ISSN: 2474-3658

Nworgu et al. J Infect Dis Epidemiol 2022, 8:276
DOI: 10.23937/2474-3658/1510276
Volume 8 | Issue 8
Open Access

Journal of
Infectious Diseases and Epidemiology

ORIGINAL ARTICLE

# Toxicity and Antiplasmodial Assessments of *Chromolaena odorata* Leaf Extract on *Plasmodium berghei*-Infected Mice

***Confidence Ogechi Nworgu[1], Kinsley Elele[1] and Elias Adikwu[2*]***

[1]*Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria*

[2]*Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria*

***Corresponding author:** *Elias Adikwu, Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State, Nigeria, Tel: +2347068568868*

## Abstract

**Introduction:** This study assessed the toxicity and antiplasmodial profile of the ethanolic leaf extract of *Chromolaena odorata* on *Plasmodium berghei*-infected mice.

**Methods:** The extract was screen qualitatively and quantitatively for phytochemical constituents. Adult Swiss albino mice (20-23 g) of n = 5/group were inoculated with Plasmodium berghei intraperitoneally and were orally treated with the extract (250, 500 and 1000 mg/kg) and CQ (10 mg/kg) (Standard) daily, respectively. In the sub-acute toxicity study, mice (n = 5/group) were treated with the extract (250, 500 and 1000 mg/kg) daily for 28 days, respectively. After treatments, blood samples were collected and examined for percentage parasitemia, inhibition and biochemical parameters.

**Results:** The extract contains flavonoids, alkaloids, steroids, tannins, terpernoids, glycosides and saponins. It has high flavonoids (9.04 mg) quantity and low steroids (0.41 mg) quantity. The acute toxicity study of the extract showed no mortality with 3162 mg/kg as the lethal dose 50. The extract exhibited significant ($p < 0.05$) curative, suppressive and prophylactic antiplasmodial activities in a dose-related fashion when compared to parasitized control. Curatively, the extract (250, 500 and 1000 mg/kg) produced 84.81%, 90.70% and 95.63% parasitemia inhibitions, respectively while CQ produced 94.31% parasitemia inhibition. MST was increased by the extract in a dose-dependent fashion when compared to parasitized control. The altered haematological parameters were restored by the extract in a dose-dependent fashion when compared to parasitized control. The acute toxicity study of the extract (250, 500 and 1000 mg/kg) significantly impaired renal and liver function biomarkers in a dose-related fashion when compare to normal control at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

**Conclusion:** Chromolaena odorata leaf extract has promising antiplasmodial activity.

## Keywords

Chromolaena odorata, Leaf, Extract, Toxicity, Antiplasmodial, Mice

## Introduction

Malaria is one of the serious infectious diseases of primary health concern. It is highly poses a significant socioeconomic burden on most African and Asian countries were its incidence is very high [1,2]. It affects nearly half of the world's population and causes deaths yearly, especially, among children in Africa [3]. Malaria is managed through vector control methods and drugs for both treatment and prevention. The use of artemisinin-based combination therapies (ACTs) has contributed to substantial and appreciable decline in malaria-related deaths; but the emergence of drug resistance threatens the use of ACTs [3]. Also, the cost and toxicities caused by some antimalarial drugs are significant challenges in malaria treatment [4]. In developing nations with high malaria burden the aforementioned challenges created decisions for the alternative use of materials of plant origin as remedy for malaria [5].

There are information on age-long folkloric uses of plants as rich sources of medicines. It is recorded that over 80% of the world population rely medicines from plants. The past decade the global use of herbal

**Citation:** Nworgu CO, Elele K, Adikwu E (2022) Toxicity and Antiplasmodial Assessments of *Chromolaena odorata* Leaf Extract on *Plasmodium berghei*-Infected Mice. J Infect Dis Epidemiol 8:276. doi.org/10.23937/2474-3658/1510276

**Accepted:** August 10, 2022: **Published:** August 12, 2022

medicines has significant increase due to proven efficacy and safety [5]. Most communities in different localities in the world have various folkloric knowledge on medicinal plants, their utilization, management and conservation [6]. Plant products and secondary metabolites are alternative agents for malarial treatment. Medicinal substances of plant origin have been used to treat malaria for thousands of years by humans indifferent parts of the world. Quinine the first antimalarial drug was isolated from *Cinchona* (Rubiaceae) species, while artemisinin was extracted from *Artemisia annua* [7].

