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# Developmental stages influence *in vivo* antimalarial activity of aerial part extracts of *Schkuhria pinnata*

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A R T I C L E I N F O

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Antimalarial
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Toxicity

A B S T R A C T

*Ethnopharmacological relevance:* Malaria remains a dire health challenge, particularly in sub-Saharan Africa. In Uganda, it is the most ordinary condition in hospital admission and outpatient care. The country's meager health services compel malaria patients to use herbal remedies such as *Schkuhria pinnata* (Lam.) Kuntze ex Thell (Asteraceae). Although *in vivo* studies tested the antimalarial activity of *S. pinnata* extracts, plant developmental stages and their effect at different doses remain unknown.

*Aim of the study:* This study aims to determine the effect of the plant developmental stage on the antimalarial activity of *S. pinnata* in mice and to document the acute oral toxicity profile.

*Methods:* Seeds of *S. pinnata* were grown, and aerial parts of each developmental stage were harvested. Extraction was done by maceration in 70% methanol. The antimalarial activity was evaluated using chloroquine-sensitive *Plasmodium berghei* on swiss albino mice, in a chemosuppressive test, at 150, 350, and 700 mg/kg, p.o. Standard drugs used were artemether-lumefantrine (0.57 + 3.43) mg/kg and chloroquine (10 mg/kg) as positive controls. Distilled water at 1 mL/100g was used as a negative control. The Lorke method was adopted to determine the acute toxicity of extracts.

*Results:* The flowering stage extract had a maximum suppression of parasitemia at 700 mg/kg (68.83 ± 4.49%). Extract at other developmental stages also significantly suppressed the parasitemia (in the ascending order) fruiting (50.71 ± 1.87%), budding (54.92 ± 7.56%), vegetative (55.39 ± 2.01%) compared to the negative control (24.7 ± 2.7%), $p < 0.05$. Extracts from all developmental stages increased survival time, with the flowering stage having the highest survival time at 20.33 ± 0.88 days. All extracts had an $LD_{50}$ of 2157 mg/kg, implying that extracts are safe at lower doses.

*Conclusion:* Together, our findings revealed that the *S. pinnata* extracts at the flowering stage had superior antimalarial activity compared to other plant developmental stages. Extracts from all developmental stages have demonstrated a dose-dependent suppression of malarial parasites and increased survival time with an $LD_{50}$ of 2157 mg/kg. Thus, for better antimalarial activity, local communities could consider harvesting *S. pinnata* at the flowering stage. Further studies are needed to isolate pure compounds from *S. pinnata* and determine their antimalarial activity.

## 1. Introduction

Malaria, a mosquito-borne infection, remains a leading cause of morbidity and mortality, particularly in the tropical African regions (WHO, 2020). In 2019, an estimated 228 million malaria infections and 405,000 malaria-related death occurred globally, with Africa accounting for 94% of all cases and death (WHO, 2020). About 13 sub-Saharan African nations shared three-quarters of the malaria cases, with Uganda

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being among the top five nations that accounted for 51% of global total malaria incidents (WHO, 2018). According to Uganda's annual health sector performance report, malaria is the most ordinary condition among hospital outpatients (29.8%) and admissions (34.9%), claiming 150 lives daily (MOH, 2020).

Vulnerable Uganda's economy and meager health services compel malaria patients to use herbal remedies such as *Schkuhria pinnata* (Lam.) Kuntze ex Thell (Asteraceae) as their first-line therapy (Anywar et al., 2016; Okello and Kang, 2019; Tabuti et al., 2012; Tugume et al., 2016). Several other factors are implicated in the general trend of increased use of herbal medicines to manage malaria and other ailments: widespread availability of herbal medicines and their perceived efficacy; high cost and increased side effects associated with the conventional medicine, to name just a few (Adia et al., 2014; Mohammadi et al., 2020). For example, local communities in the Cegere sub-county, Apac district in Northern Uganda, prepare the *S. pinnata* infusion by boiling fresh leaves either singly or mixed with other herbs such as Aloe vera (L.) Burm. f. (Xanthorrhoeaceae) or Baccharoides adoensis (Sch.Bip.ex. Walp) H. Rob (Asteraceae) and take orally one teaspoonful (children) or tablespoonful (adults) three times daily (Anywar et al., 2016). The ethnomedical use of *S. pinnata* for malaria was also reported in Uganda's neighboring country-Kenya among the Kikuyu community (Omara, 2020; Njoroge and Bussmann, 2006).

The validation of traditional claim was reported in recent animal studies of the antimalarial and antinociceptive activity of *S. pinnata* whole plant extracts (Sesaazi et al., 2021; Waiganjo et al., 2020). Researchers observed significant antimalarial activity in animals at a dose of 100 mg/kg of methanol, dichloromethane, and water extracts of the *S. pinnata* whole plant (Waiganjo et al., 2020). Although this study tested different fractions of the whole plant, plant developmental stages and their effect at different doses remain unknown.

A plant developmental stage has a profound effect on the quality and quantity of different bioactive compounds. These variations, in turn, affect the plant's biological activity, such as antimalarial, antipyretic, and toxicity (Bhandari et al., 2019). Thus, there is a need to determine the *in vivo* antimalarial activity and toxicity profile of extracts from different developmental stages of *S. pinnata*.

Therefore, this study determined the effect of the plant developmental stage on the antimalarial activity of *S. pinnata* in mice and documented the acute oral toxicity profile. In addition, the study contributed to understanding the optimal harvesting stage to ensure maximum antimalarial activity and local community safety.

## 2. Materials and methods

### 2.1. Chemicals and parasites

Artemether-lumefantrine and chloroquine diphosphate standards, Giemsa-stain were purchased from Toronto research chemicals (Toronto, Canada). Chloroquine (CQ)-sensitive *Plasmodium berghei* ANKA strain was obtained from BEI Resources, USA.

### 2.2. Plant collection and extraction

A fresh plant sample for identification was collected from National Agricultural Research Organization (NARO), Kawanda, Uganda, located at $0^0$ 25′ 14.0″ N, $32^0$ 32′ 26.0″E, and 1300 m above sea level. Dr. Mary Namaganda from the Department of Botany, Makerere University, taxonomically identified the plant, and a voucher specimen with accession number 50926 was deposited in the Makerere herbarium.

