In vivo comparative antimalarial evaluation of three solvent leaf extracts of *Anthocleista djalonensis* against *Plasmodium berghei*

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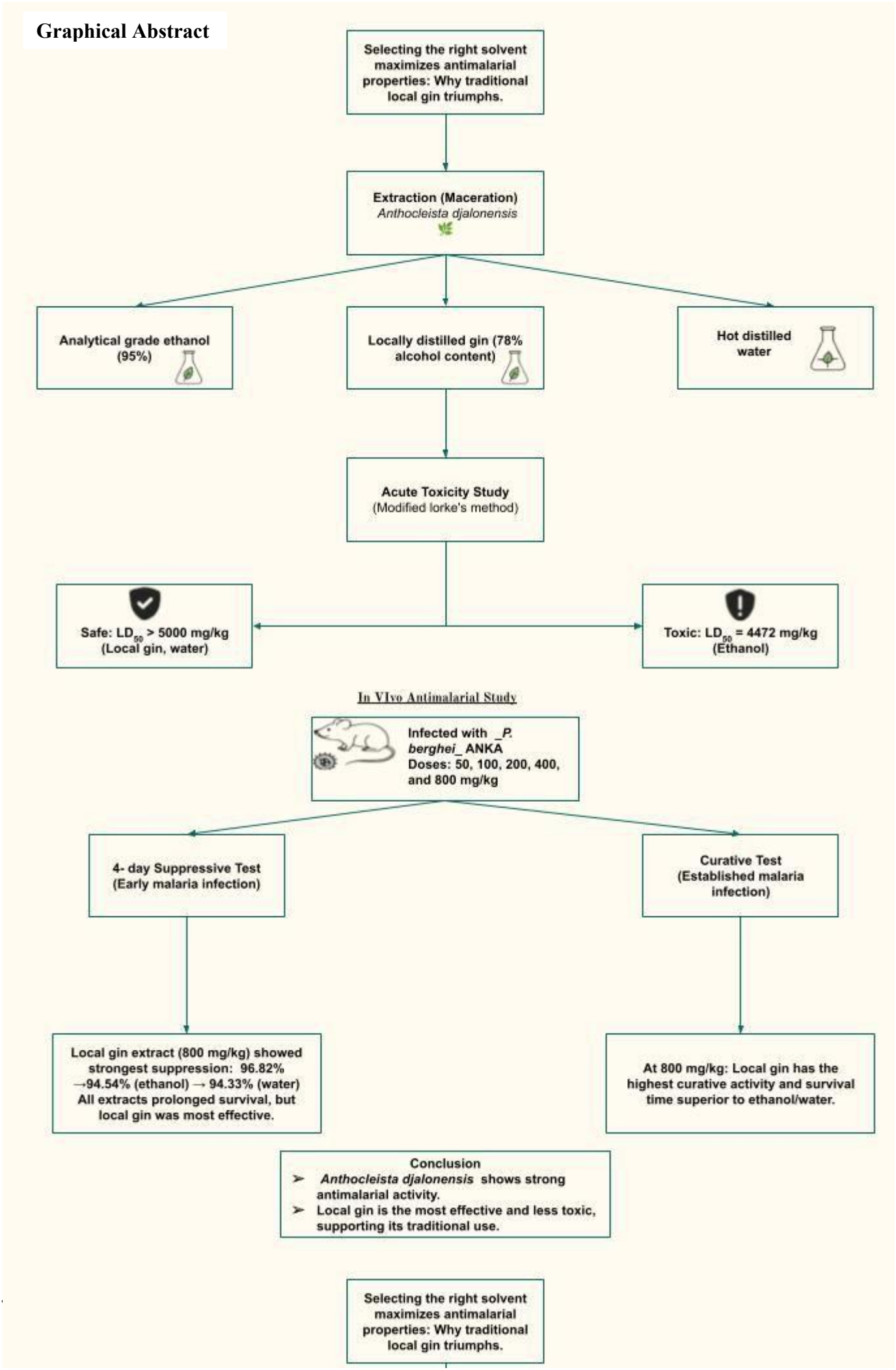
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Abstract

Solvent selection critically influences the yield and quality of bioactive compounds extracted from medicinal plants. Local gin appears to be the most widely used solvent for preparing herbal extracts locally in Nigeria, despite the side effects and toxicity. This study compared the *in vivo* antimalarial activity of three solvent extracts (95% ethanol, water, and local gin (78% alcohol) from *Anthocleista djalonensis* leaves against *Plasmodium berghei* ANKA strain. The extracts were obtained by cold maceration. Oral actute toxicity (LD_{50}) was evaluated using modified Lorke's (1983) method with doses up to 5000 mg/kg. Antimalarial efficacy was assessed using the 4-day suppressive and curative tests in infected mice at doses of 50, 100, 200, 400, and 800 mg/kg. The ethanol extract proved to be toxic (LD_{50} = 4472 mg/kg), while the local gin and aqueous extracts were non-toxic (LD_{50} > 5000 mg/kg). In the suppressive assay, all extracts significantly reduced parasitemia and prolonged the survival, with the local gin extract at 800 mg/kg showing the uppermost suppression (96.82%), surpassing the ethanol (94.54%) and aqueous (94.33%) extracts, and displaying no statistically significant difference ($p < 0.05$) from the positive control at the same dose. In the curative assay, the local gin extract at 800 mg/kg offered the greatest curative action and survival time, with a clear dose-dependent response detected for all extracts. These findings confirm the plant's strong antimalarial properties, with solvent choice influencing results. The local gin was most effective, confirming its traditional use, while water attained slightly lower efficacy with decreased toxicity.

Keywords: Antimalarial activity, *Anthocleista djalonensis, Plasmodium berghei,* Solvent extraction efficiency, Local gin solvent.

Graphical Abstract



Introduction

Malaria, a mosquito-borne parasitic disease caused by Plasmodium species, remains a major global public health challenge, particularly in tropical and subtropical regions, where millions of cases and thousands of deaths occur annually (WHO, 2021). Although artemisinin-based combination therapies (ACTs) have been effective in malaria management, the emergence of drug-resistant *Plasmodium falciparum* strains threatens their efficacy (Barman and Goswami, 2025). While the Mosquirix vaccine marks a significant advancement, its limited availability highlights the need for alternative treatment strategies (Stanisic and Good, 2023).

