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Original Research Article

In Vivo anti-Plasmodium Potential of *Aloe barbadensis* Anthraquinones and its Combination with Amodiaquine

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ABSTRACT

Antimalarial drug resistance is a critical global health challenge. This study investigates the antiplasmodial activities of anthraquinones and their combination with amodiaquine. Twenty-five experimental mice were examined in five groups, each receiving varying concentrations of the compound, either singly or in combination with 40 mg/kg amodiaquine. All the solvent fractions significantly inhibited Plasmodium parasitemia in the order of methanol > ethyl acetate > n-hexane ($P < 0.01$). The highest reduction in parasitaemia (0.53±0.20 parasites/µL) was recorded with the methanolic fraction at 200µg/kg, while the lowest (3.7±1.2 parasites/µL) was observed with n-hexane at 800 µg/kg. At a dose of 200 µg/kg, the compound exhibited significant prophylactic and suppressive activities ($P < 0.01$). The combination of 200 µg/kg anthraquinones and 40 mg/kg amodiaquine exhibited an optimum suppression rate, while the least (67.4%) was recorded with 800 µg/kg. Curative synergy was significantly observed between the standard drug and anthraquinones at 800 µg/kg ($P < 0.01$). The study advocates further studies on the mechanism behind the antiplasmodium activity of the combination and its safety for human consumption.

Keywords: *Aloe barbadensis*, Antiplasmodium, Anthraquinones, Amodiaquine, Combination Therapy.

Introduction

Malaria, a serious parasitic infection often spread by mosquitoes (female Anopheles), poses a significant global health threat, especially among antenatal women and kids under five years.[1,2,3,4] The global population at risk of malaria is approximately 3.5 billion, while about 435,000 deaths are recorded annually.[5,6] Artemisinin-based combination therapy (ACT) was recommended by the World Health Organisation (WHO) as the preferred and first-line therapy for uncomplicated malaria in endemic countries. However, with the growing rate of artemisinin-resistant malaria in Southeast Asia and with the recent report in Africa, there is concern that the trend may soon increase on the continent, where the burden of falciparum malaria is much higher.[7] In view of the declining efficacy of anti-malarial drugs including ACT, coupled with limited efficacy of the newly discovered anti-malarial vaccine, regular search for replacement of the failing drugs is crucial to curtail the frequency of the infection. *Aloe barbadensis*, commonly known as Aloe vera, is a medicinal plant frequently utilised by traditional herbalists in Nigeria for treating fevers. Previous studies on the antiplasmodium activities of methanolic extract and fraction of *A. barbadensis* have shown promising results on the anti-plasmodium activity of the plant, justifying further research into its potential as a natural anti-plasmodium agent.[8,9]

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Apart from *A. barbadensis*, several indigenous plants are often used for the traditional treatment of malaria and other diseases.[10–12]
Studies have confirmed the anti-Plasmodium activity of *Artemisia annua*,[13,14] *Cinchona ledgeriana*,[15,16] *Terminalia arjuna*, [17–20] and *Zingiber officinale*.[21] Previous studies have attributed the antimalarial activity of *A. barbadensis* mainly to the presence of anthraquinones in the plant.[22,23,24]. The compound was also reported to be composed of alloin A and B, aloe-emodin and chrysophanol processing anti-plasmodium properties.[25]
Preclinical investigation is the foremost approach to assessing the efficacy of ethnobotanical products. Similarly, four-day suppressive, chemoprophylactic, and curative tests have also been widely employed to study the veracity of antimalarial claims of new medicinal products.[26,27] This study aimed to investigate the efficacy of *Aloe barbadensis* solvent fractions, anthraquinones extract and its combination with amodiaquine against the plasmodium parasite.

Materials and Methods

Plant collection and preparation of extracts
Ethical approval was sought and obtained from the Ethical Review Committee of the University of Medical Sciences, Ondo, via a letter dated June 3rd, 2022, with reference number NUREC/TR/UNIMED-HREC-OndoSt/22/06/21, before the commencement of the study. The leaves of the *Aloe barbadensis* plant were collected between March and April 2023 from Oke-Aluko farm located at Latitude 8.4733° North and Longitude 5.6080 ° East of Ilorin, Kwara State, Nigeria. Identification and authentication of the plant were performed by an expert in the Plant and Biotechnology Unit, University of Medical Sciences, Ondo, followed by the issuance of voucher number UNIMED/PBTH/0067.
Following proper washing of the leaves under running tap water, they were decontaminated with 70% ethyl alcohol to remove the contaminants, after which they were thoroughly rinsed with sterile distilled water and then air-dried before drying in an oven at 60 °C. [28]

The dried leaves were pulverised with an electric blender (Excella, VTCL, China). The powdery form was weighed, re-dried, and weighed again until a constant weight was obtained.
Twenty grams (20g) of the pulverised leaves were separately soaked in 1000 ml of 95% methanol and distilled water, respectively, for 48 hours at 25–27 ℃. The methanol and water soaks were filtered through Whatman filter paper no. 1, and the filtrate was evaporated to dryness using a rotary evaporator (Rotavapor, Switzerland) under reduced pressure at 40℃. This dried extract was kept at 4 ℃ for fractionation using different solvents. Both crude extracts were properly dried before they were subjected to antiplasmodial screening in vitro against the chloroquine-sensitive Pf65K strain. Crude extract with higher antiplasmodial activity (methanolic extract) was selected for fractionation.

