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From rural practice to scientific evidence: synergistic antiplasmodial and antioxidant properties of combined *Ageratum conyzoides* and *Bidens pilosa* extracts

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Abstract

Aim: This study evaluates the in vitro and in vivo antiplasmodial, hemolytic, and antioxidant activities of a combined extract of *Ageratum conyzoides* (*A. conyzoides*) and *Bidens pilosa* (*B. pilosa*), a traditionally used but scientifically unvalidated combination.

Methods: Plant leaves were extracted via aqueous decoction and cold maceration, combining equal parts to mimic traditional preparation. In vitro antiplasmodial activity against the chloroquine-sensitive *Plasmodium falciparum* 3D7 (Pf3D7) strain was assessed using the SYBR Green I assay. Cytotoxicity was evaluated via hemolysis test, and antioxidant potential using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)], and FRAP (ferric ion reducing antioxidant potential) assays. The most potent combination was tested for acute toxicity and curative antimalarial activity in a rodent model.

Results: Extract yields ranged from 6.6% (cold maceration extract of *B. pilosa*) to 29.2% [aqueous decoction extract of combination (Cd)]. Extracts showed moderate to mild in vitro antiplasmodial activity [IC_{50} (median inhibitory concentration): 24.8–96.6 µg/mL], with the aqueous Cd showing potential synergism [CI (combination index) < 1]. No significant cytotoxicity was observed (< 10% hemolysis).

Moderate to good antioxidant activity was found in DPPH [SC_{50} (median scavenging concentration): 134.65–307.55 µg/mL] and ABTS assays (SC_{50}: 92.23–183.45 µg/mL), with Cd showing the highest activity. FRAP values were low. The Cd extract demonstrated no significant acute toxicity up to 5,000 mg/kg and significant in vivo antimalarial activity, achieving 65% parasite inhibition at 200 mg/kg/day. It also prolonged survival time, with a maximum survival of 28 days at 200 mg/kg/day.

Conclusions: This preliminary investigation suggests that combined extracts of *A. conyzoides* and *B. pilosa* exhibit noteworthy in vitro and in vivo antiplasmodial activity against the tested strains. Further studies are warranted to validate these findings and develop optimized formulations as potential antimalarials.

Keywords

Malaria, herbal medicine, *Ageratum conyzoides*, *Bidens pilosa*, antimalarial, acute toxicity, polyherbal formulation

Introduction

Malaria remains one of the most devastating infectious diseases worldwide, with an estimated 249 million cases reported globally in 2022. The World Health Organization (WHO) African Region bears the greatest burden, accounting for approximately 94% of all cases (233 million) and the majority of malaria-related deaths [1]. Cameroon represents a particularly affected area, reporting about 6.5 million cases in 2022 across all ten regions of the country [1]. The disease is caused by *Plasmodium* parasites, with *P. falciparum* (*Plasmodium falciparum*) being the most prevalent and deadly species in Africa, responsible for nearly 99% of cases [2].

The pathophysiology of malaria involves complex interactions between the parasite and human erythrocytes. During the intraerythrocytic stage, the parasite metabolizes hemoglobin, releasing free heme that generates reactive oxygen species (ROS) through Fenton reactions. This oxidative stress damages cellular components and contributes to the clinical manifestations of malaria [3]. Current treatment relies heavily on artemisinin-based combination therapies (ACTs), which have significantly reduced mortality rates [4]. However, the emergence of artemisinin resistance in sub-Saharan Africa, particularly through *Pfkelch13* mutations (R561H in Rwanda and Cys469Tyr/Ala675Val/Arg561His in Uganda), threatens to reverse these gains [5, 6]. This alarming trend has prompted researchers to explore triple artemisinin combination therapies (TACTs) and investigate alternative antimalarial compounds [7].

In this context, medicinal plants represent a promising avenue for drug discovery. Traditional herbal remedies have been used for millennia to treat malaria, and many have demonstrated antiplasmodial activity in laboratory studies [8, 9]. Cameroon's rich biodiversity includes numerous plant species with documented antimalarial properties. Among these, *Ageratum conyzoides* (*A. conyzoides*) (Asteraceae; commonly known as goat weed or king grass) and *Bidens pilosa* (*B. pilosa*) (Asteraceae; commonly known as black-jack or beggarticks) stand out for their widespread traditional use and scientific evidence of efficacy [10]. *A. conyzoides*, containing bioactive constituents like terpenoids, tannins, flavonoids, saponins, and alkaloids, has demonstrated significant antiplasmodial activity in previous studies [11, 12]. Similarly, *B. pilosa*, rich in flavonoids, flavonoid glycosides, sesquiterpenes, diterpenes, polyacetylenes, aurones, and aurone glycosides, has shown potent antimalarial properties [13].

Ethnobotanical surveys underscore the profound reliance on traditional medicine for malaria treatment in Cameroon, a necessity amplified by the challenges of drug and insecticide resistance. Both *A. conyzoides* and *B. pilosa* are integral to these traditional practices, individually recognized for their roles in managing fever or explicitly treating malaria in various Cameroonian and broader African communities. Scientific investigations have largely validated their individual antiplasmodial activities, demonstrating significant parasite suppression in preclinical models [10–13]. The efficacy of these plants is rooted in their rich phytochemical composition, including flavonoids, alkaloids, polyacetylenes, and terpenoids. However, a critical observation from the literature is the absence of explicit documentation for the traditional

combined use of *A. conyzoides* and *B. pilosa* specifically for malaria treatment in Cameroonian communities. While their individual uses are confirmed, and the potential for synergy based on their complementary phytochemical profiles is theoretically strong, direct evidence of this specific polyherbal practice for malaria in Cameroon is not documented. This gap in knowledge is particularly significant given that rural populations in Cameroon, where access to conventional antimalarials is often limited by cost and availability, commonly utilize such herbal combinations as primary healthcare [14].

Plant-based combinations offer several theoretical advantages over single-plant or synthetic drugs, including: potential synergistic interactions enhancing overall antimalarial activity, possible mitigation of side effects through balanced phytochemistry, a broader spectrum of bioactive compounds potentially counteracting resistance mechanisms, and support for sustainable, culturally relevant local healthcare practices [15, 16].

This research aims to scientifically validate this traditional medicine practice by evaluating combined plant extracts of *A. conyzoides* and *B. pilosa* through: (1) in vitro assessment of antiplasmodial efficacy against chloroquine-sensitive *P. falciparum* 3D7 (Pf3D7), (2) evaluation of cytotoxicity and antioxidant properties, and (3) determination of in vivo safety and efficacy in rodent models.

Materials and methods

Plant collection and extraction

The plant material for this study consisted of leaves of *A. conyzoides* and *B. pilosa*, harvested in October 2023, from Bambili, North West region, Cameroon. The plants were identified at the National Herbarium of Cameroon, where voucher specimens were deposited under numbers 33036/HNC for *A. conyzoides* (Asteraceae) and 32989/HNC for *B. pilosa* (Asteraceae). The leaves were air-dried in the shade and ground to a fine powder using an electric blender. Extraction was performed using two aqueous methods: cold maceration and decoction. To mimic traditional preparation of the polyherbal formulation, equal quantities (1:1 w/w) of both plant powders were combined prior to extraction.