Herbal remedies have long been used in traditional medicine, offering a rich source of potential therapeutic compounds. The COVID-19 pandemic has further amplified global interest in these remedies due to their perceived health benefits. However, many lack scientific validation, particularly regarding optimal extraction methods. In traditional medicine, water and locally distilled gin (known as kai-kai or Ogogoro) are commonly used for preparing herbal treatments. The polarity of solvents influences their ability to extract different phytochemicals, water (polar solvent) primarily extracts polar compounds, while locally distilled gin (containing both water and ethanol) extracts a broader range of polar and non-polar constituents. Understanding the difference is important because the therapeutic efficacy of herbal preparations depends on the solvent's extraction efficiency. For malaria treatment, it remains unclear whether the active antimalarial compounds are primarily polar, non-polar, or both.

Anthocleista djalonensis A. Chev, a medicinal plant from the Gentianaceae family, is traditionally used across Africa to treat malaria, typically prepared as a decoction or maceration (Adebayo and Olamide, 2022). Previous studies confirm its antiplasmodial activity against *Plasmodium falciparum* and *Plasmodium berghei*, yet there is limited research comparing the effectiveness of traditional solvents (water and locally distilled gin) with laboratory-grade ethanol, which remains the standard solvent in most existing studies on extracting bioactive compounds. While ethanol is known for its broad extraction efficiency, its toxicity makes it unsuitable for direct use in traditional preparations. This study addresses this gap by comparing the efficacy of water, local gin, and ethanol extracts of *Anthocleista djalonensis* leaves in inhibiting and treating *Plasmodium berghei* infection *in vivo*. The findings will provide scientific validation for traditional solvent choices and identify the most effective extraction solvent for antimalarial compounds.

Materials and methods

Determination of ethanol content in local gin

The percentage of ethanol (alcohol) in the local gin used for this study was determined using a simple distillation apparatus. 100 ml of local gin was measured using a graduated cylinder and transferred to a round-bottom flask. The flask was connected to a distillation head and a condenser which was placed on a heating mantle and switched on to heat the local gin. The ethanol (having a lower boiling point than water) evaporated and travelled through the condenser, where it cooled and condensed back into liquid form. This condensed ethanol was collected in a separate measuring cylinder. The heating continued until no further ethanol was distilled. The volume of collected ethanol was recorded to determine the alcohol content of the local gin.

Plant collection and extraction

The leaves of *Anthocleista djalonensis* were collected from its natural habitat in Nnamdi Azikiwe University Main Campus, Agu Awka, Anambra State on April 2023, during the morning hours. The plant material was identified and authenticated by Mrs Onwunyili Amaka of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikwe University, and the voucher number PCG/474/9/057. The collected leaves were washed, air-dried at room temperature, and then ground into a fine powder. The preparation of crude extracts followed the same procedure as detailed in (Chineze *et al.,* 2024*)*. The pH of the solvents was first determined using a pH meter to understand the chemical environment in which the extraction occurred. The powdered leaves were extracted by maceration using three different solvents: local gin, hot distilled water and 95% analytical grade ethanol. Each of the solvents (2000 ml) was used to soak 150g of the powdered leaves for 24 hours. The mixture was doubly filtered with muslin cloth and Whatman No 1 filter paper (Whatman®, England). The ethanol filtrate was concentrated under reduced pressure using a rotary evaporator (Buchi Rotavapor R-200, Switzerland) while the distilled water and local gin extracts were freeze-dried. The resulting concentrated crude extracts were stored in a refrigerator at 4 ˚C until used for experiment. The percentage yield of each extract was calculated using the formula:

$$\text{Percentage yield} = \frac{\textit{weight of dried extract}}{\textit{weight of pulverized leave used in maceration}} \times 100$$

Experimental animal and parasites

Healthy Swiss Albino mice 7 - 8 weeks old weighing 24 - 30 g of both sexes were obtained from the Animal House of the Department of Pharmacology, University of Science and Technology, Enugu State, Nigeria. The animals were kept in clean and well ventilated cages with a 12-hour light-dark cycle at room temperature, and provided with pellet diet (grower feeds by Top Feeds Nigeria Limited) and water *ad libitum*. Animals were allowed to acclimatize to the laboratory condition for a week before beginning the experiment. The rodent parasite (*Plasmodium berghei*) used was of ANKA strain obtained from Nigeria Institute of Medical Research, Lagos, Nigeria. The parasite was maintained by serial passage in mice in the laboratory.

Acute oral toxicity test

Swiss Albino mice (24 - 30 g) of both sexes were used. The median lethal dose (LD_{50}) was determined for each of the extracts using modified Lorke's method (Lorke, 1983) as described by (Ibrahim *et al*., 2024). This method has two phases which are phases 1 and 2 respectively.

Phase 1: In this phase, nine animals were randomly assigned to each of the three test groups: the local gin extract group, the ethanol extract group, and the water extract group. Each group was further divided into three sub-groups, with three animals in each sub group. Members of each of the three sub-groups were administered doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg of each of the crude extract respectively. Administration of extracts was done orally with a cannula attached to a graduated syringe. The general behavior of the individual animals was observed continuously for 30 minutes after the treatment and then periodically for 4 hours and thereafter over a period of 24 hours, for signs of behavioral changes as well as mortality that may suggest toxicity.

Phase 2: This phase involved the use of four animals per extract sample. These animals were distributed into sample groups of one animal per group. The animals were then administered doses based on the result obtained from the first

phase. Doses selected were 2000 mg/kg, 3000 mg/kg, 4000 mg/kg and 5000 mg/kg. The mice were also observed for signs of toxicity. The mice were further observed for up to 14 days following treatment for any signs of toxicity and death. Any adverse effects, such as weakness, stretching, gasping, fatigue, and body weight loss in the treated groups were noted. The oral median lethal doses (LD_{50}) were calculated as;

$$LD_{50} = \sqrt{D0 \times D100}$$

Where; D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality

The above calculation of the lethal dose (LD_{50}) represents the dose at which 50% of the animal exhibits toxicity or mortality. This calculation will help establish the acute toxicity level of the extracts.