Preparation of solvent fractions
Ninety-nine percent (99%) Ethyl acetate (Fumman Chemicals, Lagos, Nigeria) 99.5% methanol (Fumman Chemicals, Lagos, Nigeria), and 99% n-hexane (Multi-dimension Chemicals, Lagos, Nigeria) were used as the solvents to fractionate the methanolic crude extract. Twenty grams (20g) of the extract was dissolved in 450 mL of n-hexane, and the mixture was shaken gently; the filtrate (hexane fraction) was separated and collected in a 500 mL beaker as a hexane fraction. The procedure was repeated twice, and the hexane filtrates were pooled together. After the separation of the hexane fraction, the same procedure was repeated for the remaining solvents. The filtration of each fraction was concentrated with a rotary evaporator followed by drying in an oven at 50℃. Each fraction was tested for anti-Plasmodium activity, and the fraction with the highest chemoprophylactic, suppressive and curative anti-Plasmodium activities was identified[5].

Extraction of anthraquinones from the methanolic fraction
In this study, the methanolic fraction was identified as the most effective antiplasmodial agent. Anthraquinone was extracted from the solvent fraction as earlier illustrated by Tan et al [25]. Briefly, the fraction was adjusted to an acidic pH of 3 by adding five drops of 1M HCl (Fumman Chemicals, Lagos, Nigeria). Five millilitres of ethyl acetate was added to 5 mL of the acidified fraction, and the mixture was shaken gently to allow separation into the organic phase (upper layer) and aqueous (lower layer) phase in a separating funnel. Five millilitres of the aqueous solution was separated and added to another 5 mL of ethyl acetate to recover the anthraquinones content. The resultant mixture in the organic phase was then removed, and five drops of 1M NaOH (Fumman Chemicals, Lagos, Nigeria) were added to increase the pH to 8.9 (basic). Five millilitres of the basic mixture were mixed with 5 mL of ethyl acetate again and passed through the mobile phase of the column chromatography to separate the anthraquinones from other compounds in the mixture. The extracted anthraquinones were evaporated with a Soxhlet rotary evaporator (Rotavapor, Switzerland) and subjected to chromatographic purification using silica gel (0.04-0.06, Merck), chromatographic purification, freeze-dried, weighed and administered accordingly.

Preparation of the experimental animals and parasite inoculum.
The efficacy of the extracts was evaluated based on the assessment of their chemoprophylactic, suppressive and curative antiplasmodial activities on *P. berghei*-infected Swiss albino mice. Chloroquine phosphate (Emzor Pharma, Nigeria), a *Plasmodium*-sensitive drug, was prepared by dissolving 0.5 grams of the salt in 10 mL of distilled water, resulting in final doses of 25 mg/kg body weight. The chloroquine-sensitive NK65 strain of *P. berghei* was obtained from the Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research, Lagos, Nigeria, and amodiaquine (Merck, Germany) was used in this study. Amodiaquine at the concentration of 40 mg/kg was employed as adopted in the previous study by Olliaro and Mussano.[29] Ethical considerations was maintained in the handling and treatment of the animals throughout the period of the experimentation in conformity with the guidelines of National Institute of Health and Institute for Laboratory Animal Research.[30] A donor mouse was infected with *P. berghei* and used for parasite inoculum preparation. Each test mouse was inoculated intraperitoneally with 0.1 mL of the infected blood containing about 1×10^6 *P. berghei* parasitised red blood cells.
Determination of the doses for Aloe barbadensis anthraquinones and amodiaquine
The most effective range of concentrations employed in this study was determined based on the knowledge of IC_{50} (50% maximum inhibitory concentration) of *A. barbadensis* and amodiaquine against *Plasmodium falciparum* from previous studies. According to Balogun et al. [31], the IC_{50} of amodiaquine against *P. falciparum* was 24.2 mg/mL, while Kumar et al.[32] reported that 0.289 – 1086 µg/mL of *A. barbadensis* inhibited 50% of the Plasmodium parasite *in vivo*.