*Chromolaena odorata (L)*(*C. odorata*)is one of herbs that belongs to Asteraceae, sunflower family [8]. It is an ornamental plant considered to be one of the most invasive environmental weeds of roadsides, wastelands and other exposed areas in the world [9]. It is a flowering shrub that is native to Central and North America, and was later introduced to Africa, Asia, and Australia [10]. *C. odorata* is known by other names such as baby tea, bitter bush, Armstrong's weed, butterfly weed, devil weed, king weed, Christmas bush, Siam weed, eupatorium, paraffin weed and turpentine weed [11]. It contains bioactive compounds such as steroids, flavonoids, alkaloids, tannins and saponins, which can act singly or synergistically to exert different biological effects [12]. In sub-Saharan Africa and it is use for the treatment of different ailments and disease conditions such as diabetes, inflammation, wounds and fever [12]. In Benin and Ghana, infusion from fresh leaves of *C. odorata* is used to treat malaria [13]. It has been shown to have an *in-vitro* antiplasmodial activity [14] with limited *in-vivo* antiplasmodial studies. This study as certain the toxicity and antiplasmodial profile of the ethanolic leaf extract of *C. odorata* on *Plasmodium berghei* infected mice.

## Materials and Methods

### Collection and identification of plant materials

*C. odorata* was obtained from Ignatius Ajuru University, Rumuolumeni Obio/Akpor Local Government Area, Rivers State, Nigeria (Latitude of 4.49'4" and longitude of 6.51'24) *C. odorata* was deposited in Rivers State University Botanical garden where it was identified by a plant taxonomist.

### Preparation of ethanolic extract of *Chromoleana odorata* leaf

The ethanolic extract of *C. odorata* was prepared as described by Bligh and Dyer, 1959 [15] with slight modification. The fresh leaves of *C. odorata* was washed in clean water and blended using porcelain and electric blender. The leaves were weighed before grinding. The blended paste of *C. odorata* was macerated in ethanol solvent (100%) and allowed to stand overnight after which, the slurry was filtered using Whitman filter paper. The filtrate was the concentrated in a rotary evaporator (45 °C) and the residue was dried under reduced pressure to determine the dry weight of the residue and the percentage extraction yield was calculated. The extract was properly stored in an air tight container in a refrigerator until needed.

### Phytochemical analysis of *Chromolaena odorata* leaf extract

Phytochemical analysis of the extract of *C. odorata* was performed using standard procedures as reported by Trease and Evans (1989) [16] and Harborne (1998) [17]. The extract was examined for alkaloids, anthraquinones, saponins, flavonoids, cardiac glycosides, tannins, steroids and terpernoids.

### Acute toxicity evaluation of *Chromolaena odorata* leaf extract

The median lethal dose ($LD_{50}$) of the extract was determined using the method described by Alaribe, et al. [18]. Sixteen mice (25 g-28 g) of n = 4/group were used. The mice were subjected to 24 hours starvation before the oral administration of the extracts (1000, 2000, 3000 and 5000) dissolved in Tween-80 (20%), respectively. The control received only Tween-80 (20%). Physical observations such as degree of restiveness, aggressions and calmness were observed. The mice were also observed for toxicity and fatalities within 72 hours. The $LD_{50}$ was calculated using the modified formula reported by Enegide, et al. [19] $LD_{50} = \sqrt{ab}$

a = least tolerable dose;

b = maximum tolerable dose.

### Sub-acute toxicity study of *Chromolaena odorata* leaf extract

Twenty five Swiss albino mice were grouped into 5 of n = 5/group and were treated with normal control (Normal saline 0.2 mL), solvent control [Tween 80 (20%)], extract (250 mg/kg, 500 mg/kg and 1000 mg/kg), respectively for 28 days. Thereafter, blood samples were collected and evaluated for renal and liver biochemical markers.

### Ethical Consideration

Ethical approval was granted the research and ethics committee of the Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education.

### Acquisition of mice, parasites and mice inoculation

Donor mice parasitized with *P. berghei* (NK65) were obtained from the Faculty of Basic Clinical Science, University of Port Harcourt, Rivers State, Nigeria. Adult mice of both sexes (25 to 28 g) were used were obtained from the aforementioned school. They were housed in wooden/wire gauze cages and acclimated for 2 weeks with access to water freely before use. The mouse

were handled according to United States of America guidelines for the use of laboratory animals (2003). The mice were inoculated intraperitoneally with blood samples (0.2 mL) containing *P. berghei* ($1x10^7$) collected from the donor mice through cardiac puncture.

