**Sida acuta** Burm.f. leaves ethanol extract ameliorates haematological and biochemical alterations induced by *Plasmodium berghei* ANKA-65 in mice

Osmond Chukwuma Enechi 1, Christian Chijioke Amah 1, Innocent Uzochukwu Okagu 2*, Pamela Chidinma Onioniwu 1, Alice Chinwendu Nweke 1, Theophilus Chukwudi Ugwuanyi 1, Emmanuel Arinzechukwu Ajibo 1, Anthonia Chiamaka Nweze 1 and Blessing Chiamaka Chukwurah 1

**Abstract**

**Background:** Malaria has continued to be a threat to man and his wellbeing, especially Africans and Asians. New antimalarial drugs are urgently needed to mitigate malaria treatment failure due to resistant *Plasmodium* species. Medicinal plants used by indigenous Nigerians for treating fever and malaria such as *Sida acuta* Burm.f. (Malvaceae) could be a promising source of lead compounds for developing new generations of antimalarial drugs. The effects of ethanol extract of *S. acuta* leaves (EESAL) on malaria parasitemia, haematological and biochemical status of *P. berghei*-infected mice were investigated, using the 4-day curative test.

**Methodology:** EESAL was prepared by maceration method. The phyto-constituents and acute toxicity profile of the extract were evaluated using standard protocols. In addition, malaria parasitemia and chemo-suppression, and indicators of haematological and biochemical status of *P. berghei*-infected mice treated with EESAL were assessed.

**Results:** At 200, 400 and 600 mg/kg/d b.w., p.o doses for 4 consecutive days, EESAL significantly (*p* < 0.05) decreased parasitaemia and suppressed malaria parasite by 89.64%, 95.95% and 97.38%, respectively comparable to negative control. The reduction in percentage malaria parasitemia by EESAL is comparable to Artemether (140 mg/kg/d b.w., p.o) used as standard antimalarial drug in this study. The packed cell volume (PCV), haemoglobin (Hb) concentration, and red blood cell (RBC) and white blood cell (WBC) counts of negative control are significantly (*p* < 0.05) higher than normal control. However, parasitized-EESAL-treated mice have significantly (*p* < 0.05) higher PCV value, Hb concentration and RBC and WBC counts than negative control. Similarly, treatment of parasitized mice with EESAL restored some indicators of the antioxidant, lipid peroxidation, lipid profile and liver status altered by malaria. In addition, EESAL was tolerable up to 5000 mg/kg b.w., p.o.

**Conclusion:** These results indicate that the EESAL possesses antimalarial activity and normalizes alterations in haematological and biochemical status of malaria-infected mice.

**Keywords:** *Sida acuta* Burm.f., *Plasmodium berghei*, Malaria, Haematological indices, Biochemical parameters
Introduction
Malaria is a parasitic disease caused by *Plasmodium species* that causes high annual numbers of hospital visits and deaths globally with higher impact in Sub-Saharan Africa and South-East Asia [1]. Out of the 228 million cases of malaria globally in 2018, 213 million are from the WHO African Region, and Nigeria contributed 25% of this number [2]. This high burden of the disease demands concerted interventions to reduce the impact on man to barest minimum. Current efforts to eliminate malaria have been hampered by antimalarial resistance [3, 4], and worrisome adverse effects of current antimalarials [5]. This situation necessitates the hunt for new, cheap and effective antimalarial drugs especially from natural products such as plants to avoid getting to an era when malaria will become incurable.

Alterations in the haematological profile such as lymphopenia, anaemia, thrombocytopenia, eosinopenia, neutropenia, monocytes and leucocytosis are prominent indicators of severity of malaria infection [6]. Recently, oxidative stress has been identified as one of the complications in malaria [7]. Furthermore, the existence of dyslipidemia in malaria is well recognized. Mohanty et al. [8] reported significant elevation in total cholesterol (CHOL) and low-density lipoproteins (LDL) while high density lipoproteins (HDL) and triacylglycerols (TAGs) decreased significantly. It was also reported that the extent of dyslipidemia is dependent on the severity of the disease [9, 10]. Similar studies in both vivax and falciparum malaria patients reported that concentrations of HDL, LDL, and CHOL decreased significantly but TAGs increased in malaria-infected patients than in healthy control [11–15]. These reports generally support the opinion that malaria induces dyslipidaemia and hence, a good antimalarial agent should, in addition to clearing malaria parasite, restore homeostasis in lipid status.

Medicinal plants employed in treating fever and malaria by traditional healers is suggested to be potential sources of a new generation of antimalarials. *Sida acuta* Burm.f. (Malvaceae), also known as Wirewood and *Udo* in English and Igbo languages, respectively, is among the common plants traditionally used for treating malaria in Nigeria and other parts of Africa [16–19]. Extracts from the plant have been shown to have larvicidal effects on *Anopheles stephensi* [20], anti-plasmodial [21], analgesic [22], antibacterial [23], antioxidant [24], anxiolytic and neuromodulatory [25] activities. The present research was designed to reveal some of the phytochemical constituents and investigate the acute toxicity profile and in vivo malaria-curative activity of the ethanol extract of *S. acuta* leaves (EESAL). The effect of ESAL on some altered haematological and biochemical (lipid profile, antioxidant and lipid peroxidation and liver status) parameters of *P. berghei*-infected mice was also investigated.

Materials and methods
Chemicals and instruments
Instruments used were weighing balance (Vickas Ltd., England), centrifuge (Vickas Ltd., England), microscope (SMDM-1030, Ted Pella Inc. USA) and spectrophotometer (E312 Model) (Jenway, UK) while reagents for the biochemical experiments were products of Randox (USA). Solvents used (ethanol, ethyl acetate, chloroform and n-hexane) were products of JHD (India) while other chemicals were products of Sigma-Aldrich (USA). All chemicals used were of analytical grade, received in good condition and were freshly prepared before use.

Study animals
Fifty-four adult Swiss albino mice of average body weight of 30 ± 5 g were used for this study; 18 and 36 mice for acute toxicity and antimalarial studies, respectively. The mice were maintained on commercial feed (Chukun Feeds Nig. Ltd.) and water ad libitum. The study was approved by the Faculty of Biological Sciences Committee on Research and Bioethics (UNN/FBS/EC/1022). The study animals were ethically handled throughout the experimental period in accordance with the institutional, national and international ethical recommendations for care and use of laboratory animals.