The choice of Amodiaquine in the combination therapy
Amodiaquine was chosen in the combination therapy because the drug is relatively cheaper than many antimalarial drugs, which will make the combination therapy more affordable in resource-limited settings. Also, the choice of the combination was based on its already established potency as a standard antimalarial drug. [29] It has also been reported that it is capable of synergistic activities when in combination with other antimalarial drugs, like artesunate or sulfadoxine-pyrimethamine.[33] Amodiaquine in combination will make it more difficult for the parasites to develop resistance.
Twenty-five (25) pure strains of adult Swiss albino mice were obtained from the animal house of the University of Medical Sciences Ondo. The animals were allowed acclimatisation to the laboratory environment for one week, and fed with a mice pellet diet (Ladokun Farms, Ibadan, Nigeria) and water *ad libitum*. This was carried out before randomisation into various experimental groups of five (5) animals per group based on the body weight of the mice and was designated as groups B (200 µg/kg), C (400 µg/kg), and D (800 µg/kg), with E (25 mg/kg Chloroquine) and A (Distilled water) as positive and negative controls, respectively.

Suppressive Treatment
A four-day suppressive test (D0, D1, D2, and D3) strategy earlier described by Misganaw et al.[5] was adopted. The animals were grouped into cohorts of five animals per cage. Group A, which served as the negative control, received a sterile placebo, while groups B, C, and D were separately administered aqueous and methanolic extracts of *A. barbadensis* at doses of 200 µg/kg/day, 400 µg/kg/day, and 800 µg/kg/day, respectively. Group E served as the positive control and received 25 mg/kg chloroquine. On the D0, erythrocytes infected with 10^6 inoculum of *P. berghei* were administered to every mouse intraperitoneally. The extracts were then orally administered to the animals two hours after inoculation on day 0 (D0), followed by daily administration from day 1 to day 3 using an oral cannula. The five remaining groups of animals were administered with 200 µg/kg/day, 400 µg/kg/day, and 800 µg/kg/day of the solvent fractions, respectively, including positive and negative control groups.
A venous blood sample was collected from the tail of each mouse three days after treatment, prepared smears on clean slides, allowed to dry, fixed with ethanol, stained with 3% Giemsa stain for 45 minutes, and examined under a microscope (CX23LFS1K, Olympus, Japan) using the oil immersion objective to determine parasite density. The percentage of parasite suppression relative to the negative control was evaluated using the formulae (equations 1 and 2).

$$\%\ suppresion = \frac{Mean\ parasitaemia\ in\ Negative\ control - Mean\ parasitaemia\ in\ test}{Mean\ parasitaemia\ in\ the\ Negative\ group}\ x\ 100$$

Equation 1

$$\%\ \text{Parasitaemia} = \frac{\text{Number of parasitized RBC}}{\text{The total number RBCs counted}} \times 100$$

Equation 2

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Chemoprophylactic treatment.
The same protocol of animal grouping and experimentation for suppressive treatment was followed in chemoprophylactic treatment but

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with a modification that the animals were administered the extracts and methanolic fraction for D0 to D3 before inoculation of infected erythrocytes on day 4. Three days after inoculation, blood samples were collected, processed and examined as described in suppressive treatment.

The percentage chemoprophylaxis of the extracts and fractions against the parasite density was also calculated using the formula (equation 3):

$$Mean\ \%\ Chemoprophylaxis = \frac{Mean\ parasitaemia\ in\ Negative\ group - Mean\ parasitaemia\ in\ test}{Mean\ prasitaemia\ in\ the\ Negative\ group} \text{ x } 100$$

Equation 3

Curative Treatment

The curative potential of the methanolic fraction of *A. barbadensis* and its combinations with amodiaquine was evaluated in this study by closely adopting the protocol outlined by Misganaw et al. [5]. Briefly, five groups of infected mice, each consisting of five animals, were utilised. After inoculation with Plasmodium parasites, 72 hours was allowed for manifestation of the parasite before treatment was initiated. Animals were grouped as earlier described in the 4-day suppressive test and were administered the methanol fraction at doses of 200 µg/kg, 400 µg/kg, and 800 µg/kg, sterile distilled water and 25 mg/kg of chloroquine, respectively. Treatment with the fraction was administered for seven consecutive days (D0-D6). The same procedure was repeated for the combinations of 200 µg/kg and 40 mg Amodiaquine, 400 µg/kg with 40 mg Amodiaquine, and 800 µg/kg with 40 mg Amodiaquine, respectively. Throughout this period, blood smears were prepared daily from each animal, fixed and stained as previously described. Parasite density was determined by dividing the parasites counted by 200 (white blood cells differentiated) and the product multiplied by 8000 according to equation 4.

$$\text{Parasite density} = \frac{\text{No of parasite}}{200\ \text{WBC}} X\ 100$$

Equation 4

Blood samples were collected from the tails of the experimental animals, thick films made in duplicates from the blood collected from the tails of the experimental animals, stained using the Giemsa technique and examined under an oil immersion lens of a light microscope (CX23LFS1K, Olympus, Japan) for the stage of malaria parasite present. The percentage of schizonts present in the erythrocytes was calculated.

Statistical analysis

The data obtained were subjected to statistical analysis using SPSS software version 23 (SPSS Inc., Chicago, III., USA). Analysis of variance (ANOVA) was conducted on the parasite density in different solvent fractions and between the treatments and the controls. Significant variation in parasitaemia in different solvents necessitated the post hoc Tukey test conducted to further determine the solvent that significantly exhibited the highest anti-Plasmodium activity. P-value < 0.01 was considered significant.

