



Phytochemical Composition and Antimalarial Activity of Methanol Leaf Extract of *Crateva adansonii* in *Plasmodium berghei* Infected Mice

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The need for new compounds active against malaria parasites is made more urgent by the rapid spread of drug-resistance to available antimalarial drugs. The crude methanolic leaf extract of *Crateva adansonii* was investigated for its antimalarial activity against *Plasmodium berghei* (NK65) infected mice. A total of 15 mice were intraperitoneally infected with chloroquine sensitive *P. berghei* strain and divided into 5 equal groups, group 1 served as negative control (untreated), groups 2,3 and 4 were given 200, 400 and 600 mg/kg *Crateva adansonii* methanolic leaves extract respectively while group 5 served as positive control and was given 5 mg/kg chloroquine for five days. The phytochemical constituents of the plant extract were evaluated to elucidate the possibilities of their antimalarial effects. The extract produced a significant dose dependent decrease in the level of parasitaemia when compared to infected untreated group. Also, the extract at dose of 400 mg/kg and 600 mg/kg produced significant increase in body weight and PCV of the infected mice as compare to mice treated with 200 mg/kg of extract and infected untreated group. Phytochemical screening showed that the leaves extract contains alkaloids, anthraquinones,

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tannins, flavonoids, saponins cardiac glycosides and steroids. It is concluded that *Crateva adansonii* could serve as a possible source of antimalarial compounds.

Keywords: *Crateva adansonii*; antimalarial; phytochemical; parasitarmia; *Plasmodium berghei*.

1. INTRODUCTION

Malaria is a chronic endemic disease that obstructs social and economic development. It is the leading cause of mortality and morbidity around the world with an estimated 225 million malaria cases and 781,000 deaths being reported globally in 2009 [1]. Most of the reported malaria cases occur in tropical and subtropical regions where the atmospheric condition (temperature and rainfall) are favourable for the development of vectors and parasite [2], the mortality occurs mostly in young children and pregnant women [3]. The attack of malaria during pregnancy stage usually results into severe anemia and impairment of fetal nutrition which contribute to the low birth weight, premature delivery, mental retardation and 60% miscarriages [4]. Malaria is caused by a protozoan of the genus *Plasmodium* transmitted to the vertebrate by female Anopheles mosquitoes in the vertebrate host, the sexual blood forms of the parasite are the life cycle stage that are responsible for the morbidity and mortality of plasmodia infections [5]. Four species of malaria parasite cause disease in humans: *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium vivax* and *Plasmodium ovale* whereas 3 species give rise to considerable natural morbidity, only *P. falciparum* results in high mortality [6], as a result of prevalence, virulence and drug resistance. The continuous spread of the *Plasmodium falciparum* resistance to the commonly used anti-malarial drug including the newly introduced Artemisinin Combination Therapy (ACT) has resulted in resurgence in treatment failure [7], and hence the need to intensify research in the area of development of new anti-malarial drugs especially from medicinal plants with traditional antimalarial reputation.

Crateva adansonii DC, also known as *Crateva religiosa* or sacred garlic pear, belongs to the family Capparaceae. The plant is in high demand, especially its leaves for the treatment of ear infections. The bark is widely used for stomach troubles and held to have tonic properties. In Senegal the roots figure in several treatments for syphilis, jaundice and fever. Agboke and coworkers claimed the antimicrobial properties of its leaves [8]. Two phytoconstituents had also

been isolated and identified as oleanolic acid and 4-epi-hederagenin [9].

In order to scientifically validate the medicinal claims of *Crateva adansonii* and determine its effectiveness as a potential source of new antimalarial agents, the present study was undertaken to evaluate its phytochemical composition and *in vivo* anti-malarial properties against *Plasmodium berghei* infected mice.

2. MATERIALS AND METHODS

2.1 Plant Collection

Fresh leaves of *Crateva adansonii* were collected from Baddegi, Niger State, Nigeria. It was identified and authenticated by a Botanist in the Department of Biological Science, Federal University of Technology Minna, Niger State.

2.2 Experimental Animal

Swiss albino mice weighing between 20-25 g were used in this study. The animals were obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria. They were housed in plastic cages with saw dust bed and given standard laboratory diet and water *ad-libitum*. They were then allowed to acclimatize for two weeks to their new environment before the initiation of the experiments.