January 2020, an experimental plot was set up in Bushenyi district located at $0^0$ 36′ 59.814″ S, $30^0$ 39′ 20.442″ E, elevation: 1417.7 m above sea level. The *S. pinnata* seeds were planted and monitored. Aerial parts of the *S. pinnata* plant were obtained at different developmental stages, i.e., vegetative, budding, flowering, and fruiting at 25, 32, 43, and 73 days respectively, from the day of germination.

Plant samples were shade dried to a moisture content of 9.3%, pulverized to a fine powder using an electric grinder. About 200 g of powdered sample for each developmental stage was macerated in 2000 mL of 70% methanol for 72 h with intermittent agitation at room temperature (26 ± 2 °C) (Nureye et al., 2018). The methanol extraction solvent was selected based on a previous study that demonstrated that the methanol fraction of the *S. pinnata* whole plant had better antimalarial activity than water (Waiganjo et al., 2020). The extracts were filtered through a Whatman no.1 filter paper and concentrated in vacuo using a rotary evaporator (IKA® RV10) at 45 °C. The dried crude extracts obtained at vegetative (VG), budding (BB), flowering (FL), and fruiting (FR) stages were stored in a plastic airtight container at 4 ± 2 °C until use.

### 2.3. Qualitative phytochemical screening and chemical fingerprinting

Qualitative phytochemical screening for saponins, flavonoids, anthraquinones, quinones, alkaloids, tannins, terpenoids, cardiac glycosides, phenols, and steroids was performed according to standard methods (Adusei et al., 2019; Evans, 2009).

The HPLC method was used for establishing chemical fingerprints of each *S. pinnata* developmental stage's extract as previously described (Peter et al., 2021). Briefly, the chromatographic system consisted of a Shimadzu LC-10AT equipped with an SPD-20A UV/VIS detector (Tokyo, Japan), communicator CBM-20A (Tokyo, Japan), and degassing unit DGU-$20A_{5R}$ (USA) with an isocratic binary system of the mobile phase. Chromatography separation was performed on a Lunar® $C_{18}$ column (5 μm; 250 × 4.6 mm; Phenomenex, USA) maintained at a temperature of 40 °C in a Shimadzu column oven (CTO-20AC, Tokyo Japan). The mobile phase used was methanol: water containing 0.01% trifluoroacetic acid: acetonitrile (60:30:10 v/v). A flow rate of 1.0 mL/min and volume injected was 10 μL at a pressure of 2596.17 psi. Peak detection was done at a wavelength of 370 nm. For HPLC fingerprinting, samples were prepared by dissolving 10 mg of dried extract in 10 mL of methanol in a shaking water bath for 30 min at 40 °C. All samples and mobile phase were filtered through 0.45 μm membrane filters (EZ-Pak®, France) before loaded into the HPLC system.

### 2.4. Acute oral toxicity

Acute oral toxicity was performed in two phases. For the first phase, 39 female mice were randomly assigned to 13 groups of 3 mice per group (Lorke, 1983). Mice in groups I - III was dosed orally with extract VG, groups IV-VI dosed with BB, groups VII-IX dosed with FL, while groups X-XII dosed with FR. All extracts were given at the same dose levels of 10, 100, 1000 mg/kg by oral gavage at a volume of 1 mL/100g. Observations for apparent signs of toxicity, including discomfort, reduced respiration rate, hair erection, and food intake, were done continually for 48 h and later over 14 days. Since no mortality occurred in the first phase, the testing proceeded to the second phase, in which 16 mice were similarly assigned to 16 groups of one mouse per group.

Mice in groups I - IV were orally given extract VG, groups V-VIII was given extract BB, groups IX-XII was given extract FL, while groups XIII-XVI was given extract FR, but at an increased dose level of 1000, 1600, 2900, 5000 mg/kg body weight. Close observation for mortality within 48 h and later over 14 days was done.

The Median lethal dose ($LD_{50}$) was computed using equation (1) (Alson et al., 2018) below;

$$LD_{50} = \sqrt{A\ XB} \quad (1)$$

where A is the highest dose with no mortality and B is the lowest dose with mortality.

### 2.5. In vivo antimalarial assay

The 4-day suppressive test was adopted for *in vivo* antimalarial activity assessment of the extracts described in a previous study (Peters et al., 2002).

#### 2.5.1. Animals

Swiss albino mice of both sexes, aged 7–8 weeks and weighing 18–22 g, were obtained from an animal research facility at Mbarara University of Science and Technology, Uganda. The animals were kept in separate cages and maintained under standard environmental conditions of 12 h (light/dark cycle). They had free access to water and standard food pellets. They were acclimatized for two weeks before treatments. Animals were cared for according to the principles stipulated in the "Guide for the Care and Use of Laboratory Animals" (NRC, 1996).

#### 2.5.2. Preparation of infected red blood cells suspension

Chloroquine (CQ)-sensitive Plasmodium berghei ANKA were maintained by serial blood passage from infected to uninfected mice weekly (Mekonnen, 2015). Blood was collected from the donor mouse through cardiac puncture under anesthesia diethyl ether. The blood was diluted with normal saline (0.9% w/v sodium chloride) to prepare a standard inoculum ($1 \times 10^7$) of parasitized erythrocytes per mL.

#### 2.5.3. Inoculation of parasites and animal dosing of extracts

A total of 90 mice were intraperitoneally inoculated with 200 μL of parasitized erythrocyte suspension (Huang et al., 2015). Two hours post-infection, inoculated mice were randomly assigned to 15 treatment groups with six mice per group as follows.

Groups I, II, and III received VG extract at 150, 350, and 700 mg/kg.

Groups IV, V, and VI received BB extract at 150, 350, and 700 mg/kg.

Groups VII, VIII, and IX received FL extract at 150, 350, and 700 mg/kg.

Groups X, XI, and XII received FR extract at 150, 350, and 700 mg/kg.

Groups XIII and XIV received Artemether-Lumefantrine at 4 mg/kg and Chloroquine at 10 mg/kg, respectively.

Groups XV was a negative control that received distilled water at 200 μL.

Group XVI was a normal control ($n = 4$) that received distilled water at 200 μL.

The initial testing doses for the extracts were estimated from the results of acute oral toxicity testing. All interventions were given by oral gavage daily for four days.