### Curative antiplasmodial evaluation of *Chromolaena odorata* leaf extract

It was determined using the protocol explained by Ryley and Peters [20]. Thirty albino mice (25-28g) of 6 groups of n = 5/group were parasitized with *P. berghei* ($1x10^7$) and allowed for 3 days. The groups which served as normal control and parasitized control were treated with the solvent [Tween 80 (20%)], while the standard control was treated with CQ (10 mg/kg) for 3 days. Other parasitized groups were treated with the *C. odorata* leaf extract (250 mg/kg, 500 mg/kg and 1000 mg/kg), respectively for 3 day. During treatment, on day 1, 3, and 5, tails blood samples were collected from the mice on slides and thin blood smears produced. The blood smear were air dried, washed and stained. Percentage of parasitemia was determined by counting the parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Percentage parasitemia and inhibitions were calculated using the formula below.

$$PP = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} * 100$$

PP = Percentage parasitaemia,

PRBC = parasitized red blood cells, and

RBC = red blood cells

$$APP = \frac{\text{APPC-APPT}}{\text{APPC}} * 100$$

APP: Average percentage parasitemia

APPC: Average percentage parasitemia in control group

APPT: Average percentage parasitemia in test group.

### Suppressive antiplasmodial evaluation of *Chromolaena odorata* leaf extract

It was evaluated using the method reported by Knight and Peters [21]. Twenty five Swiss albino mice were grouped into 5 of n = 5/group and inoculated with *P. berghei* ($1x10^7$). After 2 hr, the mice were treated with the *C. odorata* leaf extract 250 mg/kg, 500 mg/kg and 1000 mg/kg, respectively for 3 day. The parasitized and the standard controls were treated with normal saline (0.2 ml) and CQ (10 mg/kg) for 3 days, respectively. On day 4, blood smears were produced on slides, air dried, and washed. The slides were stained and examined for percentage of parasitemia and inhibitions as stated above.

### Prophylactic antiplasmodial evaluation of *Chromolaena odorata* leaf extract

It was performed as reported by Peters [22]. Twenty five Swiss albino mice were grouped into 5 of n = 5/group and were treated with the *C. odorata* extract (250 mg/kg, 500 mg/kg and 1000 mg/kg), respectively for 3 day. The parasitized control and the standard control were treated with normal saline (0.2 ml) and CQ (10 mg/kg) for 3 days, respectively. On day 4, the mice were inoculated with *P. berghei* ($1x10^7$). After, 24 hours, tail blood smears were produced on slides, air dried, and washed. The slides were stained and examined for Percentage parasitemia and inhibitions as stated above.

### Determination of mean survival time

In the curative study, from the inception of infection until death, mortality of each mouse was monitored and recorded in days. Mean survival time (MST) of mice in each group was determined as shown below.

$$MST = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

### Evaluation of biochemical markers

Samples of blood collected from the curative mice group were evaluated for hemoglobin (Hb), packed cell volume (PCV), neutrophils (NEU), red blood cells (RBC), lymphocytes (LYM) monocytes (MON), white blood cells (WBC), Aspartate aminotransferase (AST), alkaline phosphatase, alanine aminotransferase (ALT), Creatinine (CR), urea (UR), total bilirubin (TBL) and total protein (TP) using laboratory reagents according to the manufacturer specifications.

### Statistical analysis

Results as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) and Tukey's tests were used for data analysis with the aid of Graph Pad Prism (Version 5.0, Graph Pad Software Inc., La Jolla, California, U.S.A). P-values < 0.05, < 0.01 < 0.001 were used as significance.

## Results

### Phytochemical and toxicity Screening of *Chromolaena odorata* leaf extract

The qualitative screening of *C. odorata* leaf extract shows that it contains alkaloids, cyanogenic glycosides, flavonoids, saponins, steroids, tannins and terpernoids (Table 1). The quantitative screening of *C. odorata* leaf extract shows that alkaloids (6.23 mg), flavonoids (9.04 mg), and alkaloids (6.23 mg) were present in high quantities whereas saponins (1.24 mg) and steroids (0.41 mg) were present in low quantities (Table 2).