2.3 Parasite

A chloroquine-sensitive strain of *Plasmodium berghei* (NK-65) was obtained from the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria and maintained by re-infestation via intraperitoneal injection of infected blood suspension (0.2 ml) containing about 1×10^7 suspension of *P. berghei* parasitized red blood cells.

2.4 Sample Preparation and Extraction Procedure

The collected fresh leaves of *Crateva adansonii* were washed with clean water and air dried. The dried sample was grounded using a grinder mill. Extraction of plant material was performed by

soxhlet extraction using methanol. The resulting methanol extract was concentrated in a water bath and stored in a refrigerator until required.

2.5 Determination of Yield of Extract

The percentage yield of methanol leaf extract of *Crateva adansonii* was determined by weighing the coarse sample before extraction and the methanol leaf extract of *Crateva adansonii* after concentration and then calculated using the formula.

Percentage yield (%) =

$$\frac{\text{Weight (g) of the concentrated extract}}{\text{Weight (g) of the } \textit{Crateva adansonii} \text{ leaf}} \times 100$$

2.6 Phytochemical Analysis

Methanol leaves extract of *Crateva adansonii* was characterized for phytochemical composition including alkaloids, anthraquinones, tannins, flavonoids, saponins, cardiac glycosides, steroids and phlobatannins according to the methods Harborne, [10], and Sofowora [11].

2.7 In vivo Antimalarial Study

2.7.1 Curative test

Evaluation of the curative potential of the crude extract in Peter's test was carried out according to the method described by Ryley and Peters [12]. On Day 0, standard inocula of 1×10^7 infected erythrocytes were inoculated in mice intraperitoneally. Seventy-two hours later, mice were randomly divided into their respective groups and dosed accordingly once daily for five days. The extract was dissolve in normal saline.

Group I Mice were given 200 mg/kg b.w of methanol leaves extract of *C. Adansonii*.

Group II Mice were given 400 mg/kg b.w of methanol leaves extract of *C. Adansonii*.

Group III Mice were given 600 mg/kg b.w of methanol leaves extract of *C. Adansonii*.

Group IV Mice received 5 mg chloroquine /kg body weight.

Group V Mice were given normal saline/kg body weight.

2.7.2 Daily parasitaemia count

On each day a drop of blood were collected from the tail of each rat, smeared unto a microscopic slide to make thin films, stained with 10% Giemsa stain and examined microscopically to monitor the parasitaemia level.

2.7.3 Determination of packed cell volume (PCV)

The capillary tubes were filled with blood to about 1cm or two-third (2/3) of its length and the vacant end of each of the capillary tubes was sealed by plastic seal or sealer to protect the blood level from spilling. The tubes were placed in haematocrit centrifuge with seal side towards the periphery and then centrifuge for 5-6 minutes. The percentage of packed cell volume or haematocrit was read directly from haematocrit reader [13].

2.8 Statistical Analysis

Data were analyzed using statistical package for social science (SPSS) version 16 and presented as means \pm SEM. Comparisons between different groups was done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $P < 0.05$ were considered as statistically significant as described by Mahajan, [14].

3. RESULTS

3.1 Extract Yield

The percentage yield of leaves extract of *Crateva adansonii* is shown in Table 1. The yield of methanol leaves extract of *Crateva adansonii* was 25.09%.

3.2 Phytochemicals

Table 2 shows the result of qualitative phytochemical composition of methanol leaf extract of *Crateva adansonii*. The results revealed the present of alkaloids, anthraquinones, tannins, flavonoids, saponins cardiac glycosides and steroids, however, phlobatannins were absent.