Standard drug
Artemether (a combination of artemether and lumenfantrin, 80:480 w/w, Ajanta Pharma Ltd., India) was the standard antimalarial of choice in this study.

Extraction of plant materials
In June of 2019, fresh leaves of *S. acuta* Burm.f. (Malvaceae) were collected from its natural habitat in Opanda-Nimbo, Enugu State, Nigeria and were authenticated by Mr. Alfred Ozioko, a taxonomist of the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State (voucher number - Intercedd/160). This was compared with features available in the plant database (http://www.theplantlist.org/) for confirmation. Two weeks air-dried leaves of *S. acuta* plant were powdered and the powder was extracted thrice with ethanol by cold maceration method. Filtrates from the extraction chambers were dried (rotary evaporator) (Ted Pella Inc. USA) and spectrophotometer (E312 Model) (Jenway, UK) while reagents for the biochemical experiments were products of Randox (USA). Solvents used (ethanol, ethyl acetate, chloroform and n-hexane) were products of JHD (India) while other chemicals were products of Sigma-Aldrich (USA). All chemicals used were of analytical grade, received in good condition and were freshly prepared before use.
Phytochemical analysis of EESAL
The quantitative secondary metabolites in EESAL were determined using standard procedures described by Trease and Evans [28] and Harborne [29] as per [30]. The procedures are briefly presented below.

Detection and quantification of tannins in EESAL
Using ferric chloride assay, the presence of tannins in the extract was determined using the method of the methods of Trease and Evans (1989). Extract (0.1 g) was stirred with 10 ml of distilled water and then filtered. The appearance of blue-black precipitate when three drops of 1% ferric chloride solution were added to 2 ml of the filtrate indicated the presence of tannins. Thereafter, the amount of total tannins in the sample was quantified using the method of Harborne (1973). To 5 ml of the filtrate above in a test tube, 0.1 N ferric chloride in 0.1 N HCl and 0.008 M potassium ferricyanide were added and mixed. The absorbance of the mixture was read at 720 nm. The tannin content of the extract was extrapolated from a tannic acid standard curve.

Detection and quantification of alkaloids in EESAL
The alkaloids in EESAL were detected by Dragendorff’s reagent test following the methods of Trease and Evans (1989). Briefly, 0.1 g of EESAL was boiled with 5 ml of 1% aqueous HCl on a water bath for 10 min and filtered. The appearance of orange-red precipitate on addition of 2 drops of Dragendorff’s reagent to 1 ml of the filtrate indicated the presence of alkaloids. Following the method of Harborne (1973), the quantity of total alkaloids in EESAL was determined. EESAL (0.1 g) was macerated in 20 ml of 20% H2SO4 in ethanol (1:1) for 30 min and was filtered. To 1 ml of the filtrate, 5 ml of 60% H2SO4 was added and after 5 min, 5 ml of 0.5% formaldehyde in 60% H2SO4 was also added, mixed and allowed to stand for 3 h. The absorbance of the mixture was read at a wavelength of 550 nm. The alkaloid content was extrapolated from an atropine standard curve.

Detection and quantification of glycosides in EESAL
The presence of glycosides in EESAL was detected using Fehling’s test as described by Trease and Evans (1989). Briefly, 0.1 g of EESAL was mixed with 30 ml of distilled water and heated on a water bath for 5 min, cooled and was filtered. To 5 ml of the filtrate, a mixture of 0.2 ml of each of Fehling’s solutions A and B was added drop by drop till pH changes to alkaline (pH ~ 8). The appearance of a brick-red precipitate on heating in water bath for 2 min indicated the presence of glycosides. To 0.1 g EESAL, 20 ml of distilled water and 2.5 ml of 15% lead acetate were added, mixed and allowed to stand for 30 min and was filtered. To 2 ml of the filtrate, 2.5 ml of chloroform was added and shaken vigorously and allowed to stand for 5 min. The lower layer was collected into a beaker and was evaporated to dryness in a water bath. Thereafter, 3 ml of glacial acetic acid, 0.1 ml of 5% ferric chloride and 0.25 ml of concentrated H2SO4 were added to the beaker, mixed very well and allowed to stand in a dark chamber for 2 h. Thereafter, absorbance of the resultant mixture was measured at 530 nm against the reagent blank (not containing EESAL).

Detection and quantification of terpenoids in EESAL
The existence of terpenoids in EESAL was detected by acetic anhydride test following the procedure of Trease and Evans (1989). EESAL (0.1 g) was dissolved in 5 ml of ethanol and 1 ml of acetic anhydride and drops of concentrated H2SO4 were added. A change in colour from pink to violet indicated the presence of terpenoids. To determine the quantity of terpenoids in the sample, a quantity (0.1 g) of EESAL was macerated in 20 ml of ethanol for 10 min and was filtered. To 1 ml of the filtrate, 1 ml of 5% phosphomolybdic acid solution was added and shaken, followed by gradual addition of 1 ml of concentrated H2SO4. The mixture was left to stand for 30 min and thereafter, 2 ml of ethanol was added and absorbance was measured at 700 nm against the reagent blank. The terpenoid content was estimated as follows;

\[
\text{Terpenoid content (mg/100g)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the sample} - \text{Absorbance of the blank}} \times 100
\]

Detection and quantification of flavonoids in EESAL
Using the lead acetate test, the presence of flavonoids in EESAL was detected as per Trease and Evans (1989). To 5 ml of the filtrate from the tannins test, 3 ml of lead acetate solution was added. The formation of yellowish precipitate indicated the presence of flavonoids. To quantify the amount of total flavonoids in EESAL, 0.1 g of the sample was macerated in 20 ml of ethyl acetate, and the suspension was filtered. To 5 ml of the filtrate, 5 ml of dilute ammonia was added, mixed and allowed to separate into two layers. The upper layer of the mixture, containing the flavonoids was collected and the absorbance was read at 510 nm using ethyl acetate as the blank. The concentration of flavonoids in the extract was extrapolated from a catechin standard curve.

Detection and quantification of steroids in EESAL
To 0.1 g of EESAL, 2 ml of acetic acid was added and mixed thoroughly. The solution was cooled in ice followed by the addition of concentrated tetraoxosulphate (VI) acid
(H₂SO₄). The formation of a violet ring which turns bluish on addition of acid indicated the presence of steroids. To quantify the content of steroids in EESAL, 0.1 g of the sample was macerated in 20 ml of ethanol and filtered. To 2 ml of the filtrate, 2 ml of chromogen solution (4 ml ferric chloride stock made up to 24 ml with concentrated H₂SO₄) and 1 ml of 0.5% potassium hexacyanoferrate (III) solution were added, mixed and the mixture was left to stand for 30 min in a water bath set at 70°C. Thereafter, the mixture was cooled and made up to 10 ml with distilled and the absorbance was measured at 780 nm against the reagent blank (prepared by adding all the reagents except in the sample test tube except the extract).

