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

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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 examined qualitatively and quantitatively for phytochemical constituents. Adult Swiss albino mice (25-28 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 parasitaemia, inhibition and biochemical parameters.

Results: The extract contains flavonoids, alkaloids, steroids, tannins, terpenoids, glycosides and saponins. It has high flavonoids (9.04 mg) and low steroids (0.41 mg) quantities. The lethal dose 50 was found to be >5000 mg/kg. 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% parasitaemia inhibitions, respectively while CQ produced 94.31% parasitaemia 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) showed significantly impaired renal and liver function biomarkers in a dose-related fashion when compared 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 causes a significant socioeconomic burden on most African and Asian countries where 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 on medicines from plants. In the past decade the global use of herbal

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 in different 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, it is used for the treatment of different ailments 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 assessed 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 of Education, Rumuolumeni, Obio/Akpor Local Government Area, Rivers State, Nigeria (Latitude of 4.49'4" and longitude of 6.51'24). It was deposited in Rivers State University Botanical Garden where it was identified by a plant taxonomist.

Preparation of ethanolic extract of *Chromolaena odorata* leaf

The ethanolic extract of *C. odorata* leaf was prepared as described by Bligh and Dyer, 1959 [15] with slight modifications. The fresh leaves of *C. odorata* were washed in clean water and blended using porcelain and electric blender. The blended paste of *C. odorata* was macerated in ethanol solvent (100%), and allowed to stand overnight and was filtered using Whitman filter paper. The filtrate was concentrated using a rotary evaporator (45 °C) and the residue was dried under reduced pressure. The weight of the residue was

determined and the percentage yield 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 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 terpenoids.

Acute toxicity evaluation of *Chromolaena odorata* leaf extract

The median lethal dose (LD₅₀) of the extract was determined using the method described by Alaribe, et al. [18] and Chindeu et al. [19]. Twenty mice (25 g-28 g) of n = 4/group were used. The mice were subjected to 24 hr starvation before the oral administration of the extract (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.

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

Twenty five adult Swiss albino mice were grouped into 5 of n = 5/group and were orally 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) daily for 28 days, respectively. Thereafter, blood samples were collected and evaluated for renal and liver biochemical markers.

Ethical Consideration

Ethical approval was granted by the Research Ethics Committee of the Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rivers State.

Acquisition of mice, parasites and mice inoculation

Donor mice parasitized with *P. berghei* (NK65) were obtained from the Faculty of Basic Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria. Adult Swiss albino mice of both sexes (25 to 28 g) 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 mice 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* (1x10⁷) 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 Swiss albino mice of 6 groups of $n = 5/\text{group}$ were parasitized with *P. berghei* (1×10^7) 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 4 days. Other parasitized groups were treated with the *C. odorata* leaf extract (250 mg/kg, 500 mg/kg and 1000 mg/kg), respectively for 4 day. During treatment, on day 1, 3, and 5, tail blood samples were collected from the mice on slides and thin blood smears produced. The blood smear were air dried, washed and stained. Percentage parasitama was determined by counting the parasitized erythrocytes out of 200 erythrocytes in random fields of the microscope. Percentage parasitama and inhibitions were calculated using the formula below.

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

PP = Percentage parasitama,

PRBC = Parasitama, red blood cells

RBC = Red blood cells

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

APP: Average percentage parasitama

APPC: Average percentage parasitama of negative control

APPT: Average percentge parasitama of treated 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 $n = 5/\text{group}$ and inoculated with *P. berghei* (1×10^7). After 2 hr, the mice were treated with the *C. odorata* leaf extract (250 mg/kg, 500 mg/kg and 1000 mg/kg) for 4 days, respectively. The parasitized and the standard controls were treated with normal saline (0.2 ml) and CQ (10 mg/kg) for 4 days, respectively. On day 5, blood smears were produced on slides, air dried, and washed. The slides were stained and examined for percentage parasitama 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 $n = 5/\text{group}$ and were treated with the *C. odorata* leaf extract (250 mg/kg, 500 mg/kg and 1000 mg/kg), for 4 days, respectively. The parasitized control and the standard control were treated with normal saline (0.2 ml) and

CQ (10 mg/kg) for 4 days, respectively. On day 5, the mice were inoculated with *P. berghei* (1×10^7). After, 24 hr, tail blood smears were produced on slides, air dried, and washed. The slides were stained and examined for percentage parasitama 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 mice in the curative group were evaluated for hemoglobin (Hb), packed cell volume (PCV), neutrophils (NEU), red blood cells (RBC), lymphocytes (LYM) monocytes (MON), white blood cells (WBCs), aspartate aminotransferase (AST), alkaline phosphatase, alanine aminotransferase (ALT), creatinine (CR), urea (UR), total bilirubin (TB), uric acid and total protein (TP) using laboratory reagents according to the manufacturer's specifications.

Statistical analysis

Results as mean \pm 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 and < 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 terpenoids (Table 1). The quantitative screening of *C. odorata* leaf extract shows that 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 the acute toxicity study, *C. odorata* leaf extract (1000, 2000, 3000 and 5000 mg/kg) did not produce mortality, but at 5000 mg/kg notable physical changes occurred, which include lethargy, decreased locomotor activity, diarrhoea and pilo erection (Table 3). In the sub-acute toxicity study, *C. odorata* leaf extract (250, 500 and 1000 mg/kg) administered for 28 days significantly increased serum AST, ALT, ALT, TB, CRE, UR and uric acid levels in a dose-related fashion at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively when compared

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

| Phytochemicals | <i>C. Odorata</i> leaf |
|----------------------|------------------------|
| Alkaloids | +++ |
| Anthraquinones | – |
| Cyanogenic glycoside | + |
| Flavonoids | +++ |
| Saponins | + |
| Steroids | + |
| Tannins | ++ |
| Terpernoids | ++ |