### Toxicity study of *Chromolaena odorata* leaf extract

In acute toxicity study the *C. odorata* extract (1000, 2000, 3000, 4000 and 5000 mg/kg) did not produce mortality, but at 5000 mg/kg dose notable physical changes occurred, which include lethargy, decreased locomotor activity, diarrhoea and pilo erection (Table 3). In the sub-acute toxicity study, *C. odorata*

**Table 1:** Qualitative phytochemical composition of *Chromolaena odorata* leaf extract.

| Phytochemicals | *C. Odorata* leaf |
|---|---|
| Alkaloids | +++ |
| Anthraquinones | _ |
| Cyanogenic glycoside | + |
| Flavonoids | +++ |
| Saponins | + |
| Steroids | + |
| Tannins | ++ |
| Terpernoids | ++ |

**Table 2:** Quantitative phytochemical composition of *Chromolaena odorata* leaf extract.

| Phytochemicals | Quantity (mg) |
|---|---|
| Alkaloid | 6.23 |
| Anthraquinones | _ |
| Cyanogenic glycoside | 0.31 |
| Flavonoids | 9.04 |
| Saponins | 1.24 |
| Steroids | 0.41 |
| Tannin | 4.52 |
| Terpernoids | 3.01 |

**Keys:** -: absent, + : Low, ++ : Moderate, +++ : High.

**Table 3:** Acute toxicity evaluation of *Chromolaena odorata* leaf extract in mice.

| Group | No of mice | Dose (Mg/kg) | Mortality |
|---|---|---|---|
| 1 | 4 | 1000 | 0/4 |
| 2 | 4 | 2000 | 0/4 |
| 3 | 4 | 3000 | 0/4 |
| 4 | 4 | 5000 | 0/4 |

**Table 4:** Effect of *Chromolaena odorata* extract on liver biomarkers of mice.

| Treatment (mg/kg) | AST (U/L) | ALT (U/L) | TBL(U/L) | ALP (U/L) |
|---|---|---|---|---|
| Control | 28.00 ± 2.57 | 27.00 ± 2.15 | 5.67 ± 0.21 | 25.67 ± 2.33 |
| CO 100 | 40.67 ± 3.85[a] | 39.00 ± 2.36[a] | 7.33 ± 0.44[a] | 31.33 ± 2.61[a] |
| CO 250 | 57.00 ± 5.50[b] | 59.67 ± 2.64[b] | 12.67 ± 0.63[b] | 42.80 ± 4.37[b] |
| CO 250 | 71.33 ± 6.61[c] | 78.33 ± 1.36[c] | 16.67 ± 0.24[c] | 68.67 ± 4.55[c] |

Data as mean ± SEM. n = 5, CO: *Chromolaena odorata* extract, AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; TBL: Total Bilirubin; ALP: Alkaline Phosphatase; [a]Significant difference from control at $p < 0.05$; [b]Significant difference from control at $p < 0.01$; [c]Significant difference from control at $p < 0.001$.

**Table 5:** Effect of *Chromolaena odorata* extract on renal biomarkers of mice.

| Treatment (mg/kg) | TP g/dL | UREg/dL | CRE g/dL | Uric acid g/dL |
|---|---|---|---|---|
| Control | 3.65 ± 1.45 | 2.43 ± 0.12 | 60.67 ± 6.33 | 1.67 ± 0.38 |
| CO 250 | 3.10 ± 1.33[a] | 3.07 ± 0.18[a] | 70.67 ± 4.33[a] | 1.57 ± 0.16[a] |
| CO 500 | 2.51 ± 2.08[b] | 3.67 ± 0.12[b] | 63.67 ± 6.63[b] | 2.07 ± 0.27[b] |
| CO 1000 | 2.06 ± 1.15[c] | 4.52 ± 0.15[c] | 61.33 ± 7.89[c] | 2.61 ± 0.32[c] |

Data as Mean ± SEM. n = 5; TP: Total Protein, URE: Urea; CRE: Creatinine; CO: *Chromolaena odorata* Extract; [a]Significant difference from control at $p < 0.05$; [b]Significant difference from control at $p < 0.01$; [c]Significant difference from control at $p < 0.001$.

extract (250, 500 and 100 mg/kg) administered for 28 days significantly increased serum AST, ALT, ALT, TB, Creatinine, urea and uric acid levels in a dose-related fashion at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively when compared to normal control (Table 4 and Table 5). On the other hand, *C. odorata* extract (250, 500 and 100 mg/kg) administered for 28 days significantly decreased total protein in a dose-related fashion when compared to normal control (Table 4 and Table 5).