#### 2.5.4. Determination of percentage parasitemia and chemosuppression

Parasitemia levels were examined on day four by counting parasitized and non-parasitized erythrocytes in Giemsa-stained blood smears (Nureye et al., 2018). The number of parasitized erythrocytes was observed in eight random views under a light microscope at 100× oil immersion objective lens. Mean percentage parasitemia and percentage chemosuppression were calculated using equations (2) and (3) below (Alson et al., 2018);

$$Mean \cdot Percentage \cdot Parasitemia = 100\left\{\frac{Na}{Nb}\right\} \quad (2)$$

where *Na* is the total number of parasitized erythrocytes, while *Nb* is the sum of parasitized and non-parasitized erythrocytes in the eight views.

$$Percentage \cdot chemosuppression = 100\left\{\left(\frac{P-Q}{P}\right)\right\} \quad (3)$$

P is the mean percentage parasitemia of the negative control group, and Q is the mean percentage parasitemia of the test group.

Also, initial and final body weight and rectal temperature were recorded on day 0 and day 3. Survival time after treatment was monitored daily for 28 days (Nureye et al., 2018).

### 2.6. Hematological analysis

Blood samples were collected through cardiac puncture of three anesthetized mice from each treatment group. Hematological parameters were analyzed using an automated hematology analyzer (Haema Ana.1, Beckman coulter AC-T-5diff CP) in the clinical and research laboratory at Mbarara University of Science Technology. The analyzed hematological indices were; white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT), mean hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width (RDW), platelets (PTL), mean platelet volume, neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

### 2.7. Statistical analysis

Data were entered into the statistical package for social sciences (SPSS) version 21 (New York, USA). Percentage parasitemia was presented into mean and standard deviations. The one-way analysis of variance (ANOVA) followed by Tukey HSD Post-Hoc test was applied to test the difference in mean percentage parasitemia and chemosuppression parasitemia level between treatment groups at 0.05 level of significance.

## 3. Results and discussion

### 3.1. Phytochemical profile of S. pinnata extracts

#### 3.1.1. Qualitative phytochemical profile of S. pinnata extracts from four developmental stages

The phytochemical investigation on *S. pinnata* methanol extracts indicated saponins, flavonoids, anthraquinones, alkaloids, tannins, terpenoids, cardiac glycosides, phenols, steroid glycosides in all developmental stages. Conversely, quinones were absent in all stages (Table 1). Interestingly, the flowering and budding stage had a similar chemical profile characterized by consistently moderate amounts of all major groups except for steroids present in trace amounts. The majority of these phytochemical classes, except for cardiac glycosides and anthraquinones, were also reported in an ethanol extract of *S. pinnata* aerial part (Sesaazi et al., 2021). However, saponins and anthraquinones in our extract are contrary to the findings from the previous study (Beseni et al., 2019). Such difference could likely be due to the reliability of screening tests used. Although some studies indicated several major classes of phytochemicals present in *S. pinnata*, there is no report of the isolated compounds investigated for their antimalarial activities. Thus, these phytochemical groups could be responsible for the folkloric medicinal claims of this plant.

**Table 1**
Phytochemical groups screened in *S. pinnata* methanolic extracts.

| Phytochemical group | Plant's developmental stages | | | |
|---|---|---|---|---|
| | Vegetative | Budding | Flowering | Fruiting |
| Saponins | ++ | ++ | ++ | ++ |
| Flavonoids | ++ | ++ | ++ | ++ |
| Anthraquinones | + | ++ | ++ | + |
| Quinones | - | - | - | - |
| Alkaloids | ++ | ++ | ++ | ++ |
| Tannins | ++ | ++ | ++ | ++ |
| Terpenoids | ++ | ++ | ++ | ++ |
| Cardiac glycosides | ++ | ++ | ++ | ++ |
| Phenols | + | ++ | ++ | ++ |
| Steroids | + | + | + | + |

+traces present; ++ moderate; - absent.

#### 3.1.2. Chemical fingerprints of S. pinnata extracts from four developmental stages

The HPLC chromatograms of extracts from each developmental stage of *S. pinnata* are presented in Fig. 1 and supplementary materials S1. About nine peaks were noted in each of the three developmental stages, i.e., the vegetative, budding, and fruiting stages. However, the flowering stage had only seven peaks. Interestingly, the nature of peaks as analyzed by considering retention time has not significantly changed (Fig. 1).

### 3.2. Acute oral toxicity

In the first phase of the acute oral toxicity test, no apparent signs of toxicity, such as hair erection, signs of discomfort, food or water intake changes in all groups that received different extracts of different development stages. Moreover, there was no mortality in all test groups. However, in the second phase, animals in all the 16 test groups showed toxicity at a dose of 1600 mg/kg. Initially, animals had hair erection, a sign of discomfort, decreased eating habit. The onset of toxicity signs was apparent from the second day of administration. Then, animals observed for 14 days, and no death occurred in all the groups. Conversely, animals at a 2900 mg/kg dose showed severe toxicity signs within 24 h of observations, and death occurred within 48 h. Thus, the $LD_{50}$ was calculated as 2157 mg/kg for extracts from all the developmental stages.

Our findings indicated that at a lower dose, the extracts are relatively safe in animals. However, higher doses showed significant toxicity signs and mortality across all developmental stages of the *S. pinnata*. Our findings are contrary to those reported in another study where the $LD_{50}$ of ethanol extract of *S. pinnata* was 1224.8 (95% CI; 952.2–1575.3) (Sesaazi et al., 2021). These discrepancies in $LD_{50}$ could be explained by the different extraction solvents used. Based on these acute toxicity findings, the minimum starting dose for efficacy study was estimated.

### 3.3. Antimalarial activities of Schkuhria pinnata extracts

All *S. pinnata* extracts, i.e. (VG), (BB), (FL) and (FR) at an oral dose of 700 mg/kg significantly reduced the parasitemia levels ($p < 0.05$) in test mice to 11.02 ± 0.49, 11.13 ± 1.87, 7.70 ± 1.11, and 12.17 ± 0.46% respectively compared to the negative control (24.7 ± 2.72%) as indicated in Table 2. Percentage chemosuppression significantly increased dose-dependently in the same order of extract mention. Although the antimalarial activities did not show significant difference with the developmental stage, the extract obtained at the flowering stage (FR) had better antimalarial activity at all dose levels with percentage chemosuppression as follows: 38.45 ± 2.81 at 150 mg/kg, 55.2 ± 2.50 at 300 mg/kg, and 68.83 ± 4.49% at 700 mg/kg. However, these percentage chemosuppression levels were significantly lower than chemosuppression of positive controls (p < 0.05) i.e. ACT at 4 mg/kg (96.51 ± 0.15%) and CQ at 10 mg/kg (94.74 ± 0.73%).

