were scientifically validated for their anti-plasmodial/antimalarial activities [37].

In Ghanaian Traditional Medicine, *Senna siamea* is a widely used medicinal plant for treating malaria [18]. The aqueous decoction of its root has been a common remedy. The leaves have been traditionally used to treat malaria [16]. The aqueous extract of the root showed a weak *in vitro* antiplasmodial activity [19]. The ethanol and chloroform leaf extracts exhibited antimalarial properties and yielded antiplasmodial alkaloids, cassiarin A and B in a previous study [16]. The cassiarin series, cassiarin A-B [24] from the leaves and cassiarin C-E from flowers [30], also showed antiplasmodial activity. Lupeol from the plant also showed antiplasmodial activity [29]. Many anthraquinones including emodin, Chrysophanol, and Cassiamin A-C were isolated from the plant [16]. *S. siamea* is a tropical plant, indigenous to southeast Asia, India, and Sri Lanka. It is widely distributed in Africa (Ethiopia, Ghana, Nigeria, Zambia), Latin America and Oceania [7]. It is used to manage other diseases such as gastrointestinal disorders, diabetes, dysentery [8].

The traditional use of S. siamea root in malaria treatment is not backed by scientific evidence, particularly *in vivo* studies, which highlights the need for further research into its antimalarial properties. This work, therefore aimed to evaluate the antimalarial activity of the aqueous root extract of *S siamea* using murine models, and isolate constituents from the most active organic fraction of the root.

Materials and methods

Reagents, solvents, and equipment

Analytical grade solvents including petroleum ether, ethyl acetate, chloroform and methanol were obtained from VWR chemicals BDH (France). Immersion oil and Giemsa stain were obtained from UK Chemical (Kumasi, Ghana). Silica gel (70–230 mesh) and Precoated aluminium support (Merck silica gel 60 F254) were obtained from Merck KGaA (Darmstadt, Germany). Artesunate was purchased from Phyto-Riker, Accra, Ghana.

Equipment included a Mettler Toledo electronic balance (ME204 ME, Vietnam), oven (United Kingdom) and Rotary evaporator (Stuart SRC4, COLE-PARMER Ltd., UK)

Plant material collection and processing

The plant material was harvested in March 2021 at Kwahu Asakraka in Ghana's Eastern Region (06°37.356'N/ 000°41.393'W). It was authenticated by Prof. Merlin Mensah of the Department of Herbal Medicine, KNUST. A voucher specimen with number, KNUST/HM1/2021/R024, was stored at the herbarium of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST.

The outer scaly cover of the root was scraped and the root subsequently washed and cut into smaller sizes, and air dried under shade for 14 days with occasional turning. The air-dried material was ground into a coarse powder with a mechanical grinder.

Extraction of plant material

About 500 g of the dried powdered material was decocted in 1.5 L of water (mimicking the traditional method of preparation) for 30 mins. The mixture was sieved through cotton wool and the filtrate was further filtered through Whatman No 1 filter paper (Whatman®, England). The filtrate was freeze-dried and the dried extract was kept in a desiccator until needed for use.

The powdered root was also serially Soxhlet-extracted with organic solvents comprising petroleum ether, ethyl acetate and methanol. The most active against plasmodium parasite would be used to isolate the bioactive compounds in the root.

Table 1

Table 1
Plant extracts and yield.

Sample	%Yield
Senna siamea aqueous	3.45
Senna siamea methanol fraction	6.50
Senna siamea ethyl acetate fraction	3.30
Senna siamea petroleum ether fraction	0.67

Qualitative phytochemical screening

The powdered root was tested for the presence of various classes of secondary metabolites including alkaloids, tannins, glycosides, triterpenoids, phytosterols, coumarins and flavonoids using standard methods [13].

Experimental animals

Mixed sexes of Swiss albino rats and mice were purchased from the Noguchi Memorial Institute for Medical Research (NMIMR) of the University of Ghana, Legon, Accra. The animals were accommodated in the Department of Pharmacology's animal house, KNUST. They were exposed to a 12:12 dark-to-light cycle for 7 days, and offered unhindered access to clean water and a diet made of pellets prior to the experiment.