## Curative antiplasmodial activity of *Chromolaena odorata* leaf extract

In the curative study, *C. odorata* leaf extract produced dose-related decreases in percentage parasitemia in day 1, 2 and 3 with significant difference at $p < 0.05$ when compared to parasitized control (Table 6). The decreased percentage parasitemia produced by *C. odorata* extract at 1000 mg/kg was not different from CQ. *C. odorata* extract produced 84.81%, 90.70% and 95.63% inhibitions at 250, 500 and 1000 mg/kg, respectively while CQ produced 94.31%parasitamia inhibition (Table 6).

## Suppressive and prophylactic antiplasmodial activities of *Chromolaena odorata* leaf extract

In the suppressive and curative antiplasmodial studies, *C. odorata* extract in a dose-dependent fashion significantly decreased percentage parasitemia at

**Table 6:** Curative effect of *Chromolena odorata* leaf extract on parasitized mice.

| Treatment (Mg/kg) | Day 1 | Day 2 | Day 3 | % Inhibition Day 3 | Mortality (Days) |
|---|---|---|---|---|---|
| PCO | 14.35 ± 1.16 | 25.70 ± 02.98 | 44.19 ± 2.02 | - | 9.21 ± 0.34 |
| CQ 10 | 10.49 ± 1.36[a] | 2.99 ± 0.32[a] | 2.51 ± 0.08[a] | 94.31 | 34.61 ± 3.72[a] |
| CO 250 | 12.42 ± 1.01[a] | 8.35 ± 0.66[ab] | 6.71 ± 0.29[ab] | 84.81 | 17.34 ± 2.11[ab] |
| CO 500 | 15.11 ± 0.25[ab] | 6.37 ± 0.98[ab] | 4.11 ± 0.46[ab] | 90.70 | 23.52 ± 2.61[ab] |
| CO 1000 | 9.71 ± 0.99[a] | 4.23 ± 0.55[ab] | 1.93 ± 0.08[ab] | 95.63 | 32.22 ± 3.00[a] |

PCO: Parasitized control; CQ: Chloroquine; CO: *Chromolaena odorata* extract; Data as mean ± SEM (Standard error of mean) [a]Significant different ($p < 0.05$) when compared to Parasitized control; [b]Significant different ($p < 0.05$) compared to CQ.

**Table 7:** Suppressive and prophylactic antiplasmodial activities of *Chromolena odorata* leaf extract on parasitized mice.

| Treatment (mg/kg) | Suppressive % Parasitemia | % Inhibition | Prophylactic % Parasitemia | % Inhibition |
|---|---|---|---|---|
| PCO | 32.35 ± 3.16 | - | 28.99 ± 2.02 | - |
| CQ 10 | 1.22 ± 0.45[a] | 96.23 | 1.10 ± 0.08[a] | 96.21 |
| CO 250 | 4.62 ± 0.67[ab] | 85.71 | 3.35 ± 0.29[ab] | 88.44 |
| CO 500 | 2.21 ± 0.83[ab] | 93.17 | 1.70 ± 0.46[ab] | 94.14 |
| CO 1000 | 1.27 ± 0.61[a] | 96.07 | 1.12 ± 0.08[a] | 96.14 |

NCO: Parasitized Control; CQ: Chloroquine, CO: *Chromolaena odorata* Extract; Data as mean ± SEM (Standard error of mean); [a]Significant different ($p < 0.05$) when compared to Parasitized control; [b]Significant different ($p < 0.05$) compared to CQ.

