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# ANTIMALARIAL ACTIVITY OF N-BUTANOL FRACTION OF ETHANOL LEAF EXTRACT OF *COMBRETUM HYPOPILINUM DIEL* AGAINST PLASMODIUM BERGHEI INFECTED MICE

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## ABSTRACT

The incidence of resistance among currently available antimalarial drugs, as well as the high economic cost of malaria, has prompted researchers to look for novel antimalarial molecules. As a result, the current study was proposed to evaluate the antiplasmodial activity (in vivo) of – butanol fraction of Combretum hypopilinum diel based on the plant's traditional claims. A cold maceration procedure using 70% methanol was employed to obtain a crude extract from Combretum hypopilinum diel leaf. N - butanol, and pure water were used to fractionate the hydro ethanol extract. Standard procedures (OECD) were followed for an acute oral toxicity test. The antimalarial effects of the plant at 100, 200, and 400 mg/kg doses were investigated using three rodent malaria models (4-day suppressive, curative (rane's), and prophylactic tests. To compare results between groups, a one-way ANOVA with Post Hoc Dunnett was used, and a paired t–test was used to compare pre- and post-treatment (PCV and bodyweight). In a 4-day suppressive investigation, all doses of the n-butanol fraction of crude extract suppressed parasitemia significantly ($P < 0.001$) as compared to the negative control. The crude extract had the greatest chemosuppressive effect (40%) at a 400 mg/kg dose. In Rane's test, all doses of the n-butanol fraction of crude extract produced substantial ($P < 0.001$) curative effects as compared to the negative control. In the prophylactic test, all doses of the n-butanol fraction of crude extract significantly suppressed ($P < 0.001$) parasitemia as compared to the negative control. According to this study, the n-butanol fractions of Combretum hypopilinum diel leaf contain antimalarial activity with a substantial suppressive effect.

## 1.0 INTRODUCTION

### 1.1 Background of Study

Malaria is a communicable illness transmitted through the bite of an infected female Anopheles mosquito carrying the parasite (Bizuneh *et al.*, 2023). Malaria is a prevalent and perilous protozoan disease that results in numerous hospitalizations and fatalities among Africans annually. (Tadege *et al.*, 2023). Human malaria is caused by five protozoan species belonging to the Plasmodium genus: *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax,* and *P. knowlesi*. Among these, P. falciparum and P. vivax are the primary culprits, with P. falciparum being highly lethal and prevalent in Africa, while P. vivax is less dangerous but more widespread (Uzor, 2020).

According to the World Health Organization (WHO), approximately 3.3 billion individuals reside in malaria-endemic areas, putting them at risk of contracting the disease. (Uzor, 2020). Nearly all malaria cases (96%) worldwide are concentrated in 29 countries, with Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), and Mozambique (4%) accounting for nearly half of all cases (World Malaria Report, 2022). The majority of malaria cases (around 80%) and deaths (around 90%) occur in sub-Saharan Africa, with children under the age of 5 and pregnant women being particularly vulnerable (Uzor, 2020).

Malaria has been addressed through the administration of various medications such as quinine, chloroquine, amodiaquine, mefloquine, and artemisinin derivatives, among others (Uzor, 2020). Studies have indicated that more than 80% of the global population relies on plant-based medicine to meet their basic healthcare needs (Ahmed *et al.*, 2022). This effort is exacerbated by

limited access to modern healthcare services in rural areas, leading to a significant reliance on traditional medicine due to its accessibility (Gyasi *et al.*, 2020). In some African countries like Ghana, the preference and utilization of traditional medicine are driven by factors such as the scarcity of drugs and modern facilities in rural areas (Kretchy *et al.*, 2021).

The increasing resistance of malaria parasites to available treatments, including Artemisinin-based Combination Therapy (ACT), poses a significant challenge in the control and management of malaria. (Uzor, 2020). Additionally, limited access to modern healthcare services in rural areas leads to a reliance on traditional medicine, including the use of medicinal plants, as an accessible alternative. (Gyasi *et al.*, 2020; Kretchy *et al.*, 2021). Therefore, there is a need to explore the potential of medicinal plants, such as *Combretum hypopilinum Diels*, for the development of new antimalarial compounds to overcome drug resistance and improve treatment outcomes.