Ethical considerations

The *in vivo* experiments were conducted in line with the Institution's standard operating procedures. At the end of the experiments, animals were euthanised with diethyl ether, and the carcass buried in a designated location. The KNUST Ethics Committee for research using animals approved the protocol for the conduct of the experiment (ID KNUST 002).

Acute toxicity test

The aqueous extract was assayed for acute toxicity using a total of three (3) Swiss albino rats. The limit test was adopted since *S. siamea* has a long history of traditional medicine use without demonstrable toxicity [27]. Rats were dosed with a single 2000 mg/kg body weight of extract using a stomach tube after they were starved overnight but with unrestricted access to water. Feeding was delayed for another 3 h, during which time the rats were monitored at 30-minute intervals for the first 1 hour. They were then monitored occasionally for the next 24 h, with specific attention paid to the first 4 h. Thereafter, observation was made daily for the next 13 days for indicators of toxicity including rigidity, sleepiness, abnormal secretion, hair erection and death.

Parasite passage and inoculation

Chloroquine-sensitive ANKA *Plasmodium berghei* were passaged in doner Swiss albino rats. Parasitised rats were euthanised by cervical dislocation and the blood collected into heparinized vacutainer tubes via cardiac puncture [5]. The blood was diluted with physiological saline (0.9 %) to yield 5×10^7 parasite-infected RBC per millilitre of blood. Experimental mice were inoculated with about 0.2 mL diluted blood containing approximately 1×10^7 infected RBC and accordingly used for the *in vivo* antimalarial study.

Peter's four-day suppressive antimalarial test

The suppressive antimalarial properties of the aqueous extract of *Senna siamea* was assessed using the Peter's 4-day suppressive assay [31]. Three (3) hours post-inoculation, infected-mice, randomly divided into 6 groups of 5, were treated with 50, 100, 200 and 400 mg/kg/day of the aqueous extract, 4 mg/kg/day of artesunate (positive control) and 2 mL normal saline/day (negative control) for four (4) days. On the 5th day of treatment, thin blood smears were prepared by taking blood sample from the tail vein of the mice. Parasitaemia was determined by counting the number of parasitized red blood cells out of total red blood cells in five randomly chosen fields of the slide using a light microscope (Leica DM750, Wetzlar-Germany). % Parasitaemia and percentage parasitaemia suppression were estimated using the formulae:

$$\%\ parasitaemia = \frac{number\ of\ parasitized\ erythrocytes}{total\ number\ of\ erythrocytes\ counted} \times 100\%$$

$$\%\ \text{Parasitaemia} - \text{suppression}: \frac{A - B}{A} \times 100\%$$

where A is the mean % parasitaemia of the negative control group and B, the mean % parasitaemia of the test group.

Body weight changes in animals during treatment were determined and the animals trucked for mortality over 30 days. Survival curves were plotted and median survival time determined [2].

Rane's curative test

The curative antimalarial potential of the aqueous extract of S. siamea was evaluated according to the method by Nardos et al. [26]. Seventy-two (72) hours following parasite inoculation, mice with established parasitaemia were randomly divided into six groups of 5. These groups were dosed with 50, 100, 200 and 400 mg/kg/day of extract, 4 mg/kg/day of artesunate (positive control) and 2 ml of normal saline (negative control) daily for 4 consecutive days. % Parasitaemia was determined on day 5 of treatment using thin blood smears, and percentage parasitaemia clearance estimated. Body weight changes in mice during treatment was determined. Mice were

monitored for survival over 30 days and a survival curve constructed and the median survival time extrapolated.

Fractionation of the ethyl acetate fraction and isolation of phytoconstituents

Following the *in vitro* antiplasmodial assay of the different organic (petroleum ether, ethyl acetate and methanol) fractions of the root, the ethyl acetate fraction emerged the most active. This fraction was, thus, subjected to series of chromatographic fractionations to obtain the bioactive constituents. About 34 g was chromatographed on a silica gel (70–230 mesh) column by gradient elution (petroleum ether/ethyl acetate/methanol) to afford 8 fractions (SE1–SE8). Fraction SE1 was similarly fractionated on silica gel (70–230 mesh) column with an isocratic Pet. Ether/ethyl acetate; 9/1 solvent system to yield a precipitate (250 mg). The precipitate was washed in Pet. Ether/ethyl acetate (9.5:0.5) to yield an orange compound, SR1 (80 mg). Fraction SE3 was also column chromatographed on silica gel (70–230 mesh) and eluted with gradient solvent systems comprising petroleum ether and ethyl acetate to obtain the compound SR2.