### Phenol content (mg/100g)

\[
\text{Phenol content} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the sample - Absorbance of the blank}} \times 100
\]

### Acute toxicity test

The acute toxicity profile of the plant extract was determined in two phases using a method described by Lorke [31]. A total of 18 mice were used for this test. In phase I, nine mice were divided into three groups of three mice each (groups 1–3 received 10, 100 and 1000 mg/kg b.w of EESAL, respectively). The mice were observed for 24 h for signs of toxicity and lethality. Thereafter, the remaining nine mice were randomized into three groups of three mice each (groups 4–6 received 1600, 2900 and 5000 mg/kg b.w of EESAL, respectively). These mice were again observed for 24 h for signs of toxicity and lethality. Body weight of the mice 24 h post-EESAL administration were measured and compared with the initial body weight.

### Parasite inoculation

*Plasmodium berghei*-infected blood from a donor mouse was used for inoculum preparation. The blood was diluted serially in Alsever’s solution to make a suspension containing about 1 × 10⁷ infected RBCs in every 0.2 mL suspension. The mice in test groups (groups 2–6) were intraperitoneally inoculated with 0.2 mL of this suspension to initiate infection. Malaria parasite infection was confirmed 72 h post-infection.

### Experimental design for malaria curative study

Thirty-six adult Swiss mice used for this study were divided into six groups of six mice each as follows:

- **Group 1 = Normal control** (administered with the vehicle, normal saline)
- **Group 2 = Negative control** (inoculated with malaria parasite and untreated)
- **Group 3 = Standard drug control** (*P. berghei*-parasitized + 140 mg/kg b.w. of Artemether).
- **Group 4 = *P. berghei*-parasitized + 200 mg/kg b.w. of EESAL.
- **Group 5 = *P. berghei*-parasitized + 400 mg/kg b.w. of EESAL.
- **Group 6 = *P. berghei*-parasitized + 600 mg/kg b.w. of EESAL.

Treatment lasted for 4 days (from day 0-day 3) and on day 4, overnight fast blood sample was collected from each of the mouse for parasitaemia count, haematological and biochemical (lipid peroxidation, antioxidant and liver status, and lipid profile) analyses. The three doses were selected based on activity-guided dose selection from pilot study.

### Determination of malaria parasitemia in parasitized mice

Malaria parasitaemia was determined by methods described earlier [32, 33] and the percentage malarial parasitemia and chemosuppression were calculated using the relations below:

\[
\%\text{Malaria Parasitemia} (\text{MP}) = \frac{\text{Number of parasitized RBCs}}{\text{Total number of RBCs counted}} \times 100
\]

\[
\%\text{Malaria Chemosuppression} = \frac{\text{Day0 MP} - \text{Day3 MP}}{\text{Day0 MP}} \times 100
\]

### Determination of haematological and some biochemical status of experimental mice

Haematological indices were determined by previous methods [34, 35]. Aspartate and alanine aminotransferases (AST and ALT) and alkaline phosphatase (ALP)
activities were assayed using previously described methods [36, 37] as contained in the Randox commercial kits. Serum total bilirubin [38], CHOL [39], and HDL and TAG concentrations were determined using colorimetric methods [40] as contained in Randox commercial kits. Serum LDL level was determined as the difference between CHOL and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethylene glycol monomethyl ether. The extent of lipid peroxidation (level of malondialdehyde (MDA)) was measured as per known protocol [41]. Superoxide dismutase (SOD) and catalase (CAT) activities were assayed using methods earlier described [42, 43], respectively while the reduced glutathione (GSH) concentration was determined according to method described by Kings and Wootton [44].

**Statistical analysis**

Primary data from the laboratory were analyzed using one way analysis of variance in Statistical Product and Service Solution version 20.0 (IBM Inc., USA) and Dunnett’s post hoc test was used to compare means across the groups. The results were presented as mean ± standard deviation (SD) in tables and mean values with \( p < 0.05 \) were considered significant.

**Results**

**Phytochemical composition of EESAL**

As shown in Table 1, the phytochemical compositions of EESAL include tannins (573.28 ± 30.74 mg/100 g), terpenoids (161.75 ± 3.12 mg/100 g), steroids (0.41 ± 0.08 mg/100 g), phenols (3115.16 ± 143.23 mg/100 g), alkaloids (4.62 ± 1.04 mg/100 g), flavonoids (2051.44 ± 84.32 mg/100 g) and glycosides (108.54 ± 12.71 mg/100 g).

**Acute toxicity profile of the extract**

The result of acute toxicity test (Table 2) shows that EESAL was not lethal even at the highest dosage (5000 mg/kg b.w., p.o) administered. Similarly, there was no significant body weight and behavioural changes within the 24 h observation period post-extract administration. This shows that the extract is tolerable and hence, the median lethal dose (LD_{50}) of the extract was therefore assumed to be higher than 5000 mg/kg body weight.

**Effect of EESAL on malaria parasitemia of *Plasmodium berghei*-infected mice**

After 72 h post inoculation (before the administration of the extract), there was no significant (\( p > 0.05 \)) difference among the percentage parasitemia of the test mice. However, after the treatment of parasitized mice with artemether (group 3) and graded doses of EESAL (groups 4–6), the percentage malaria parasitemia were significantly (\( p < 0.05 \)) lower than negative control (Table 3). Similarly, EESAL and standard drug suppressed malaria parasite count by comparable percentage, suggesting that EESAL has good antimalarial activity.

**Effect of EESAL on some haematological indices of *Plasmodium berghei*-infected mice**

The pack cell volume (PCV), red blood cell (RBC) and white blood cells (WBC) counts, and haemoglobin (Hb) concentration of negative control mice were lower than normal control. However, administration of graded doses of EESAL to parasitized mice caused significantly (\( p < 0.05 \)) higher PCV, RBC count and Hb concentration when compared with negative control (Table 4).