Results and Discussion

Anti-Plasmodium activities of the crude extracts

In this study, the chemoprophylactic, suppressive and curative anti-Plasmodium potentials of the *Aloe barbadensis* anthraquinone and its combination with amodiaquine were investigated. A statistically insignificant reduction in parasitaemia was generally observed with a 4-day suppressive test at concentrations of 200, 400 and 800 µg/kg of the aqueous extract (Figure 1a). The findings from the 4-day suppressive test with the methanol extract of *Aloe barbadensis* however revealed a significantly reduced parasitaemia ($p < 0.05$). Treatment with 200 µg/kg, 400 µg/kg and 800 µg/kg concentrations showed a reduction in the parasite density to 3.8 ± 3.45, 2.2 ± 1.79, and 2.98 ± 1.07, respectively (Figure 1b), implying that 400 µg/kg was the most effective concentration of the methanolic extract. As shown in Figure. 1c, a statistically significant reduction was observed in the parasitaemia levels in the chemoprophylactic test ($p<0.05$). The 800 µg/kg dose of *A. barbadensis* produced the highest rate of inhibition in parasite density (80.8%), followed by 200 µg/kg, with 64% and 27.2% at a dose of 400 µg /kg. Kumar et al. [34] previously documented the antimicrobial potential of *Aloe barbadensis*. Subsequently, Sánchez et al. [35] reported that the pharmacological effects of the major constituents of *Aloe vera*, could be attributed to bioactive compounds that are responsible for its antimicrobial activities.

Earlier reports on the anti-Plasmodium activity of *A. barbadensis* extracts had indicated that a medicinal preparation must suppress parasitaemia by 30% to be considered active.[9,36] The present findings indicated that the aqueous extract of *A. barbadensis* with the highest inhibition rate of 27.1 % in the 4-day suppressive test is not active against the Plasmodium parasite.

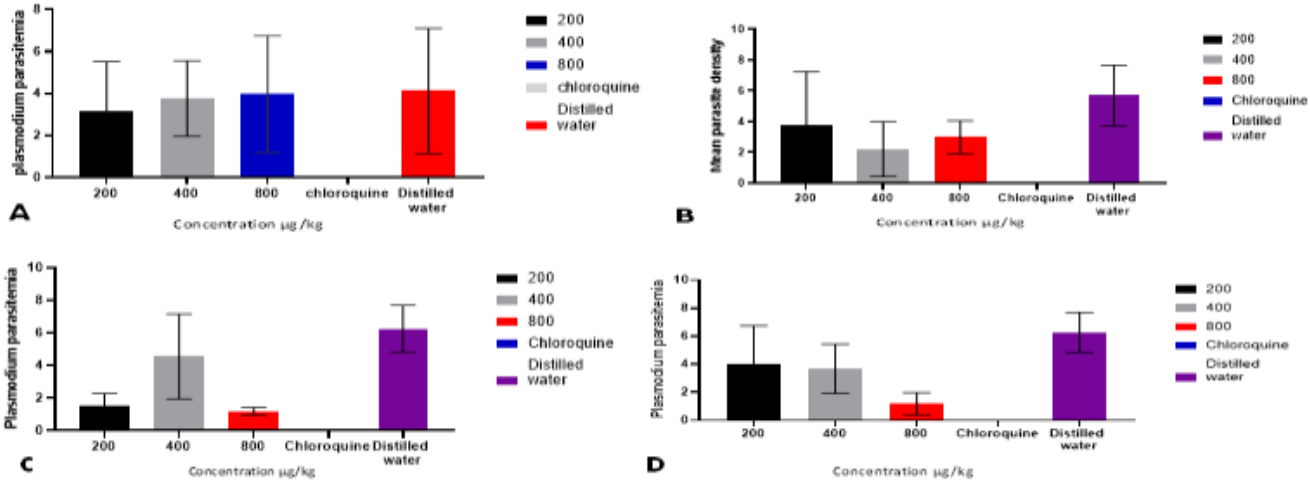

Figure 1: a. Effect of *A. barbadensis* aqueous extract on *P. berghei*-infected mice in a 4-Day Suppressive Test. **b.** Effect of *A. barbadensis* methanolic extract on *P. berghei*-infected mice in a 4-Day Suppressive Test. **c.** Chemo-prophylactic effect of different concentrations of *A. barbadensis* aqueous leaf extract on plasmodium parasite density. **d.** Chemo-prophylactic effect of different concentrations of *A.barbadensis* methanolic leaf extract on plasmodium parasite density.

The variation in chemoprophylactic activities at different doses of methanolic extract of *A. barbadensis* on the parasitemia inhibition is shown in Figure. 1d.