Ten grams of individual plant powder, or 10 g of the combined powder (5 g of each plant), underwent cold maceration in 100 mL of distilled water for 24 h. The mixtures were intermittently agitated via manual shaking during this period. After that, it was filtered using a Whatman filter paper to obtain the filtrate. The decoction was done by boiling 10 g of each plant powder and 10 g of the combination (5 g of each plant powder) in 100 mL of distilled water for 15 min. It was allowed to cool, then filtered using a Whatman filter paper to obtain the filtrate. All filtrates were oven-dried at 50°C for 48 h to obtain dried aqueous extracts. Extracts were stored at 4°C until use.

All filtrates were oven-dried at 50°C for 48 h to obtain dried aqueous extracts. This temperature was selected to preserve heat-sensitive bioactive compounds while ensuring efficient water evaporation, consistent with WHO guidelines for herbal material processing [17].

Extraction yields were calculated as [18]:

$$\text{Yield } (\%) = \frac{\text{Weight of dried extract (g)}}{\text{Weight of initial plant material (g)}} \times 100$$

In vitro antiplasmodial activity of *A. conyzoides* and *B. pilosa* extracts on Pf3D7 chloroquine-sensitive strain

The in vitro antiplasmodial activity was evaluated using the SYBR Green I fluorescence-based assay, which quantifies parasite DNA [19].

The Pf3D7 strains, procured from BEI-Resources (https://www.beiresources.org/), were cultured in human O+ erythrocytes [20] in complete RPMI (Roswell Park Memorial Institute)1640 medium (Sigma Aldrich, USA) containing 25 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (Sigma Aldrich, Switzerland), 0.50% (w/v) Albumax I (Gibco, Thermo Fisher Scientific, New Zerland), sodium bicarbonate (Sigma Aldrich, USA), glucose (PanReac AppliChem, USA), 1× hypoxanthine (Gibco, USA), and 20 µg/mL gentamicin (Biowest, USA) [21]. Daily culture maintenance included medium replacement and

microscopic examination of Giemsa-stained (10% v/v) (Carl ROTH, Germany) thin blood films using 100× magnification. Parasite synchronization was performed 48 h before assays using 5% (w/v) sorbitol (Sigma Aldrich, France) to obtain predominantly ring-stage parasites [22].

Stock solutions of extracts were prepared at 100 mg/mL in 100% DMSO (dimethyl sulfoxide) (Th. Geyer GmbH & Co. KG, Germany), with vortexing employed to ensure complete dissolution. Positive control (artemisinin) was prepared at 10 mM in 100% DMSO. Five-fold serial dilutions in incomplete RPMI 1640 generated intermediate plates with extract concentrations of 1 mg/mL to 1.6 µg/mL and positive control concentrations of 10 µM to 16 nM.

The SYBR Green I assay [19] measured antiplasmodial activity. Test plates (96-well) received 90 µL of synchronized parasite culture (2% parasitemia, 1% hematocrit) per well in triplicate. 10 µL of extract or positive control solution was added from the intermediate plate, resulting in final concentrations of 100 µg/mL to 0.16 µg/mL for extracts and 1 µM to 0.0016 µM for artemisinin (Sigma Aldrich, Germany). Negative controls (100% growth) contained inoculum with solvent (0.1% DMSO final concentration); positive controls contained artemisinin. Plates were incubated at 37°C under 5% CO_2 (carbon dioxide) for 72 h.

After incubation, 100 µL SYBR Green I lysis buffer [containing SYBR Green I dye (Sigma Aldrich, Germany), Tris-base (Sigma Aldrich, Germany), EDTA (ethylenediaminetetraacetic acid) (PanReac AppliChem, USA), Triton X-100 (Sigma Aldrich, Germany), and saponin (Sigma Aldrich, Germany)] was added. Plates were incubated for 1 h in the dark to facilitate erythrocyte lysis and DNA binding. Fluorescence was measured (Infinite M200, Tecan, Switzerland) at 485 nm excitation and 538 nm emission. Parasite inhibition rate (IR) was calculated as:

The IR was calculated using the following formula:

$$\text{Inhibition } (\%) = \frac{F_{\text{neg}} - F_{\text{test}}}{F_{\text{neg}}} \times 100$$

Where:

F_{neg}: represents the fluorescence of the negative control.

F_{test}: represents the fluorescence of the test sample.

IC_{50} (median inhibitory concentration) values were determined from the inhibition (%) using GraphPad Prism 8.0.1 software, fitting sigmoidal concentration-response curves. The antiplasmodial activity of the extracts was classified according to Kamaraj et al. [23], where promising activity is defined as $IC_{50} \leq 10$ µg/mL, good activity as $10 < IC_{50} \leq 20$ µg/mL, moderate activity as $20 < IC_{50} \leq 40$ µg/mL, mild activity as $40 < IC_{50} \leq 70$ µg/mL, and inactive as $IC_{50} > 70$ µg/mL.

Combination effects were assessed by fractional inhibitory concentration (FIC_{50}) and combination index (CI) [24].

The FIC_{50} for each extract in the combination was calculated using the following formula (A: *A. conyzoides*; B: *B. pilosa*):

$FIC_{50}A$ = IC_{50} of extract A alone/IC_{50} of extract A in combination

The same formula was applied to calculate the FIC_{50} for extract B.

The CI was then calculated by summing the FIC_{50} values for both extracts:

$CI_{A/B} = FIC_{50}A + FIC_{50}B$

The CI value was used to determine the nature of the interaction between the two extracts:

CI < 1: indicates a trend towards synergism, suggesting that the combined effect is greater than the sum of the individual effects.

CI > 1: indicates a trend towards antagonism, suggesting that the combined effect is less than the sum of the individual effects.

In vitro hemolysis test of individual and combined extracts on human erythrocytes

The cytotoxicity of plant extracts on human erythrocytes was assessed using an in vitro hemolysis assay [25]. Healthy erythrocytes from O+ donors were collected, separated, and a 4% hematocrit suspension was prepared in RPMI 1640 medium (Sigma Aldrich, Germany). Equal volumes (500 µL) of this suspension and each extract (500 µg/mL) were incubated in triplicate in 1.5 mL Eppendorf tubes for 3 h at 37°C with 5% CO_2. Positive (0.5% Triton X-100; Sigma Aldrich, Germany) and negative (RPMI 1640 medium) controls were included. Following centrifugation at 2,500 rpm, 200 µL of the supernatant was transferred to 96-well plates, and absorbance was measured at 540 nm using a Tecan spectrophotometer. The hemolysis rate was then calculated using the absorbance values, using the formula:

$$\text{Hemolysis rate} = \frac{A_{\text{sample}} - A_{\text{negative control}}}{A_{\text{positive control}} - A_{\text{negative control}}} \times 100$$

A_{sample}: represents the absorbance of the sample.

$A_{\text{negative control}}$: represents the absorbance of the negative control (cells with no hemolytic agent).

$A_{\text{positive control}}$: represents the absorbance of the positive control (100% hemolysis, cells treated with Triton X-100).

Antioxidant tests

The in vitro antioxidant capacity of the extracts was evaluated through free radical scavenging {DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] assays} and ferric ion reducing antioxidant potential (FRAP) assay.