In vivo antimalarial activity of isolated compounds

The isolated compounds, identified as Chrysophanol (SR1) and Cassiamin A (SR2), were assessed for antimalarial activity in the Rane's curative assay. Two (2) groups of 5 infected mice were treated with 10 mg/kg compound daily for 4 days. The negative and positive control groups (5 mice each) received 2 ml normal saline and 4mg/kg artesunate. A day following the last treatment, mice were humanely killed by cervical dislocation and blood collected via cardiac puncture into EDTA vacutainer tubes. Thin blood smears were prepared, and the % parasitaemia and % parasitaemia clearance subsequently estimated.

Haem concentration was determined by mixing 10 μL blood with 250 μL of 10 % Sodium dodecyl sulfate (SDS) (w/v) and 250 μL of 1 M NaOH. The mixture was sonicated for 10 mins to lyse the red blood cells, denature and dissolve the haem. The mixture was incubated at room temperature for 2 h and absorbance was read at 404 nm wavelength. The concentration of haem, in mmol/ml, *C*, was estimated according to Asakura *et al.*, [3].

$$C = \frac{A}{\varepsilon \times L}$$

Where C = Haem concentration (mol/L), A = absorbance, ε = Haem molar absorption coefficient (taken to be $9.08 \times 10^4\ M^{-1}cm^{-1}$), L = path length.

Data analysis

Data was analysed using Graph Pad Prism. (GraphPad Software version 8.0.2, San Diego, CA, USA) and presented as mean ± SD. Comparisons were made against negative controls as well as among treatment groups using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. Body weight changes were compared using two-way ANOVA followed by Dunnett's multiple comparison tests. The results were considered statistically significant at a 95 % confidence level.

Results

Phytochemical screening

The root contained anthraquinones, phytosterols, triterpenoids, coumarins, saponins, reducing sugars, tannins and flavonoids. Alkaloids were absent.

Acute oral toxicity the aqueous extract

The aqueous extract caused no observable physical or behavioural changes such as sleepiness, rigidity, diarrhoea, hair erection and

Table 2
Suppressive and curative activities of the extract in *P. berghei*-infected mice.

	SUPPRESSIVE TEST			CURATIVE TEST			
Dose (mg/kg)	% Pst on day 4	% SUPP	MST (days)	% Pst on day 3	% Pst on day 7	% CLE	MST (days)
NC	62.80±9.88	0.00	8.0	66.32±8.55	69.04±8.25	0.00	9.0
50	23.80±2.41	62.10±2.58	12.0	68.33±5.48	27.72±6.23	59.84±2.47	12.0
100	20.79±3.26	66.90±3.46	18.0	72.07±10.20	20.14±1.71	70.82±6.21	16.0
200	12.51±4.18	80.08±2.05	25.0	57.79±6.20	17.85±2.87	74.14±9.54	24.0
400	7.52±1.85	88.02±1.46	26.0	60.99±4.22	13.14±4.22	80.96±3.25	25.0
PC (4)	1.66±0.34	97.36±0.25	30.0	63.23±8.26	6.77±1.50	90.19±1.23	27.0

Values presented as mean±SD, N = 5, % Pst: %Parasitaemia, % SUPP: % parasitaemia suppression, % CLE: %Parasitaemia clearance, MST: Median survival time, NC: Negative Control group, PC: Artesunate.

abnormal secretion for 24 hours. Additionally, no mortality was witnessed within the 2-weeks of observation. Hence, the LD_{50} was estimated to be greater than 2000 mg/kg extract.