Treatment with extract at 200 µg/kg showed a suppression rate of 36%, while 400 µg/kg recorded 41.3%, and 800 µg/kg exhibited 81.3% suppression compared to the positive control, which produced a 100% suppression rate. As the concentration of the extract increased, so also the suppressive activities increased. The low activity of aqueous extract exhibited in this study is consistent with previous reports on the aqueous fractions of other plant species.[18,19] On the other hand, the present findings revealed a significantly high chemoprophylactic rate of the aqueous extract (80.8%) at a concentration of 800 µg/kg/day but the suppression was independent of the concentration of the extract. Similarly, methanolic extract competed favourably with plasmodium-sensitive chloroquine efficacy with respect to chemoprophylactic activity against the plasmodium parasite by recording an inhibition rate of 81.3 % at the concentration of 800 µg/kg/day compared to 100.0% rate recorded by the positive control. Also, in the suppressive test, the methanol extract exhibited the highest anti-plasmodium suppressive activity (64.3%) at 400 µg/kg/day. Methanol extract exhibited a dose-dependent activity in the prophylactic test, whereas the result of the suppressive test of the extract was independent of concentration. The assessment of the activities of the two crude extracts reveals that methanolic extract is more potent as an anti-Plasmodium suppressive and chemoprophylactic preparation than the aqueous. These findings are consistent with past studies that reported up to a 73% reduction in parasitaemia in mice infected with *P. berghei*. Also, reports have shown that *Aloe barbadensis* methanolic extract is capable of inhibiting the parasite growth and development at 50 µg/mL. [34,37,38] Similarly, IC_{50} values ranging from 10 to 40 µg/mL against *Plasmodium berghei* have been documented.[37]

Anti-Plasmodium activities of solvent fractions

Our findings on the activity of the solvent fractions against Plasmodium parasites revealed that the anti-Plasmodium activity of the fractions can be influenced by the choice of solvents for extraction or fractionation because different solvents can extract varying levels of bioactive compounds in the medicinal plant. At varying concentrations, the solvent fractions inhibited parasite density significantly in the order of methanol > ethyl acetate > n-hexane ($P < 0.01$). The highest reduction in parasite density (0.53 ± 0.20 parasites/µL) was recorded with the methanolic fraction at 200 µg/kg, while the lowest (3.7 ± 1.2 parasites/µL) was observed with n-hexane solvent at 800 µg/kg after the four-day suppressive test. This implies that all the solvents used for fractionation in this study extracted varying levels of anti-Plasmodium compounds from the plant. Earlier findings by van Zyl and Viljoen [39] reported that several methanol extracts exhibited antimalarial activity, inhibiting 50% of *Plasmodium falciparum* growth (IC_{50}) at concentrations ranging from 32 to 77 µg/mL. In another study conducted by Nassar and Oyewole [8] *Aloe barbadensis* extract was reported to exhibit parasitaemia clearance rates of 6.3%, 18.3%, and 32.0% at a dose of 400 mg/kg; 5.3% to 28.7% at 600 mg/kg; and 4.0% to 22.3% at 800 mg/kg, respectively. The effect of *A. barbadensis* fractions on parasite density in a 4-day suppressive test is shown in Table 1. The results depicted that the *A. barbadensis* methanolic fraction recorded the highest reduction in parasite density (0.53 ± 0.20) at a concentration of 200 µg/kg, while the least reduction of 3.7 ± 1.2 was documented with n-hexane solvent at a concentration of 800 µg/kg when compared to the negative control. All the concentrations of the fractions significantly reduced the parasite density as compared to the distilled water in the order of methanol > ethyl acetate > n-hexane ($P < 0.01$).

Table 1 depicts the percentage suppressive rate of *A. barbadensis* fractions on parasite density. The highest suppression of parasite density was recorded with methanol solvent (87.7%), closely followed by Ethyl Acetate fraction (87.6%). While n-hexane solvent exhibited the lowest suppression rate at 67.4%

At concentrations of 200, 400, and 800 µg/kg, the methanolic fraction depicted parasitaemia suppression rates of 87.7%, 73.5%, and 38.21%, respectively.

Table 1: Effect of *Aloe barbadensis* fractions on suppression of parasite density

Fractions	Dosage in µg/kg body weight			Controls	
	200	400	800	Distilled water	Chloroq-uine
Ethyl Acetate (A)	0.56 (87.6%)	1.3 (69.8%)	3.3 (21.3%)	4.3 (0.0%)	0 (100.0%)
Methanol (B)	0.55 (87.7%)	1.4 (73.6%)	1.9 (38.2%)	4.3 (0.0%)	0 (100.0%)
n-Hexane (C)	1.56 (67.4%)	1.8 (62.6%)	3.7 (20.1%)	4.3 (0.0%)	0 (100.0%)

P-value =0.001.

Note: Distilled water = Negative Control, Plasmodium sensitive Chloroquine (CQ) = Positive Control.