Standard antimalarial drug

Artemether/lumefantrine (A/L) was used as the standard antimalarial drug in this study. Based on previous research by (Georgewill and Adikwu, 2022) we administered the drug orally by gavage at doses of 2.3 mg/kg for artemether and 13.7 mg/kg for lumefantrine. These doses are designed to ensure the drug concentrations in the bloodstream are sufficient to effectively clear the malaria parasites in the mice while maintaining safety.

Experimental design (Number of animals used)

The experimental design includes three test groups (local gin extract, ethanol extract and water extract), each tested with five different doses of the extract, and two control groups (positive and negative). Each group contained an initial sample size of five (5) animals. To account for potential attrition, an attrition rate of 10% was considered to ensure that any potential loss of animals during the experiment would not compromise the integrity of the results. The corrected sample size per group was calculated using the method described by (Charan and Kantharia, 2013).

$$\text{Corrected sample size} = \frac{initial\ sample\ size}{1 - (\frac{\%\ attrition}{100})}$$

Substituting the given values:

$$\text{Corrected sample size} = \frac{5}{1 - (\frac{10}{100})} = 5.556 \approx 6$$

The final sample size per group was six (6) animals; this ensured that even with a 10% attrition rate, the study would retain at least five animals per group, thereby maintaining the statistical power and integrity of the experiment. The total number of animals required for both assays combined was 204 animals (102 animals for the curative assay and 102 animals for the suppressive assay). Additionally, 15 mice were used in the pilot study, and 39 mice were used for the acute oral toxicity study. Therefore, a total of 258 mice were used to complete the study.

Animal grouping and dosing

In both models (4-day suppressive and curative test), animals were grouped into seven groups of six animals each for each extract. The test groups (groups 1, 2, 3, 4 and 5) animals were treated orally with extract doses of 50, 100, 200, 400 and 800 mg/kg respectively. The doses were determined based on the outcomes of the acute toxicity studies and the pilot study carried out. Groups 6 and 7 animals served as the negative control (distilled water, 10 ml/kg) and positive control (artemether/lumefantrine, 2.3/13.7 mg/kg).

Inoculation of mice

Inoculation of the mice was carried out using a reported method (Misganaw *et al;* 2020*)*. Albino mice previously infected with *Plasmodium berghei* and having a parasitemia level of 50 - 60 % were used as donors. Blood samples were collected from the donor mice via

their retro-orbital plexus into heparinized tubes. The blood was then diluted with normal saline (0.9 %) based on the parasitemia of the donor mice, and the red blood cells (RBC) counted in such a way that 1 ml of blood contained $5x10^7$ infected erythrocytes. Thereafter, 0.2 ml of blood, containing $1x10^7$ *Plasmodium berghei* - infected erythrocytes, was injected through the intraperitoneal (ip) route for each mouse.

Determination of antimalarial activity

4-day suppressive test

The suppressive activities of the extracts were evaluated by Peter's 4-day suppressive test (Peter, 1975) against mice infected with *Plasmodium berghei*. A total of one hundred and two mice (102) were inoculated on the first day (day 0) with $1x10^7$ parasitized erythrocytes from *Plasmodium berghei*. Two hours after infection, the mice were randomly divided into five test groups and two control groups, with six mice per cage for each extract, as described in the Animal Grouping and Dosing section. Afterwards, treatment was continued for an additional three consecutive days (until day 3). The determination of parasitemia was conducted on day four of the experiment (96 hours post-infection). Body weights were measured before infection and at the end of the experiment. Other parameters measured at the end of the experiment include packed cell volume (PCV) and haemoglobin (Hb).

Rane's curative test

The curative potential of the extracts was evaluated using (Ryley and Peters, 1970). On the first day (day 0), the mice were injected intraperitoneally with a standard inoculum of $1x10^7$ *Plasmodium berghei*-infected erythrocytes. Following confirmation of parasitemia 72 hours later (day 3), the mice were divided into seven groups, each consisting of six mice, for each extract. These groups were treated with the prepared leaf extracts of local gin, ethanol and distilled water and administered at doses of 50, 100, 200, 400 and 800 mg/kg. Artemether/lumefantrine (2.3/13.7 mg/kg) was given to the positive control, and distilled water (10 ml/kg) was given to the negative control group. The treatment lasted for 4 days (day 7) at a single dose per day, after which blood smears were collected and examined microscopically to monitor the parasitemia level. Body weights were measured before infection and at the end of the experiment. Other parameters measured at the end of the experiment include packed cell volume (PCV) and haemoglobin (Hb).

Determination of parasitemia

Parasitemia was determined by counting the number of infected RBCs (a minimum of three fields per slide) using a light microscope (MB23-0 T, China). Thin blood smears were prepared from the tail snip of each mouse on the fifth day for the suppressive test, and from day 3 after infection was established to day 7 for the curative test. The microscopic slides were dried, fixed with absolute methanol, and stained with 10 % Giemsa at pH 7.2 for 10 minutes. The slides were then washed gently with distilled water and air-dried at room temperature. Finally, the slides were viewed under a light microscope using an oil immersion objective (× 100 magnification power) by taking an average of six fields. Percentage parasitemia was calculated by counting infected red blood cells and total red blood cells from Giemsa-stained thin blood films. The modified formula from (Peters and Robinson, 1992) was used.

$$\text{Percentage parasitemia} = \frac{\textit{Number of parasitized RBC}}{\textit{Total number of RBC counted}} \times 100$$

The percentage suppression of parasitaemia was calculated for each tested doses by comparing the parasitaemia in infected controls with those that received different doses of the test extracts.