**Effect of EESAL on liver status in *Plasmodium berghei*-infected mice**

The ALT, ALP and AST activities and total bilirubin concentration of *P. berghei*-infected and untreated mice (negative control) were significantly (\( p < 0.05 \)) higher than normal control. After 4 consecutive days oral treatment with graded doses of EESAL and artemether, *P. berghei*-infected mice in groups 3–6 normalized the liver status (by returning the values of the liver function markers of parasitized-EESAL-treated mice to levels of mice in normal control) (Table 5).

**Effect of EESAL on lipid peroxidation and antioxidant parameters of *Plasmodium berghei*-infected mice**

It was observed that negative control had significantly (\( p < 0.05 \)) higher MDA concentration than normal control. Conversely, negative control had significantly (\( p < 0.05 \)) lower SOD and CAT activities, and GSH concentration than normal control. When malaria-parasitized mice were administered EESAL and artemether, there was a significant (\( p < 0.05 \)) reduction in MDA concentration and elevation in SOD and CAT activities and GSH concentration than negative control. Furthermore, compared to normal control, the lipid peroxidation and
antioxidant parameters of parasitized-EESAL-treated mice were not significantly different, suggesting that EESAL has reversed the alterations in lipid peroxidation and antioxidant status (Table 6).

**Effect of the EESAL on lipid profile of *Plasmodium berghei*-infected mice**

Malaria induced significantly \((p < 0.05)\) higher serum concentrations of CHOL, LDL, cholesterol and TAGs, but significantly \((p < 0.05)\) lower serum HDL concentration in negative control compared to normal control. When administered graded doses of EESAL and artemether for 4 days, parasitized-EESAL-fed mice had significantly \((p < 0.05)\) reduced CHOL, LDL and TAGs but significantly \((p < 0.05)\) increased HDL concentration compared to negative control (Table 7).

**Discussion**

The use of plants as a source of nutrients and drugs is as old as man. Plant products have provided many therapeutic agents, and many conventional drugs have their roots traced from natural sources [45]. The isolation of these natural products is usually based on their uses in traditional practice. *S. acuta* Burm.f. is used by rural Nigerian dwellers for treating malaria, fever among others [46]. This study examined the ability of crude ethanol leaf extract of *S. acuta* (EESAL) to reduce malaria parasite load and modulate alterations in haematological and biochemical status markers of *P. berghei*-infected mice. The presence of phytochemicals such as alkaloids, terpenoids, glycosides, flavonoids, phenols, steroids and saponins in EESAL suggest that the extract might be bioactive and elicit some pharmacological effects like antioxidant and antimalarial activities [47]. In addition, the lack of any morphological, behavioural and body weight change, and mortality in mice orally administered EESAL up to 5000 mg/kg b.w after 24 h showed that the extract is tolerable and safe for consumption. This suggests that the LD50 of EESAL is above 5000 mg/kg b.w. The variation between our result and that of Konaté et al. [22] could be attributed to differences in the solvent of extraction and location of source of the plant. It is documented that substances whose LD50 are above 5000 mg/kg b.w. p.o are considered safe and non-toxic [48].

The antimalarial activity of EESAL, characterized by significant \((p < 0.05)\) dose-dependent decrease in percentage malaria parasitemia and increase in parasite chemosuppression in parasitized-EESAL-treated mice when compared with negative control could be due to the phytoconstituents of the extract. Extracts of some plants (such as Fagara zanthoxyloides and Erythrina senegalensis leaves and Peltophorum pterocarpum stem bark) that are rich in phytochemicals also present in EESAL have been shown to have antimalarial effects, although with

| Phase | Group (Treatment) | Mortality | Behavioral and body weight changes |
|-------|------------------|-----------|-----------------------------------|
| **Phase I** | | | |
| Group 1 (10 mg/kg b.w. EESAL) | 0/3 | Not significant |
| Group 2 (100 mg/kg b.w. EESAL) | 0/3 | Not significant |
| Group 3 (100 mg/kg b.w. EESAL) | 0/3 | Not significant |
| **Phase II** | | | |
| Group 4 (1600 mg/kg b.w. EESAL) | 0/3 | Not significant |
| Group 5 (2900 mg/kg b.w. EESAL) | 0/3 | Not significant |
| Group 6 (5000 mg/kg b.w. EESAL) | 0/3 | Not significant |

| Table 2: Acute toxicity profile of the extract |
|-----------------|-----------------|-----------------|
| Groups (Treatment) | Mortality | Behavioral and body weight changes |
|-------------------|-----------|-----------------|
| **Phase I** | | | |
| Group 1 (10 mg/kg b.w. EESAL) | 0/3 | Not significant |
| Group 2 (100 mg/kg b.w. EESAL) | 0/3 | Not significant |
| Group 3 (100 mg/kg b.w. EESAL) | 0/3 | Not significant |
| **Phase II** | | | |
| Group 4 (1600 mg/kg b.w. EESAL) | 0/3 | Not significant |
| Group 5 (2900 mg/kg b.w. EESAL) | 0/3 | Not significant |
| Group 6 (5000 mg/kg b.w. EESAL) | 0/3 | Not significant |

| Table 3: Effect of EESAL on malaria parasitemia of *Plasmodium berghei*-infected mice |
|-----------------|-----------------|-----------------|
| Groups | Day 0 Malaria parasitaemia (%) | Day 4 Malaria parasitaemia (%) | Chemosuppression (%) |
|-----------------|-----------------|-----------------|-----------------|
| Normal control | 0.00 ± 0.00<sup>a</sup> | 0.00 ± 0.00<sup>a</sup> | – |
| Negative control | 45.75 ± 8.18<sup>b</sup> | 81.75 ± 2.06<sup>c</sup> | – |
| MP + 140 mg/kg b.w. of Artemether | 42.50 ± 6.45<sup>a</sup> | 10.50 ± 4.12<sup>a</sup> | 75.29 ± 3.61<sup>b</sup> |
| MP + 200 mg/kg b.w. EESAL | 48.25 ± 3.64<sup>a</sup> | 5.00 ± 0.82<sup>a</sup> | 89.64 ± 5.74<sup>b</sup> |
| MP + 400 mg/kg b.w. EESAL | 49.50 ± 4.20<sup>a</sup> | 2.00 ± 0.22<sup>a</sup> | 95.95 ± 3.71<sup>b</sup> |
| MP + 600 mg/kg b.w. EESAL | 47.75 ± 3.86<sup>a</sup> | 1.25 ± 0.50<sup>a</sup> | 97.38 ± 1.87<sup>c</sup> |