DPPH radical scavenging assay

Antioxidant activity was determined using the DPPH assay [26]. Extract stock solutions (100 mg/mL in sterile water) and 0.02% (w/v) DPPH (Sigma Aldrich, India) in ethanol were prepared. Serial dilutions (from 500 to 15.625 µg/mL) were dispensed in a 96-well plate. After adding 75 µL DPPH solution to 25 µL sample, the plates were incubated for 30 min in the dark. Absorbance was measured at 517 nm using a microplate reader (Infinite M200, Tecan, Switzerland). Gallic acid (Sigma Aldrich, Germany) (from 50 to 1.5625 µg/mL) served as a positive control, with DPPH solution alone as a negative control. Tests were performed in triplicate. The IR was calculated using the following formula:

$$\text{IR}\left(\%\right) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Where:

A_{control}: represents the absorbance of the blank solution (without the sample).

A_{sample}: represents the absorbance of the test sample.

The SC_{50} (median scavenging concentration) values were determined using GraphPad Prism 8.0.1 software.

ABTS radical trapping assay

The plant extracts $ABTS^+$ radical cation scavenging ability was assessed as previously described [27].

ABTS (PanReac AppliChem, USA) (7 mM) and potassium persulfate (PanReac AppliChem, USA) (2.45 mM) were mixed (16 h, dark) to generate $ABTS^+$ stock solution. Then, 25 µL of extract (from 500 to 15.625 µg/mL) or positive control dilutions were treated with 75 µL $ABTS^+$ solution. After 30 min of dark incubation, absorbance was measured at 734 nm (Infinite M200, Tecan, Switzerland). Gallic acid was the positive control; $ABTS^+$ solution alone was the negative control. IR and SC_{50} values were calculated as above.

FRAP assay

The ferric-reducing antioxidant capacity of the plant extracts was evaluated using the FRAP assay [28]. Different dilutions of extracts (from 500 to 15.625 µg/mL) and standards (25 µL each) were dispensed into 96-well plates, followed by the addition of 25 µL Fe^{3+} solution (1.2 mg/mL $FeCl_3$; Sigma Aldrich, Germany). After 15 min dark incubation at 25°C, 50 µL *ortho*-phenanthroline solution (0.2% w/v) was added. Plates were incubated for 15 min at 25°C, and absorbance was measured at 505 nm (Infinite M200, Tecan, Switzerland). Negative control consisted of 25 µL of ethanol + 25 µL of Fe^{3+} + 50 µL of *ortho*-phenanthroline (Kem Light Laboratories Pvt. Ltd, India), while gallic acid served as the positive control (as described above). IR and SC_{50} values were determined as above.

Antioxidant activity was classified using standardized criteria [29] where very high = IC_{50} < 50 µg/mL, active = 50–100 µg/mL, moderately active = 101–250 µg/mL, less active = 251–500 µg/mL, and inactive > 500 µg/mL.

Acute toxicity test of the most active combined extract (Cd)

An acute oral toxicity study of Cd (decoction extract of combination) was conducted following OECD (Organisation for Economic Co-operation and Development) Test Guideline 423 [30]. Nine adult, non-pregnant female mice were randomly assigned to three groups (n = 3 per group). Group 1 (control) received distilled water (10 mL/kg), and groups 2 and 3 received single oral doses of Cd extract at 2,000 mg/kg (E2000) and 5,000 mg/kg (E5000), respectively. A 12-hour pre-treatment and 4-hour post-treatment non-water fast was implemented. Following administration via esophageal tube, animals were observed for signs of toxicity for 4 hours, with particular attention given to the first 30 minutes, and daily thereafter for 14 days. Observations included behavioral changes (e.g., aggressiveness, mobility, tremors, convulsions), coat changes, and body weight fluctuations. At study termination, animals were euthanized, and the liver, kidneys, spleen, lungs, and heart were excised, weighed, and subjected to macroscopic examination.

Evaluation of the in vivo curative antimalarial activity of the most active combined extract (Cd)

Parasite amplification

Plasmodium berghei (*P. berghei*) (NK-65) from Bei-Resource (https://www.beiresources.org/) was grown in vivo by taking blood from infected rats to non-infected rats [20]. Briefly, parasites that were cryopreserved in the –80°C freezer were thawed in a water bath at 37°C, and then injected intraperitoneally (i.p.) into a healthy rat. A Giemsa-stained thin blood smear was made from the tail vein blood of the infected rat, three days after inoculation. The slide was examined under the 100× objective of the light microscope (HumaScope Classic, Humans), using immersion oil, and the parasitemia was estimated [number of parasites per 100 RBCs (red blood cells) in three different fields]. When the parasitemia was about 30%, the rat was sacrificed, and the blood was collected and injected into other healthy animals.

Preparation of parasite inoculum

Preparation of parasite inoculum from infected donor rats was done as follows: donor rats were anesthetized and euthanized via terminal overdose of diazepam (30 mg/kg) and ketamine (100 mg/kg) administered i.p. [31]. Death was confirmed by the absence of pedal reflex and cessation of respiration. The thoracic cavity was subsequently opened, exposing the heart, and blood was then collected via post-mortem cardiac puncture into heparinized tubes. Physiological saline (0.9%) was used for the dilution of the blood, such that each 0.5 mL of the aliquot contains about 1×10^6 infected RBCs.

The total volume of inoculum (V_t) to be prepared was calculated using the following formula:

$$V_t = \frac{\text{NRBCs in rats} \times P_i \times V_c \times \text{Desired dose volume}}{[P]}$$

Where:

V_t = Total volume (in mL) of the prepared inoculum stock solution. This stock is prepared such that each 0.5 mL aliquot contains 1×10^6 parasites.

NRBCs (nucleated RBCs) in rats = RBC level in rats (9.6×10^6 RBCs/mL)

P_i = Initial parasitemia of the donor animal (expressed as a decimal).

V_c = Volume of blood collected from the donor animal (in mL).

[P] = The target number of parasites desired per inoculation dose (1×10^6 parasites).

Desired dose volume = The volume of the single inoculation dose (0.5 mL).

Parasite inoculation and drug administration

The curative assay was carried out using Rane's test for curative activity, an 8-day test [20, 32]. On the first day (day 0), each rat was injected i.p. with 0.5 mL standard inoculum of 1×10^6 *P. berghei* infected erythrocytes. 72 h later (day 3), the parasitemia was estimated, and the rats were divided into six groups, four rats per group. There were 3 control groups and 3 treatment groups of four rats each. The most active combined extract, Cd was assessed for its in vivo activity. Treatment was given orally, once daily for 5 days, from day 3 to day 7. The treatment groups, G1, G2, and G3, received doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively. The positive control group received 10 mg/kg/day of artemisinin at 1 mg/mL. The negative control group received (10 mL/kg) distilled water. Giemsa-stained thin blood films were prepared from the tail of each rat from day 5 to 8, to monitor the parasitemia level. The body weights of the rats were measured after infection on day 3 and after treatment on day 8. The parasitemia per 100 RBCs [Parasitemia (%)] was calculated using the following formula:

$$\text{Parasitemia (\%)} = \left(\frac{\text{Number of parasitized RBCs}}{\text{Total number of RBCs counted}}\right) \times 100$$

The IR (%) was calculated to assess the reduction in parasitemia using the formula:

$$\text{IR (\%)} = 100 - \left(\frac{\text{Mean parasitemia treated}}{\text{Mean parasitemia of control}} \times 100\right)$$

The rats were monitored for 30 days after treatment, and their relative survival time was noted, as one of the parameters used to assess the effectiveness of the plant combination. A longer survival time indicates better curative activity.