**Table 8:** Effect of *Chromolaena odorata* extract on the haematological parameters of parasitized mice.

| Treatment (mg/kg) | PCV | HB | RBC | WBC | NEU | LYM | MON |
|---|---|---|---|---|---|---|---|
| Control | 49.75 ± 5.79 | 15.02 ± 0.26 | 4.85 ± 0.12 | 3.35 ± 0.19 | 73.25 ± 8.31 | 23.00 ± 0.40 | 1.00 ± 0.40 |
| PCO | 12.00 ± 1.08[a] | 5.82 ± 0.49[a] | 2.12 ± 0.14[a] | 12.10 ± 0.72[a] | 60.75 ± 6.56 | 31.75 ± 2.70[a] | 2.50 ± 0.28[a] |
| CQ | 43.50 ± 3.55[b] | 13.80 ± 0.23[b] | 4.42 ± 0.14[b] | 5.20 ± 0.19[b] | 72.75 ± 6.07 | 21.25 ± 3.48[b] | 0.50 ± 0.28[b] |
| CO 250 | 22.33 ± 2.20[bc] | 7.60 ± 0.26[bc] | 3.07 ± 0.17[bc] | 10.10 ± 0.20[bc] | 65.33 ± 8.67 | 23.67 ± 2.67[b] | 2.00 ± 0.11[bc] |
| CO 500 | 27.33 ± 2.20[bc] | 9.46 ± 0.43[bc] | 3.43 ± 0.09[bc] | 5.80 ± 0.05[b] | 68.33 ± 7.20 | 22.67 ± 3.89[b] | 1.00 ± 0.32[bc] |
| CO 1000 | 33.67 ± 3.88[bc] | 11.33 ± 0.23[bc] | 3.77 ± 0.09[bc] | 3.97 ± 0.08[bc] | 69.00 ± 6.73 | 21.67 ± 2.89[b] | 0.67 ± 0.03[ab] |

PCO: Parasitized Control; CQ: Chloroquine; CO: *Chromolaena odorata* Extract; Data as mean ± SEM (Standard error of mean); [a]significant different ($p < 0.05$) when compared to the normal control; [b]Significant different ($p < 0.05$) when compared to parasitized control; [c]Significant different ($p < 0.05$) compared to CQ.

$p < 0.05$ when compared to parasitized control. No significant difference in percentage parasitemia was observed in *C. odorata* extract (1000 mg/kg) when compared to CQ (Table 7). In the prophylactic study, the following inhibitions; 88.44% and 94.14% 96.14% were produced by C. *odorata* extract (250, 500 and 1000 mg/kg), respectively whereas CQ produced 98.85% parasitemia inhibition (Table 7).

### Effect of *C. odorata* leaf extract on haematological parameters of parasitized mice

*P.berghei*-infected mice showed decreased PCV, HB, RBCs and increased WBCs, MON and LYM when compared to normal control at $p < 0.05$ (Table 8). *C. odorata* extract (250, 500 and 100 mg/kg) increased PCV, HB, WBCs and decreased WBCs, MON and LYP when compared to the parasitized control at $p < 0.05$. *C. odorata* extract (1000 mg/kg) increased PCV, HB,WBCs and decreased WBCs, MON and LYM when compared to CQ at $p < 0.05$ (Table 8).

## Discussion

The use of medicinal plants in the treatment of ailments is part of the culture of the indigenous people of African. From time immemorial, humans have relied on plants as sources of medicines to treat diseases and provide for different health needs [23]. For decades, plants have provide medicines for the malaria treatment with the discovery of two major antimalarial drugs-quinine and artemisinin, which are used worldwide. Thus, medicinal plants have potential as new sources of antimalarial drugs [24]. The extraction of bioactive compounds from medicinal plants based on ethno medical data or on traditional use is a promising step toward the development of new antimalarial drugs [25]. This study assessed the toxicity and antiplasmodial profile of the ethanolic leaf extract of *C. odorata* on *Plasmodium berghei*-infected mice. *In-vivo* model was used as compared with an *in-vitro* model to account for a prodrug effect and the involvement of the immune system in the eradication of infection [26]. The primate model provide a better prediction for the assessment of