Curative and suppressive assays of the aqueous extract on P. berghei-infected mice

The aqueous extract had an ED_{50} of 194.98 mg/kg in the suppressive test and 100 mg/kg in the curative test. It produced significant ($p < 0.05$) suppression and clearance of parasites in the suppressive and curative tests respectively (Table 2). The higher percentage parasitaemia suppression recorded in the suppressive test was 88.02±1.46 % while the highest percentage clearance in the curative study was 80.96±3.25 %, all at the highest dose of 400 mg/kg. The extract prolonged the survival of the treated mice (Table 2). At the highest dose of 400 mg/kg, the median survival time (MST: the time taken for half the population of the mice treated at the dose of 400 mg/kg of extract to die) was 26 days in the suppressive assay and 25 days in the curative assay. The survival curve (Kaplan-Meier plot) of the suppressive and curative tests (Fig. 1) shows the survivability of the infected mice over a 30-day period. Survival was extended for the treated groups relative to the untreated.

Effect of the aqueous extract (SA) on body weight

Weight loss associated with malaria infection was averted in the extract-treated mice in a non-dose dependent manner. With the exception of mice treated at the dose of 400 mg/kg, all mice in the curative assay gained significant weights ($p < 0.05$) relative to those in the suppressive test. However, the negative control group experienced weight reduction in both the suppressive and curative assays (Fig. 2).

Identification of isolated compounds

SR1 was obtained as an orange amorphous powder of 95.5 % purity (by UPLC). It melts between 193 and 195 °C. It was established as $C_{15}H_{10}O_4$ by the MS data with a peak at m/z 255.03 $(M + H)^+$ and proposed molecular weight of 254. The IR absorption bands at 3386.91 and 1621.08 (APPENDIX A.6) were assigned to phenol-OH and ketone (C = O) functional groups. The thin layer chromatogram showed a single yellow spot (R_f =0.7; Petroleum ether: EtoAc [9:1]) under UV (365 nm) which turned red when sprayed with alcoholic KOH. Careful analysis of the data coupled with the NMR data including ^1H, ^{13}C, HSQC, COSY and HMBC (APPENDIX A.1 – A.5) led to the resolution of SR1 as 1,8-dihydroxy-3-methylanthracene-9,10-dione trivially known as Chrysophanol. The spectra data of SR1 is consistent with the literature values for Chrysophanol [29,33,38,43].

SR2 was obtained as an orange amorphous powder of 78.42 % (by UPLC). The molecular formula was established as $C_{30}H_{18}O_9$ by the MS data with a peak at m/z 521.09 $(M + H)^-$. The proposed molecular weight was 522. The IR absorption bands at 3500, 1619.33 were ascribed to phenol-OH and ketone groups (APPENDIX B.6). The thin layer chromatogram showed a discrete single brown spot under UV (365 nm) which turned red when sprayed with alcoholic KOH (R_f = 0.68, Petroleum ether: EtoAc [6:4]). Analysis of the NMR data including ^1H, ^{13}C, HSQC, COSY and HMBC (APPENDIX B.1 – B.5) led to the resolution of SR2 as 1,1′,6,8,8′-pentahydroxy-3,3′-dimethyl-[2,2′-bianthracene]−9,9′,10,10′-tetraone commonly known as Cassiamin A. The spectra data of SR2 is consistent with the literature data [9,20,22,24]. Fig. 3 shows the structures of the isolated compounds.

Chrysophanol (SR1)

^1H NMR (500 MHz, $CDCL_3$) δ: 12.05 (1H, 8-OH), 11.93 (1H, 1-OH), 7.03 (1H, d, J = 1, H-2), 7.62 (1H, t, J = 8.5 Hz, H-3), 7.76 (1H, dd, J = 8.5 Hz, H-4), 7.58 (1H, d, J = 1 Hz, H-5), 7.24 (1H, dd, J = 8.5 Hz, H-7), 2.42 (3H, s, CH_3).

^{13}C NMR δ: 162.8 (C-1), 124.5 (C-2), 137.0 (C-3), 120.0 (C-4), 133.3 (C-4a), 121.5 (C-5), 149.4 (C-6), 124.7 (C-7), 162.5 (C-8), 113.8 (C-8a), 192.6 (C-9), 115.9 (C-9a), 182.0 (C-10), 133.7 (C-10a), 22.4 (CH_3).