Statistical analysis

Statistical analyses utilized Microsoft Excel for calculating IRs, while GraphPad Prism 8.0.1 facilitated advanced testing. For in vitro antiplasmodial (IC_{50}) and antioxidant (SC_{50}) assays, non-linear regression analysis (log[inhibitor] vs. normalized response, variable slope) was applied to dose-response data, with results expressed as mean ± standard deviation (SD) of replicate experiments. In vivo efficacy studies, one-way ANOVA (analysis of variance) followed by Tukey's post hoc test compared parasitemia levels and body weight changes across groups; survival curves were analyzed using the Log-rank test. For the acute toxicity study, one-way ANOVA with Tukey's test evaluated body weight trajectories and relative organ weights (organ weight/final body weight × 100%), with results reported as mean ± standard error of the mean (SEM). Clinical signs and mortality were documented descriptively. Across all inferential tests (ANOVA, Tukey's, Log-rank), significance was defined at $p < 0.05$.

Results

Yields of extraction

A total of six aqueous extracts, four individual extracts, and two combined extracts (containing equal parts of both plants) were prepared from the leaves of *A. conyzoides* and *B. pilosa* through cold maceration and decoction. The Cd had the highest extract yield (29.15%) while the cold maceration extract of *B. pilosa* (Bm) had the lowest extraction yield (6.63%) (Table 1).

Table 1. Extraction yields of *A. conyzoides* and *B. pilosa*

Extract	Full meaning	Mass of dry extract (mg)	Yield (%)
Am	Cold maceration extract of *A. conyzoides*	970	9.7
Bm	Cold maceration extract of *B. pilosa*	663	6.6
Ad	Decoction extract of *A. conyzoides*	1,569	15.7
Bd	Decoction extract of *B. pilosa*	1,860	18.4
Cm	Cold maceration extract of combination	987	9.9
Cd	Decoction extract of combination	4,230	29.2

Ad: decoction extract of *A. conyzoides*; Am: cold maceration extract of *A. conyzoides*; Bd: decoction extract of *B. pilosa*; Bm: cold maceration extract of *B. pilosa*; Cd: decoction extract of combination; Cm: cold maceration extract of combination

Antiplasmodial activity of crude extracts and their combination

The individual and combined plant extracts of *A. conyzoides* and *B. pilosa* showed in vitro antiplasmodial activity with IC_{50} values ranging from 24.84 µg/mL to 96.59 µg/mL (Table 2).

Table 2. IC_{50} values of individual and combined extracts on Pf3D7

Extracts	IC_{50} (µg/mL) on Pf3D7 (mean ± SD)
Am	33.61 ± 1.53
Bm	53.37 ± 1.73
Ad	45.96 ± 1.66
Bd	68.58 ± 1.84
Cm	96.59 ± 1.98
Cd	24.84 ± 1.40
Artemisinin (µM)	0.02 ± 0.0007

Each value represents the mean ± SD; *n* = 3. Ad: decoction extract of *A. conyzoides*; Am: cold maceration extract of *A. conyzoides*; Bd: decoction extract of *B. pilosa*; Bm: cold maceration extract of *B. pilosa*; Cd: decoction extract of combination; Cm: cold maceration extract of combination; IC_{50}: median inhibitory concentration; Pf3D7: *Plasmodium falciparum* 3D7; SD: standard deviation

The combinations of extracts demonstrated enhanced antiplasmodial activity. Specifically, the cold maceration extract of combination (Cm) showed a trend towards antagonism (CI > 1), while the Cd exhibited higher activity (IC_{50}: 24.84 µg/mL) and a trend towards synergism (CI < 1) (Table 3).

Table 3. Median fractional inhibitory concentration and combination index of combined antiplasmodial extracts

Extract combination	$FIC_{50}A$ (µg/mL)	$FIC_{50}B$ (µg/mL)	CI (µg/mL)
Cm	2.87	1.81	4.68
Cd	0.54	0.36	0.9

Cd: decoction extract of combination; Cm: cold maceration extract of combination; CI: combination index; $FIC_{50}A$: median fractional inhibitory concentration for *A. conyzoides*; $FIC_{50}B$: median fractional inhibitory concentration for *B. pilosa*

Given its superior in vitro antiplasmodial activity and synergistic effect, the Cd was selected as the most active combination for further in vivo studies.

Effect of the extract on RBCs

The individual and combined extracts were screened for their hemolytic potential on normal human erythrocytes, and all extracts showed no cytotoxic activity (< 10% hemolysis). The individual extracts, Am (cold maceration extract of *A. conyzoides*), Bm, Ad (decoction extract of *A. conyzoides*), and Bd (decoction extract of *B. pilosa*), showed a hemolytic potential ranging from 1.62% to 3.81%. The combined extracts, Cm and Cd, showed a hemolytic potential of 2.29% and 2.62% respectively. The positive control, 0.5% Triton X-100, exhibited 92.07% hemolysis (Figure 1).

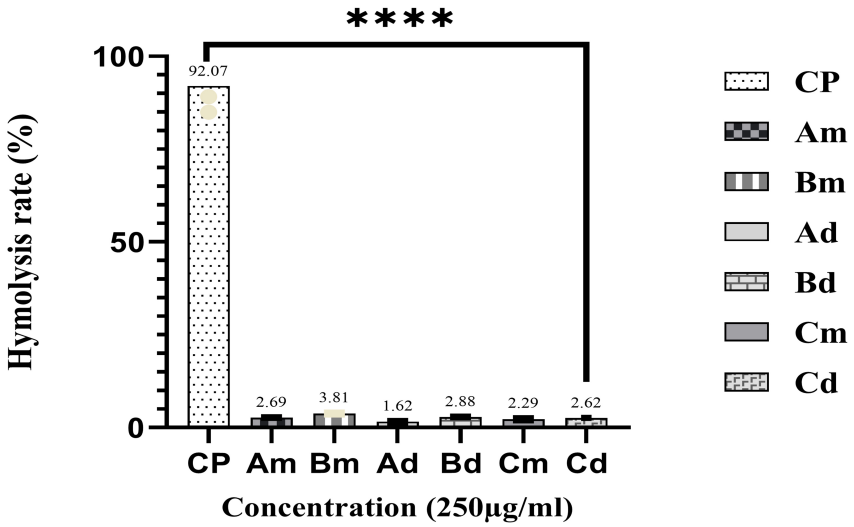

Figure 1. Hemolytic effect of extracts on human erythrocytes. Each value represents the mean ± SD; *n* = 3. Ad: decoction extract of *A. conyzoides*; Am: cold maceration extract of *A. conyzoides*; Bd: decoction extract of *B. pilosa*; Bm: cold maceration extract of *B. pilosa*; Cd: decoction extract of combination; Cm: cold maceration extract of combination; CP: positive control-0.5% Triton X-100. Significant ****$p < 0.0001$, when compared to the positive control.

All the extracts showed no toxicity on human erythrocytes, with hemolysis percentages ranging from 1.62% to 3.81%. The lowest hemolysis was observed with the Ad, and the highest was observed with the Bm. The Cd, which showed a higher antiplasmodial activity and was chosen for the in vivo activity, had a hemolysis percentage of 2.62%.

Antioxidant potential of the tested extracts

The antioxidant capacity of various plant extracts (individual and combined) was evaluated using three common assays: DPPH and ABTS radical scavenging assays and FRAP reducing power analysis. All extracts demonstrated concentration-dependent antioxidant activity (Table 4).