potential antimalarial agents, but the rodent was used in this study because it is the first step used to screen most *in-vivo* antimalarial activities of test compounds [27]. The rodent model has been also validated through the discovery of several conventional antimalarial agents. *P. berghei* was used in this study since it is frequently used for the assessment of potential antimalarial agents in rodent model and has higher accessibility. Due to the sensitivity and significant suppression of *P. berghei* by CQ, it was used as the standard [27]. The 4-day suppressive test, which evaluates the activity of a test compound on early malaria infection, and Rane's test, which estimates the curative ability of a test compound on established infection, are the two adopted methods frequently used for the screening of potential antimalarial agents [28]. In the qualitative screening the extract was found to harbour notable phytochemicals of paramount importance, which include alkaloids, saponins, tannins, flavonoids, terpenoids and steroids. Similarly, Igboh, et al. [29] reported the presence of the aforementioned phytochemicals in *C. Odorata* leaf extract. Quantitatively the extract contains higher quantities of flavonioids and alkaloids with low quantities of saponins and steroids. No mortality was observed in the acute toxicity evaluation of *C. odorata* extract which shows it may be safe. But physical observations such as piloerection, lethargy, diarrhoea and reduced locomotor activity were observed at the highest dose of the extract (5000 mg/kg). The sub-acute toxicity assessment of *C. odorata* for 26 days shows altered levels of renal and liver function biomarkers. This shows that long-term use of the extract may impair renal and liver function. In the 4 day suppressive study, the extract exhibited notable suppressive activity against *P. Berghei* in a dose- dependent fashion, which was at par with CQ at 1000 mg/kg of the extract. The extract also showed pronounced curative and prophylactic activities in a dose-related fashion, which were similar to CQ at the highest dose of the extract. Similarly, funmilayo, et al. [30] reported the antiplasmodial activity of the methanolic leaf extract of *C. odorata* on *P. berghei*-infected mice. *In-vivo* antiplasmodial activity can be classified as moderate, good and very good if an extract displayed a respective percent parasite suppression equal to or greater than 50% [31]. *C. odorata* produced 84.81 % parasitemia suppression at the least dose, which indicates a very good antiplasmodial activity. MST of the mice were prolonged by the extract in the curative study, maximum prolongation of MST was observed at the highest dose of the extract, which was similar to effect produced by CQ. This further add credence to the suppression of *P. berghei,* by the *C. odorata* extract resulting in decreased overall pathologic effect of the parasite on the mice. On the haematological parameters, anaemic condition was observed in the parasitized mice marked by low PCV, RBCs and HB levels. The observed anaemic conditions in parasitized mice was curtailed by *C. odorata* extract in a dose-related fashion. Studies associated malaria-related anaemia to the destruction of infected RBCs and erythropoietic suppression by parasites [32], thus the *C. odorata* extract might have curtailed *P. berghei*-induced anaemia by inhibiting the aforementioned mechanism. The antiplasmodial activity of *C. odorata* extract can be attributed to its phytochemical constituents. Phytochemicals have been associated with antiplasmodial activity through different possible mechanisms. These mechanisms include endoperoxidation by terpenes, disruption of the ability of the parasite to detoxify heme into nontoxic malaria pigment by alkaloids, blocking protein synthesis by alkaloids and chelation of nucleic acid base pairing by flavonoids. It also include the immunomodulatory effects of steroids and flavonoids, and the free radical scavenging effects of tannins [33].

## Conclusion

The observations in this study showed that *C. odorata* leaf extract may be a source for the development of new plant-based antimalarial agents which support it folklore use for the treatment of malaria. This study suggest further studies on the fractionation, isolation and characterization of the active principle responsible for the observed antiplasmodial activity.

## References

1. Joseph OA, Samson TO (2020) Antiplasmodial Efficacy of *Anacardium occidentale* in Albino Mice Infected with *Plasmodium berghei.* J Fam Med Dis Prev 6: 123.
2. Sato S (2021) Plasmodium-a brief introduction to the parasites causing human malaria and their basic biology. J Physiol Anthropol 40: 1.
3. Phillips M, Burrows J, Manyando C, Huijsduijnen RH, Voorhis WC, Wells TNC, et al. (2017) Malaria. Nat Rev Dis Primers 3: 17050.
4. AlKadi HO (2007) Antimalarial drug toxicity: A review. Chemother 53: 385-391.
5. Kunle O F, Adache AA, Omoregie EH (2013) Medicinal plants used for the treatment of malaria in Rukuba, Bassa Local Government Area of Plateau State, Nigeria. Intern J Basic App Sci 2: 134-138.
6. Alebie G, Urga B, Worku A (2017) Systematic review on traditional medicinal plants used for the treatment of malaria in Ethiopia: Trends and perspectives. Malar J 16: 307.
7. Adebayo JO, Krettli AU (2011) Potential antimalarials from Nigerian plants: A review. J Ethnopharmacol 133: 289-302.
8. Sirinthipaporn A, Jiraungkoorskul W (2017) Wound healing property review of Siam Weed, *Chromolaena odorata*. Pharmacogn Rev 11: 35-38.
9. Chakraborty AK, Rambhade S, Patil U (2011) Chromolaena odorata (L.): An overview. J Pharm Res 43: 573-576.
10. Zahara M (2019) Description of *Chromolaena odorata* L. R.M King and H. Robinson as medicinal plant: A Review. IOP Conf Ser: Mater Sci Eng, 506: 012022.
11. Vijayaraghavan K, Rajkumar J, Seyed MA (2017) Efficacy of *Chromolaena odorata* leaf extracts for the healing of rat excision wounds. Veter Med 62: 565-578.