ANOVA, $p = 0.001$ for all Percentage parasitemia and Chemosuppression; Turkey for multiple comparisons of percentage parasitemia $^{abc}P = 0.001$ for VG, BB, FL, FR at 150, 350, 700 mg/kg Vs ACT at 4 mg/kg, CQ at 10 mg/kg, DW at 200 mg/kg; $^{d}P = 0.048$ for VG at 150 mg/kg Vs VG at 700 mg/kg; $^{e}P = 0.029$ for BB at 150 mg/kg Vs BB 700 mg/kg; $^{f}P = 0.001$ for FL at 150 mg/kg Vs FL at 700 mg/kg.

Turkey for multiple comparisons for chemosuppression, $^{12}P = 0.001$ for VG, BB, FL, FR at 150, 350, 700 mg/kg Vs ACT at 4 mg/kg, CQ at 10 mg/kg, DW at 200 mg/kg; $^{3}P = 0.049$ for VG at 150 mg/kg Vs VG at 350 mg/kg; $^{4}P = 0.002$ for VG at 150 and VG at 700 mg/kg; $^{5}P = 0.001$ for BB at 150 mg/kg Vs BB at 700 mg/kg; $^{6}P = 0.001$ for FL at 150 mg/kg and FL at 700 mg/kg.

Our findings that *S. pinnata* methanolic extract significantly suppressed malaria parasites in mice agree with Waiganjo and colleagues' study that found *S. pinnata* whole plant methanolic extract at 100 mg/kg orally had a chemosuppression of 38.31 ± 6.31% (Waiganjo et al., 2020). This chemosuppression was similar to that observed in our FL extract at 150 mg/kg (38.45 ± 2.81%). Although Waiganjo's experiment did not indicate the harvesting stage, the studied whole plant was likely harvested at the flowering stage. Interestingly, the flowering stage had a consistently superior chemosuppresion effect across doses tested compared to other developmental stages in our study.

Studies identified that extraction solvents significantly influence the extraction efficiency of active constituents from plant species irrespective of the plant's developmental stages (Zhang et al., 2018). Such solvent effect, in turn, affects the biological activity of the plant extract. For example, researchers found that a methanolic extract of *S. pinnata* moderately suppressed parasitemia at 100 mg/kg compared to water extract that had poor suppression of malaria parasitemia at the same dose (Waiganjo et al., 2020). However, considering that water is the primary solvent used to prepare extracts traditionally, such traditional practices likely failed to tape *S. pinnata*'s inherent capacity to manage malaria. This interpretation warrants further studies to ascertain the influence of solvent on *S. pinnata*'s developmental stage. Conversely, an aqueous *S. pinnata* whole plant extract had a higher chemosuppression at 64.22 ± 4.43%, compared to methanolic extract 49.0 ± 3.61% at 100 mg/kg (Muthaura et al., 2007). These findings are contrary to those obtained in our study at 150 mg/kg, which had a low chemosuppression of 38.45 ± 2.81% (Fig. 2). Several factors could be implicated, including the difference in extraction solvent used, developmental stages, extraction methods, and study design. Therefore, it is likely that the water extraction solvent has a better extraction efficiency of phytochemical constituents with antimalarial activity than methanol. Clearly, the evidence that solvents, particularly methanol and water, influence

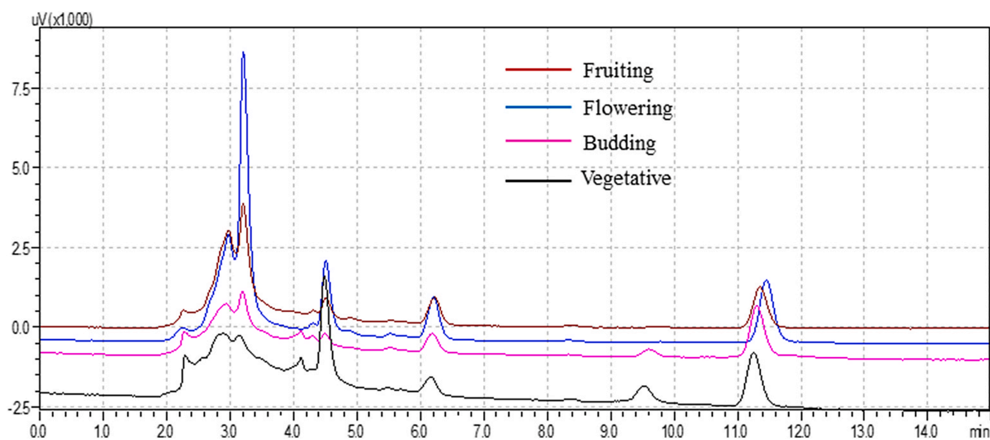


**Fig. 1.** The HPLC chromatograms for *S. pinnata* extract from four developmental stages.

**Table 2**
In vivo antimalarial activities of Schkuhria pinnata vegetative, budding, flowering, and fruiting extracts on Plasmodium berghei ANKA.

| Extract | Extract dose (mg/kg) | | | | | |
|---|---|---|---|---|---|---|
| | 150 | | 350 | | 700 | |
| | % Parasitemia | % Chemosuppression | % Parasitemia | % Chemosuppression | % Parasitemia | % Chemosuppression |
| VG | 16.53 ± 0.78$^{abcd}$ | 33.08 ± 3.14$^{1234}$ | 12.3 ± 0.68$^{abc}$ | 50.19 ± 2.75$^{123}$ | 11.02 ± 0.49$^{abcd}$ | 55.39 ± 2.01$^{124}$ |
| BB | 16.92 ± 0.92$^{abce}$ | 31.50 ± 3.75$^{125}$ | 13.38 ± 0.57$^{abc}$ | 45.82 ± 2.31$^{12}$ | 11.13 ± 1.87$^{abce}$ | 54.92 ± 7.56$^{125}$ |
| FL | 15.20 ± 0.69$^{abcf}$ | 38.45 ± 2.81$^{126}$ | 11.07 ± 0.62$^{abc}$ | 55.2 ± 2.50$^{12}$ | 7.70 ± 1.11$^{abcf}$ | 68.83 ± 4.49$^{126}$ |
| FR | 15.57 ± 1.3$^{abc}$ | 36.97 ± 5.44$^{12}$ | 13.26 ± 0.71$^{abc}$ | 46.25 ± 2.88$^{12}$ | 12.17 ± 0.46$^{abc}$ | 50.71 ± 1.87$^{12}$ |
| ACT (0.57 + 3.43 mg/Kg) | 0.86 ± 0.47$^{a}$ | 96.51 ± 0.15$^{1}$ | 0.86 ± 0.47 $^{a}$ | 96.51 ± 0.15$^{1}$ | 0.86 ± 0.47 $^{1}$ | 96.51 ± 0.15$^{1}$ |
| CQ (10 mg/Kg) | 1.3 ± 0.18$^{b}$ | 94.72 ± 0.73$^{2}$ | 1.3 ± 0.18$^{b}$ | 94.72 ± 0.73$^{2}$ | 1.3 ± 0.18 $^{2}$ | 94.72 ± 0.73$^{2}$ |
| DW (200 μL) | 24.7 ± 2.72$^{c}$ | 0.00 | 24.7 ± 2.72 $^{c}$ | 0.00 | 24.7 ± 2.72 $^{c}$ | 0.00 |