Results are expressed in Means ± SD (n = 6); At \(p < 0.05\), mean values with different letters of the alphabets (a, b, c, d ...) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different.
Table 4 Effect of the EESAL on some haematological indices in Plasmodium berghei-infected mice

| Groups                        | Haematological indices                  |
|-------------------------------|-----------------------------------------|
|                               | PCV (%)                                 |
| Normal control                | 40.75 ± 1.26<sup>b</sup>                |
| Negative control              | 33.75 ± 3.50<sup>a</sup>                |
| MP + 140 mg/kg b.w. of Artemether | 36.75 ± 3.30<sup>ab</sup>               |
| MP + 200 mg/kg b.w. EESAL     | 41.75 ± 2.36<sup>c</sup>                |
| MP + 400 mg/kg b.w. EESAL     | 43.70 ± 3.50<sup>c</sup>                |
| MP + 600 mg/kg b.w. EESAL     | 43.50 ± 1.29<sup>c</sup>                |

|                               | RBC (× 10<sup>6</sup>)                  |
|                               | 10.43 ± 0.32<sup>b</sup>                |
|                               | 8.16 ± 0.65<sup>a</sup>                 |
|                               | 9.82 ± 0.69<sup>b</sup>                 |
|                               | 10.64 ± 0.70<sup>b</sup>                |
|                               | 10.25 ± 0.06<sup>b</sup>                |
|                               | 10.61 ± 0.34<sup>b</sup>                |

|                               | WBC (×10 mm<sup>3</sup>)                |
|                               | 10,525.00 ± 25.00<sup>a</sup>           |
|                               | 10,025.00 ± 573.73<sup>a</sup>          |
|                               | 11,025.00 ± 741.08<sup>b</sup>          |
|                               | 10,275.00 ± 95.74<sup>ab</sup>          |
|                               | 14,025.00 ± 602.07<sup>c</sup>          |

|                               | Hb (g/dl)                               |
|                               | 10.86 ± 0.35<sup>c</sup>               |
|                               | 9.53 ± 0.21<sup>a</sup>                |
|                               | 11.13 ± 0.34<sup>c</sup>               |
|                               | 11.33 ± 0.46<sup>cd</sup>              |
|                               | 11.70 ± 0.20<sup>d</sup>               |

Results are expressed in Means ± SD (n = 6); At p < 0.05, mean values with different letters of the alphabets (a, b, c, d ...) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different.

RBC red blood cells, PCV packed cell volume, WBC white blood cells and Hb hemoglobin concentration.

lower chemosuppression than S. acuta [49–51]. For example, plant-derived flavonoids, alkaloids and terpenoids have been shown to exhibit potent antimalarial activities [52–54].

The observed significant (p < 0.05) reduction in total haematological status (PCV value, Hb concentration, and RBC and WBC counts) in this study agrees with the results of previous studies both in human [55–57] and rodent [58] malaria. This suppression in RBC count, PCV and Hb concentration could be linked to the invasion of erythrocytes by malaria parasites, mechanical devastation of parasitized red cells and the use of Hb as nutrient source during erythrocytic stage of the parasite life cycle [59]. These haematological aberrations may have resulted from the presence of malaria parasites in the host; malaria parasites release adhesion molecules on the surface of parasitized erythrocytes leading to vascular adhesion. It also leads to the adhesion of both parasitized and non-parasitized erythrocytes, increasing RBCs destruction by splenic clearance [60]. In addition, malaria induces dyserythropoiesis, mitigating restoration of erythropoietic balance [61]. Therefore, the elevation in haematological indices of parasitized-EESAL-treated mice showed that the administration of EESAL ameliorated alterations in haematological status induced by malaria. This ameliorative effect suggests that one or more of the phytoconstituents of the extract is haematopoietic. It could also mean that the clearance of malaria parasite boosted the drive to maintain haematological homeostasis in the host.

Table 5 Effect of the EESAL on the liver function parameters in Plasmodium berghei-infected mice

| Groups                        | Liver function markers                  |
|-------------------------------|-----------------------------------------|
|                               | ALT (IU/L)                              |
| Normal control                | 20.25 ± 2.63<sup>a</sup>                |
| Negative control              | 36.50 ± 3.42<sup>b</sup>                |
| MP + 140 mg/kg b.w. of Artemether | 25.50 ± 1.91<sup>b</sup>               |
| MP + 200 mg/kg b.w. EESAL     | 20.50 ± 1.73<sup>c</sup>                |
| MP + 400 mg/kg b.w. EESAL     | 26.25 ± 2.63<sup>b</sup>                |
| MP + 600 mg/kg b.w. EESAL     | 20.00 ± 3.74<sup>a</sup>                |

|                               | ALP (IU/L)                              |
|                               | 64.50 ± 3.42<sup>b</sup>                |
|                               | 95.25 ± 6.70<sup>b</sup>                |
|                               | 62.25 ± 4.35<sup>b</sup>                |
|                               | 67.25 ± 4.35<sup>b</sup>                |
|                               | 64.75 ± 3.50<sup>a</sup>                |
|                               | 62.25 ± 2.63<sup>a</sup>                |

|                               | AST (IU/L)                              |
|                               | 37.00 ± 2.58<sup>a</sup>                |
|                               | 73.25 ± 10.63<sup>b</sup>               |
|                               | 41.25 ± 0.96<sup>a</sup>                |
|                               | 36.75 ± 2.99<sup>a</sup>                |
|                               | 34.50 ± 3.11<sup>a</sup>                |
|                               | 35.25 ± 2.50<sup>a</sup>                |

|                               | T. bil (mg/dl)                          |
|                               | 0.45 ± 0.12<sup>b</sup>                |
|                               | 0.65 ± 0.08<sup>c</sup>                |
|                               | 0.52 ± 0.06<sup>b</sup>                |
|                               | 0.41 ± 0.08<sup>cd</sup>               |
|                               | 0.44 ± 0.02<sup>ab</sup>               |
|                               | 0.37 ± 0.06<sup>a</sup>                |

Results are expressed in Means ± SD (n = 6); At p < 0.05, mean values with different letters of the alphabets (a, b, c, d ...) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different.

AST aspartate aminotransferase, ALP alanine aminotransferase, ALP alkaline phosphatase, T. bil total bilirubin.
suggests that EESAL has normalization effect on hepatocyte integrity of parasitized mice. Hepatic damage from malaria has been linked with oxidative stress, suggesting that the modulation of hepatocytes in this study by the extract could be linked with its antioxidant phytoconstituents.