Table 4. Antioxidant activity of individual and combined extracts on DPPH, ABTS, and FRAP

Samples	DPPH (SC_{50}), mean ± SD (µg/mL)	ABTS (SC_{50}), mean ± SD (µg/mL)	FRAP (SC_{50}), mean ± SD (µg/mL)
Ad	199.60 ± 0.00	183.45 ± 0.85	2,185.00 ± 7.00
Am	307.55 ± 1.05	101.35 ± 1.25	5,070.50 ± 64.50
Bd	184.80 ± 1.50	109.05 ± 0.55	2,700.00 ± 55.00
Bm	226.45 ± 1.35	127.9 ± 0	5,441.50 ± 47.50
Cd	134.65 ± 2.05	92.23 ± 0.51	1,884.50 ± 56.50
Cm	164.95 ± 0.85	124.25 ± 0.65	9,116.00 ± 15.00
PC	1.84 ± 0.03	1.63 ± 0.09	11.98 ± 0.05

Each value represents the mean ± SD; *n* = 3. ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; Ad: decoction extract of *A. conyzoides*; Am: cold maceration extract of *A. conyzoides*; Bd: decoction extract of *B. pilosa*; Bm: cold maceration extract of *B. pilosa*; Cd: decoction extract of combination; Cm: cold maceration extract of combination; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric ion reducing antioxidant potential; PC: positive control-gallic acid; SC_{50}: median scavenging concentration; SD: standard deviation

Individual extracts show varied antioxidant capacities. Bd has the highest DPPH activity among individual extracts at 184.80 µg/mL. Cold maceration extract of *A. conyzoides* (Am) shows the best ABTS activity among individual extracts at 101.35 µg/mL. For FRAP, Ad is the best individual extract at 2,185.00 µg/mL, though still higher than Cd. While the Cd has the best reducing power among plant extracts (lowest FRAP SC_{50} at 1,884.50 µg/mL), the Cm shows the lowest reducing power, with the highest FRAP SC_{50} at 9,116.00 µg/mL.

The Cd demonstrates the strongest antioxidant activity among all plant-based samples. It shows the lowest SC_{50} values for DPPH (134.65 µg/mL), ABTS (92.23 µg/mL), and FRAP (1,884.50 µg/mL). This suggests a potential synergistic effect from combining these plants via the decoction method.

The decoction method (Ad, Bd, Cd) generally appears to yield better antioxidant activity compared to cold maceration (Am, Bm, Cm). This is particularly evident in the combined extracts, where Cd consistently outperforms Cm across all three assays.

This study suggests that combining these specific plant extracts and preparing them using Cd significantly enhances their antioxidant properties.

Acute toxicity test results of the most active combined extracts of Cd in mice

Effects on some clinical parameters

Table 5 details the effects of Cd extract administration on clinical parameters in mice. At 2,000 mg/kg, no behavioral abnormalities (aggressiveness, chills) were observed, with responses mirroring the normal control group. At 5,000 mg/kg, animals maintained normal stool consistency, auditory/tactile sensitivity, and baseline mobility beyond the initial 30-minute period where transient drowsiness and reduced mobility occurred. Critically, no mortality was observed at either dose during the 14-day observation period, indicating an oral LD_{50} (lethal dose 50%) exceeding 5,000 mg/kg.

Table 5. Effects of the Cd extract on some clinical signs

Parameters	NC			E2000			E5000		
	30 min	4 hours	14 days	30 min	4 hours	14 days	30 min	4 hours	14 days
Number of deaths	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Tremor	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Convulsions	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Aggressiveness	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Mobility	3/3	3/3	3/3	3/3	3/3	3/3	0/3	3/3	3/3
Somnolence	0/3	0/3	0/3	0/3	0/3	0/3	2/3	0/3	0/3

Cd: decoction extract of combination; E2000 and E5000: healthy mice treated with the extract at a dose of 2,000 and 5,000 mg/kg; NC: healthy mice treated with distilled water

Effects of Cd on weight change

Figure 2 below shows the effects of the administration of the aqueous extract on the weight evolution of mice. According to this figure, there were slight, non-significant changes ($p > 0.05$) in body weight from day 0 to day 14 in mice treated with plant extract at 5,000 mg/kg compared to those treated with distilled water.

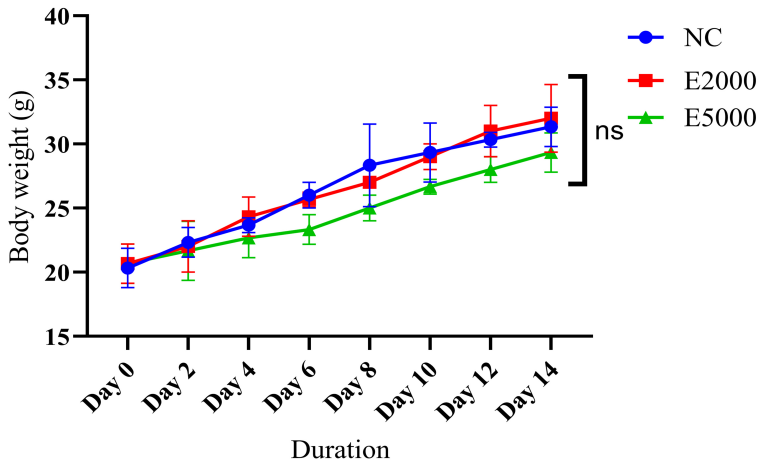

Figure 2. Effects of the extract on the weight evolution of acutely toxic mice. The graph depicts the mean body weight (± SEM, $n = 3$) for each treatment group: normal control (NC, distilled water), Cd, E2000 (2,000 mg/kg extract), and Cd, E5000 (5,000 mg/kg extract). ns, not significant, when compared to the NC or between different doses. SEM: standard error of the mean

Figure 2 illustrates the effects of Cd administration on the body weight evolution of mice over a 14-day period. Analysis of the figure reveals that mice treated with the 5,000 mg/kg extract (E5000) exhibited slight, non-significant variations in body weight compared to the distilled water control (NC) throughout the 14-day observation period.

Effects of Cd on the relative mass of selected organs involved in toxicity

Figure 3 depicts the effects of Cd administration on the relative organ weights (liver, kidneys, spleen, heart, and lungs) in mice following acute toxicity testing. The graph compares the relative organ masses of mice treated with single oral doses of 2,000 mg/kg and 5,000 mg/kg of the extract to those of the control group administered distilled water. After a 14-day observation period, no statistically significant differences in the relative weights of the aforementioned organs were observed between the extract-treated groups and the control group.