Cassiamin A (SR2)

^1H NMR (500 MHz, DMSO)δ:2.16 (6H, s, 3-CH_3, 3′-CH_3), 6.53 (1H, d, J = 2.4 Hz, H-7), 7.14 (1H, d, J = 2.4 Hz,H-5), 7.36 (1H, d, J =

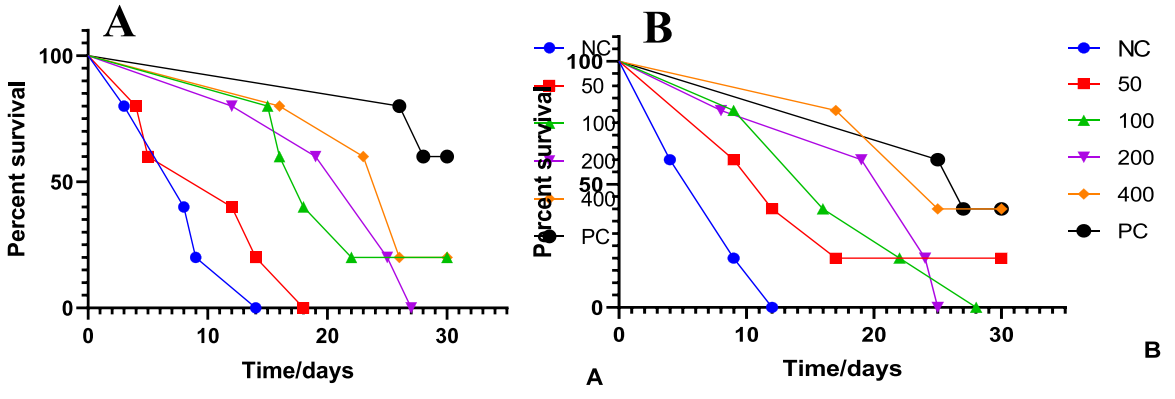

Fig. 1. Survival curves for mice in Peter's suppressive test, **A,** and Rane's curative test, **B.**

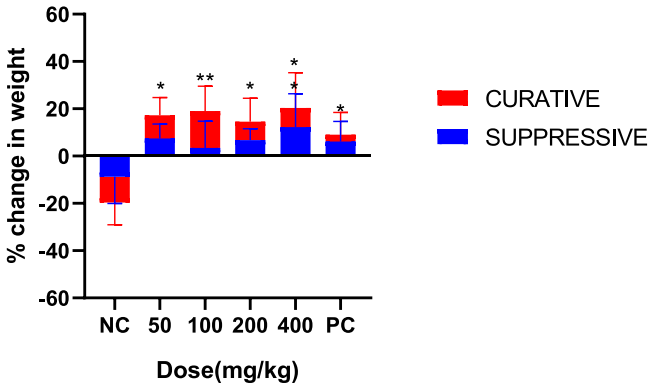

Fig. 2. Percentage change in weight of infected-mice during treatment. Values are significantly different at $*p < 0.05$, $**p < 0.005$ compared to the negative control group.

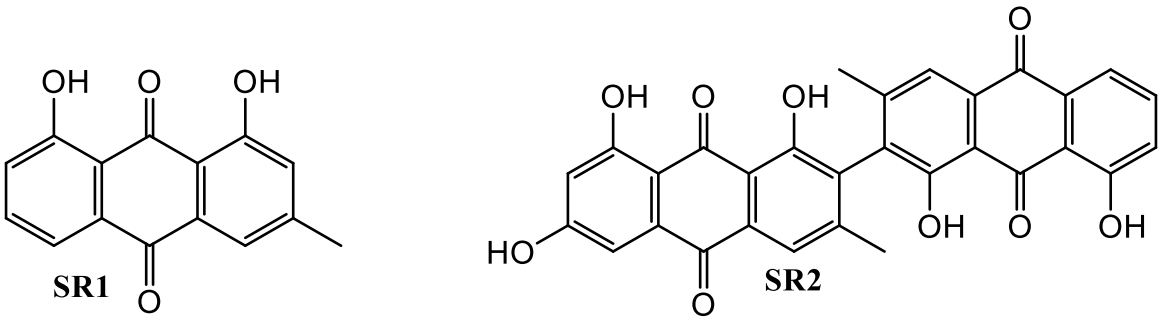

Fig. 3. Structure of SR1 (Chrysophanol) and SR2 (Cassiamin A).