The World Health Organization (WHO) recommends ACT for malaria treatment, but there have been documented cases of parasite resistance to this therapy (Uzor, 2020). Resistance to existing malaria treatments, including ACT, is a growing concern (Bizuneh *et al.*, 2023; Uzor, 2020). Given the high burden of malaria in Africa, the development of effective antimalarial drugs is crucial for reducing the impact of this disease on public health. *Combretum hypopilinum Diels* is a medicinal plant with significant importance in traditional medicine in Africa (Hussaini *et al.*, 2021). Exploring the medicinal properties of this plant and identifying its active compounds could lead to the discovery of new antimalarial agents. Furthermore, this research aligns with the global effort to identify novel chemotherapeutic agents from natural sources to combat drug resistance in malaria treatment (Uzor, 2020).

The scope of this research project focuses on investigating the antimalarial potential of *Combretum hypopilinum Diels*, a medicinal plant used in traditional medicine. The study aims to explore the plant's efficacy against the malaria parasite, ***Plasmodium falciparum***, through in vitro assays and phytochemical analyses. The research will involve collecting samples of *Combretum hypopilinum Diels* from selected regions in Africa, extracting bioactive compounds, conducting antimalarial activity assays, assessing cytotoxicity, and investigating the mechanisms of action of the active compounds. The objectives of this study are;

- To identify, collect, extract and fractionate bioactive compounds from *Combretum hypopilinum diels*
- To conduct preliminary phytochemical analyses of ethanol leaf extract of *Combretum hypopilinum diels.*
- To determine acute toxicity of the extracts using OECD Method.
- To evaluate the antimalarial activity of *Combretum hypopilinum diel* extracts through suppressive and curative studies.
- To compare the antimalarial activity of the extracts and isolated compounds from *COMBRETUM HYPOPILINUM DIEL s* with standard antimalarial drugs to determine their potency and potential as alternative treatment options.
- To provide scientific evidence supporting the traditional use of *Combretum hypopilinum Diels* for the treatment of malaria and contribute to the development of novel antimalarial drugs.

## 2.0 MATERIALS AND METHOD

### 2.1 Plant material

The plant material used in this study was *Combretum hypopilinum diel. Fresh* leaf of the plant was collected from the Malumawa, Gadau village of Itas–Gadau Local Government, Bauchi State of Nigeria, in June 2023. The plant was identified and authenticated by Dr. Umar Aminu Muhammad, a botanist at the Biological Science Department of Bauchi State University Gadau, with a voucher specimen number **BASU0060** deposited at the university herbarium. The collection of plant material was carried out in compliance with the relevant regulations and permissions from the local authorities. Proper care was taken to ensure that the plant collection did not cause any harm to the environment or the population of *Combretum hypopilinum diel.*

### 2.2 Laboratory animal and parasites

In this research, an animal model (healthy Swiss albino mice of either sex, aged 6 to 8 weeks, with a weight range of 18 to 25 grams) was employed to evaluate the in vivo antimalarial activity of the extracts.

### 2.3 Equipment and Apparatus

Measuring cylinder, microscopes, weighing Balance, syringe, oral gavage, cages, medical adhesive tapes, test tubes, filter paper, gloves, mask, markers, conical flask, motor and pestle, capillary tubes, glass slides, dry oven, water bath, separation funnel, containers.

### 2.4 Chemicals and Drugs

Ethanol, chloroform, ethyl acetate, n-butanol, n-hexane, aqueous residues, standard antimalarial drugs (chloroquine, pyrimethamine), Standard reagents, including tests for alkaloids, flavonoids, tannins, and other phytochemicals, were used to identify the presence of specific compounds in the plant extracts

### 2.5 METHODOLOGY

#### 2.5.1 Plant material preparation

The plant materials (Fresh leaves of *Combretum hypopilinum diel*) were carefully collected, thoroughly washed with distilled water to remove impurities, and dried at a controlled temperature (40°C) until a constant weight was obtained. The dried plant materials were ground into a coarse powder using a motor and pestle

and are stored in airtight containers until further use to prevent degradation of bioactive compounds.