Percentage suppression (%) = $\frac{Percentage\ parasitemia\ in\ negative\ control - Percentage\ parasitemia\ in\ treated\ group}{Percentage\ parasitemia\ in\ negative\ control} \times 100$

Determination of mean survival time

The mean survival time for each group in both models was determined by finding the average survival time (days) of the mice. The mice were checked daily and the number of surviving days from inoculation to death were recorded for each mouse in treatment and control groups. The average survival days from the date of infection for a period of 28 days were calculated (Alaribe *et al,* 2021). The mean survival time (MST) was estimated for each group using the following formula;

Mean survival time (MST) = $\frac{Total\ number\ of\ days\ mice\ survived}{Total\ number\ of\ mice}$

Monitoring of body weight changes

The body weight of each mice in all groups were taken before infection (day 0) and after treatment (day 4) in the 4-day suppressive test while in the curative test it was measured on day 3 after infection was established and on day 7 after treatment. The body weight of each mouse was taken with the aid of a sensitive analytical weighing balance (METTLER AL 204, China) to observe if the test extracts prevented body weight loss that is commonly observed in infected mice.

Body weight change = *Body weight day 4 – body weight day 0*

Percent body weight change = $\frac{Body\ weight\ day\ 4 - body\ weight\ day\ 0}{Body\ weight\ day\ 4} \times 100$

Determination of packed cell volume

The packed cell volume (PCV) of the mice was measured to assess the effectiveness of the crude extracts in preventing the destruction of red blood cells (hemolysis) resulting from increasing levels of *Plasmodium* parasites in the bloodstream.

Blood was withdrawn from the tail of each mouse using heparinized capillary tubes, where heparinized ends were filled with blood to 3/4th of their height. The capillary tubes were carefully sealed with non-heparinized ends using sealing clay and then placed in a centrifuge with the sealed ends facing outward. Centrifugation was done at 12,000 rpm for five minutes using a microhematocrit centrifuge (Hettichhaematokrit, Germany) After centrifuging, the capillary tubes were removed from the machine, and the PCV was measured using a micro hematocrit reader (Hawksley, England) PCV was measured both before inoculating the parasite (day 0) and on day 4 in Peters's 4-day suppressive test. In the case of the curative test, PCV was measured on the third day after infection was established and on the last day of treatment, on the seventh day. PCV is a measure of the proportion of RBCs to plasma in the whole blood and is determined using the relationship shown below (Misganaw *et al.,* 2020).

PCV = $\frac{Volume\ of\ erythrocyte\ in\ a\ given\ volume\ of\ blood}{Total\ blood\ volume\ examined} \times 100$

Haemoglobin quantification

The haemoglobin content of the whole blood was quantified using Drabkin's reagent ($K_3Fe(CN)_6$ 200 mg/l, KCN 50 mg/l, $NaHCO_3$ 1 g/L, pH 8.6). The procedure is based on the oxidation of haemoglobin to methaemoglobin in the presence of alkaline potassium ferricyanide. The methemoglobin reacts with potassium cyanide to form cyanmethemoglobin. The colour intensity measured at 540 nm is proportional to the total haemoglobin concentration. To all tubes—blank and samples—5 ml of Drabkin's solution was added, followed by 20 ul of whole blood added

to the sample tubes, while distilled water was added to the blank tube. The tubes were mixed and allowed to stand for 15 minutes at room temperature. The absorbance of tests was read at 540 nm after blanking, and the total haemoglobin concentration (mg/ml) was derived from the calibration curve of cyanmethaemoglobin (Kenechukwu, 2020).

Statistical analysis

All experimental data were analyzed using Windows SPSS version 23.0. Results for the studied parameters were expressed as mean ± standard error of mean (SEM). Statistical analysis was conducted using One-way analysis of variance (ANOVA), followed by pairwise multiple comparison using Tukey's Honestly Significant Difference (HSD) test (Tukey-kramer), which was utilized to compare the measured parameters' results within and between groups. The analysis was performed with 95 % confidence interval and p-values less than 0.05 were considered to be statistically significant. The analysis was conducted in triplicate.

Results

pH measurement of the extraction solvents

The pH values obtained for the solvents were carried out using a multi-point calibration method as presented in **Table 1.**

Table 1: pH values of Extraction Solvents

Solvent	pH value
Ethanol (95% analytical grade)	7.2
Distilled water	5.4
Local gin	4.2

Percentage of ethanol (alcohol) in local gin solvent

The analysis of the local gin solvent used in this study revealed an ethanol content of 78% (v/v) and a water content of 22% (v/v) as determined by simple distillation.

Yield of extracts

The percentage yield of the crude extracts of *Anthocleista djalonensis*is leaves was calculated using $\frac{Final\ weight\ of\ dried\ extract\ (g)}{Initial\ weight\ of\ powdered\ leaves\ (g)} \times 100$

The percentage yield presented in **Table 2** showed that the Local gin solvent yielded more than ethanol and water extract.

Table 2: Percentage yield of the extracts *Anthocleista djalonensis* leaf

Solvent type	Initial Weight of Powdered leaves (g)	Final Weight of Dried Extract (g)	Percentage Yield (%)
Ethanol	150	14.1	9.4
Hot distilled water	150	25.6	17.1
local gin	150	30.8	20.5

Acute oral toxicity study

The acute oral toxicity study, investigates the toxicity of *Anthocleista djalonensis* leaves extracts administered orally to mice with doses of 10, 100, 1000, 2000, 3000, 4000 and 5000 mg/kg. In the initial phase (phase 1) of the study, the oral administration of the all studied extracts (10, 100 and 1000 mg/kg) showed no physical signs of toxicity or mortality. This was consistent for all three studied extracts: distilled water, local gin, and ethanol extracts. As the doses increased to 2000, 3000, 4000 5000 mg/kg (phase 2), differences in the extracts' toxicity profiles became evident. The ethanol extract showed severe physical signs of toxicity. The signs include writhing, gasping, palpitation, stretching, decreased respiratory rate and death. All mice treated with 5000 mg/kg dose of the ethanol extract died. The LD_{50} (lethal dose for 50% of the population) of ethanol extract was calculated to be at 4472 mg/kg. The local gin and water extracts showed mild behavioral signs of toxicity that lasted 1- 4 hours after administration with no death recorded. The signs include sedation, weakness, loss of appetite and restlessness. The LD_{50} of both local gin and water extract was estimated to be above 5000 mg/kg.