The involvement of lipids in the development and complications associated with malaria is a matter of recent scientific discussion. Alterations in lipid metabolism in malaria infection, characterized by variation in lipid profile of malaria infected persons when compared with uninfected, have been well documented [8, 11–13]. The reduction in HDL concentration and elevation in CHOL, LDL and TAGs concentration in Negative control when compared with normal control agrees with the reports of earlier studies [50, 58]. In mice infected with *P. chabaudi*, Kluck et al. [65] reported higher CHOL and lower TAGs levels when compared to control. It was further shown that this dyslipidaemia in malaria is exhibited by the inhibition of the AMPK-ACC pathway via reduced 5′AMP-activated protein kinase (AMPK) phosphorylation. The significantly lower HDL level in parasite control mice when compared with normal control might be attributed to a reduction in cholesterol transport, cholesterol esterification by lecithin cholesterol acyltransferase, and inhibition of lipid metabolizing liver enzyme by a parasite factor such as using lipids in the synthesis of haemozoin [66]. In addition, parasite activities might have inhibited HDL-synthesizing enzymes. On the other hand, the elevation in TAGs level might be attributed to an elevation in VLDL synthesis in the liver, due to liver damage as observed in this study. In addition, the hypertriglyceridaemia could be attributed to elevated secretion of TAGs in serum and a decline in its clearance by cholesterol ester transfer proteins. As suggested by Khovidhunkit et al. [67], it can be deduced from the above observations that alterations in lipid metabolism in malaria are essentially due to disease-associated mechanisms. In general, the effects of malaria infection in serum lipid status are dependent on malaria parasite species, severity of infection, history of parasite infection, and other host genetic factors. The reduction in levels of CHOL, LDL and TAGs and elevation in HDL levels of parasitized-EESAL-treated mice towards normal control showed that the phytoconstituents of the extract might have normalized the lipid status of the parasitized mice.

The increase in the extent of lipid peroxidation which was estimated by the concentration of MDA in negative control when compared with control in this study agrees

| Table 6 | Effect of the EESAL on lipid peroxidation and antioxidant parameters of *Plasmodium berghei*-infected mice |
|---|---|---|---|---|
| Groups | Lipid peroxidation and antioxidant parameters | | | |
| | MDA Conc. (mg/dl) | SOD activity (IU/L) | CAT activity (IU/L) | GSH Conc. (mg/dl) |
| Normal control | 2.68 ± 0.99b | 9.63 ± 0.42b | 0.65 ± 0.02b | 0.21 ± 0.05a |
| Malaria control | 6.05 ± 0.68c | 4.18 ± 0.38a | 0.16 ± 0.05b | 0.10 ± 0.01b |
| MP + 140 mg/kg b.w. of Artemether | 2.25 ± 0.70ab | 9.52 ± 0.74b | 0.66 ± 0.06b | 0.26 ± 0.07a |
| MP + 200 mg/kg b.w. EESAL | 2.31 ± 0.39ab | 9.94 ± 0.58b | 0.68 ± 0.18b | 0.22 ± 0.14a |
| MP + 400 mg/kg b.w. EESAL | 2.28 ± 0.30ab | 9.95 ± 0.39b | 0.72 ± 0.10b | 0.24 ± 0.05a |
| MP + 600 mg/kg b.w. EESAL | 1.54 ± 0.59a | 10.19 ± 0.19b | 0.68 ± 0.06b | 0.25 ± 0.03a |

Results are expressed in Means ± SD (n = 6); At p < 0.05, mean values with different letters of the alphabets (a, b, c, d …) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different.

*MDA* malondialdehyde, *SOD* Superoxide dismutase, *CAT* catalase, *GSH* reduced glutathione

| Table 7 | Effect of the EESAL on lipid profile of *Plasmodium berghei*-passaged mice |
|---|---|---|---|---|
| Groups | Concentration of lipid profile panels | | | |
| | LDL (mg/dl) | HDL (mg/dl) | CHOL (mg/dl) | TAG (mg/dl) |
| Normal control | 13.75 ± 3.86ab | 63.75 ± 2.63b | 77.25 ± 2.50a | 94.50 ± 5.26b |
| Malaria control | 72.25 ± 3.10d | 29.25 ± 2.50a | 99.25 ± 7.46b | 110.75 ± 2.75c |
| MP + 140 mg/kg b.w. of Artemether | 20.25 ± 2.63bc | 68.00 ± 4.32bc | 95.75 ± 4.79b | 82.00 ± 6.32a |
| MP + 200 mg/kg b.w. EESAL | 16.50 ± 1.00abc | 73.50 ± 8.19c | 94.25 ± 4.03b | 77.75 ± 8.34a |
| MP + 400 mg/kg b.w. EESAL | 18.00 ± 2.16bc | 70.75 ± 5.10c | 92.00 ± 3.56b | 81.00 ± 4.08a |
| MP + 600 mg/kg b.w. EESAL | 13.00 ± 2.94a | 73.75 ± 5.44c | 94.25 ± 5.12b | 82.25 ± 9.12a |

Results are expressed in Means ± SD (n = 6); At p < 0.05, mean values with different letters of the alphabets (a, b, c, d …) as superscripts in a column are statically different while mean values with similar alphabets are not statistically different.

*CHOL* total cholesterol, *HDL* high density lipoprotein, *TAGs* triacylglycerols, *LDL* low density lipoprotein
with an earlier study in human malaria patients. In agreement with our findings, a reduction in antioxidant status (activities of SOD and level of vitamin C) when compared to healthy control was reported in *P. falciparum* infected Yemen patients [68]. Similarly, Fabbri et al. [69] showed that *P. vivax* malaria patients have elevated lipid peroxidation status (MDA level) and reduced antioxidant status than healthy control. Similarly, the lower SOD and CAT activities in Negative control mice than normal control is consistent with the findings of D’Souza et al. [70] who also reported that the reduction in the activities of the above antioxidant enzymes was more in *P. falciparum* malaria patients than *P. vivax* malaria patients. It was also reported that the increase in MDA level in *P. falciparum* malaria patients was much more when compared to *P. vivax* malaria patients [71]. In this study, the significant \( p < 0.05 \) decrease in the MDA concentration and elevation in the activities of antioxidant enzymes of parasitized mice treated with EESAL compared with Negative control mice could be explained in two ways. Firstly, the extract might be rich in mineral contents that serve as cofactors to these enzymes and hence, increasing their activities. In addition, the phytoconstituents of the extract such as flavonoids which have antioxidant effects could have limited the level of lipid peroxidation. Selenium has been shown to boost antioxidant status and reduce the level of lipid peroxidation in malaria [72]. This is also supported by an earlier study [73] that vitamin E ameliorated *P. berghei*-induced anaemia and pathological alterations in serum biochemistry.