### 2.5.2 Extraction of Bioactive Compounds

To extract the bioactive compounds from the plant material, the coarse powdered leaf of *Combretum hypopilinum diel was* subjected to maceration using an extraction method. The extraction solvent (ethanol) was selected based on polarity and previous reports on the effective extraction of bioactive compounds from *Combretum* species. A volume of 7000 mL of 70% ethanol was added to 1000g of the coarse powdered plant material in a container, which was then sealed tightly and kept at room temperature for 72 hours (3 days) with intermittent shaking and agitation. After 72 hours, the extract was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a water bath at 40°C, followed by further drying using a dry oven. The resulting dried crude ethanol extract weighed 122 grams, was stored in amber glass vials until further analysis to protect them from light and oxidative degradation.

$$\text{percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of the crude leaaf}} \times 100$$

### 2.5.3 Phytochemical Analysis

The preliminary phytochemical screening of the ethanol extract of *Combretum hypopilinum diel* was performed to identify the presence of various phytoconstituents using standard qualitative tests as described by (Harborne 1984; 1998; 2003; El-Olemyl *et al.,* 2004; Shaik and Patil, 2020). The extract was tested for the presence of alkaloids, flavonoids, tannins, saponins, phenols, steroids, and terpenoids.

**Testing for Flavonoids**

- Sodium Hydroxide Test: Add a few drops of 10% sodium hydroxide to an aqueous solution of the n-butanol fraction of ethanol extract. The formation of a yellow color indicates the presence of flavonoids (Choi 2004; El-Olemyl *et al.,* 2004; Harborne, 1998)

**Testing for Cardiac Glycosides**

- Keller Killani's Test: to 2ml of the extract, 2ml of 3.5% FeCl3 was added and allowed to stand for one minute. 2ml of conc. $H_2SO_4$ was then carefully poured down the wall of the tube to form a lower layer. A reddish-brown ring formed at the interface indicating the presence of cardiac glycosides.

**Testing for Saponins**

- Frothing Test: Shake 1 mL of the n-butanol fraction of ethanol leaf extract of *Combretum hypopilinum diel* vigorously with 3 mL of distilled water for 30 seconds. Allow it to stand vertically for 30 minutes. The formation of persistent honeycomb froth indicates the presence of saponins.

**Testing for Tannins**

- Ferric Chloride Test: Add 3 drops of ferric chloride solution to 1 mL of the n-butanol fraction of ethanol leaf extract of *Combretum hypopilinum diel* solution. The formation of a greenish-black precipitate indicates the presence of condensed tannins.
- Lead Sub-Acetate Test: Dissolve 0.5g of the n-butanol fraction of ethanol leaf extract of *Combretum hypopilinum diel* in 2 mL of water, then add 3 drops of lead sub-acetate solution. Observe the formation of a black-green colored precipitate, which indicates the presence of tannins.

**Testing for Steroids and Triterpenes**

- Salkowski test: To 2ml of extract, 2ml of chloroform was added, and 2ml of sulphuric acid was carefully added to form a lower layer. A reddish-brown color formed at the interface, indicating the presence of a steroidal ring (Harbone, 2003).
- Lieberman-Burchard's Test: Mix 1 mL of the chloroform solution of the extract with an equal volume of acetic acid anhydride. Add 1 mL of concentrated sulfuric acid along the side of the test tube to form a lower layer. Immediate observation and observation over one hour are necessary. A brownish-red color at the interphase indicates the presence of triterpenes, while a blue-green color in the upper layer indicates the presence of steroids.

**Testing for Alkaloids**

- Wagner's Test: Add two drops of Wagner's reagent to a few mL of the filtrate. The presence of a reddish-brown precipitate confirms the presence of alkaloids.
- Dragendoff's Test: Add two drops of Dragendoff's reagent to a few mL of the filtrate. The presence of an orange-red precipitate indicates the presence of alkaloids.

**Testing for Anthraquinones**

- Bontrager's Test: Add 5 mL of chloroform to 500 mg of the extract in a dry test tube, stopper it, and shake it for at least 5 minutes. Filter the mixture and shake the filtrate with an equal volume of 10% ammonia solution. Observe the upper aqueous layer for a bright pink color, indicating the presence or absence of free anthraquinones.