Table 1. The percentage (%) yield of methanolic leaves extract of *Crateva adansonii*

<i>Crateva adansonii</i>	Weight (g)
Leaf powder	50.00
Methanol extract	12.547
Extract yield (%)	25.09

Table 2. Qualitative phytochemical composition of methanol leaf extract of *Crateva adansonii*

Phytochemicals	Inference
1. Alkaloids	++
2. Cardiac glycosides	++
3. Anthraquinones	++
4. Steroids	++
5. Tannins	+
6. Saponins	+++
7. Flavonoids	++
8. Reducing sugars	++
9. Phlobatannins	-

Key: (-) absent, (+) slightly present, (++) moderately present, (+++) highly present

3.3 Antimalarial Study

3.3.1 Parasitaemia count

The average daily parasitaemia level of the *Plasmodium berghei* infected mice treated with methanol leaves extract of *Crateva adansonii* are shown in Fig. 1 and Table 3. The average daily parasitaemia of infected mice treated with methanol leaves extract of *Crateva adansonii* at doses of 400 and 600mg/kg were significantly ($P < 0.05$) reduced (37.71 and 40.41% inhibition)

when compare with the negative control over the period of the experiment. The average daily parasitaemia of infected mice treated with chloroquine was significantly ($P < 0.05$) reduced (54.51% inhibition) when compared with extract treated groups. However no significant ($p > 0.05$) difference in the level of parasitaemia count of infected mice treated with 200mg/kg leaf extract of *Crateva adansonii* as compared with the control group.

3.3.2 Body weight changes

Effect of methanol leaves extract of *Crateva adansonii* on body weight of *Plasmodium berghei* infected mice is shown in Fig. 2 the body weight of the infected untreated mice and infected treated with 200 mg/kg of *Crateva adansonii* show significant decrease in body weight after 5 days of treatment. However the infected mice treated with 400 mg/kg, 600 mg/kg of *Crateva adansonii* as well as those treated with 5 mg/kg chloroquine show significant increase in body weight after 5 days of treatment.

3.3.3 Packed cell volume

Effect of methanol leaves extract of *Crateva adansonii* on PCV of *Plasmodium berghei* infected mice are shown in Fig. 3 the PCV of *P. berghei* infected untreated mice and infected treated with 200 mg/kg of *Crateva adansonii* show significant decrease in PCV after 5 days of treatment. However the infected mice treated with 400 mg/kg, 600 mg/kg of *Crateva adansonii* as well as those treated with 5 mg/kg chloroquine show significant increase in PCV after 5 days of treatment.

Table 3. Average parasitaemia count of *Plasmodium berghei* infected mice treated with methanol leaves extract of *Crateva adansonii*

Groups	Day 3	Day 4	day 5	Day 6	Day 7	% parasite reduction
Chloroquine	44.40±3.45 ^a	32.60±3.11 ^a	22.10±2.23 ^a	15.12±2.45 ^a	3.50±0.47 ^a	54.51
200 mg/kg <i>C. adansonii</i>	44.30±2.14 ^a	44.76±4.56 ^b	51.97±3.45 ^c	56.82±4.34 ^c	58.98±5.66 ^c	-
400 mg/kg <i>C. adansonii</i>	43.10±4.31 ^a	37.90±4.44 ^a	33.10±3.45 ^b	29.70±2.34 ^b	17.4±1.19 ^b	37.71
600 mg/kg <i>C. adansonii</i>	44.10±4.03 ^a	35.30±3.60 ^a	31.90±2.50 ^b	28.20±2.11 ^b	14.70±1.34 ^b	40.41
control	46.20±3.45 ^a	47.00±3.67 ^b	50.9±6.35 ^c	55.68±4.01 ^c	59.01±5.67 ^c	-

Values are $M \pm SEM$ of triplicate determination; Values along the same column with different superscripts are significantly different ($p < 0.05$)