4-day Suppressive test (early malaria infection)

The effect of the three solvent leaf extracts of *Anthocleista djalonensis* on percentage parasitemia and survival time in *Plasmodium berghei* infected mice in the 4-day suppressive test

As shown in **Table 3**, all three extracts (local gin, ethanol, and water) significantly ($p < 0.05$) suppressed parasitemia at every tested dose (50 – 800 mg/kg) compared to the negative control, with suppression exceeding 92% at higher doses (200 – 800 mg/kg) for local gin and ethanol extracts. The local gin extract demonstrated the strongest suppression across all doses, followed by ethanol and water. While artemether/lumifantrine (positive control) achieved 100% suppression, the local gin extract at 800 mg/kg showed no significant difference ($p < 0.05$) from the standard drug, indicating comparable efficacy. All extracts significantly prolonged mean survival time (MST) compared to the negative control, though local gin was consistently superior, showing significant differences even at the lowest dose (50 mg/kg), where ethanol and water had no effect. MST efficacy ranked as follows: local gin > ethanol > water.

Table 3: Parasitemia and survival time of infected mice treated with local gin, ethanol and water extracts of the leaf of *Anthocleista djalonensis* in the 4-day suppressive test

Treatment Groups	Mean Percentage Parasitemia at Day 4 (%)	Percentage Suppression (%)	Mean Survival Time (Days)
Negative Control	37.34 ± 0.50	-	9.60 ± 0.50
A/L	0.00 ± 0.00	$100^{a,d,e}$	$30.00 \pm 0.00^{a,c,d,e}$
800 mg/kg			
Local gin	1.19 ± 0.40	96.98^{a}	$24.80 \pm 0.58^{a,b,}$
Ethanol	2.04 ± 0.35	$94.54^{a,b}$	$21.40 \pm 0.87^{a,b}$

Water	2.12 ± 0.50	94.33[a,b]	19.00 ± 0.89[a,b,c]
400 mg/kg			
Local gin	1.13 ± 0.25	96.82[a,e]	24.00 ± 0.89 [a,b,d,e]
Ethanol	1.98 ± 0.30	94.70 [a,b,e]	20.80 ± 1.01 [a,b,c,e]
Water	4.32 ± 0.43	88.43 [a,b,c,d]	17.20 ± 0.86 [a,b,c,d]
200 mg/kg			
Local gin	1.39 ± 0.45	96.28[a,e]	22.00 ± 0.63 [a,b,e]
Ethanol	2.93 ± 0.44	92.16 [a,b,e]	20.40 ± 0.76 [a,b,e]
Water	6.21 ± 0.50	83.36 [a,b,c,d]	14.20 ± 0.73 [a,b,c,d]
100 mg/kg			
Local gin	2.79 ± 0.45	92.54[a,b,d,e]	20.00 ± 0.84 [a,b,d,e]
Ethanol	6.15± 0.42	83.52 [a,b,c,e]	14.80 ± 0.58 [a,b,c]
Water	10.51 ± 0.57	71.85 [a,b,c,d]	12.20 ± 0.80 [a,b,c]
50 mg/kg			
Local gin	6.19 ± 0.55	83.43 [a,b,d,e]	15.6 ± 0.68 [a,b,d,e]
Ethanol	11.91 ± 0.50	68.09 [a,b,c,e]	10.00 ± 0.55[b,c]
Water	18.11 ± 0.49	51.50 [a,b,c,d]	8.80 ± 0.49[b,c]

Data are expressed as mean ± SEM, n = 6, ANOVA followed by pairwise multiple comparison using Tukey's HSD test (Tukey-Kramer); values with a superscript are statistically significant when compared to the treatment of the same dose. The letter represents: a, compared to negative control; b, to Artemether/Lumefantrine (A/L) (2.7/13.2 mg/kg); c, to the local gin extract; d, to the ethanol extract; e, to the water extract; $p < 0.05$.

The effect of the three solvent leaf extracts of *Anthocleista djalonensis* on changes in body weight, packed cell volume, and haemoglobin concentration in *Plasmodium berghei* infected mice in the 4- days suppressive test .

Table 4 summarizes changes in mean body weight, packed cell volume (PCV), and hemoglobin (Hb) concentration on day 4. Initial body weights (day 0) were similar across all groups ensuring a fair basis for comparison. By day 4, the negative control group lost 13% body weight, while the positive control group gained 6% increase. The local gin extract demonstrated a dose-dependent weight gain, with 800 and 400 mg/kg showing no significant difference ($p < 0.05$) from the positive control. The ethanol extract caused weight gain only at 800 mg/kg but reductions at lower doses (50 –

400 mg/kg). The water extract treated group showed a dose-dependent weight loss at all doses. At 50 mg/kg, all extracts showed no statistically significant difference from the negative control.

For PCV and Hb concentration, the positive control significantly outperformed ($p < 0.05$) the negative control. All extract showed a dose-dependent effects on both PCV and Hb. Both local gin and ethanol extracts at 800 mg/kg showed significant protection of PCV and Hb, comparable to the positive control. Lower doses of the extracts (50 – 100 mg/kg) had no statistically significant effect compared to the negative control.

