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Original article

Vitex doniana, in Vitro Antioxidant, Membrane Stabilization Potential and Protective Impact Against *Plasmodium berghei*-Passaged Mice

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Abstract

Background and objectives: *Vitex doniana* Sweet (Lamiaceae) is used to treat various ailments, including respiratory infections, liver diseases, anaemia and jaundice. This study assessed the in vitro antioxidant and membrane stabilization potential, as well as the protective impact of semi-purified solvent fractions of *V. doniana* leaves against *Plasmodium berghei*-passaged mice. **Methods:** Dried leaves were extracted with ethanol, followed by fractionation using a solvent-gradient system of increasing polarity (hexane, ethyl acetate, and methanol), and the concentrated fractions were obtained. Forty-two mice were randomly divided into seven groups as: group 1 (normal control), group 2 (disease control, untreated), while groups 3 to 7 received the standard drugs (artequick and chloroquine) and combined *V. doniana* fraction (VDF, 100 mg/kg) at varying ratios. **Results:** Comparatively to *V. doniana* extract, the fractions (F6, F8) displayed considerable antioxidant activity by scavenging $O_2^{\bullet-}$, $OH^{\bullet}$ and DPPH radicals, and effectively reduced Fe^{3+} to Fe^{2+}. VDF (1:1) at different concentrations (200, 400, 600 µg/mL) inhibited erythrocyte haemolysis by 91.29±3.61%, 80.52±0.13%, 75.68±1.45% and 80.57±0.94%, respectively. Also, the VDF in synergy with artequick and chloroquine decreased parasitaemia levels by 4.25±0.25% and 4.65±0.28% compared to the disease control (7.93±1.61%). The combined fractions significantly normalized the plasma calcium concentration (1.85±0.17 mg/dL, 1.65±0.21 mg/dL, 1.72±0.23 mg/dL, 1.65±0.22 mg/dL) for groups 3 to 6 compared to the disease control (1.30±0.09 mg/dL), while the bodyweights presented no significant change in all experimental groups. **Conclusion**: The results indicate the promising potential of *V. doniana* as a drug candidate in managing malarial infection.

Keywords: antioxidant; membrane stability; mice; *Plasmodium berghei*; *Vitex doniana*

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Introduction

Malaria is a vector-borne parasitic infection caused by *Plasmodium* species and remains a deleterious infectious disease worldwide [1,2]. The disease mainly resulted from five *Plasmodium* species: *P. falciparum, P. vivax, P. ovale, P. malariae, and P. knowlesi.* From epidemiological studies, differences in the patterns of malaria cases, disease severity, and mortality rate exist across diverse geographical regions [3]. In 2019, the world recorded 229

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million malaria cases, resulting in 409,000 deaths. Although malaria cases are reported in 87 countries globally, Africa had about 94% of the total malaria cases and deaths, especially in sub-Saharan Africa [4]. The disease claims more than 400 thousand lives each year, largely in Africa, and children below 5 years old are the most vulnerable and die from this curable disease [5]. With a record of 60 million malaria cases, Nigeria has contributed 27% of malaria cases and 23% of malaria deaths worldwide, the highest rate among malaria-endemic countries. Both malaria cases and deaths increased in 2019 compared to 2018, a trend that is normally observed in countries with high malaria incidences [4].

The pathophysiology of malaria has been suggested to be in part influenced by oxidative stress [6]. To maintain cellular integrity, red blood cells infected with the *Plasmodium* parasite must eliminate reactive oxygen species (ROSs). The parasite produces a large number of redox-active species during its growth and multiplication. By using these redox-active compounds, the parasite produces free heme (Fe/ferroporphyrin IX-FP), as well as reactive oxygen and nitrogen species (ROSs, RNSs). Additionally, the host immune system produces ROS during malaria infection, contributing significantly to oxidative stress [7]. Antioxidants are molecules that donate electrons to electron-deficient compounds or molecules, protecting them from the damaging effects of reactive oxygen species (ROS) [8]. It is widely known that plants contain antioxidant molecules, especially those that have high amounts of phenolic compounds. Hence, natural antioxidants from foods, particularly fruits and vegetables, are becoming increasingly studied. Studies on animals have shown that plants and compounds with antioxidant activity may ameliorate malarial infection from progressing by inhibiting parasite growth and preventing subsequent complications [9].