The findings also indicated that the highest suppression of parasite density was recorded with methanolic solvent (87.7%), closely followed by the ethyl acetate fraction (87.6%), while n-hexane solvent exhibited the lowest suppression rate at 67.4%. The anti-plasmodium properties of *Aloe barbadensis* are dose-dependent, with increasing efficacy observed at higher concentrations.[6,8] Gebremariam et al.[27] reported that latex from *Aloe melanacantha* significantly suppressed *Plasmodium berghei* parasitaemia in infected mice at doses of 100, 200, and 400 mg/kg, resulting in parasitaemia suppression rates of 14.9%, 29.0%, and 43.2%, respectively (IC_{50} = 22.63 mg/mL). These previous reports implied suppressive and curative potential of the plant and corroborate our findings in this present study. Comparatively, the methanolic fraction recorded the highest suppression rate (87.7%) with the lowest parasite density (0.53 ± 0.20) at a concentration of 200 µg/kg, thereby providing scientific evidence for the antimalarial claim of the plant by traditional healers. The present finding agrees with the results from similar studies in which efficacy was also documented with methanol as a solvent.[11,40] Comparing the suppressive activities of the fractions by solvent, the study revealed insignificant variation in the activities of methanol and ethyl acetate at a concentration of 200 µg/kg ($P > 0.01$), whereas methanol and n-hexane solvents depicted a significant variation at the same concentration of the fraction ($P < 0.01$) in the suppressive test. These present findings inferred that methanol and ethyl acetate solvents are equally efficacious at the concentration of 200 µg/kg.

Anti-Plasmodium combinatorial activity of the anthraquinones and amodiaquine

Our findings on the anti-Plasmodium activity of anthraquinones showed that as the concentration of anthraquinones extracted from the methanolic fraction increases, the suppressive activity on the parasite also increases in agreement with the dose-dependent antimalarial suppressive activity earlier reported on methanolic.[5,6,8,27,41] Table 2 shows the parasite density for each day with varying concentrations of the anthraquinones extract in a 7-day curative study. The order of curative activities of the *A. barbadensis* anthraquinones was 6,800±20 < 8,268±14 < 25,900±16 for 800 µg/kg, 200 µg/kg, and 400 µg/kg respectively, against 131,586±81 for negative and 0 for positive controls.

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Table 2: Curative efficacy of Anthraquinones on mean malarial parasite density

	Anthraquinones Dosage in µg/kg-1 body weight				
Days	200	400	800	Chloroquine	Distilled water
D0	580±39	18,265 ±27	25321 ± 21	27688 ±22	16867±93
D1	32,460 ±27	39,684 ±35	79,038 ±62	9,588 ± 78	13,737±14
D2	42,377±39	55,208±20	109,017±75	764±87	30,450±16
D3	41,324±27	47,197±25	31,010±15	0	58,712±34
D4	22,267±10	28,249±15	52,297±59	0	88.931±43
D5	15,053±65	26,601±19	11,396±24	0	98,759±74
D6	8,268±14	25,900±16	6,800±20	0	131,586±81

Daily parasite density with varying concentrations of the Anthraquinones (from methanolic fraction) in a 7-day curative study.

The effect of the combination of *A. barbadensis* anthraquinones and 40 mg/kg of amodiaquine on *Plasmodium* parasite-infected mice in a suppressive test is shown in Table 3. The optimum suppression rate against the *Plasmodium* parasite was observed with the combination of 200 µg/kg of anthraquinones, while the lowest suppression rate was reported with the concentration of 800 µg/kg. Table 4 shows the effect of variations in the concentration of combined anthraquinones and 40 mg amodiaquine in a curative test, with respect to the positive and negative controls.

The combinatorial effect of *A. barbadensis* Anthraquinones and 40mg Amodiaquine on *Plasmodium* Parasite density in a 4-day suppressive test. The optimum suppression rate against the *Plasmodium* parasite was observed with the combination of 200 µg/kg of Anthraquinones, while the lowest suppression rate was reported with the concentration of 800 µg/kg.

Table 3: Effect of the combination of *Aloe barbadensis* Anthraquinones and 40 mg Amodiaquine on *Plasmodium* Parasite density in a 4-day suppressive test.

Combined Fractions	Parasite Density	% Parasite Inhibition
200 µg Anthraquinones + 40 mg Amodiaquine	0.00	100.0
400 µg Anthraquinones+ 40 mg Amodiaquine	0.7 ± 0.90	87.8
800 µg Anthraquinones + 40 mg Amodiaquine	1.9 ± 1.0	66.7
Plasmodium Sensitive Chloroquine (25 mg)	0.00	100.0
Distilled Water (10 ml)	5.7 ± 1.97	0.00
P-Value (ANOVA)	0.001	

The findings depicted non-dose-dependent activities of the combination from 200 µg/kg to 400 µg/kg to 800 µg/kg. A significant reduction in the parasite density was observed from D0 to D6 at the concentration of 800 µg/kg when zero parasite density was recorded over the 7-day investigation. The anti-Plasmodium chemoprophylaxis of *A. barbadensis* anthraquinones also affirmed that the compound has anti-plasmodium potential, thereby reducing the risk of malaria, especially in the endemic regions including Nigeria.[27] With the anti-Plasmodium chemoprophylaxis potential of the compound, the development of new antimalarial drugs is possible in the near future that can serve as a preventive measure for travellers, immigrants, or residents of malaria-endemic communities. Figure 2a shows the combinatorial effect of 200 µg/kg of anthraquinones and 40 mg amodiaquine on the parasite density over 7 days of treatment with respect to positive and negative controls.