### 2.6 Acute Toxicity Studies

Acute toxicity testing of the crude extract and its fractions was assessed following the guidelines outlined by the Organization for Economic Co-operation and Development 423 (OECD). This internationally recognized framework provides a standardized approach to evaluating the potential toxic effects of substances on animals. Six healthy non-pregnant female mice were used to evaluate the toxicity profile of the n-butanol fraction of hydroethanol extract and fractions. Each mouse was fasted for 4 hours before and 2 hours after

administration of the test substances. Initially, a 2000 mg/kg dose of the crude extracts was administered via oral gavage to one mouse from each group. These mice were observed for 24 hours for any behavioral changes such as changes in skin fur, convulsion, food intake, lacrimation, tremor, salivation, diarrhea, and mortality. As no deaths or significant behavioral changes were observed in the first mouse, the same dose was administered to another four mice in each group. Each animal received a single dose and was closely observed for 4 hours with 30-minute intervals, followed by daily monitoring for 14 consecutive days for signs and symptoms of toxicity.

### 2.7 PHARMACOLOGICAL STUDIES

#### 2.7.1 Parasite Inoculation

Initially, the percentage parasitemia level was established in donor mice by collecting blood from their tails, which had been previously infected with *Plasmodium berghei.* The blood was smeared on frosted microscope slides, fixed with absolute methanol, stained with Giemsa stain, and examined under a microscope. When the donor mice parasitemia reached an appropriate concentration, their blood was collected in a heparinized container through a retro-orbital procedure. The collected blood was diluted with 0.9% normal saline to achieve a concentration of 5 × $10^7$ *Plasmodium berghei-infected* red cells in 1 milliliter of blood (Fidock *et al.,* 2004). Each mouse received 0.2 milliliters of this diluted blood containing 1 × $10^7$ *Plasmodium berghei-infected* erythrocytes via intraperitoneal injection.

#### 2.7.2 Suppressive Test

The suppressive test aimed to determine the ability of the extract to prevent the establishment of malaria infection in experimental animals. Animals were infected with Plasmodium parasites and immediately treated with the extract. For the evaluation of the crude extract and its fraction, twenty-five infected mice were randomly divided into five groups, each consisting of five mice. Group I served as the negative control and received 0.2 milliliters of distilled water, while Group II, the positive control, received chloroquine at a daily dose of 10 mg/kg. The remaining mice in Groups III, IV, and V were treated with the n-butanol fraction of hydroethanol extract at daily doses of 100, 200, and 400 mg/kg, respectively. All test drugs were administered orally via oral gavage. Treatment began 2 hours after infection on day 0 and continued daily until day 3. On day 4, blood smears were prepared, and the number of parasites was determined under a microscope using an oil immersion objective with 100× magnification power. Parameters such as percentage parasitemia and percentage suppression were computed using the following formulas:

$$\text{Parasitemia density} = \frac{\text{Number of parasitised red blood cell}}{\text{Total number of red blood cell}} \times 8000$$

$$\%\ \text{Suppression} = \frac{\%\ \text{parasitemia in negative control} - \%\ \text{parasitemia in treatment}}{\%\ \text{parasitemia in negative control}} \times 100$$

#### 2.7.3 Curative Test

The curative test assessed the extract's capacity to alleviate malaria symptoms in animals with established infections. Animals were first infected with Plasmodium parasites and subsequently treated with the extract. For the evaluation of the crude extract and its fraction, twenty-five infected mice were randomly divided into five groups, each consisting of five mice. Group I served as the negative control and received 0.2 milliliters of distilled water, while Group II, the positive control, received chloroquine at a daily dose of 10 mg/kg. The remaining mice in Groups III, IV, and V were treated with the n – n-butanol fraction of the hydroethanol extract of *Combretum hypopilinum diel* at daily doses of 100, 200, and 400 mg/kg, respectively. All test drugs were administered orally via oral gavage. Animals are infected on day 0, and treatment begins 72 hours after infection and continues daily for 3 days. On day 7, blood smears were prepared, and the number of parasites was determined under a microscope using an oil immersion objective with 100× magnification power. Observations included changes in parasitemia, body weight, and packed cell volume, to determine the extract's curative potential.