8.1 Hz,H'−7), 7.14–7.80(4H, m, H-4, H'−4, H'−5, H'−6); 11.43 (1H, 6′-OH), 11.84 (1H, 8′-OH), 11.99 (1H, 8-OH), 12.20 (1H, 1′-OH), 12.37 (1H, 1-OH).

^{13}C NMR δ:158.97 (C-1), 130.14 (C-2), 147.67 (C-3), 120.84 (C-4), 109.05 (C-5), 146.74 (C-6), 107.97 (C-7), 165.71(C-8), 189.81 (C-9), 181.26 (C-10), 113.62(C-1a), 132.44 (C-4a), 108.94 (C-5a), 135.16 (C-8a), 158.83 (C'−1), 130.14 (C'−2), 146.74 (C'−3), 120.84 (C'−4), 137.44(C'−5), 119.39 (C'−6), 124.45 (C'−7), 161.32 (C'−8), 191.84 (C'−9), 181.23 (C'- 10), 113.91 (C'−1a), 132.38 (C'−4a), 133.29 (C'−5a), 115.89 (C'- 8a), 20.01 (3-CH_3, 3′-CH_3)

Curative antimalaria activity of isolated compounds

SR1 and SR2 exerted significant ($p < 0.05$) antimalarial activity (Table 3). Both compounds produced comparable percentage parasitaemia clearance of 68.90 % and 70.25 % respectively (Table 4). They also have a comparable effect on the haem content of the erythrocytes. Haem concentration in treated mice was higher than that in the untreated mice, attributable to haem degradation prevention in treated mice. Similarly, mice treated with either compound had comparably higher haematocrit levels, suggestive of haemolysis inhibition caused by the compounds. However, that of untreated mice were significantly ($p < 0.05$) lower possibly attributable to increased haemolysis caused by the parasite.

Table 3
Effect of compounds on % parasitaemia clearance, haem concentration and haematocrit of *P. berghei*-infected mice.

Test substance	% CLE	Haem Conc. (mmol/L)	% Haematocrit
NC	0.00	2740±835.5023	40.68±3.57
SR1	68.90±5.30	3156±409.30	49.28±1.71*
SR2	70.25±8.27	3413±613.80	46.50±3.06*
PC	90.19±3.15	ND	ND

% CLE; % clearance. Values were presented as Mean±SD, N = 5, NC = negative control group, PC = positive control. Compounds were dosed at 10 mg/kg and artesunate at 4 mg/kg. Values are significantly different at $*p < 0.05$ compared to the negative control group.

Discussion

This study evaluated the antimalarial properties of the aqueous extract of *S. siamea* root in murine models. Chromatographic purification of the ethyl acetate fraction, the most active organic solvent fraction, led to the isolation of two anthraquinone compounds, Chrysophanol and Cassiamin A. The extract and the compounds showed notable antimalarial activity.

Parasitaemia suppression of 88.02 % and clearance of 80.96 % coupled with ED_{50} values of 194.98 mg/kg and 100 mg/kg in the suppressive and curative studies make the extract a noteworthy remedy for the treatment of both early and established malaria infections. Nevertheless, a greater potency [ED_{50}= 34.7 mg/kg] reported for the ethanol leaf extract [12], makes the leaf extract a more viable alternative for malarial treatment in traditional medicine, as its use could encourage a more sustainable use of the plant.

The extract, notably, prevented weight loss often associated with malaria infection. Conditions such as appetite loss, disturbed metabolic function and hypoglycaemia often accompany malaria [28] and lead to malaria-related degenerative consequences [6] including weight loss. The aqueous extract averted all these conditions, and together with the reduction of parasitaemia, led to prolonged survival of treated mice.