Table 4: Effects of different concentrations of *Aloe barbadensis* Anthraquinones in combination with 40 mg Amodiaquine on the parasite density

	Dosage of the combinations in µg/kg 1 body weight				
Days	Combination 1	Combination 2	Combination 3	Chloroquine	Distilled Water
D0	1,580±37	19,285 ±29	23,334 ± 21	27,688 ±22	16,867±93
D1	35,474 ±49	38,984 ±31	77,036 ±42	9,588 ± 78	13,737±14
D2	29,377±71	23,248±32	56,017± 43	764±87	30,450±16
D3	19,324±13	28,697±27	37,048±28	0	58,712±34
D4	12,377±27	24,749±25	25,205±63	0	88,931±43
D5	4,853±35	27,921±27	23,324±34	0	98,759±74
D6	1,767±29	15,900±19	0	0	31,586±81

Key:
Combination 1 = 200 µg/kg A. *barbadensis* Anthraquinones + 40 mg Amodiaquine
Combination 2 = 400 µg/kg A. *barbadensis* Anthraquinones + 40 mg Amodiaquine
Combination 3 = 800 µg/kg A. *barbadensis* Anthraquinones + Amodiaquine

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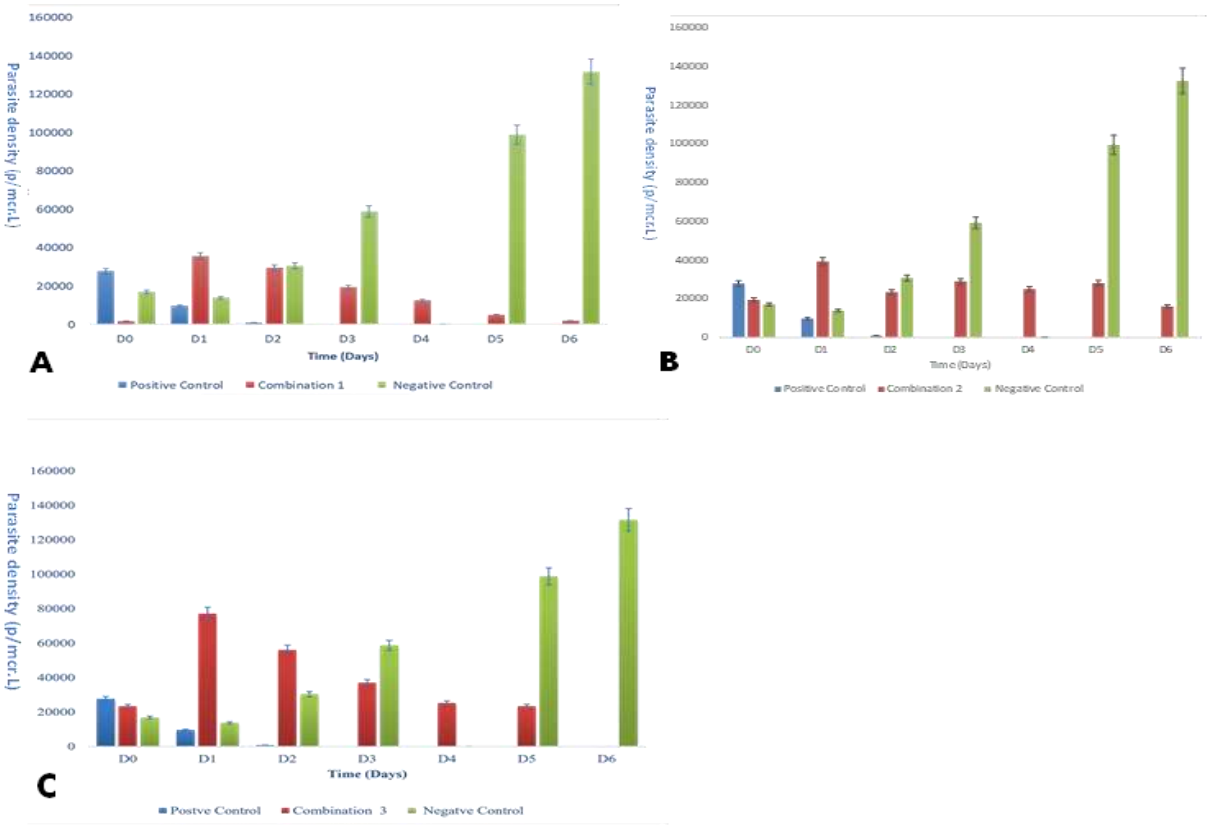

Figure 2: a. Shows the combinatorial effect of 200 µg/kg *A.barbadensis* Anthraquinones and 40 mg Amodiaquineon the parasite density. **b.** Depicts the combinatorial effect of 400µg/kg A. *barbadensis*Anthraquinonesand 40 mg Amodiaquine on the parasite density **c.**combinatorial effect of 800 µg/kg methanolic of *A. barbadensis* fraction and 40 mg Amodiaquine on the parasite density.