Results stated as Mean ± SEM. VG=Vegetative, BB = budding, FL=Flowering. FR=Fruiting. Positive controls: ACT = Artemisinin-based Combination Therapy, CQ=Chloroquine. Negative control: DW = Distilled water.

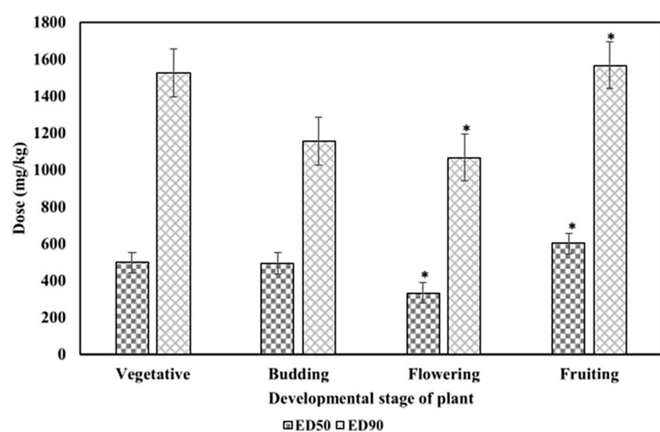


**Fig. 2. The effective doses of *Schkuhria pinnata* extract at different developmental stages.** Data are expressed as Mean ± S.E.M. $*p < 0.05$.

*S. pinnata*'s antimalarial activity in animals is contradictory that warrants further studies (Waiganjo et al., 2020; Muthaura et al., 2007).

*S. pinnata* could be an excellent source of antimalarial agents at the flowering stage. Frequently, flowering stages have high phytochemical content, such as polyphenols, terpenes, and flavonoids, as previously reported (Masoko and Masiphephethu, 2019). These compounds, particularly terpenoids (e.g., artemisinin) and flavonoids (e.g., quercetin) in *Artemisia annua* L. (Compositae), are responsible for the antimalarial activities with flavonoids potentiate the antimalarial activity of artemisinin (Ferreira et al., 2010). Furthermore, flavonoids have anti-inflammatory and antioxidant properties (Ferreira et al., 2010; Orabueze et al., 2020). Moreover, pure compounds such as sesquiterpene lactones isolated from *S. pinnata* had anti-inflammatory activity (Kudumela et al., 2019). The antioxidant and anti-inflammatory activities are crucial in malaria treatment, particularly in controlling fever and modulation of immune response to malaria infection. Thus, the antimalarial activity observed in the *S. pinnata* could be the resultant synergetic effects of several phytochemicals present in this plant.

### *3.4. Effect of Schkuhria pinnata extracts on body weight, temperature, and survival time*

Our findings demonstrated that extracts from all developmental stages promoted weight gain, averted body temperature increase, and prolonged survival time in dose-dependent patterns compared to the negative control (Table 3). Interestingly, the flowering stage had a significantly large survival time than the rest of the developmental states. For example, at the flowering stage, a dose of 700 mg/kg had a mean survival time of 20.33 ± 0.88 days lower than that observed in standard drugs chloroquine and Artemisinin-based combination therapy 28.00 ± 0.00 days each, but more prominent than the rest of developmental stages (Table 3). Furthermore, previous studies indicated that reducing malaria parasites increases animals' average survival time due to the reduction of the condition's pathological effects (Mulisa et al., 2018). Frequently, 12 days increase in survival time is regarded as a minimum cut-off time for an active antimalarial principle (Mulisa et al., 2018). In light of this standard practice, our study demonstrated that medium to high doses irrespective of developmental stages had significant survival time increments ranged from 10.67 ± 0.88 to 20.33 ± 0.88 days. Conversely, the lowest doses of extracts marginally prolonged the

**Table 3**
Weight, temperature, and survival time measurements from the chemosuppressive test of Schkuhria pinnata at four developmental stages.