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

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

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

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* leaf extract on liver biomarkers of mice.

| Treatment (mg/kg) | AST (U/L) | ALT (U/L) | TB (g/dL) | ALP (U/L) |
|-------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Control | 28.00 ± 2.57 | 27.00 ± 2.15 | 5.67 ± 0.21 | 25.67 ± 2.33 |
| CO 250 | 40.67 ± 3.85 ^a | 39.00 ± 2.36 ^a | 7.33 ± 0.44 ^a | 31.33 ± 2.61 ^a |
| CO 500 | 57.00 ± 5.50 ^b | 59.67 ± 2.64 ^b | 12.67 ± 0.63 ^b | 42.80 ± 4.37 ^b |
| CO 1000 | 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* leaf extract, AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; TBL: Total Bilirubin; ALP: Alkaline Phosphatase; ^aSignificant difference from control at p < 0.05; ^bSignificant difference from control at p < 0.01; ^cSignificant difference from control at p < 0.001.

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

| Treatment (mg/kg) | TP g/dL | UR mg/dL | CRE mg/dL | Uric acid mg/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, UR: Urea; CRE: Creatinine; CO: *Chromolaena odorata* leaf extract ^aSignificant difference from control at p < 0.05; ^bSignificant difference from control at p < 0.01; ^cSignificant difference from control at p < 0.001.

to normal control (Table 4 and Table 5). On the other hand, *C. odorata* leaf extract (250, 500 and 1000 mg/kg) administered for 28 days significantly decreased TP 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 parasitama on day 1, 3 and 5 with significant difference at p < 0.05 when compared to parasitized control (Table 6). The decreased percentage parasitama produced by *C. odorata* leaf extract at 1000 mg/kg was not different

from CQ. *C. odorata* leaf extract produced 84.81%, 90.70% and 95.63% inhibitions at 250, 500 and 1000 mg/kg, respectively while CQ produced 94.31% parasitama inhibition (Table 6).

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

In the suppressive and curative antiplasmodial studies, *C. odorata* leaf extract in a dose-dependent fashion significantly decreased percentage parasitama at p < 0.05 when compared to parasitized control. No significant difference in percentage parasitama was observed in *C. odorata* leaf extract (1000 mg/kg) when compared to CQ (Table 7). In the prophylactic study, the

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

| Treatment (mg/kg) | Day 1 | Day 3 | Day 5 | % Inhibition Day 5 | 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* leaf extract; n=5; Data as mean ± SEM (Standard error of mean) ^aSignificant different (p < 0.05) when compared to parasitized control; ^bSignificant different (p < 0.05) when compared to CQ.

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

| Treatment (mg/kg) | Suppressive % Parasitamaia | % Inhibition | Prophylactic % Parasitamaia | % 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* leaf extract; Data as mean ± SEM (Standard error of mean); ^aSignificant different (p < 0.05) when compared to parasitized control; ^bSignificant different (p < 0.05) when compared to CQ.

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

| Treatment (mg/kg) | PCV | HB | RBCs | WBCs | NEU | LYM | MON |
|-------------------|----------------------------|----------------------------|---------------------------|----------------------------|----------------------------|---------------------------|---------------------------|
| Control | 49.75 ± 5.79 | 15.02 ± 0.26 | 4.85 ± 0.12 | 3.35 ± 0.19 | 60.25 ± 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 | 130.75 ± 6.56 ^a | 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 ^b | 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} | 80.33 ± 8.67 ^b | 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 ^b | 22.67 ± 3.89 ^b | 1.50 ± 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} | 65.00 ± 6.73 ^b | 21.67 ± 2.89 ^b | 1.06 ± 0.03 ^{ab} |

PCV: Packed Cell Volume; HB: Hemoglobin; RBCs: Red Blood Cells; WBCs: White Blood Cells; NEU: Neutrophils; LYM: Lymphocytes; MON: Monocytes; PCO: Parasitized Control; CQ: Chloroquine; CO: *Chromolaena odorata* leaf extract; n=5; Data as mean ± SEM (Standard error of mean); ^asignificant different (p < 0.05) when compared to the normal control; ^bSignificant different (p < 0.05) when compared to parasitized control; ^cSignificant different (p < 0.05) when compared to CQ.

following inhibitions; 88.44% and 94.14% 96.14% were produced by *C. odorata* leaf extract (250, 500 and 1000 mg/kg), respectively whereas CQ produced 98.85% parasitemia inhibition (Table 7).

Effect of *Chromolaena odorata* leaf extract on haematological parameters of parasitized mice

P. berghei-infected mice showed decreased PCV, HB, RBCs and increased WBCs, MON, NEU and LYM when compared to normal control at p < 0.05 (Table 8). However, *C. odorata* leaf extract (250, 500 and 1000 mg/kg) increased PCV, HB, WBCs and decreased WBCs, MON, NEU and LYM when compared to the parasitized control at p < 0.05. *C. odorata* leaf 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 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 *p berghei*-infected mice. *In-vivo* model was used as compared with *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 provides a better prediction for the assessment of potential antimalarial agents, but the rodent was used for 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 were used. The two adopted methods are 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 flavonoids 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 signs 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* leaf extract for 28 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 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, Afolayan, 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* leaf produced 84.81% parasitaemia 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* leaf 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* leaf 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* leaf extract might have curtailed *P. berghei*-induced anaemia by inhibiting the aforementioned mechanism. The antiplasmodial activity of *C. odorata* leaf 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 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 its folklore use for the treatment of malaria. This study suggests further studies on the fractionation, isolation and characterization of the active principle responsible for the observed antiplasmodial activity.

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