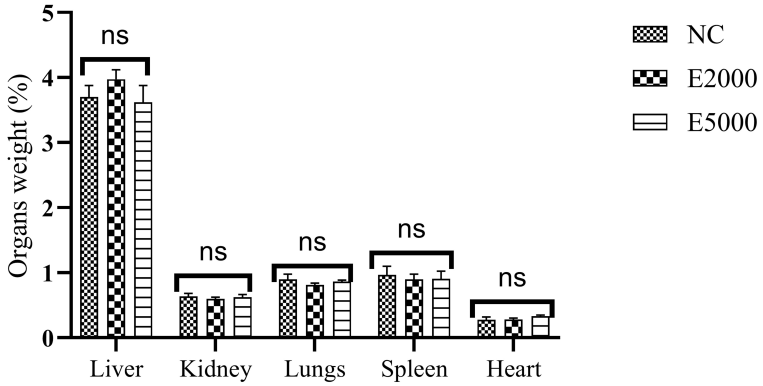

Figure 3. Effects of the extract on the relative weight of organs in acute toxicity. Each value represents the mean ± SEM; *n* = 3; NC: healthy mice treated with distilled water; E2000 and E5000: healthy mice treated with extract at a dose of 2,000 and 5,000 mg/kg. ns, not significant, when compared to the NC or between different doses. SEM: standard error of the mean

Curative activity and antiplasmodial activity of the most active combined extracts of Cd in rats

Effect of Cd on parasitemia

The Cd demonstrated significant dose-dependent suppression of parasitemia in *P. berghei*-infected mice. Following a 5-day treatment, Cd at 200 mg/kg/day achieved 65% parasite IR ($p < 0.001$ vs. negative control), with dose-dependent efficacy observed across doses (Table 6). After treatment, it was observed that there was a significant parasite suppression ($p < 0.0001$) in all the treatment groups, when compared to the negative control, with the high dose (200 mg/kg) exhibiting the best activity (Figure 4). The IR (%) for the treatment groups ranged from 59.09% to 65.15%, with the positive control noting an IR of 67.68%.

Table 6. Effect of combined extracts (Cd) on parasitemia in *P. berghei* infected rats

Treatment groups	Inhibition (%)	Mean survival time (day)
G1 (50 mg/kg)	59.09	17.8 ± 3.57
G2 (100 mg/kg)	60.61	24 ± 3.03
G3 (200 mg/kg)	65.15	27.8 ± 2.25
NC (DW)	0	4.5 ± 0.65
PC (Art)	67.68	28.8 ± 0.95

Results are expressed as mean ± SEM, *n* = 4; NC, negative control (DW; distilled water); PC, positive control (Art; artemisinin); G1: 50 mg/kg, G2: 100 mg/kg, G3: 200 mg/kg. Cd: decoction extract of combination; SEM: standard error of the mean

The curve shows the changes in parasitemia for the treatment days (day 3 to day 8). On the last day (day 8), the parasitemia was seen to increase for the negative control group (10 mg/kg distilled water) and was seen to significantly decrease for group 1 (G1: 50 mg/kg, $p < 0.0001$), group 2 (G2: 100 mg/kg, $p < 0.0001$), group 3 (G3: 200 mg/kg, $p < 0.0001$), and the positive control (10 mg/kg artemisinin, $p < 0.0001$), when compared to the negative control.

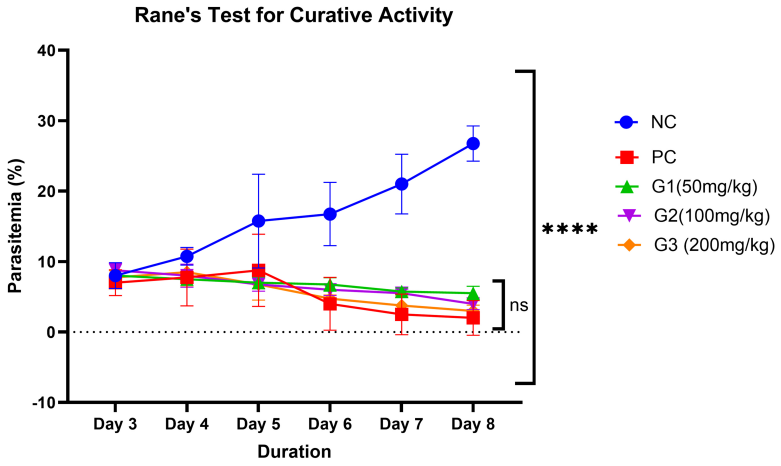

Figure 4. Effect of combined extracts; Cd on parasitemia in *P. berghei* infected rats. Each value represents the mean ± ESM; $n = 4$, $p < 0.0001$. G1: 50 mg/kg, G2: 100 mg/kg, G3: 200 mg/kg. Cd: decoction extract of combination; NC: negative control (DW; distilled water); PC: positive control (Art; artemisinin); ns, not significant; significant ****$p < 0.0001$, when compared to the NC

Effect of Cd on the body weight of *P. berghei*-infected rats

The body weights of the rats were noted on day 3 before treatment and day 8 post-treatment (Table 6). All three doses slightly prevented body weight loss, as compared to the negative control group (Figure 5).

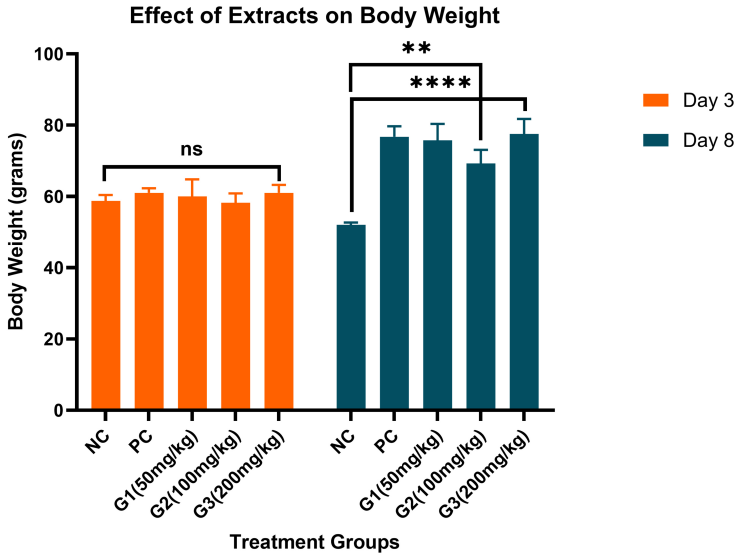

Figure 5. Effect of combined extract, Cd on body weights on day 3 and day 8. Each value represents the mean ± SEM; $n = 4$. G1: 50 mg/kg, G2:100 mg/kg, G3: 200 mg/kg. NC, negative control (DW; distilled water); PC, positive control (Art; artemisinin). ns, not significant; **$p < 0.001$; ****$p < 0.0001$, when compared to the NC

There was a reduction in body weight observed for rats in the negative control group (received distilled water) compared to the rats in the treatment groups, which recorded an increase in body weight during the course of the treatment. Group 1 recorded an average weight increase of 15.8 g, group 2 recorded an average increase of 11 g, group 3 recorded an average increase of 16.5 g, and the positive control group recorded an average increase of 15.8 g.

Effect of Cd on the survival time of *P. berghei* infected rats

The survival time, which was taken for 30 days post-treatment, revealed that the medium and high doses of the extract (100 mg/kg and 200 mg/kg, respectively) affected a significantly prolonged survival time ($p < 0.05$) when compared to the negative control. The positive control (artemisinin) equally affected a significant prolonged survival time ($p < 0.001$) when compared to the negative control (Figure 6). The rats

were observed for 30 days after treatment, and the survival time (days) ranged from an average of 17 days to 28 days for the treatment groups (Table 6).