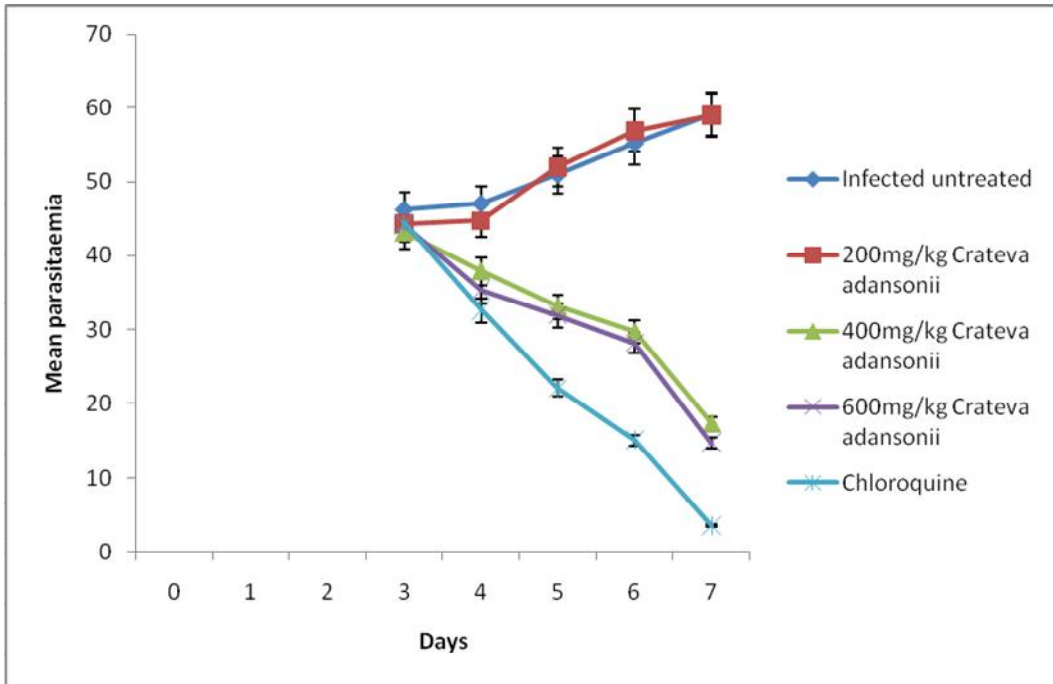


Fig. 1. *In vivo* antiplasmodial activity of methanol leaves extract of *Crateva adansonii* against *Plasmodium berghei* infected mice: Each point is a Mean±SEM of triplicate determination

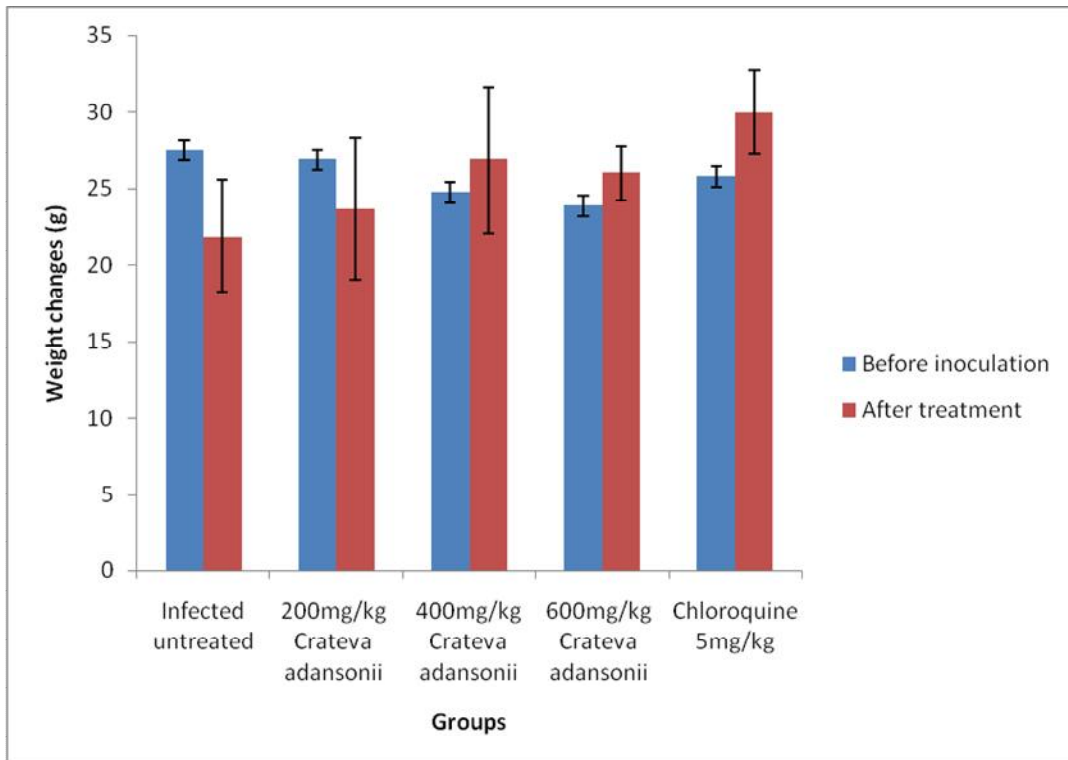


Fig. 2. Effect of methanol leaf extract of *Crateva adansonii* on body weight of *P. berghei* infected mice. Values are M±SEM of triplicate determination