| Extract | Animal grouping | Weight (g) (n = 5) | | | Temperature (°C) (n = 5) | | | Survival time (day) (n = 3) |
|---|---|---|---|---|---|---|---|---|
| | | D0 | D4 | % Change | D0 | D4 | % Change | |
| Vegetative | VG150 | 21.47 ± 0.79 | 21.10 ± 0.79 | −1.85 ab*** | 38.08 ± 0.13 | 37.78 ± 0.13 | 2–0.79a* | 9.00 ± 0.57bc |
| | VG350 | 21.24 ± 1.17 | 23.15 ± 2.09 | 7.12a*** | 38.04 ± 0.18 | 37.82 ± 0.28 | 2–0.59a** | 14.33 ± 0.88abc*** |
| | VG700 | 20.42 ± 1.47 | 23.78 ± 2.49 | 12.96a*** | 37.84 ± 0.40 | 37.74 ± 0.16 | −0.27a** | 17.00 ± 0.58abc |
| Budding | BB150 | 22.68 ± 0.51 | 22.00 ± 0.57 | −3.26abc*** | 37.46 ± 0.29 | 37.66 ± 0.27 | 0.51 ab*** | 8.33 ± 0.88bc* |
| | BB350 | 22.73 ± 1.43 | 22.21 ± 1.41 | −2.34abc*** | 37.92 ± 0.23 | 37.72 ± 0.15 | −0.53a** | 10.67 ± 0.88abc*** |
| | BB700 | 20.47 ± 1.04 | 21.35 ± 1.55 | 3.28a*** | 37.70 ± 0.24 | 37.44 ± 0.16 | −0.69a** | 12.33 ± 0.88abc* |
| Flowering | FL150 | 24.21 ± 0.59 | 25.90 ± 0.95 | 6.33 ab*** | 36.30 ± 0.66 | 36.48 ± 0.22 | 0.51a*** | 10.67 ± 1.66abc*** |
| | FL350 | 23.69 ± 1.67 | 26.86 ± 2.26 | 11.26a*** | 37.50 ± 0.38 | 36.96 ± 0.39 | −1.48 | 13.33 ± 0.88abc*** |
| | FL700 | 23.35 ± 0.86 | 25.76 ± 1.41 | 8.91a*** | 37.52 ± 0.11 | 37.26 ± 0.15 | −0.7a** | 20.33 ± 0.88bc |
| Fruiting | FR150 | 23.82 ± 0.38 | 23.64 ± 0.42 | −0.83 ab*** | 37.27 ± 0.07 | 36.96 ± 0.13 | −0.82a** | 9.00 ± 0.57bc** |
| | FR350 | 24.48 ± 0.73 | 25.58 ± 0.51 | 4.37a*** | 37.16 ± 0.16 | 37.10 ± 0.23 | 2–0.17a** | 11.33 ± 0.88abc*** |
| | FR700 | 24.72 ± 0.32 | 26.68 ± 0.63 | 7.26a*** | 37.52 ± 0.25 | 37.04 ± 0.24 | −1.2a** | 16.67 ± 1.76abc*** |
| ACT | ACT4 | 23.44 ± 0.71 | 26.49 ± 1.84 | 11.49ac*** | 37.26 ± 0.37 | 37.66 ± 0.28 | 1.04a*** | 28.00 ± 0.00a*** |
| CQ | CQ10 | 22.63 ± 1.04 | 25.08 ± 1.05 | 9.82a*** | 37.92 ± 0.23 | 37.72 ± 0.16 | 2–0.53a** | 28.00 ± 0.00a*** |
| DW | DW200 | 23.78 ± 0.89 | 19.6 ± 0.61 | −21.41b*** | 37.66 ± 0.28 | 36.50 ± 0.38 | 2–4.91bc** | 5.33 ± 0.00bc*** |

Values were expressed as mean ± S.E.M. Statistical significance; $***P < 0.001$, $**P < 0.01$, $*P < 0.05$; a = compared to negative control; b = compared to ACT 4 mg/kg; c = compared to CQ 10 mg/kg; Do = pre-assaying value on day 0; D4 = post-assaying value on day 4.

mean survival time to $9.00 \pm 0.57$, implying extract at lower doses are less active.

Our findings indicated that vegetative extract at a dose of 700 mg/kg and flowering stage extract at a dose of 350 mg/kg had a maximum proportion of weight gain at 12.96 and 11.26, respectively. This gain is close to those observed in animals that received standard treatment (Table 3). The increase in body weight could be due to the amelioration of malaria infection. A mice study indicated that malaria infection is associated with weight loss, anemia, and body temperature change (Mekonnen, 2015). Thus, therapies that treat malaria are likely to improve body weight and temperature. Generally, malaria causes anorexia; thus, increased body weight could be linked to appetite boosters in the extracts, as reported in another study (Mekonnen, 2015). Therefore, gain in appetite during treatment with *S. pinnata* would be beneficial, especially in children and pregnant women who are the most affected by malaria.

### *3.5. Hematological parameters of mice treated with Schkuhria pinnata extracts*

Our study findings indicated that *S. pinnata* extracts from all developmental stages were protective against plasmodia's negative effects on blood composition (Table 4). The malaria-infected mice had a significant increase in the mean number of white blood cells ($11.7 \pm 0.64 \times 10^9$/L) compared to the normal control group ($8.00 \pm 0.86 \times 10^9$/L). This rise in white blood cells is often seen in infections such as malaria as the body tries to mount an immune response against the disease. More abnormalities were observed through RBC reduction ($6.11 \pm 0.31 \times 10^{12}$/L), HGB ($10.67 \pm 0.46 \times 10^{12}$/L), HCT ($31.73 \pm 2.22$%) and MCH ($18.03 \pm 0.69$ pg) in test mice relative to RBC ($8.87 \pm 0.13 \times 10^{12}$/L), HGB ($14.53 \pm 0.71 \times 10^{12}$/L), HCT ($43.00 \pm 0.85$%) and MCH ($15.80 \pm 0.23$ pg) in normal group. The prevention of hemolysis was attributed to active ingredients present in the medicinal extract. Often, hematological analyses are conducted to confirm the profound effects of malaria infection, especially on erythrocytes (Zeeba et al., 2014).

Results reported as mean ± SEM: Mean values carrying identical superscripts are insignificant at 0.05 level.

## 4. Conclusion

Together, our findings revealed that the *S. pinnata* extracts at the flowering stage had superior antimalarial activity compared to other plant developmental stages. Extract from all developmental stages has demonstrated a dose-dependent suppression of malarial parasites and increase survival time with an $LD_{50}$ of 2157 mg/kg. Thus, for better antimalarial activity, local communities could consider harvesting *S. pinnata* at the flowering stage. Further studies are needed to isolate pure compounds from *S. pinnata* and determine their antimalarial activity and their effect on liver and kidney functions.

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## Ethical consideration

The research protocol was approved by the Mbarara University of Science and Technology Research Ethics Committee (MUREC 1/7). The experimental animals were handled and cared for according to "Guide for the Care and Use of Laboratory Animals."