### 2.8 Statistical analysis

The data collected were expressed as mean ± SEM. Results were presented as graphs and tables where appropriate. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test using SPSS version 27 software. P value was set at $P \leq 0.05$.

## 3.0 RESULT AND DISCUSSION

### 3.1 Percentage Yield of Extract

The calculated percentage yield of the dried ethanol leaf extract of *Combretum hypopilinum diel* and the dried N-butanol fraction was found to be 12.2% and 14%. The dried hydroalcohol *Combretum hypopilinum diel* extract exhibited a greenish-brown color and possessed a thick, viscous nature. While the N-butanol fraction exhibited a brown color powder.

### 3.2 Preliminary Phytochemical Screening

The preliminary phytochemical screening of both the ethanol LEAF extract of *Combretum hypopilinum diel* extract and N-butanol fraction revealed the presence of key secondary metabolites, including alkaloids, flavonoids, saponins, terpenoids, glycosides, steroids, and anthraquinones. (Table 3.2).

***Table 3.2: Phytochemical Constituents of the Hydroalcohol Extract and N-Butanol Fraction of* Combretum Hypopilinum Diel.**

| Secondary Metabolites | Ethanol extract | N-butanol fraction |
|---|---|---|
| | Inference | Inference |
| Alkaloids | + | + |
| Flavonoids | + | + |
| Saponins | + | + |

| | | |
|---|---|---|
| Tannins | + | - |
| Glycosides | + | + |
| Steroids | + | + |
| Terpenoids | + | + |
| Antraquinones | + | + |

**Key (+) = Present**, (-) = **Absent**

### 3.3 Acute Toxicity Studies

Acute oral toxicity studies were conducted following Organisation for Economic Co-operation and Development (OECD) guidelines, demonstrating safety profiles of the ethanol LEAF extract of *Combretum hypopilinum diel* and N-butanol fraction. No adverse effect or mortality was observed at the selected oral dose of 2000mg/kg (limit test), indicating the 50% lethal dose ($LD_{50}$) value of *Combretum hypopilinum diel* is more than 2000mg/kg, which supports the conclusion that the extract is relatively safe for oral consumption. Previous studies also reported that the crude extract of *Combretum hypopilinum diel did* not show any toxicity and fatality with a greater than 2000mg/kg dose (Ahmed *et al.*, 2021).

### 3.4 Antimalarial Activity of N-butanol fraction of Ethanol Leaf Extract of *Combretum hypopilinum diel*

#### 3.4.1. Four-day suppressive test

When evaluated against early infection with daily dosages of 100, 200, and 400 mg/kg/day, the N-butanol fraction of hydroalcoholic crude extract of *Combretum hypopilinum diel* had a dose-dependent chemosuppressive effect, resulting in parasitemia suppression of 22.2%, 25.2% and 40%, respectively (Figure 3.4b). Even at the lowest dose examined, the chemo suppression generated by the N-butanol fraction of hydro alcoholic crude extract of *Combretum hypopilinum diel* was statistically significant ($P < 0.001$). Furthermore, as compared to the negative control group, they also prevented body weight loss and a decrease in PCV caused by an increase in parasitemia (Table 3.4a) and (Figure 3.4a). However, when compared to the conventional (chloroquine) drug, they showed decreased parasitemia suppression effect ($P < 0.001$). (Figure 3.4b).

***Table 3.4a: Packed cell volume and body weight of* PLASMODIUM BERGHEI–infected *mice treated with N-Butanol fraction of* COMBRETUM HYPOPILINUM DIEL *in a 4 – 4-day suppressive test.***

| Group (mg/kg) | Packed cell volume (PCV) | | Body weight (g) | |
|---|---|---|---|---|
| | Day 0 | Day 4 | Day 0 | Day 4 |
| **N/S 10ml/kg** | 48.20 ± 7.35 | 36.40 ± 0.81* | 24.80 ± 0.37 | 27.60 ± 0.50* |
| **NBCH 100** | 48.00 ± 0.89 | 46.5 ± 2.92 | 25.60 ± 1.24 | 24.60 ± 0.93 |
| **NBCH 200** | 49.40 ± 1.24 | 47.20 ± 1.80 | 24.80 ± 0.37 | 27. 60 ± 0.51 |
| **NBCH 400** | 53.00 ± 3.93 | 49.40 ± 1.30 | 19.00 ± 1.48 | 19.80 ± 2.22 |
| **CQ 10** | 49.40 ± 2.11 | 51.20 ± 2.17 | 20.20 ± 0.49 | 20.80 ± 0.58 |