The highest reduction in the parasite density was recorded on day 6 (1,767 ± 29), while the lowest was documented on day 1 (35,474 ± 49). The combinatorial effect of 400 µg/kg of the compound with 40 mg amodiaquine on the parasite density over the 7-day treatment with controls is depicted in Figure 2b. The highest reduction in the parasite density was recorded on Day 6 (15,900 ± 19), while the lowest was documented on Day 1 (38,984 ± 31). Fig. 2c depicts the effect of an 800 µg/kg concentration of the compound in combination with 40 mg amodiaquine on the parasite density over the seven-day treatment using chloroquine and distilled water as controls. The highest reduction of the parasite density (zero) was recorded at D6, while the lowest was documented at D1 (77,036 ± 42). The findings on the combinatorial activity of the anthraquinones and amodiaquine against the Plasmodium parasite revealed optimal inhibition of the parasite density after administration of combined *A. barbadensis* anthraquinones and 40 mg/kg amodiaquine at the lowest concentration of 200 µg/kg/day, while the least (64.5%) was reported with 800 µg/kg of the compound with 40 mg amodiaquine. The suppressive synergistic activity was more pronounced at 200 µg/kg/day, whereas the highest curative synergy was recorded at the concentration of 800 µg/kg/day. According to Habte et al., [42] any extract, fraction or compound that produces anti-Plasmodium inhibition ≥ 50% at a dose of 500-250, 250-100, and ≤ 100 mg/kg/day should be rated as moderate, good, and very good, respectively. Therefore, the efficacy of anthraquinones may be rated moderate to a good antimalarial suppressive agent when administered singly and rated very good in combination therapy. Given the emphasis of the WHO on combination therapy, the anthraquinone from *Aloe barbadensis* could be administered in combination with other existing standard antimalarial drugs to enhance efficacy and reduce the risk of antimalarial drug resistance.[43] If the potential of the compound is properly harnessed, the severity and number of malaria cases will be reduced, ultimately leading to a reduction in morbidity and mortality rates. Though the mechanisms of action are not yet fully established, the combination might have inhibited various stages of the parasite's growth and reproduction, altered the permeability and integrity of parasite membranes and ultimately led to the death of the parasite. Anthraquinones, a component of the combination, may modulate the host's immune response, thereby enhancing the host's natural defences against malaria. Therefore, further studies on the anti-oxidation, inhibition of protein synthesis, immune modulation, and interference with the invasion of enterocytes should be conducted to explore the mechanism of action of the compound against malaria parasites.[44]

The synergistic effect of the combination observed in this study may serve as baseline information and a guide for further research on *A. barbadensis* anthraquinones in combination with the existing antimalarial drugs. The compound may enhance the absorption and bioavailability of amodiaquine thereby preventing drug resistance. The combination may also provide an affordable and accessible treatment option for consumers, particularly in low-resourced economies. The anti-Plasmodium efficacy of the combination could contribute immensely to the global efforts towards controlling malaria, thereby aligning with the WHO's goal to eliminate malaria by 2050, as similar synergy has been reported when *hydroxyethylamine-phthalimide* was combined with chloroquine or artemisinin.[37] Since this study was limited to *in vivo* anti-Plasmodium efficacy using rodent strains of the Plasmodium parasite, replication of the study by an in vitro approach on human Plasmodium parasite strains is strongly advocated. Further study on the therapeutic window for the anthraquinones compound to understand how the dose-dependence activity observed in this study impacts the potential for resistance development is also recommended. Lastly, pharmacokinetic and pharmacodynamic studies of the combination to evaluate drug interactions and toxicity studies to assess the adverse effects are suggested.

Conclusion

This study has demonstrated a promising prophylactic, suppressive and curative anti-Plasmodium activity of anthraquinones when administered singly and in combination with 40 mg/kg amodiaquine.

The synergistic effect of the combination was more prominent on chemo-prophylactic inhibition of parasite density at 200 µg/kg concentration of the anthraquinones while optimum curative synergy was observed between the standard drug and the compound at 800 µg/kg. The suppressive activity of the compound was dose-dependent, whereas the curative was independent of the concentration of the compound. Further studies to unveil the mechanisms behind the anti-Plasmodium activity of the combination and the toxicity effect of the compound are strongly advocated.

Conflict of Interest

The author declares no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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