12. Olawale F, Olofinsan K, Iwaloye O (2022) Biological activities of *Chromolaena odorata*: A mechanistic review. South Afri J of Bot 144: 44-57.

13. Tonzibo Z F, Wognin E, Chalchat JC, Guessan YTN (2007) Chemical investigations of *Chromolaena odorata* L. King Robinson from Ivory Coast. J Ess oils-Bearing Plants 10: 94-100.

14. Okunji C, Jackson JE, Tally JD, Roth N, Iwu MM, et al. (2000) *In vivo* antimalarial and antileishmanial activities of 4, 5-dihydroxy-7-methoxy flavones from *E. odoratum*. Am J Trop Med Hyg 62: 296-399.

15. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911-917.

16. Trease GE, Evans WC (1989) Pharmacognosy. W.B Scandars Company Ltd, London, England, 269-300.

17. Harborne JB (1998) Phytochemical methods guide to modern technique of plant analysis. (3rd edn), Chapmen all Hall, London, England.

18. Alaribe CSA, Corker HAB, Shode FO (2011) Antiplasmodial and phytochemical investigation of leaf extract of *Anthocleista vogelii*. J Nat Prod 5: 60-67.

19. Chinedu E, Arome D, Ameh FS (2013) A new method for determining acute toxicity in animal models. Toxicol Int 20: 224-226.

20. Ryley J, Peters W (1970) The antimalarial activity of some quinolone esters. Ann Trop Med Parasitol 64: 209-222.

21. Knight D, Peters W (1980) The antimalarial activity of N-benzyloxydihydrotriazines. I. The activity of clociguanil (BRL 50216) against rodent malaria, and studies on its mode of action. Ann Trop Med Parasitol 74: 393-404.

22. Peters W (1975) The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of Plasmodium berghei in screening for blood schizontocidal activity. Ann Trop Med Parasitol 69: 155-171.

23. Frimpong EK, Asong JA, Aremu AO (2021) A review on medicinal plants used in the management of headache in Africa. Plants (Basel) 10: 2038.

24. Kweyamba PA, Zofou D, Efange N, Assob JC-N, Kitau J, et al. (2019) *In vitro* and *in vivo* studies on anti-malarial activity of *Commiphora africana* and *Dichrostachys cinerea* used by the Maasai in Arusha region, Tanzania. Malar J 18: 119.

25. Newman DJ (2008) Natural products as leads to potential drugs: An old process or the new hope for drug discovery? J Med Chem 51: 2589-2599.

26. Hilou A, Nacoulma OG, Guiguemde TR (2006) *In vivo* antimalarial activities of extracts from *Amaranthus spinosus* L. and *Boerhaavia erecta* L. in mice. J Ethnopharmacol 103: 236-240.

27. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S (2004) Antimalarial drug discovery: Efficacy models for compound screening (supplementary documents). Trends Parasitol 15: 19-29.

28. Belay WY, GurmuAE, Wubneh ZB (2018) Antimalarial Activity of Stem Bark of *Periploca linearifolia* during early and established *Plasmodium* infection in mice. Evid Based Complement Alternat Med 2018: 4169397.

29. Igboh MN, Ikewuchi CJ, Catherine C (2009) Chemical profile of *Chromolaena odorata* L. (King and Roobison) leaves. Pakistan J Nutri 8: 521-524.

30. Afolayan FID, Adegbolagun OM, Irungu B, Kangethe L, Orwa J, et al. (2016) Antimalarial actions of *Lawsonia inermis, Tithonia diversifolia* and *Chromolaena odorata* in combination. J Ethnopharmacol 191: 188-194.

31. Fentahun S, Makonnen E, Awas T, Giday M (2017) *In vivo* antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei*-infected mice. BMC Complement Altern Med 17: 13.

32. Lamikanra AA, Brown D, Potocnik A, Casals-Pascual C, Langhorne J, et al. (2007) Malarial anemia: Of mice and men. Blood 110:18-28.

33. Kifle ZD, Adinew GM, Mengistie MG, Gurmu AE, Enyew EF, et al. (2020) Evaluation of antimalarial activity of methanolic root extract of *Myrica salicifolia* a rich (Myricaceae) against *Plasmodium berghei*-infected mice. J Evid Based Integr Med 25: 2515690X20920539.