The use of natural and traditional medicines is widespread in malaria-endemic countries [10]. Plant-based bioactive compounds have remained a major source of biologically active compounds, and indigenous remedies have developed many significant drugs [11]. In recent times, the scientific validity of several medicinal plants for malaria treatment has been established by in vitro or in vivo tests [12]. In Nigeria, *Vitex doniana* Sweet (Lamiaceae), commonly known as black plum (Figure 1), is a common herbal condiment and contains potential drug compounds. In different regions of Nigeria, the local preparations of *V. doniana* are used as medications to treat various diseases, including liver disease, anemia, and jaundice [13,14]. Studies have shown that *V. doniana* possesses properties such as antimicrobial, induction of aphrodisiac, analgesic and anti-inflammation, antioxidant, hypoglycaemic, anti-hypertensive, as well as anti-hyperglycaemic and anti-hyperlipidaemic activities [15-18]. Generally, the important steps to utilize biologically active compounds from plant sources are extraction, pharmacological screening, isolation and characterization of bioactive compounds, toxicological, and clinical evaluation [19]. Therefore, investigating the pharmacological properties of natural products from herbal plants may pave way for discovering novel compounds [20,21]. Hence, the present study seeks to evaluate the in vitro antioxidant and membrane stabilization potential of *V. doniana* leaves fractions, as well as its anti-plasmodial impact against *P. berghei* infection.

Figure 1. *Vitex doniana* leaf (photograph by authors)

Material and Methods

Ethical considerations

All animal experiments were conducted in accordance with the US National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996) and were approved by the Institutional Animal Research Ethics Committee, University of Agriculture Makurdi, Nigeria (BCH/UAM/21/0008).

Chemicals

The chemicals used in this study were of analytical grade, and were purchased from Sigma-Aldrich® (St. Louis, MO, USA).

Plant material

Fresh *Vitex doniana* Sweet leaves were collected randomly from the site in Makurdi, Benue State, in 2021. They were identified at the Department of Botany, Federal University of Agriculture, Makurdi, Nigeria and authenticated at the International Centre of Ethnomedicines and Drugs Development, Nigeria (voucher specimen InterCEDD/207). The fresh leaves were shade-dried for several days and pulverized into fine powder. The pulverized material (500 g) was macerated in ethanol (2.5 L) for 48 hours, filtered with a Whatman No. 1 filter paper and the filtrate was concentrated using a rotary evaporator (I.K.A., Germany) at an optimum temperature of 40 °C. The extraction process was repeated thrice more. Using SiO_2 gel column (100-200 mesh), the *Vitex doniana* crude extract was column partitioned and eluted using a solvent gradient-system ($CH_3(CH_2)_4CH$, EtOAc and MeOH) of increasing polarity (Table 1). *Vitex doniana* fractions (F6, F8), which gave the best results in the preliminary activity-guided study, were concentrated and used in this study.

Table 1. Solvent-gradient for column chromatography

Solvent system	Ratio	Volume (mL)	Fraction
Hexane	100%	200	1
Hexane: ethyl acetate	75%: 25%	200	2
Hexane: ethyl acetate	50%: 50%	200	3
Hexane: ethyl acetate	25%: 75%	200	4
Ethyl acetate	100%	200	5
Ethyl acetate: methanol	75%: 25%	200	6
Ethyl acetate: methanol	50%: 50%	200	7
Ethyl acetate: methanol	25%: 75%	200	8
Methanol	100%	200	9

Animals

Swiss albino mice weighing between 20 g and 28 g of either sex were used for the study. The animals were housed at 25±2 °C under a 12-h light/dark cycle maintained on standard chick grower's pellet (Guinea Feed Nigeria, Ltd.) with free access to water ad libitum. The animals were allowed to acclimatize for 14 days prior to the study.

In vitro antioxidant study

The extract and fractions (F6, F8) of *V. doniana* leaves were evaluated for antioxidant activity using established in vitro models. The scavenging activity of DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals was determined using the method described by Chowdhury et al. [22]. The superoxide radical scavenging activity was determined by the method described by Kavimani et al. [23]. The hydrogen peroxide free radical scavenging activity was determined by the previously described method of Gulcin et al. [24]. The ferric-reducing power capacity was determined by the method described by Benzie and Strain [25].