In a recent study, Ajayi et al. [74] showed that extracts of *Vernonia amygdalina* and *Tithonia diversifolia* at 400 mg/kg suppressed *P. berghei* in mice by 75% and 66%, respectively which were lower than 95.95% recorded in the present study. In addition, Muluye et al. [75] showed that root extract of *Euphorbia abyssinica* at 200, 400 and 600 mg/kg respectively gave 66.87%, 84.94% and 93.69% chemosuppression of *P. berghei* infection in mice which were however, lower than the suppression by *S. acuta* extract in the present study. A similar lower chemosuppression malaria parasite (71.93%, 80.97% and 85.64% at 100, 200, and 400 mg/kg) was recorded by *Terminalia mantaly* stem bark water extract [76]. Furthermore, Biruk et al. [77] reported that at 400 mg/kg each, aqueous-methanol and water extracts of *Terminalia brownie* bark had lower chemosuppression (61.2 and 51.1%, respectively) compared with the results of this study. The higher malaria chemosuppression by EESAL relative to many other plant extracts, and its ability to abrogate alterations in hematology and biochemical status of malaria-passaged mice has made a potential source of new antimalarial agent(s). Interestingly, some *Sida* secondary metabolites including stigmasterol and its derivative, stigmasterol-3-O-β-D-glucopyranoside, have been shown to exhibit antimalarial properties [53, 78]. Similarly, the presence of high levels of flavonoids, alkaloids, terpenoids and tannins which have been known to have antimalarial and chemoprotective activities could be responsible for the findings of the present study [27, 79, 80]. This study has some limitations such as the inability to track the specific compounds responsible for the antimalarial activities of EESAL and characterize them and inability to evaluate the specific mechanism of killing the parasites by EESAL. Hence, this study is considered a preliminary study which has opened the door for further in-depth investigation of the active ingredients through activity-guided fractionation and characterization using chromatographic and spectroscopic techniques which are currently going on in our laboratory. However, findings of this study generally demonstrate that EESAL has antimalarial property, modulates oxidative stress, and rescues haematological aberrations and abrogated dyslipidemia and hepatic dysfunction associated with malaria.

**Conclusion**

From the above results, it can be concluded that by reducing parasite density in infected mice, EESAL exhibits antimalarial properties. The findings also showed that EESAL normalized the haematological and some biochemical status altered by malaria. This suggests that the plant is a potential reserve of novel antimalarial agents; hence, isolation of specific components of the extract responsible for the above biological activities using bioassay-guided fractionation is warranted. In addition, the specific molecular mechanism(s) of action of the extract and its active compounds are unknown and hence, recommended for further investigations.

**Abbreviations**

EESAL: Ethanol extract of *Sida acuta* leaves; PCV: Packed cell volume; Hb: Haemoglobin; RBC: Red blood cell; WBC: White blood cell; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; MDA: Malondialdehyde; CHOL: Cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TAG: Triacylglycerol/triglyceride; LD₅₀: Median lethal dose; CAT: Catalase; GSH: Reduced glutathione; SOD: Superoxide dismutase; PVs: Polyvinyl sulphone; SD: Standard deviation; PK: 5’AMP-activated protein kinase

**Acknowledgements**

Not applicable.

**Authors’ contributions**

Conceptualization, OCE and CCA; data curation and analysis, IUO, PCO, CAN, TCU, EAA, ACN and BCC; writing—original draft preparation, OCE, CCA and IUO; writing—review and editing, IUO and CCA. All authors have read and agreed to the published version of the manuscript.

**Funding**

Not applicable.

**Availability of data and materials**

Available from the corresponding author upon reasonable request.
Declarations

Ethics approval and consent to participate
The study protocols were approved by the Faculty of Biological Sciences Committee on Research Ethics, University of Nigeria, Nsukka (UNN/FBS/EC/102). Other national and international guidelines for the care and use of animals for research were strictly followed in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Biochemistry, Ethnomedicinal and Pharmacological Biochemistry Unit, University of Nigeria, Nsukka, Enugu State, Nigeria.
2Department of Biochemistry, Medical Parasitology, Toxicology and Drug development Unit, University of Nigeria, Nsukka, Enugu State, Nigeria.

References

1. World Health Organization (WHO). 2017 world malaria report factsheet. Geneva; 2018. https://www.who.int/who-news-room/fact-sheets/detail/malaria. Accessed 28 Sept 2019.

2. World Health Organization (WHO). World malaria report 2019. 2019. https://www.who.int/malaria/publications/world-malaria-report-2019/en/. Accessed 5 Feb 2020.

3. Fairhurst RM, Dondorp AM. Artemisinin-resistant Plasmodium falciparum malaria. Microbiol Spectr. 2006;4(3):12–21.

4. Suresh N, Haldar K. Mechanisms of artemisinin resistance in Plasmodium falciparum. Curr Opin Pharmacol. 2018;42:46–54. https://doi.org/10.1016/j.coph.2018.06.003.

5. Luo Y, Che M, Liu C, et al. Toxicity and related mechanisms of Plasmodium–Volta regional hospital, Ghana. Adv Hematol. 2020;2019:9369758:1–6. https://doi.org/10.1155/2020/9369758.

6. Sakzabre D, Asiamah EA, Akorsu EE, Abaka-Yawson A, Dika ND, Kwasie DA, et al. Ethnomedicinal, phytochemical and anti-malarial activities of Ocimum gratissimum and Sida acuta. African J Tradit Complement Altern Med. 2015;26(2):209–15. https://doi.org/10.1016/j bjpa.2015.09.011.

7. Enechi E, Akpabio BN, Akpabio AN, et al. Ethnomedicinal, phytochemical and anti-malarial activities of Ocimum gratissimum and Sida acuta. Afr J Tradit Complement Altern Med. 2015;12(1):61–5. https://doi.org/10.1016/j.ajcp.2014.03.007.