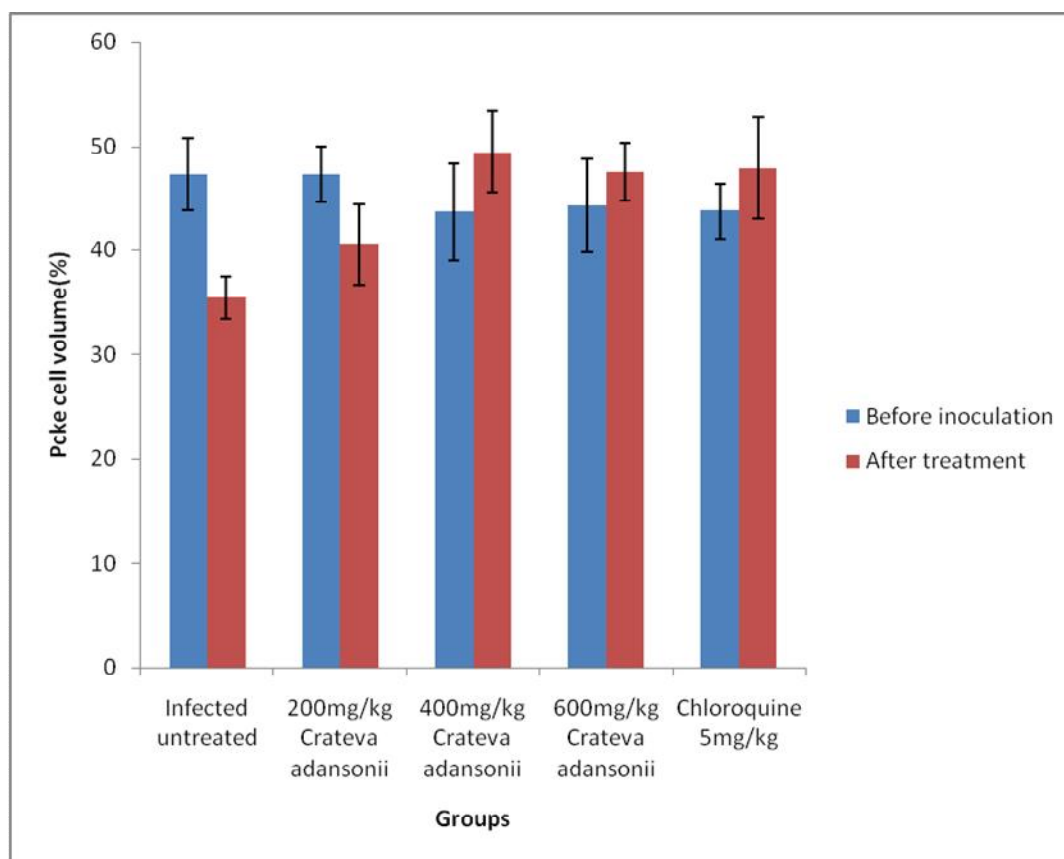


Fig. 3. Effect of methanol leaves extract of *Crateva adansonii* on PCV of *P. berghei* infected mice. Values are M±SEM of triplicate determination

4. DISCUSSION

Plants used in treatment of diseases are said to contain active compounds called phytochemicals some of which are responsible for their characteristic odours, pugnencies and colour while others give to a particular plant its virtues as food, medicinal or poisonous [15]. There is considerable interest by phytochemists to identify the therapeutic agent contained in a medicinal plant in order to establish the basis for their uses in traditional medical practice.

This study revealed the presence of various medicinal important phytochemicals including alkaloids, anthraquinones, tannins, flavonoids, saponins cardiac glycosides and steroids in methanolic leaves extract of *Crateva adansonii*.

Flavonoids are compounds with a widespread occurrence in the plant kingdom which have also been detected in *Artemisia* species. They are reported to have exhibited significant *in vitro*

antimalarial activity against *P. falciparum* [16]. Their presences in *Crateva adansonii* extract could justify the antimalarial activities exhibited by the plant extract.