In vitro membrane stabilizing study

The membrane stabilizing activity of combined *V. doniana* fractions (VDF) of F6 and F8 (1:1, w/w) was evaluated following the previously described method [26]. In brief, twelve centrifuge tubes containing 5 mL graded doses (200, 400 and 600 µg/mL) of *V. doniana* fractions dissolved in isotonic phosphate buffer solution were arranged in quadruplicate sets (4 sets per dose). Then, 0.5 mL of healthy human erythrocytes suspension was added to each tube and gently mixed. The control tubes contained 5 mL of RBCs mixed with isotonic phosphate buffer solution and 5 mL of 10 µg/mL of piroxicam, respectively. A pair of tubes was incubated at 54 °C for 20 min in a regulated water bath, whereas the other pair was maintained at −4 °C in a freezer for 20 min. Thereafter, the tubes were centrifuged at 3000 g for 10 min and absorbance of the supernatant was recorded at 540 nm using the spectrophotometer. The percentage inhibition of hemolysis was calculated using the following equation:

$$\% \text{ Inhibition of haemolysis} = 1 - \frac{\Delta \text{ absorbance of test sample (heated- unheated)}}{\Delta \text{ Absorbance of control sample (heated)-test sample (unheated)}} \times 100$$

Anti-plasmodial study

Based on the in vitro findings, VDF (F6 and F8, 1:1, w/w) was investigated further in vivo to establish the protective impact against *P. berghei* infection using the method described by Ibezim et al. [27]. In brief, the chloroquine-sensitive strain, *Plasmodium berghei* (Nk65) was obtained from the Department of Parasitology, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria, Nigeria, and was kept under standard conditions prior to experiment. Forty-two infected mice were randomly divided into 7 groups (n=6) and treated as shown below:

Group 1: normal control; group 2: disease control (untreated); group 3: received artequick (artemisinin- 62.5mg + piperaquine-375 mg) + VDF (100 mg/kg) for 7 days; group 4: received chloroquine (1000 mg/kg) + VDF (100 mg/kg) for 7 days; group 5: received artequick

(artemisinin- 62.5mg + piperaquine-375 mg) for 7 days; group 6: received chloroquine tablet (1000 mg/kg) for 7 days; group 7**:** received VDF (100 mg/kg) for 7 days.

Preparation of the inoculum from donor mice with *P. berghei* parasitaemia was established by microscopic examination of a thin blood film under oil immersion at x100 magnification and measured as percentage erythrocytes [28]. Each mouse was infected with an inoculum of approximately 10^7 parasitized erythrocytes suspension in normal saline (0.3 mL) from a donor mouse. Animals were treated for 7 consecutive days and were monitored with constant checking of the percentage of parasitemia after one day intervals. Artequick (artemisinin- 62.5mg + piperaquine-375 mg) and chloroquine tablet (1000 mg/kg) were used as the standard control of the experiment. All treatments were given orally by using a standard intragastric tube.

Determination of percentage parasitized erythrocytes

A thin smear of blood film was made from blood collected from the tail of each mouse. The smears made on the microscopic slides were fixed in methanol and stained with Giemsa, pH 7.2. The numbers of parasite erythrocytes in each field were determined thrice, and the average was used to calculate the percentage parasitaemia level of each mouse.

The percentage value of the parasitized red cells was calculated as follows:

$$\text{Percentage parasitized} = \frac{\text{Total number of infected erythrocytes}}{\text{Total number of uninfected erythrocytes}} \times 100$$

Estimation of plasma calcium concentration

The calcium ion concentration (Ca^{2+}) was determined using the modified Arsenazo III method, as previously described [29]. Reagent 1(O-cresolphthalinecomplexone 0.16 mM/L, 8-hydroxyquinoline 17 mM/L, and preservative) and reagent 2(amino-2-methyl-1-propanol (AMP) buffer (pH 10.4) at 500 mM/L) are mixed in equal proportion for working a reagent. Then 1 mL of working reagent was pipetted into three clean, dry tubes as blank, standard, and test samples. Afterwards, 10 µL of calcium standard and test samples were added into the respective test tubes keeping the blank with distilled water and mixed properly. The mixture was incubated at 37 ºC for 5 min. The absorbance of test samples and standard were measured against the reagent blank at the wavelength of 630 nm; hence, the calcium concentration was calculated from the formula:

$$\text{Plasma calcium concentration (mg/dL)} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times 100$$

Statistical analysis

Results were expressed as mean ± SEM using the Statistical Product and Service Solutions (SPSS) software (version 21.0, SPSS Inc., USA). This was followed by the one-way Analysis of variance (ANOVA) to determine differences between means, and the new Duncan's multiple range test was used for multiple comparisons of the means [24]. Values of $p<0.05$ were considered significantly different from each other.