8. Agbodeka K, Gbekley HE, Karou SD, Anani K, Agbonnon A, Tchacondo T, et al. Ethnobotanical study of medicinal plants used for the treatment of malaria in the plateau region, Togo. Phcog Res. 2016;8(5):12–8. https://doi.org/10.4103/0974-8490.178246.

9. Abat JK, Kumar S, Mohanty A. Ethnomedicinal, phytochemical and ethnopharmacological aspects of four medicinal plants of Malvaceae used in Indian traditional medicines: a review. Medicines. 2017;4(4):75. https://doi.org/10.3390/medicines4040075.

10. Builders MI. African traditional antimalarials: a review. Pharm Chem J. 2017;4(6):78–97.

11. Ignacimuthu S, Ayyanar M, Sankara-Siramann K. Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India). J Ethnobiol Ethnomed. 2006;2(1):25–31. https://doi.org/10.1186/1742-4699-2-25.

12. Aanith N, Murugan K, Madhiyazhagan P, et al. Studies on the effect of Sida acuta and Vernonia zinnioides against the malarial vector, Anopheles stephensi and malarial parasite, Plasmodium berghei. Int J Pure Appl Zool. 2014(2):1:51–60.

13. Banouzi JT, Prado R, Menan H, Valentin A, Roumestan C, Malliè M, et al. Studies on medicinal plants of Ivory Coast: investigation of Sida acuta for in vitro antiplasmodial activities and identification of an active constituent. Phytomedicine. 2004;11(4):338–41. https://doi.org/10.1078/09447110495245.

14. Konaté K, Bassolé HN, Hilou A, et al. Toxicity assessment and analgesic activity investigation of aqueous acetone extracts of Sida acuta Burm f. and Sida cordifolia L. (Malvaceae), medicinal plants of Burkina Faso. BMC Complement Altern Med. 2012;12:120. https://doi.org/10.1186/1472-6882-12-120.

15. Mbaijuka CS, Obuegu B, Ochê KC, Nnadi DC. The antibacterial activity of leaf extracts of Ocimum gratissimum and Sida acuta. Int J Microbiol Res. 2014(5):2:124–9. https://doi.org/10.14713/ijmr.2014.1249.

16. Krishnaveni A, Ezhilarsan B, Iyappan A, Sathali AAH. Preliminary phytochemical screening and in vitro antioxidant activity of Sida acuta Burm. Int J Res Pharmaco Pharmacother. 2018;2(1):157–65.

17. Babaliche P, Gubba P. Variation in common serum lipid parameters in patients with malaria in Indian traditional medicines: a review. Medicines. 2017;4(4):26–9. https://doi.org/10.3390/medicines4040075.

18. Builders MI. African traditional antimalarials: a review. Pharm Chem J. 2017;4(6):78–97.

19. Ignacimuthu S, Ayyanar M, Sankara-Siramann K. Ethnobotanical investigations among tribes in Madurai District of Tamil Nadu (India). J Ethnobiol Ethnomed. 2006;2(1):25–31. https://doi.org/10.1186/1742-4699-2-25.

20. Aanith N, Murugan K, Madhiyazhagan P, et al. Studies on the effect of Sida acuta and Vernonia zinnioides against the malarial vector, Anopheles stephensi and malarial parasite, Plasmodium berghei. Int J Pure Appl Zool. 2014(2):1:51–60.

21. Banouzi JT, Prado R, Menan H, Valentin A, Roumestan C, Malliè M, et al. Studies on medicinal plants of Ivory Coast: investigation of Sida acuta for in vitro antiplasmodial activities and identification of an active constituent. Phytomedicine. 2004;11(4):338–41. https://doi.org/10.1078/09447110495245.

22. Konaté K, Bassolé HN, Hilou A, et al. Toxicity assessment and analgesic activity investigation of aqueous acetone extracts of Sida acuta Burm f. and Sida cordifolia L. (Malvaceae), medicinal plants of Burkina Faso. BMC Complement Altern Med. 2012;12:120. https://doi.org/10.1186/1472-6882-12-120.

23. Visser BJ, de Vries SG, Vingerling R, et al. Serum lipids and lipoproteins in children with malaria. Curr Opin Pharmacol. 2018;42:46–54. https://doi.org/10.1016/j.coph.2018.06.003.

24. Babaliche P, Gubba P. Variation in common serum lipid parameters in patients with malaria in Indian traditional medicines: a review. Medicines. 2017;4(4):26–9. https://doi.org/10.3390/medicines4040075.

25. Balbiche P, Gubb P. Variation in common serum lipid parameters in patients with malaria: a 1-year cross-sectional study. J Curr Res Sci Med. 2019;5(1):39–43. https://doi.org/10.4103/jcrsm.jcrsm_1_19.

26. Sirak S, Fola AA, Worku L, Biadgo B. Malaria parasitemia and its association with lipid and haematological parameters among malaria-infected patients attending at Metemma Hospital, Northwest Ethiopia. Pathol Lab Med Int. 2016;8:43–50. https://doi.org/10.2147/PLML.S119846.

27. Visser BJ, de Vries SG, Vingerling R, et al. Serum lipids and lipoproteins during uncomplicated malaria: a cohort study in Lambaréné, Gabon. Ann J Trop Med Hyg. 2017;66(5):1205–14. https://doi.org/10.4269/ajtmh.16-0721.

28. Chikezie CP, Okpara RT. Serum lipid profile and hepatic dysfunction in moderate Plasmodium falciparum infection. Sci J Microbiol. 2013;28(8):158–65.

29. Dungdung A, Jalavadi VM, Yadav UP. Study of serum lipid profile in Plasmodium falciparum malaria. Int J Res Med Sci. 2018;6(1):3673–7. https://doi.org/10.18203/2320-6012.ijmr20184428.

30. Omiradegun AE, Omiradegun BE. Serum apolipoprotein-A1 and cholesterol levels in Nigerian children with Plasmodium falciparum infection. Med Princ Pract. 2015;24(4):318–24. https://doi.org/10.1159/000430812.
79. Ntie-Kang F, Onguéné PA, Lifongo LL, Ndom JC, Sippl W, Mbaze LM. The potential of anti-malarial compounds derived from African medicinal plants, part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. Malar J. 2014;13(1):81. https://doi.org/10.1186/1475-2875-13-81.

80. Batista R, Júnior AJS, de Oliveira AB. Plant-derived antimalarial agents: new leads and efficient phytomedicines. Part II. Non-alkaloidal natural products. Molecules. 2009;14(8):3037–72. https://doi.org/10.3390/molecules14083037.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.