***Data are expressed as mean ± SEM, n = 5; * = p<0.001; dependent t- test was used; N/S = normal saline, CQ, chloroquine; NBCH, n – butanol fraction of* combretum hypopilinum diel; *Day 0, pre – treatment value; Day 4, post – treatment value; g, gram; mg/kg, milligram per kilogram.***

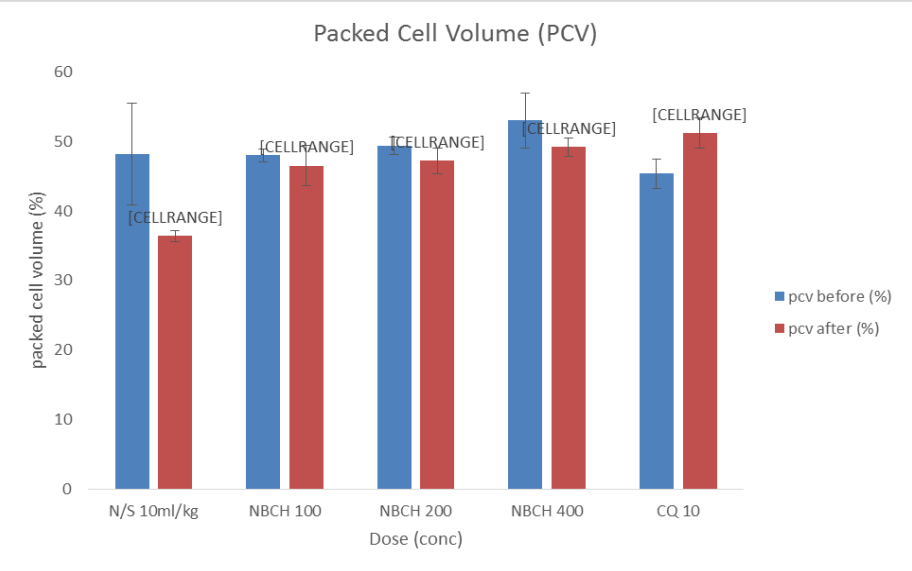


**Figure 3.4a: packed cell volume (PCV) for day 0 and day 4 suppressive test.**

**Table 3.4b: Average and percentage parasitemia of *PLASMODIUM BERGHEI–infected* mice treated with N-Butanol fraction of *COMBRETUM HYPOPILINUM DIEL* in 4 – 4-day suppressive test.**

| Test Dose (mg/kg) | Average Parasitemia (SEM ±) | Percentage Suppression (%) |
|---|---|---|
| **N/S 10ml/kg** | 432 ± 1.20 | 0 |
| **NBCH 100** | 336 ± 1.28* | 22.2 |
| **NBCH 200** | 322 ± 1.24* | 25.5 |
| **NBCH 400** | 259 ± 3.20* | 40 |
| **CQ 10** | 204 ± 3.54* | 52.7 |

**Data are presented as mean ± SEM one-way ANOVA followed by Dunnett post hoc was used. N/S = Normal Saline, CQ = Chloroquine, NBCH = N-butanol fraction of combretum hipopilinum diel, mg/kg = Milligram per Kilogram, ml/kg = mil per kilogram; n = 5; * = p<0.001.**