Results and Discussion

Figure 2 depicts the antioxidant activity of extract and fractions of *V. doniana* leaves. In the DPPH assay, the *V. doniana* extract scavenged free radicals by 13 %, followed by 18 % and 14.2% for F6 and F8, respectively. The combined *V. doniana* fractions (VDF) of F6 and F8 (1:1, w/w) showed an increase in electron-donating abilities with FRAP values of 606.3 µMFe^{2+} and 651.5 µMFe^{2+}, respectively, compared to the extract (393.9 µMFe^{2+}). Results showed $OH^{\bullet}$ scavenging activity of 18.7% for the extract, 39% and 19.3% for F6 and F8, respectively. F6 and F8 showed $O_2^{\bullet-}$ radical scavenging activity of 34.0% and 23.4%, respectively, compared to the extract (18.7%).

With a FRAP value of 1137.4 µMFe^{2+}, VDF showed the highest electron donation and radical scavenging ability against DPPH (42.8%), $OH^{\bullet}$ (49.2%), and $O_2^{\bullet-}$ (48.4%). Table 2 depicts the membrane stabilizing effect of fractions of *V. doniana* leaves. *Vitex doniana* fractions showed marked protection against heat-induced lysis of human erythrocyte membranes, as demonstrated by the high percentage of haemolysis inhibition. VDF at different concentrations (200, 400, and 600 µg/mL) showed haemolysis inhibition (%) of 80.52±0.13, 75.68±1.45 and 80.57±0.94, respectively, while the standard, piroxicam exhibited the highest inhibition of heat-induced erythrocyte haemolysis by a factor of: 91.29±3.61.

Table 3 depicts the effect of fractions of *Vitex doniana* leaves on percentage parasitaemia in *P.*

berghei-passaged mice. The parasite load of *V. doniana* fractions treated mice significantly ($p<0.05$) reduced compared to the disease control (group 2). However, groups 3 and 4, which constituted artequick and chloroquine in combination with VDF, exhibited the highest ($p<0.05$) reduction in parasite load compared with disease control. There was a significant ($p<0.05$) increase in plasma calcium concentration in the treated groups compared to the disease control (group 2). No significant ($p>0.05$) changes were observed in the body weight of the treated animals compared with the control group.

Our study clearly demonstrated considerable antioxidant potential and protective impact of *V. doniana* leaves against *P. berghei* infection in mice. Generally, research conducted so far has revealed the plant as one of the medicinally important plants utilized in folklore medicine to treat various disease conditions in Nigeria. This may be attributed to the presence of various functional components; hence, the observed effects are a consequence of the synergistic action of these components.

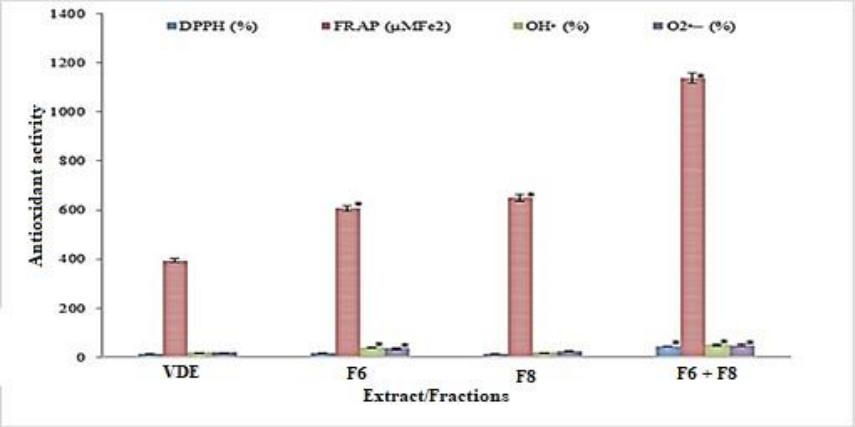

Figure 2. In vitro antioxidant activity of *Vitex doniana* leaf extract and fractions. Results are expressed as mean ± SD of three replicates; $p< 0.05$ when compared with *V. doniana* extract (VDE); *Vitex doniana* fractions (F6 and F8).

Table 2. Effect of *Vitex doniana* leaves fractions on heat-induced haemolysis of HRBCs

Treatment	Concentration (µg/mL)	Inhibition of haemolysis (%)
ontrol	-	-
Piroxicam	10	91.29±3.61[a]
VDF	200	80.52±0.13[b]
	400	75.68±1.45[b]
	600	80.57±0.94[b]

Values are presented as mean±SEM of three replica determinations. Different superscripts down the column are significantly ($p<0.05$) different compared to control. VDF: combined *V. doniana* fractions of F6 and F8 (1:1, w/w).