Table 4: Body weight changes, packed cell volume, and heamoglobin concentration of infected mice treated with local gin, ethanol and water extracts of the leaves of *Anthocleista djalonensis* in the 4-day suppressive test

Treatment Groups	Weight Before (g)	Weight After (g)	Percent Wt. Change (%)	Packed Cell Volume (%)	Haemoglobin Conc. (g/dl)
Neg. Control	25.2 ± 0.37	21.8 ± 0.58	-13.42	41.0 ± 1.14	13.7 ± 0.36
A/L	25.0 ± 0.45	26.4 ± 0.68	5.6	53.0 ± 1.30 [a]	16.93 ± 0.42 [a]
800 mg/kg					
Local gin	24.8 ± 0.58	26.6 ± 0.60	7.26 [a,e]	54.6 ± 1.33 [a]	17.4 ± 0.42[a]
Ethanol	25.0 ± 0.55	25.8 ± 0.66	3.20 [a]	52.8 ± 0.86 [a]	16.9 ± 0.27 [a]
Water	25.0 ± 0.45	23.8 ± 0.58	-4.8[c]	50.0 ± 0.89 [a]	16.0 ± 0.29 [a]
400 mg/kg					
Local gin	24.6 ± 0.75	26.0 ± 0.63	5.70 [a,e]	52.0 ± 0.95 [a,e]	16.6 ± 0.30 [a,e]
Ethanol	24.8 ± 0.73	24.4 ± 0.75	-1.61	51.6 ± 1.17 [a,e]	16.5 ± 0.37 [a,e]
Water	24.6 ± 0.68	22.6 ± 0.75	-8.13[c]	44.8 ± 1.20[b,c,d]	14.3 ± 0.38[b,c,d]
200 mg/kg					
Local gin	24.6 ± 0.74	24.8 ± 0.86	0.81[e]	50.4 ± 0.75 [a,e]	16.1 ± 0.24 [a,e]
Ethanol	25.2 ± 0.58	23.6 ± 0.60	-6.25	46.2 ± 0.86 [a,b]	14.76 ± 0.27 [a,b]
Water	25.6 ± 0.51	21.4 ± 0.60	-16.41[c]	43.4 ± 0.81[b,c]	13.87 ± 0.26[b,c]
100 mg/kg					
Local gin	24.6 ± 0.75	25.0 ± 0.55	1.63 [a,d,e]	45.0 ± 1.10 [b,e]	14.38 ± 0.35 [b,e]
Ethanol	25.4 ± 0.68	22.0 ± 0.45	-13.39 [b,c]	41.2 ± 1.16 [b]	13.16 ± 0.37 [b]
Water	25.4 ± 0.68	20.8 ± 0.37	-18.11[b,c]	36.2 ± 1.32 [b,c]	11.57 ± 0.42[b,c]
50 mg/kg					

Local gin	25.0 ± 0.45	21.6 ± 0.51	-13.6[b]	39.6 ± 0.93[b,e]	12.65 ± 0.30 [b,e]
Ethanol	25.4 ± 0.40	21.2 ± 0.37	-16.54[b]	35.0 ± 1.14[a,b]	11.18 ± 0.36 [a,b]
Water	25.4 ± 0.40	20.0 ± 0.71	-21.26[b]	32.0 ± 1.14 [a,b,c]	10.22 ± 0.36 [a,b,c]

Data are expressed as mean ± SEM, n = 6, ANOVA followed by pairwise multiple comparison using Tukey's HSD test (Tukey-Kramer); values with a superscript are statistically significant when compared to the treatment of the same dose. The letter represents: a, compared to negative control (Neg.); b, to Artemether/Lumefantrine (A/L) (2.7/13.2 mg/kg); c, to the local gin extract; d, to the ethanol extract; e, to the water extract; $p < 0.05$.

Curative test (established malaria infection)

The effect of the three solvent leaf extracts of *Anthocleista djalonensis* on parasitemia levels and survival time in *Plasmodium berghei* infected mice in the 7-day curative test

In the curative test (**Table 5**), percentage parasitemia levels on day 3 (before treatment) showed no significant differences ($p < 0.05$) among groups. By day 7, the positive control completely eradicated the parasite to an undetectable level. The 800 mg/kg local gin extract treated group showed the strongest curative activity among extracts but remained significantly less effective ($p < 0.05$) than the positive control. All extracts exhibited a dose-dependent parasitemia reduction. For mean survival time (MST), at 800 mg/kg, all extracts significantly prolonged MST ($p < 0.05$) compared to the negative control, with the local gin extract being most effective at all doses. The lower doses (50 – 200 mg/kg) had no significant effect on survival time. The order of survival time efficacy was: Positive control > local gin > ethanol ≥ water > negative control.

Table 5: Parasitemia levels and survival time of infected mice treated with local gin, ethanol and water extracts of the leaf of *Anthocleista djalonensis* in the 7-day curative test

Treatment Groups	Parasitemia (%) Day 3	Parasitemia (%) Day 7	Mean Survival Time (Days)
Negative Control	39.22 ± 1.10	41.5 ± 1.02	8.40 ± 0.51
A/L	37.67 ± 1.53	0.26 ± 0.12 [a,d,e]	30.00 ± 0.00[a,c,d,e]
800 mg/kg			
Local gin	39.92 ± 1.40	2.68 ± 0.5 [a,e]	16.80 ± 0.58 [a,b,d,e]
Ethanol	38.99 ± 1.81	3.16 ± 0.66[a,b]	13.2 ± 0.58 [a,b,c]
Water	38.26 ± 1.39	6.86 ± 0.80[a,b,c]	13.00 ± 0.55 [a,b,c]
400 mg/kg			
Local gin	38.95 ± 0.74	3.10 ± 0.46 [a,b,d,e]	15.2 ± 0.58 [a,b,d,e]

Ethanol	40.06 ± 1.07	5.98 ± 0.49 [a,b,c]	11.4 ± 0.75[a,b,c]
Water	40.19 ± 1.46	7.90 ± 0.70 [a,b,c]	9.60 ± 0.68[b,c]
200 mg/kg			
Local gin	40.88 ± 0.82	4.37 ± 0.54 [a,b,e]	10.80 ± 0.66[b]
Ethanol	39.80 ± 0.80	6.60 ± 0.58 [a,b]	10.00 ± 0.63[b]
Water	39.66 ± 0.46	8.30 ± 0.54 [a,b,c]	9.40 ± 0.75[b]
100 mg/kg			
Local gin	41.07 ± 1.36	8.10 ± 0.86 [a,b,e]	9.20 ± 0.58[b]
Ethanol	40.49 ± 0.95	10.99 ± 0.63 [a,b]	8.60 ± 0.60[b]
Water	41.72 ± 1.52	12.38 ± 0.94 [a,b]	8.80 ± 0.58[b]
50 mg/kg			
Local gin	40.44 ± 1.03	9.28 ± 0.88 [a,b,d,e]	8.80 ± 0.49
Ethanol	40.57 ± 1.50	13.30 ± 0.59 [a,b,c,e,]	8.00 ± 0.00
Aqueous	39.62 ± 1.32	18.89 ± 0.82 [a,b,c,d]	8.00 ± 0.00

Data are expressed as mean ± SEM, n = 6, ANOVA followed by pairwise multiple comparison using Tukey's HSD test (Tukey-Kramer); values with a superscript are statistically significant when compared to the treatment of the same dose. The letter represents: a, compared to negative control; b, to Artemether/Lumefantrine (A/L) (2.7/13.2 mg/kg); c, to the local gin extract; d, to the ethanol extract; e, to the water extract; $p < 0.05$.