### 3.5.2 Curative (Rane's) test

All test doses utilized during treatment resulted in considerable parasitemia suppression activities against established infection (16%, 20.5%, and 22.3% reduction at a dose of 100, 200, and 400 mg/kg, respectively), demonstrating the n – n-butanol fraction of hydroalcoholic crude extract of *COMBRETUM HYPOPILINUM DIEL* curative potential. The percentage suppression analysis revealed that the n- n-butanol fraction of hydroalcoholic crude extract treated groups (all three dosages) had a substantial parasitemia suppressive effect ($P > 0.001$) when compared to the negative control group, but were less effective ($P > 0.001$) when compared to the positive control group. Furthermore, a substantially distinct effect ($P < 0.001$) for all treatment groups) was observed in comparison to each other, demonstrating that the crude extract has a dose-dependent effect (Table 3.5b). The conventional medicine, on the other hand, was more efficacious ($P < 0.001$) than all of the test substance doses (Figure 3.5b). The n – n-butanol fraction of the hydroalcoholic crude extract of *COMBRETUM HYPOPILINUM DIEL* prevented body weight loss and PCV reduction at all doses (Table 3.5a).

***Table 3.5a: Packed cell volume and body weight of* PLASMODIUM BERGHEI–infected *mice treated with N-Butanol fraction of* COMBRETUM HYPOPILINUM DIEL *in 7–day curative test.***

| Group (mg/kg) | Packed cell volume (PCV) | | Body weight (g) | |
|---|---|---|---|---|
| | Day 0 | Day 4 | **Day 0** | **Day 4** |
| **N/S 10ml/kg** | 59.80 ± 2.78 | 46.40 ± 0.75* | 21.20 ± 0.97 | 19.20 ± 0.97* |
| **NBCH 100** | 51.60 ± 3.38 | 50.00 ± 1.11 | 22.20 ± 1.65 | 22.40 ± 2.16 |
| **NBCH 200** | 48.40 ± 2.56 | 49.00 ± 2.38 | 22.40 ± 0.68 | 22.40 ± 0.68 |
| **NBCH 400** | 64.40 ± 1.28 | 62.50 ± 1.61 | 23.60 ± 1.21 | 23.60 ± 1.40 |
| **CQ 10** | 47.20 ± 0.92 | 48.20 ± 1.16 | 20.80 ± 1.06 | 23.80 ± 1.68 |

***Data are expressed as mean ± SEM, n = 5; * = p<0.001; dependent t- test was used; N/S = normal saline, CQ, chloroquine; NBCH, n – butanol fraction of* combretum hypopilinum diel*; Day 0, pre – treatment value; Day 4, post – treatment value; g, gram; mg/kg, milligram per kilogram.***

***Table 3.5b: Average and percentage parasitemia of* Plasmodium berghei–infected *mice treated with N-Butanol fraction of* Combretum hypopilinum diel *in 7–day curative test.***

| Test Dose (mg/kg) | Average Parasitemia (SEM) | Percentage Suppression (%) |
|---|---|---|
| **N/S 10ml/kg** | 444 ± 14.61 | 0 |
| **NBCH 100** | 373 ± 8.27* | 16 |
| **NBCH 200** | 345 ± 13.31* | 20.3 |
| **NBCH 400** | 353 ± 5.20* | 22.5 |
| **CQ 10** | 234 ± 18.50* | 47.3 |

**Data are presented as mean ± SEM one-way ANOVA followed by Dunnett post hoc was used. N/S = Normal Saline, CQ = Chloroquine, NBCH = N-butanol fraction of combretum hipopilinum diel, mg/kg = Milligram per Kilogram, ml/kg = mil per kilogram; n = 5; * = p<0.001.**