Table 3. Effect of *Vitex doniana* leaves fractions on percentage parasitaemia, plasma calcium concentration and body weight in *Plasmodium berghei*-passaged mice

Groups	Parasitaemia (%)	Plasma calcium (mg/dL)	Body weight (g)
Group 1	-	1.80±0.20[a]	29.00±1.47[a]
Group 2	7.93±1.61[b]	1.30±0.09[b]	25.50±1.89[a]
Group 3	4.25±0.25[a]	1.85±0.17[a]	29.00±3.24[a]
Group 4	4.65±0.28[a]	1.65±0.21[a]	29.00±0.41[a]
Group 5	5.23±0.08[a]	1.72±0.23[a]	26.25±1.25[a]
Group 6	5.47±0.03[a]	1.65±0.22[a]	24.75±1.38[a]
Group 7	5.08±0.13[a]	1.43±0.25[ab]	29.00±2.74[a]

Results are means ± SEM of three replicate determinations. Values with different superscripts down the column are significantly ($p<0.05$) different. Group 1 = normal control; group 2 = disease control; group 3 = artequick + VDF; group 4 = chloroquine + VDF; group 5 = artequick; group 6 = chloroquine; group7 = combined *V. doniana* fractions (VDF) of F6 and F8 (1:1, w/w).

Antioxidants play a crucial role in neutralizing and scavenging free radicals, protecting against infections and degenerative diseases [30]. According to Gazdik et al. [31], the intake of high amounts of flavonoids, or compounds with antioxidant, antiproliferative, and anti-inflammatory activities, has a positive impact on human health, especially in the prevention of cancer and inflammatory diseases. The antioxidant activity of the fractions of *V. doniana* leaves was assessed in various in vitro models. DPPH, known to be a stable free radical, is widely accepted as a means for estimating antioxidants' free radical scavenging ability. DPPH receives an electron or hydrogen radical to assume a stable diamagnetic molecule [32]. The result showed the DPPH scavenging activity of fractions of *V. doniana* leaves. Superoxide radicals are produced in vivo and are harmful to cellular organelles and are precursors to other reactive species [33]. The result showed fractions of *V. doniana* leaves as scavengers of superoxide radicals. Similarly, hydrogen peroxide is among the most reactive oxygen radicals and induces severe damage to adjacent biomolecules [34]; therefore, the removal of hydroxyl radical is important for antioxidant defense in the cell, as demonstrated by fractions of *V. doniana* leaves. Iron (Fe^{3+}) reduction is often used as an indicator of electron-donating activity; hence, an important mechanism of antioxidant action as previously reported [35]. Thus, this study demonstrated the transformation of Fe^{3+} to Fe^{2+} by fractions of *V. doniana* leaves, which correlates strongly with its observed antioxidant properties.

Membrane stabilizing assessment evaluates the stability of the erythrocyte membrane to osmotic stress, which may be internal or external [36]. This study evaluated membrane permeability as a potential biomarker of oxidative membrane damage in pathologic conditions and xenobiotic-induced oxidative membrane damage in erythrocytes [37].

Plasmodium parasite remodeling during intraerythrocytic development alters the host RBC physiology, leading to increased permeability and haemolysis. In the case of *P. falciparum*, the change includes disruption of the cytoskeleton by proteins inserted into RBC membranes and an increase in plasma membrane permeability which leads to the spherical shape of the infected RBCs [38]. The structural change compromised the integrity of the host RBC and thereby reduced the osmotic stability of *Plasmodium*-infected RBCs, as observed in this study. The consequence of this reduction is an increased tendency of infected cells to be destroyed in circulation via splenic clearance and intravascular haemolysis [39,40]. Therefore, compounds capable of membrane stabilization are potent anti-inflammatory agents; hence, membrane stabilization assay is a means to investigate the anti-inflammatory abilities of biological compounds [28]. The present study observed a significant reduction in osmotic inhibition of RBC membrane haemolysis, using varying concentrations of fractions of *V. doniana* leaves. According to Anosike et *al*. [41] and Amin et al. [42], the methanol extract of garden egg (*Solanum aethiopicum*) and *Gardenia coronaria* leaves, respectively, possess anti-inflammatory activity through mechanisms of membrane stabilization. Furthermore, the in *vitro* anti-inflammatory effect of extracts of *Polyalthia longifolia* leaves was reported to ameliorate membrane fragility [43]. The above studies attest to the protection of the human erythrocyte membrane from induced heat haemolysis as observed in this study. Hence, the fraction of *V. doniana* leaves possessed anti-inflammatory properties.