The effect of the three solvent leaf extracts of *Anthocleista djalonensis* on changes in body weight, packed cell volume, and haemoglobin concentration in *Plasmodium berghei* infected mice in the 7-day curative test

The results in **Table 6** presents changes in mean body weight (days 3 and 7), packed cell volume (PCV), and hemoglobin (Hb) concentration during the curative test. The negative control group showed significant weight loss and reduced PCV and Hb levels, associated with increasing parasitemia. In contrast, the positive control (standard drug) maintained the highest PCV and Hb values. Both the local gin and water extracts demonstrated dose-dependent effects on PCV value. The group treated with local gin extract at 800 mg/kg provided significant ($p < 0.05$) protection against malaria-induced weight loss compared to both the negative control and water extract at same doses. Ethanol and local gin extracts showed dose-dependent weight reduction by day 7 (after treatment) while the water extract exhibited variable effects on body weight across tested doses.

Table 6: Weight changes, packed cell volume, and haemoglobin concentration of infected mice treated with local gin, ethanol and water extracts of the leaf of *Anthocleista djalonensis* in the 7-day curative test

Treatment Groups	Baseline body weight (g) (Day 3; before treatment)	Final body weight (g) (Day 7; After treatment)	Packed Cell Volume (%)	Haemoglobin Concentration (g/dl)
Neg. Control	25.4 ± 0.68	18.0 ± 0.89	22.4 ± 1.57	7.2 ± 0.50
A/L	25.4 ± 0.4	22.8 ± 0.8	53.2 ± 1.88	17.0 ± 0.60
800 mg/kg				
Local gin	25.6 ± 0.50	25.4 ± 0.51[a,e]	49.8 ± 1.88 [a]	15.9 ± 0.60 [a]
Ethanol	25.8 ± 0.58	23.0 ± 0.55 [a]	49.8 ± 1.11 [a]	15.9 ± 0.36 [a]
Water	25.8 ± 0.66	21.6 ± 0.68 [a,c]	45.8 ± 1.80 [a,b]	14.6 ± 0.58 [a,b]
400 mg/kg				
Local gin	25.0 ± 0.45	23.8 ± 0.58 [a]	46.8 ± 1.62 [a]	15.0 ± 0.52 [a]
Ethanol	25.4 ± 0.51	22.0 ± 0.84 [a]	43.8 ± 1.53 [a,b]	14.0 ± 0.49 [a,b]
Water	25.6 ± 0.75	20.6 ± 0.68	40.2 ± 1.11 [a,b]	12.8 ± 0.36 [a,b]
200 mg/kg				
Local gin	25.6 ± 0.75	22.8 ± 0.86 [a]	42.4 ± 1.72 [a,b]	13.5 ± 0.55 [a,b]
Ethanol	25.4 ± 0.40	21.2 ± 0.58	38.0 ± 1.55 [a,b]	12.1 ± 0.49 [a,b]
Water	25.4 ± 0.51	21.6 ± 0.75 [a]	38.0 ± 1.41 [a,b]	12.1 ± 0.45 [a,b]
100 mg/kg				
Local gin	25.4 ± 0.68	23.0 ± 0.63[a]	41.0 ± 1.30 [a,b]	13.0 ± 0.42 [a,b]
Ethanol	25.4 ± 0.51	22.0 ± 0.71[a]	40.0 ± 1.2 [a,b]	12.8 ± 0.39 [a,b]
Water	25.4 ± 0.40	21.8 ± 0.49[a]	35.8 ± 1.16 [a,b]	11.4 ± 0.37 [a,b]
50 mg/kg				
Local gin	25.0 ± 0.45	21.0 ± 0.45[a]	39.4 ± 1.17 [a,b]	12.6 ± 0.37 [a,b]
Ethanol	25.0 ± 0.45	19.6 ± 0.68[b]	34.0 ± 1.41 [a,b]	10.9 ± 0.45 [a,b]
Water	25.2 ± 0.49	18.8 ± 0.49[b]	31.6 ± 1.94[a,b]	10.1 ± 0.62[a,b]

Data are expressed as mean ± SEM, n = 6, ANOVA followed by pairwise multiple comparison using Tukey's HSD test (Tukey-Kramer); values with a superscript are statistically significant when compared to the treatment of the same dose. The letter represents: a, compared to negative control (Neg.); b, to Artemether/Lumefantrine (A/L) (2.7/13.2 mg/kg); c, to the local gin extract; d, to the ethanol extract; e, to the water extract; $p < 0.05$.