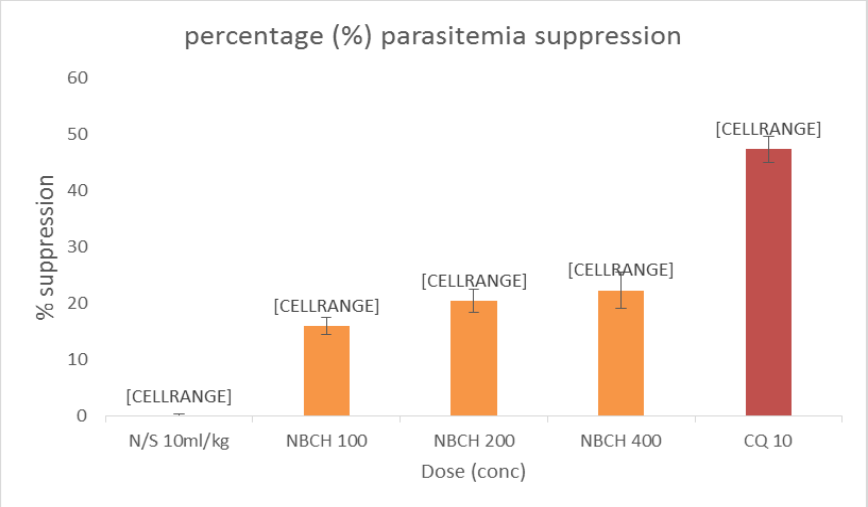


**Figure 3.5b: bar chart showing percentage parasitemia for 7-day curative (Rane's test).**

## 4.0 DISCUSSION

This study aimed to explore the effectiveness of the N-Butanol fraction extracted from *Combretum hypopilinum diel* in combating malaria through suppressive and curative (Rane's test). The findings from these evaluations unveiled varying degrees of success in suppressing the presence of malaria parasites, linked with changes observed in Packed Cell Volume (PCV), changes in body weight, as well as mean parasitemia level.

Traditionally used for treating various diseases, *Combretum hypopilinum diel* underwent an acute toxicity test in mice, demonstrating no significant adverse effects such as impaired movement, reduced motor activity, or mortality at a single dose of 2000 mg/kg over 14 days. This absence of mortality at the tested dose levels indicates the safety of the plant and supports its traditional use against various ailments, including malaria. This aligns with similar research (Hussaini *et al.,* 2021) and suggests a classification of low toxicity according to accepted toxicity standards (Badekoba *et al.*, 2023).

In the suppressive test, daily doses of 100, 200, and 400 mg/kg/day of the N-Butanol fraction exhibited a dose-dependent suppression of parasitemia by 22.2%, 25.5%, and 40%, respectively. Similar studies on medicinal plants have shown a dose-dependent antimalarial effect (Nureye *et al.*, 2021; Tadege *et al.*, 2022; Bizuneh *et al.*, 2023). Even the lowest dose significantly suppressed parasitemia compared to the negative control, although all doses showed lower suppression than the standard drug. These finding echoes research on *Commelina latifolia* (Tadege *et al.*, 2022).

In Rane's test, all doses of the N-Butanol fraction significantly suppressed parasitemia in a dose-dependent manner (16%, 20.50%, and 22.3%) compared to the negative control. However, the curative effect was lower than that of the standard drug chloroquine (Ahmed *et al.*, 2022). This discrepancy between curative and suppressive effects might be due to the extract's shorter duration of action against established infections, consistent with findings in other studies (Bizuneh *et al.*, 2023).

Malaria-induced anemia involves inhibiting erythropoiesis and the breakdown of infected red blood cells. Hence, assessing changes in PCV becomes crucial in evaluating the antimalarial activity of medicinal plants. The N-Butanol fraction prevented body weight loss and PCV decrease caused by increased parasitemia compared to negative controls in all conducted tests (suppressive, curative, and prophylactic). Secondary metabolites like flavonoids and phenols in plants can prevent malaria-induced erythrocyte hemolysis, showing significant PCV protection (Tadege *et al.*, 2022). This study is in agreement with Bizuneh *et al. (*2023 who reported the antimalarial effort of *cucumic fucofolis* roots.

## 5.0 CONCLUSION AND RECOMMENDATION

### 5.1 CONCLUSION

In conclusion, the current study provides valuable insights into the antimalarial potential of the butanol fraction of the *Combretum hypopilinum diel* extract. While displaying promising suppressive and prophylactic effects against malaria parasites, anemia, the extract's efficacy remains lower compared to the standard drug chloroquine. Nonetheless, these findings contribute to the ongoing research efforts in identifying natural sources for novel antimalarial therapies, offering prospects for further exploration and development in the field of malaria treatment and prevention.

### 5.2 Recommendation

Future investigations could focus on optimizing the extract's formulation, identifying active compounds, and exploring potential synergistic effects with other antimalarial agents. Additionally, studies elucidating the extract's mechanism of action and safety profile are warranted to pave the way for its development as a

viable alternative or adjunct to existing antimalarial treatments.

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