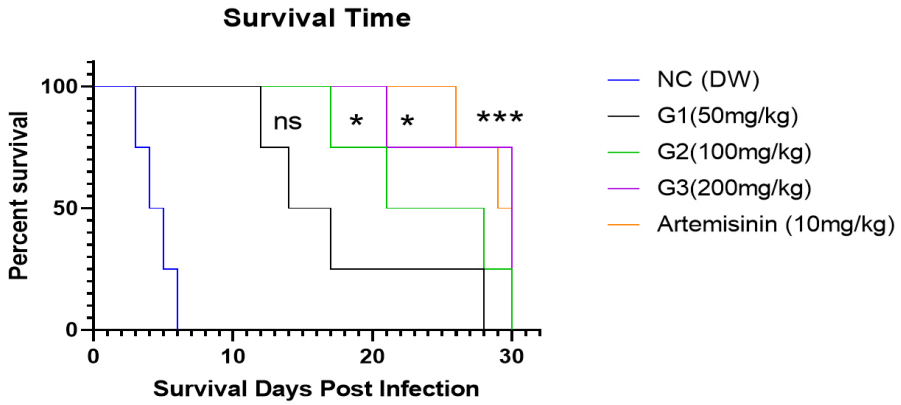

Figure 6. Effect of combined extract, Cd on survival time of *P. berghei*-infected rats. G1: 50 mg/kg, G2:100 mg/kg, G3: 200 mg/kg. NC, negative control (DW; distilled water); PC, positive control (artemisinin); ns, not significant; *$p < 0.05$; ***$p < 0.001$, when compared to the NC

The crude extract prolonged survival time in a dose-dependent manner when compared to the negative control. Group 1 demonstrated an average survival time of 17 days, surpassing the negative control's average of 4 days. Groups 2 and 3 recorded average survival times of 24 and 28 days, respectively, which were significantly higher than the negative control ($p < 0.05$). The positive control exhibited the longest survival time of 29 days, significantly outperforming even the negative control ($p < 0.0001$).

Discussion

While *A. conyzoides* and *B. pilosa* are individually recognized in traditional medicine for their antimalarial properties in Cameroon and beyond [10, 11, 33], the combined traditional use of these two species for malaria treatment, though anecdotally reported in some communities in Northwest Cameroon, currently lacks direct scientific support. Few studies, however, describe the combined use of different plant species for malaria treatment in traditional African medicine, suggesting a broader practice of polyherbal remedies [14, 24]. Our investigation reveals that aqueous extracts of both individual plants and their combinations exhibit significant antiplasmodial activity against Pf3D7, with several key findings. All extracts demonstrated notable in vitro activity (IC_{50}: 25–97 µg/mL). The Cd (IC_{50}: 24.84 µg/mL) emerged as the most potent extract, exceeding the activity of individual plant extracts (IC_{50}: 33.61–68.58 µg/mL) and suggesting enhanced efficacy through combination. Antiplasmodial activity was classified per established criteria [23]. Both *A. conyzoides* extracts (Am-maceration; Ad-decoction) demonstrated moderate activity, while *B. pilosa* extracts (Bm-maceration; Bd-decoction) showed mild activity. Among combinations, the Cd (IC_{50}: 24.84 µg/mL) exhibited moderate activity, contrasting with the mild activity of the maceration combination (Cm). CI analysis revealed Cd (CI < 1) had synergistic potential, while Cm (CI > 1) displayed antagonistic interactions. This aligns with evidence that phytochemicals can synergistically enhance bioactivity [34], supporting the traditional practice of combining these plants.

The higher antiplasmodial activity of *A. conyzoides* (IC_{50}: 33.61–45.96 µg/mL) compared to prior studies [35] may reflect chemotypic variations influenced by local environmental factors (soil composition, climate) affecting secondary metabolite production. The moderate activity of *B. pilosa* (IC_{50}: 53.37–68.58 µg/mL) is consistent with existing literature [36]. Critically, no extract exhibited significant hemolytic activity (< 10% hemolysis), confirming their safety for erythrocytes according to established cytotoxicity thresholds [25]. This non-hemolytic property is essential for potential antimalarial development. The fact that all the extracts show no toxicity on human erythrocytes could be because of the solvent used for extraction (water), which reduces the amount of toxic compounds extracted [37]. These results are similar to those reported for *B. pilosa*, which was seen to possess no toxicity on erythrocytes [38]. Similarly, *A.*

conyzoides extracts have been reported to possess low hemolytic effects, in a dose-dependent manner [39, 40].

Malaria is severe because of the development of anaemia (mainly caused by oxidative stress arising from free radicals) along with other haematological changes [41].

The antioxidant profile of the extracts reveals critical mechanistic insights and safety considerations. While all extracts demonstrated radical scavenging capacity, Cd exhibited superior activity in DPPH (SC_{50}: 134.65 µg/mL) and ABTS (SC_{50}: 92.23 µg/mL) assays. This enhancement likely stems from synergistic interactions between phenolic compounds—particularly flavonoids in *B. pilosa* and tannins in *A. conyzoides*—that function as proton donors in hydrogen atom transfer (HAT) mechanisms [42, 43]. Aqueous extraction preferentially concentrates these HAT-dominant antioxidants, explaining the significantly greater activity in DPPH/ABTS versus electron transfer (ET)-based FRAP assays ($p < 0.0001$) [44]. The observed synergism aligns with the plants' phytochemical composition. *A. conyzoides* provides caffeoylquinic acids and flavonoid aglycones acting as primary HAT donors [35]. *B. pilosa* contributes aurone glycosides and polyacetylene antioxidants that regenerate oxidized phenolics [45]. Aqueous decoction optimizes the extraction of these heat-stable, polar proton-donating compounds [46]. Our DPPH results for *A. conyzoides* corroborate prior findings [47], while *B. pilosa*'s concentration-dependent activity [48] reflects dose-responsive phytochemical interactions. Chemotypic variations across regions [35, 45] explain minor deviations from literature values.

The antimalarial efficacy of the Cd extract likely stems from a dual mechanism: direct parasite suppression by bioactive phytochemicals and mitigation of malaria-induced oxidative stress. Antioxidant-mediated protection counteracts parasitic oxidative damage [3], while specific phytochemical classes target essential *Plasmodium* pathways. Flavonoids inhibit fatty acid biosynthesis and disrupt nutrient influx (*L*-glutamine, myoinositol) in infected erythrocytes [49, 50]. Saponins induce membrane disorganization through cholesterol sequestration and protein complexation [51]. Terpenes (e.g., analogous to artemisinin) bind parasitic heme moieties [52]. This phytochemical synergy—enhanced by the extract's potent HAT-dominant antioxidant activity—collectively contributes to Cd's efficacy. Given these complementary mechanisms and a favorable safety profile, Cd was advanced for acute toxicity and curative efficacy evaluation.

The evaluation of the acute oral toxicity of Cd extract in mice revealed an LD_{50} greater than 5,000 mg/kg, indicative of low acute toxicity, as no mortality was observed. Clinical observations showed transient behavioral effects, specifically somnolence and reduced mobility, at the 5,000 mg/kg dose within the first 30 minutes, suggesting potential, reversible interactions with the central nervous system, possibly involving neurotransmitter modulation or receptor binding [53]. The absence of other clinical signs, such as changes in sensory sensitivity or autonomic function, indicates limited interference with these systems [54]. Analysis of relative organ weights showed no significant differences between treated and control groups, suggesting no major organ-specific toxicity [55]. Similarly, body weight evolution demonstrated gradual increases across all groups, with a slight reduction in the 5,000 mg/kg group, potentially due to transient somnolence affecting food intake [56]. This suggests minimal disruption of metabolic processes, though further studies on appetite-regulating hormones and metabolic pathways are warranted. Overall, the Cd extract exhibited low acute oral toxicity, with transient central nervous system effects at high doses. However, this acute toxicity assessment was limited to behavioral and macroscopic organ observations. Comprehensive organ safety evaluation requires future histopathological and biochemical verification.