Saponins are used as adjuvants in the production of vaccins [17]. Steroids are used in the stimulation of bone marrow and growth. They stimulate lean body mass and also play vital roles in the prevention of bone loss in elderly men [18]. Alkaloids have been used as CNS stimulant, topical anesthetic in ophthalmology, powerful painkillers, and antipyretic action among other use [19]. The cardiac glycoside has been used for over two centuries as stimulant in cases of cardiac failure and diseases [20]. The presence of tannins in the leaves extract of *Crateva adansonii* suggests the ability of these plants to play major roles as antifungal, antidiarrheal, antioxidant and antihemorrhoidal agent [21]. Tannins also have astringent properties, plants containing tannins have been reported to be used for healing of wounds,

varicose ulcers, hemorrhoids, frostbite and burn in herbal medicine [22].

The presence of all these phytochemicals in the leaves extract of *Crateva adansonii* is an indication that this plant, if properly screened, could yield a drug of pharmacological significance. However the absence of phlobatannins agree with early studies which also found that not all phytochemicals are present in all plants and those present differ with the solvent used in the extraction process [23].

The *in vivo* antimalarial effect of *Crateva adansonii* against *Plasmodium berghei* infected mice was evaluated. The extract showed a significant dose dependent and progressive reduction in parasitaemia with time, this is a very promising feature in the potentially of *Crateva adansonii* as an antimalarial drug. However, the antimalarial effect demonstrated by *Crateva adansonii* leaves extract was lower when compared to chloroquine. Chloroquine has been used as the standard antimalarial drug because of its established activities on *P. berghei* [24]. The *P. berghei*, a rodent malarial parasite although not able to infect man and other primate has been used in antimalarial assays because of its sensitivity to chloroquine [25]. Also the insignificant difference in the level of parasite count of infected mice treated with 200 mg/kg of the extract when compared with the control group reflect the inactivity of the extract which could be attributed to low concentration of bioactive agents at that doses.

Anemia, body weight loss and body temperature reduction are the general features of malaria-infected mice [26]. So, an ideal antimalarial agents obtained from plants are expected to prevent body weight loss in infected mice due to the rise in parasitemia. In the present study, extract of *C. adansoni* significantly prevented weight loss associated with increase in parasitemia level.

Blood parameters including packed cell volume (PCV) is used to assess anemia, erythrocytosis, hemodilution and hemoconcentration due to disease condition [13]. One of the major reasons for the development of anemia is oxidative stress [27], the immune system of the body is activated by malarial infection thereby causing the release of free radicals. In addition to this the malaria parasite also stimulates certain cells to produce reactive oxygen species (R.O.S) their by resulting in haemoglobin degradation [28]. Thus,

the significant decrease level of PCV and body weight of the untreated mice is an indication of anemic condition caused by the malarial infection. The significant increase in level of PCV and body weight in mice treated with *Crateva adansonii* at 400 and 600 mg/kg when compare with the control group is an indication of ameliorating potentials of the plant extract on the anaemia induced by the malarial infection. Though the rodent malaria model, *P. berghei* is not similar to that of human Plasmodium parasite the antiparasitic activities demonstrated by the extract especially at doses of 600mg/kg against *P. berghei* mice in this study could be an indication that the extract could possibly be effective against human malarial parasite [29].

The antimalarial activity demonstrated by this plant might be attributed to the presence of alkaloids or flavonoids which have been identified presently in this work; or even a combined action of more than one metabolite. However, the active compound(s) known to give this observed activity need to be identified.

5. CONCLUSION

The study suggests that *C. adansonii* leaf contains important phytoconstituents that could be implicated in the observed antimalarial effect of the plant. The study also represents an important preliminary demonstration of the potential of this antimalarial herbal medicine to treat human malaria. However, a bioguided fractionation of the methanolic extract of *C. adansonii* leaves aiming to isolating active compound(s) is recommended.

CONSENT

It is not applicable.

ETHICAL CLEARANCE

Ethical Clearance was given by Federal University of Technology, Minna/Nigerian Ethical Review Board (CUERB) in accordance with International standard on care and use of experimental animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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