**Table 4**
Hematological parameters of mice treated with *Schkuhria pinnata*-vegetative, budding, flowering, and fruiting extracts.

| Test drug | Dose (mg/kg) | WBC ( $\times 10^9$/L) | RBC ( $\times 10^{12}$/L) | HGB (g/dL) | HCT (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) | RDW (%) |
|---|---|---|---|---|---|---|---|---|---|
| **NC** | 0 | $8.00 \pm 0.86^{a}$ | $8.87 \pm 0.13^{b}$ | $14.53 \pm 0.71^{c}$ | $43.00 \pm 0.85^{d}$ | $48.33 \pm 0.67^{e}$ | $15.80 \pm 0.23^{f}$ | $33.43 \pm 0.29$ | $14.57 \pm 0.25^{i}$ |
| **VG** | 150 | $3.67 \pm 0.72^{a}$ | $6.82 \pm 0.31$ | $11.47 \pm 1.13^{c}$ | $32.87 \pm 1.99$ | $48.33 \pm 0.88^{e}$ | $16.73 \pm 0.88^{f}$ | $34.77 \pm 1.32^{g}$ | $14.10 \pm 0.47^{i}$ |
| | 350 | $4.17 \pm 0.48^{a}$ | $6.94 \pm 0.58^{b}$ | $11.10 \pm 1.05^{c}$ | $33.47 \pm 3.43^{d}$ | $48.00 \pm 1.15^{e}$ | $15.97 \pm 0.37^{f}$ | $33.23 \pm 0.41^{g}$ | $13.17 \pm 0.55^{i}$ |
| | 700 | $4.57 \pm 0.79^{a}$ | $7.59 \pm 0.34^{b}$ | $11.90 \pm 0.50^{c}$ | $35.97 \pm 1.79^{d}$ | $47.67 \pm 0.33^{e}$ | $15.67 \pm 0.03^{f}$ | $33.10 \pm 0.25^{g}$ | $13.77 \pm 0.23^{i}$ |
| **BB** | 150 | $4.40 \pm 0.62^{a}$ | $8.21 \pm 0.71^{b}$ | $12.67 \pm 1.08^{c}$ | $38.20 \pm 3.59^{d}$ | $46.33 \pm 0.33^{e}$ | $15.43 \pm 0.03^{f}$ | $33.23 \pm 0.27^{g}$ | $13.97 \pm 0.66^{i}$ |
| | 350 | $4.43 \pm 0.52^{a}$ | $7.61 \pm 0.38^{b}$ | $11.83 \pm 0.58^{c}$ | $35.73 \pm 1.76^{d}$ | $47.00 \pm 0.00^{e}$ | $15.53 \pm 0.13^{f}$ | $33.13 \pm 0.19^{g}$ | $13.80 \pm 0.26^{i}$ |
| | 700 | $3.93 \pm 0.96^{a}$ | $7.63 \pm 0.68^{b}$ | $11.37 \pm 1.08^{c}$ | $36.17 \pm 3.55^{d}$ | $47.33 \pm 0.67^{e}$ | $14.90 \pm 0.10$ | $31.53 \pm 0.09^{g}$ | $13.33 \pm 0.98^{i}$ |
| **FL** | 150 | $3.53 \pm 1.00^{a}$ | $7.78 \pm 0.22^{b}$ | $12.40 \pm 0.10^{c}$ | $37.80 \pm 0.95^{d}$ | $48.33 \pm 0.33^{e}$ | $15.97 \pm 0.34^{f}$ | $32.87 \pm 0.58^{g}$ | $12.37 \pm 0.14^{i}$ |
| | 350 | $2.37 \pm 0.23^{a}$ | $8.65 \pm 0.39^{b}$ | $13.57 \pm 0.58^{c}$ | $41.30 \pm 1.97^{d}$ | $48.33 \pm 0.00^{e}$ | $15.67 \pm 0.03^{f}$ | $32.87 \pm 0.18^{g}$ | $13.37 \pm 0.03^{i}$ |
| | 700 | $3.50 \pm 1.30^{a}$ | $7.79 \pm 0.19^{b}$ | $12.70 \pm 0.21^{c}$ | $37.80 \pm 0.47^{d}$ | $48.67 \pm 0.67^{e}$ | $16.33 \pm 0.23^{f}$ | $33.60 \pm 0.10^{g}$ | $12.80 \pm 0.60^{i}$ |
| **FR** | 150 | $4.90 \pm 1.27^{a}$ | $6.85 \pm 0.41$ | $11.30 \pm 0.50^{c}$ | $32.57 \pm 1.77$ | $47.33 \pm 0.33^{e}$ | $16.53 \pm 0.31^{f}$ | $34.77 \pm 0.41^{g}$ | $13.37 \pm 0.46^{i}$ |
| | 350 | $5.33 \pm 0.88^{a}$ | $7.31 \pm 0.88^{b}$ | $12.03 \pm 0.42^{c}$ | $34.93 \pm 2.42^{d}$ | $48.00 \pm 0.58^{e}$ | $16.57 \pm 0.63^{f}$ | $34.70 \pm 1.31^{g}$ | $13.10 \pm 0.40^{i}$ |
| | 700 | $6.00 \pm 2.02^{a}$ | $9.56 \pm 0.22^{b}$ | $14.70 \pm 0.31^{c}$ | $44.57 \pm 0.82$ | $46.67 \pm 0.33^{e}$ | $15.37 \pm 0.07^{f}$ | $32.97 \pm 0.09^{g}$ | $13.53 \pm 0.09^{i}$ |
| **ACT** | 4 | $2.37 \pm 0.14^{a}$ | $8.76 \pm 0.29^{b}$ | $13.60 \pm 0.55^{c}$ | $42.40 \pm 1.57^{d}$ | $48.33 \pm 0.33^{e}$ | $15.47 \pm 0.18^{f}$ | $32.00 \pm 0.35^{g}$ | $13.53 \pm 0.62^{i}$ |
| **CQ** | 10 | $4.53 \pm 0.69^{a}$ | $8.16 \pm 0.42^{b}$ | $14.00 \pm 0.36^{c}$ | $41.10 \pm 2.78^{d}$ | $49.00 \pm 0.58^{e}$ | $17.30 \pm 0.35^{f}$ | $34.73 \pm 0.33$ | $12.97 \pm 0.22^{i}$ |
| **DW (200 µL)** | | $11.7 \pm 0.64$ | $6.11 \pm 0.31$ | $10.67 \pm 0.46$ | $31.73 \pm 2.22$ | $46.00 \pm 1.00^{e}$ | $18.03 \pm 0.69$ | $37.10 \pm 0.49$ | $13.70 \pm 0.25^{i}$ |