In the curative test, all treatment doses (50 mg/kg, 100 mg/kg, 200 mg/kg) of the combined extract exhibited significant parasite suppression ($p < 0.0001$) compared to the negative control. This is consistent with the reported antimalarial properties of medicinal plants, which often demonstrate parasite IRs of 30% or more [57]. The highest dose (200 mg/kg) exhibited the most potent activity with a parasite IR of 65.15%. The extract's suppressive effect may be attributed to the presence of phytochemicals that target various pathways of the malaria parasite or indirectly boost the immune system [58].

In vivo studies that have been done on individual extracts of *A. conyzoides* and *B. pilosa* have shown that their suppressive activity is higher than their curative activity [33]. A 100% parasite suppression was reported for *B. pilosa* tested at 125 mg/kg and 500 mg/kg [33] while *A. conyzoides* demonstrated a parasite suppression of 70.46%, 82.20%, and 89.87% for the 100 mg/kg, 200 mg/kg, and 400mg/kg doses, respectively [11]. However, the curative activity of *B. pilosa* in the same study above was less than 80%, and in other studies, a curative activity of 56% was recorded [59]. *A. conyzoides* recorded a curative activity of 61%, 47%, and 37%, at tested doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively [12]. The curative activity reported for the individual extracts is seen to be lower than that of the combined extracts obtained from this study (59.09%, 60.61%, and 65.15%, for treatment doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively. Thus, we suggest that the combined plant extract is more efficient than the individual plant extracts. In vivo antiplasmodial activity can be classified as moderate, good, and very good when an extract causes a rate of inhibition ≥ 50% at doses of 500 mg/kg, 250 mg/kg, and 100 mg/kg, respectively [60]. According to this classification, with basis on the 100 mg/kg/day dose, the combined extract of interest, Cd, which showed a moderate in vitro activity, was seen to possess a good in vivo suppressive activity. This could be because the extract acts on the parasite by targeting various pathways that help to suppress the effect of the parasite; as is the case with flavonoids, which exert their antimalarial potential by targeting some functional biomolecules like proteins, enzymes, and DNA, which are essential for the parasite's survival [61].

Artemisinin, at 10 mg/kg, inhibited parasite growth by 67.68%. In comparison, chloroquine, another standard antimalarial drug, achieved 81.47% parasite suppression at the same dose and completely eradicated parasites at 25 mg/kg [62, 63]. This difference in efficacy, particularly artemisinin's lower inhibition, can be attributed to *P. berghei*'s preferential invasion of early erythrocytes within the bone marrow and spleen [64]. Parasites residing in these splenic early erythrocytes are known to be less sensitive to artemisinin treatment than those in peripheral blood. This reduced sensitivity is likely due to their ability to persist and proliferate in protected hematopoietic niches, which may contribute to parasite survival, recrudescent infection, and the development of drug resistance [65].

Body weight reduction is one of the symptoms of malaria in rats, among others like anaemia, hypothermia, and hypoglycemia [66]. All treatment doses were seen to prevent body weight loss, as compared to the negative control group. This could be because during treatment, there is parasite suppression, leading to reduced parasite effects such as appetite reduction. The rats in group 1 (received a dose of 50 mg/kg) gained more weight during the course of treatment than the rats in group 2 (received a dose of 100 mg/kg). This unexpected variation could be caused by a reduction in food intake by rats in the 100 mg/kg/day group [67]. The highest dose (group 3; received a dose of 200 mg/kg) provided the highest weight gain in the rats, compared to other treatment doses. This could be because at this dose, there was the highest parasite suppression, which led to reduced effects of the parasite, such as appetite reduction and hypoglycemia, causing the rats to regain their appetite and gain more weight [68].

The average survival time was another parameter used to assess the effectiveness of the plant combination [20]. A longer survival time indicates better curative activity. The effect of treatment doses on survival time was seen to be dose-dependent, which might be related to dose-dependent parasite suppression [68]. The medium and high dose (100 mg/kg and 200 mg/kg, respectively) treatment groups had a significant ($p < 0.05$) mean survival time when compared to the negative control group, though it was lower than that of the positive control group ($p < 0.001$). Among all treatment groups, the 200 mg/kg dose best prolonged the survival time, which is an indicator of the extract's ability to decrease the parasite's overall pathogenic effect [69].

This study validates the traditional use of combined *A. conyzoides* and *B. pilosa* extracts, demonstrating moderate in vitro antiplasmodial activity against Pf3D7 with excellent safety profiles on erythrocytes. The Cd showed enhanced in vitro efficacy and synergistic interaction, while in vivo testing revealed significant dose-dependent chemosuppression of *P. berghei*. Although direct statistical comparison to individual extracts was not performed in vivo, Cd's observed efficacy suggests potential therapeutic advantages

worthy of further investigation. Therefore, rigorous further research is indispensable to fully characterize Cd's therapeutic potential and develop it into standardized antimalarial formulations, including optimization studies, chronic toxicity assessment, and pharmacokinetic characterization.

Abbreviations

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

Ad: decoction extract of *A. conyzoides*

Am: cold maceration extract of *A. conyzoides*

ANOVA: analysis of variance

Bd: decoction extract of *B. pilosa*

Bm: cold maceration extract of *B. pilosa*

Cd: decoction extract of combination

CI: combination index

Cm: cold maceration extract of combination

CO_2: carbon dioxide

DMSO: dimethyl sulfoxide

DPPH: 2,2-diphenyl-1-picrylhydrazyl

FIC_{50}: fractional inhibitory concentration

FRAP: ferric ion reducing antioxidant potential

HAT: hydrogen atom transfer

i.p.: intraperitoneally

IC_{50}: median inhibitory concentration

IR: inhibition rate

LD_{50}: lethal dose 50%

Pf3D7: *Plasmodium falciparum* 3D7

RBCs: red blood cells

RPMI: Roswell Park Memorial Institute

SC_{50}: median scavenging concentration

SEM: standard error of the mean

WHO: World Health Organization

Declarations

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Author contributions

PVTF: Conceptualization, Supervision, Methodology, Investigation, Writing—review & editing, Validation. CAM: Investigation, Formal analysis, Writing—original draft, Writing—review & editing, Validation. KLN:

Supervision, Methodology, Writing—review & editing, Validation. MJTN, CPDD, LRTY, and ATT: Methodology, Writing—review & editing, Validation. AYK and HND: Methodology, Investigation, Writing—review & editing, Validation. MBTT and RK: Visualization, Writing—review & editing, Validation. VN and FFB: Resources, Writing—review & editing, Validation. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

The research adhered to the 2013 Helsinki Declaration for human inclusion and followed the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, along with Cameroon's National Veterinary Laboratory guidelines (No 003/19/CCS/MINEPIA/RD-NW/DDME/SSV). The University of Bamenda Institutional Review Board approved the study (Ref: 2024/0153H/UBa/IRB).

Consent to participate

Informed consent to participate in the study was obtained from participants prior to blood collection.

Consent to publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Not applicable.

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