Several factors could explain the discrepancy noted in the significant *in vivo* antimalarial activity seen in this study and the weak *in vitro* antiplasmodial activity of the extract reported earlier. *In vivo*, the phytoconstituents of extract might be metabolized into more active compounds (Uche *et al.*, 2021). These metabolites, which were absent in the *in vitro* system, could enhance antimalarial activity in animals. Moreover, animals' immune system can be potentiated by the extract [15] and these together can work in synergy, thereby enhancing the extract's overall antimalarial effect. *In vitro* studies lack this immune system component, which can lead to differences in observed activity. Furthermore, pharmacodynamic and Pharmacokinetics properties such as absorption, distribution, metabolism, and elimination of the extract in the animal can significantly impact its effectiveness [39]. These properties are inherently different *in vivo* and can lead to stronger antimalarial activity compared to the results observed *in vitro*. Also, *in vivo* conditions involve complex interactions within the animal's entire system, which can alter the effectiveness of the plant extract [34]. These interactions are not replicated in a controlled *in vitro* environment. These factors explain why it's crucial to consider both *in vitro* and *in vivo* studies when evaluating the efficacy of potential antimalarial treatments.

The two isolated compounds demonstrated remarkable activity against rodent malaria. Chrysophanol caused 68.90 % and Cassiamin A, 70.25 % parasitaemia clearance. The compounds displayed a correlation between their antimalarial activity and their number of hydroxyl groups, confirming an earlier report for hydroxyanthraquinones [42]. This relationship may explain the superior activity seen with the bianthraquinone, Cassiamin A over Chrysophanol.

Mice treated with either compound showed elevated haem concentration that correlated directly with parasitaemia clearance rate. This phenomenon suggested haem build up caused by haem polymerization inhibition by the compounds. This led to toxic haem accumulation in the erythrocyte which resulted in parasite death. This mechanism is consistent with the mode of action reported for chloroquine [36] and some anthraquinones [32]. Our findings, which show elevated haem levels and corresponding parasite clearance in compound-treated mice, further support this hypothesis.

The qualitative phytochemical analysis revealed the presence of anthraquinones and other phytochemicals such as phytosterols, triterpenoids, coumarins, saponins, reducing sugars, tannins, and flavonoids in the roots. Contrary to earlier reports of alkaloids in the leaves [24] and flowers [30], the root, on the other hand, contained no alkaloids. Chrysophanol and Cassiamin A were earlier reported in *S. siamea*. Chrysophanol was isolated from the leaves [23] and stem [29], while Cassiamin A was isolated from the leaves and stem [11]. Chrysophanol was reported to possess antiplasmodial activity against the Sierra Leonian D6 (chloroquine-sensitive) *P. falciparum* strain [1] and chloroquine-snsitive 3D7 *P. falciparum* strain [43]. Cassiamin A was, also, identified, in a molecular docking study, as a potent antimalarial agent [32]. The activity of the two compounds, confirms the earlier report which attributed the antimalarial activity of *S. siamea* plant to anthraquinones [21].

The acute toxicity results in this study coupled with the sub-acute toxicity of the aqueous root extract of *S. siamea* previously reported [17] are indications of the safety of the plant in animals.

Conclusion

This study demonstrates the antimalarial potential of the aqueous extract of *Senna siamea* root, which significantly reduced parasitaemia, prevented weight loss, and prolonged survival in mice. The isolated compounds, Chrysophanol and Cassiamin A, exhibited antimalarial property by blocking haem conversion, leading to parasite death and a significant reduction in parasitaemia. This is the first report on the antimalarial activity of the aqueous root extract of *S. siamea* and its constituents, Chrysophanol and Cassiamin A in animals. These findings support the traditional use of *Senna siamea* root decoction for malaria treatment in Ghana. However, there is the need for further clinical studies including in-depth toxicity and efficacy studies in order to explore its therapeutic potential.

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CRediT authorship contribution statement

Reinhard Isaac Nketia: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Resources. **Nkrumah Desmond:** Methodology, Formal analysis, Investigation. **Arnold Donkor Forkuo:** Methodology,

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Declaration of competing interest

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

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sciaf.2025.e02579.

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