| Test drug | Dose (mg/kg) | PLT | MPV | NE (%) | LY (%) | MO (%) | EO (%) | BA (%) |
|---|---|---|---|---|---|---|---|---|
| **NC** | 0 | $943.67 \pm 33.83^{j}$ | $6.33 \pm 0.22^{k}$ | $16.23 \pm 5.78^{l}$ | $66.60 \pm 5.60^{m}$ | $12.30 \pm 1.28^{n}$ | $0.87 \pm 0.27^{r}$ | $2.10 \pm 0.80^{t}$ |
| **VG** | 150 | $553.33 \pm 427.34^{j}$ | $9.00 \pm 0.15^{k}$ | $1.53 \pm 0.43^{l}$ | $89.03 \pm 3.07^{m}$ | $3.27 \pm 0.87^{n}$ | $0.22 \pm 0.21^{r}$ | $6.17 \pm 1.76^{t}$ |
| | 350 | $376.00 \pm 81.29^{j}$ | $8.90 \pm 0.40^{k}$ | $3.50 \pm 0.92^{l}$ | $86.40 \pm 2.26^{m}$ | $7.6 \pm 1.11^{n}$ | $0.33 \pm 0.33^{r}$ | $2.47 \pm 0.58^{t}$ |
| | 700 | $356.67 \pm 55.64^{j}$ | $9.13 \pm 0.78^{k}$ | $2.13 \pm 0.22^{l}$ | $89.20 \pm 1.95^{m}$ | $4.40 \pm 0.66^{n}$ | $0.35 \pm 0.30^{r}$ | $4.23 \pm 1.07^{t}$ |
| **BB** | 150 | $292.67 \pm 55.64^{j}$ | $8.53 \pm 0.12^{k}$ | $2.30 \pm 0.66^{l}$ | $86.53 \pm 2.35^{m}$ | $4.36 \pm 1.75^{n}$ | $0.37 \pm 0.31^{r}$ | $6.76 \pm 0.61^{t}$ |
| | 350 | $335.33 \pm 133.59^{j}$ | $8.10 \pm 0.70^{k}$ | $4.03 \pm 1.12^{l}$ | $82.77 \pm 2.77^{m}$ | $6.86 \pm 1.44^{n}$ | $0.13 \pm 0.67^{r}$ | $6.20 \pm 0.21^{t}$ |
| | 700 | $1011.67 \pm 160.67^{j}$ | $7.63 \pm 0.48^{k}$ | $7.73 \pm 2.25^{l}$ | $50.53 \pm 5.67^{m}$ | $35.77 \pm 6.85^{s}$ | $0.90 \pm 0.49^{p}$ | $5.07 \pm 1.14^{t}$ |
| **FL** | 150 | $484.00 \pm 57.14^{j}$ | $8.87 \pm 0.38^{k}$ | $6.70 \pm 1.62^{l}$ | $70.47 \pm 7.02^{m}$ | $18.80 \pm 6.16^{n}$ | $0.17 \pm 0.67^{r}$ | $3.86 \pm 0.71^{t}$ |
| | 350 | $634 \pm 142.96^{j}$ | $7.43 \pm 0.37^{k}$ | $5.13 \pm 0.68^{l}$ | $72.40 \pm 4.94^{m}$ | $18.47 \pm 5.63^{n}$ | $0.16 \pm 0.08^{r}$ | $3.83 \pm 1.36^{t}$ |
| | 700 | $316.67 \pm 46.88^{j}$ | $8.40 \pm 0.30^{k}$ | $3.07 \pm 1.22^{l}$ | $86.23 \pm 3.22^{m}$ | $4.43 \pm 0.28^{n}$ | $0.37 \pm 0.31^{r}$ | $6.20 \pm 1.79^{t}$ |
| **FR** | 150 | $480.00 \pm 131.12^{j}$ | $8.53 \pm 0.41^{k}$ | $6.36 \pm 2.36^{l}$ | $76.90 \pm 5.60^{m}$ | $8.60 \pm 2.43^{n}$ | $0.47 \pm 0.18^{r}$ | $7.67 \pm 1.21^{t}$ |
| | 350 | $517.67 \pm 186.10^{j}$ | $8.03 \pm 0.12^{k}$ | $6.07 \pm 0.89^{l}$ | $79.50 \pm 1.85^{m}$ | $7.07 \pm 2.14^{n}$ | $0.10 \pm 0.06^{r}$ | $7.23 \pm 1.23^{t}$ |
| | 700 | $337.00 \pm 90.51^{j}$ | $7.40 \pm 0.20^{k}$ | $10.70 \pm 1.20^{l}$ | $64.20 \pm 3.61^{m}$ | $18.97 \pm 2.75^{n}$ | $0.03 \pm 0.02^{r}$ | $6.10 \pm 0.50^{t}$ |
| **ACT** | 4 | $883.67 \pm 19.70^{j}$ | $7.77 \pm 0.27^{k}$ | $11.83 \pm 3.78^{l}$ | $51.13 \pm 8.01^{m}$ | $33.93 \pm 7.25^{n}$ | $0.47 \pm 0.26^{r}$ | $2.63 \pm 1.07^{t}$ |
| **CQ** | 10 | $880.33 \pm 57.79^{j}$ | $6.67 \pm 0.18^{k}$ | $16.13 \pm 4.39^{l}$ | $64.30 \pm 7.68^{m}$ | $11.37 \pm 1.80^{n}$ | $0.37 \pm 0.03^{r}$ | $2.76 \pm 1.27^{t}$ |
| **DW (200 µL)** | | $566.67 \pm 118.29^{j}$ | $7.53 \pm 0.20^{k}$ | $9.23 \pm 2.46^{l}$ | $69.33 \pm 7.40^{m}$ | $14.53 \pm 4.55^{n}$ | $0.07 \pm 0.06^{r}$ | $7.60 \pm 0.46^{t}$ |

## Author's contribution

CN conceived and drafted an earlier version of the manuscript.CN, ELP, COA, collected data, statistical analysis, and revised the manuscript's drafts. JA, GRK, EAO, PEO, CUT provided mentorship and supervision; ELP, COA, JA, GRK, EAO, PEO, CUT revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

## Declaration of competing interest

The authors declare that they have no competing interests.

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## Abbreviations

| | |
|---|---|
| BA | Basophils |
| BB | Budding |
| CQ | Chloroquine |
| EO | Eosinophils |
| FL | Flowering |
| FR | Fruiting |
| HCB | Mean corpuscular hemoglobin concentration |
| HCT | Hematocrit |
| $LD_{50}$ | Median lethal dose |
| LY | Lymphocytes |
| MCH | Mean corpuscular hemoglobin |
| MO | Monocytes |
| MOH | Ministry of health |
| MPV | Mean corpuscular volume |
| NARO | National Agricultural Research Organization |
| NE | Neutrophils |
| PHARMBIOTRAC | Pharmbiotechnology and traditional medicine center of excellence |
| PLT | Platelets |
| VG | Vegetative |
| WHO | World health organization |

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2021.114341.

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