Discussion

The present study examines the acute oral toxicity and antimalarial activities of *Anthocleista djalonensis* leaf extracts obtained using local gin (kai-kai), ethanol, and water. Their effects were evaluated in mice infected with *Plasmodium berghei*. Solvent selection significantly influences the yield and nature of bioactive compounds, which is crucial for maximizing therapeutic efficacy against malaria (Chineze *et al.*, 2024; Misganaw *et al.*, 2019). The *Plasmodium berghei* ANKA strain, a widely used experimental model due to its genetic and structural similarity with *Plasmodium falciparum*, the primary human malaria parasite was employed to establish infection. Additionally, it effectively replicates cerebral malaria, marked by microvascular sequestration of infected red blood cells and early neurocognitive deficits linked to blood-brain barrier disruption (de Lima *et al.*, 2025)

The acute oral toxicity study revealed a dose-dependent toxicity in the extracts, consistent with Paracelsus' principle that toxicity is determined by dose (Michaleas *et al.,* 2021). At lower doses (Phase 1), no toxicity was observed, indicating high safety. However, higher doses (Phase 2) demonstrated clear behavioral toxicity. The ethanol extract caused mortality at 5000 mg/kg, with an LD_{50} of 4472 mg/kg, whereas the local gin and water extracts showed no mortality at 5000 mg/kg, confirming their practical non-toxicity (LD_{50} > 5000 mg/kg) the recognized threshold for non-toxic classification (Hayes and Kobets*,* 2023). These findings support both the traditional use of *Anthocleista djalonensis* for malaria and the local preference for water and gin as extraction solvents in Nigeria.

The antimalarial efficacy of *Anthocleista djalonensis* leaf extracts was evaluated using the 4-day suppressive test (early infection) and the curative test (established infection). An *in vivo* model was used to investigate two key aspects: first, the extracts potential to act as pro-drugs (if metabolized into active compounds) and second, how the immune system contributes to combating malaria infection (Belay *et al*., 2018).

In the suppressive test, all crude extracts showed dose-dependent parasitemia reduction (**Table 3**), with significant suppression compared to the negative control, likely due to synergistic effects of bioactive components. The local gin extract showed superior efficacy (96.82% suppression at 800 mg/kg), followed by ethanol (94.54%) and water (94.33%,) extracts. These results align with prior studies on Anthocleista species (Bassay *et al.*, 2009; Odeghe *et al.*, 2012; Ogbuehi *et al*., 2014) and support the criterion that >30% parasitemia reduction indicates significant antimalarial activity (Adugna *et al.*, 2014).

The crude extracts were further evaluated for their effects on established malaria infections using the curative test. From day 3 to day 7, treatment significantly reduced parasitemia in a dose-dependent manner (**Table 5**), with the local gin extract showing the most potent activity. At 800 mg/kg, the extracts exhibited a parasitostatic effect, inhibiting parasite growth and reproduction without complete eradication, unlike the standard drug (artemether/lumefantrine), which contains isolated active compounds. These findings confirm the extract's efficacy in late-stage infections, aligning with previous reports (Misganaw *et al.*, 2020; Zeleke, 2015). While less effective than standard drugs at lower doses, the local gin extract dual suppressive-curative activity at higher dose (96.82% suppression and 87.4% curative efficacy at 800 mg/kg) supports its potential as phytotherapeutics candidate (Jampilek, 2017). This plasmodistatic effect may delay resistance development when used as adjunct therapy, offering strategic value in malaria management. These findings validate the traditional use of

Anthocleista djalonensis and local gin solvent in malaria treatment particularly relevant for malaria-endemic regions where access to standard drugs remains challenging.

Mean survival time (MST), body weight loss, and reductions in packed cell volume (PCV) and hemoglobin levels are key indicators of malaria infection. An effective antimalarial extract should prolong survival, prevent weight loss, and reverse PCV and hemoglobin decline (Birru, 2017). In this study, all crude extract doses significantly ($p < 0.05$) increased MST compared to the negative control, with the highest doses (800, 400, and 200 mg/kg) showing the most pronounced effects. The local gin extract yielded the longest MST, likely due to its higher concentration of bioactive compounds. However, the standard drug still outperformed all extract-treated groups ($p < 0.05$), correlating with maximal parasitemia inhibition. Since extracts extending MST beyond 12 days are considered effective (Kifle *et al.,* 2020), these findings support their antimalarial potential, consistent with previous reports (Atanu *et al.*, 2020; Bantie *et al.*, 2014; Sidiki *et al.*, 2023).

In the suppressive test, ethanol and water extracts reduced body weight despite suppressing parasitemia (**Table 4**). However, at 800 mg/kg, the ethanol extract increased weight, suggesting phytochemicals may influence appetite and digestion (Tucci, 2010). The local gin extract, comparable to the positive control at all doses, likely owes its efficacy to higher phytochemical concentrations (Chineze *et al*., 2024). In the curative test as shown in **Table 6**, all extracts caused significant weight loss, attributable to combined effects of infection, immune response, and treatment side effects.

The packed cell volume (PCV) is a key hematological parameter in malaria studies, reflecting erythrocyte protection against malaria-induced hemolysis (White, 2018). All extracts improved PCV in a dose-dependent manner with the local gin extract showing the strongest effect comparable to the standard drug at 800 mg/kg. This effect likely stems from phytocompounds stimulating bone marrow RBC production (Nureye *et al.*, 2021) consistent with findings in other antimalarial plants (Baah *et al.*, 2020; Uzor *et al*., 2021).

Hemoglobin, the iron-rich protein that facilitates oxygen transport and gives blood its red color, serves as a primary diagnostic marker for anemia. The negative control group exhibited low hemoglobin levels, consistent with malaria-induced anemia and its known effects on erythrocytes. As shown in **Tables 4 and 6**, higher extract doses correlated with greater parasite suppression and increased hemoglobin levels, confirming a dose-dependent efficacy which align with the findings of (Uzor *et al.*, 2021). Across all models, the local gin extract produced the highest hemoglobin levels among the crude extracts, suggesting superior antimalarial activity.

Conclusion

This study advances the understanding of solvent extraction efficiency by comparing three primary solvents used in herbal remedies. Local gin emerged as the most effective, yielding the highest concentration of active phytocompounds and demonstrating superior antimalarial efficacy. To our knowledge, this is the first study to confirm the antimalarial potential of this plant's local gin extract, validating its traditional use and its role in improving malaria treatments. The findings highlight how solvent selection critically influences the therapeutic potential of medicinal plants, suggesting that past inefficiencies may have resulted from poor solvent choices. However, inconsistencies in local gin's composition and alcohol content which may affect phytochemical yield and bioactivity warrant further investigation.

Standardizing such traditional solvents could unlock their full promise for malaria treatment in resource-limited communities.

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