For *Plasmodium* infection, the key event in the life cycle is regulated by changes in cellular levels of calcium ions, which link closely with other second intracellular messengers to form signaling networks that control important processes during pathogenesis and mosquito transmission [44]. From studies, hypocalcaemia has a prognostic correlation with malaria as it indicates complicated malaria or higher parasitaemia, and its return to normal plasma levels may indicate clinical recovery and parasite clearance [45]. In this study, the plasma calcium concentration increased in the *V. doniana* treated groups compared to the control group regarding the level of parasite load. The reduction in calcium levels in the disease control could be attributed to the trophozoites form of the parasite that accumulates calcium in its internal compartment for metabolism and gliding movement or is due to digestive and renal complications arising from malaria and an enhanced influx of calcium ions into the intracellular compartment by the *Plasmodium* parasite. The molecular process adopted by fractions of *V. doniana* leaf extract to prevent

calcium reduction in the treated groups could be adduced to the prevention of membrane haemolysis. Literature review has shown calcium ion operation as an intracellular indicator for different growth factors and hormones; hence, its homeostatic disturbance results in overload leading to alteration of cell function and death. Normally, calcium ion homeostasis is sustained by keeping cellular transport and compartmentalization systems in sync [46]; however, impairment of the processes during cell injury as observed in heat-induced membrane haemolysis results in enhanced calcium ion influx, release of calcium from intracellular storage, and inhibition of calcium ion extrusion at the plasma membrane [47]. Hence, an uncontrolled sustained rise in cytosolic calcium ion concentration differs from the temporary rapid physiological increase in response to hormones.

Conclusion

In vitro studies demonstrated that *V doniana* leaf fractions exhibited antioxidant properties and anti-inflammatory effects via membrane stabilization. In combination with artequick and chloroquine, *V. doniana* fractions synergistically enhanced plasma calcium concentration and reduced levels of plasmodium parasites. The findings indicate that the plant offers promising potential as a drug candidate for malarial infection. Further pharmacological studies are needed to determine the precise nature of the active constituents and the mechanisms underlying the reported action.

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Author contributions

Joshua Parker Elijah and Nwodo Okwesilieze Fred Chitelugo developed the ideas and supervised the study; Okpe Oche and Obi Bonaventure Chinonso performed the experiments, collected data, and wrote the first draft of the manuscript; Joshua Parker Elijah and Nwodo Okwesilieze Fred Chitelugo reviewed the first draft of the manuscript; all authors approved manuscript finalization.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

References

[1] Burda PC, Caldelari R, Heussler VT. Manipulation of the host cell membrane during *Plasmodium* liver stage egress. *mBio*. 2017; 8(2): 1–12.

[2] Arora N, Anbalagan LC, Pannu AK. Towards eradication of malaria: is the WHO's RTS,S/AS01 vaccination effective enough? *Risk Manag Healthc Policy*. 2021; 14: 1033–1039.

[3] Al-Awadhi M, Ahmad S, Iqbal J. Current status and the epidemiology of malaria in the Middle East region and beyond. *Microorganisms*. 2021; 9(2): 1–20.

[4] World Health Organization. World malaria report: 20 years of global progress and challenges. Geneva: World Health Organization, 2020.

[5] Okpe O, Agada EE, Okoduwa SIR, Upev VA, Micheal AS. Aqueous stem bark of *Mangifera indica* suppresses parasitemia and ameliorates anaemia in mice model of malarial infection. *Maleys J Biochem Mol Biol*. 2021; 3(12): 105–112.

[6] Gomes ARQ, Cunha N, Varela ELP, Brígido HPC, Vale VV, Dolabela MF, de Carvalho EP, Percário S. Oxidative stress in malaria: potential benefits of antioxidant therapy. *Int J Mol Sci*. 2022; 23(11): 1–26.

[7] Babalola AS, Jonathan J, Michael BE. Oxidative stress and antioxidants in asymptomatic malaria-positive patients: a hospital-based cross-sectional Nigerian study. *Egypt J Intern Med*. 2020; 23(23): 1–8.

[8] Gómez X, Sanon S, Zambrano K, Asquel S, Bassantes M, Morales JE, Otáñez G, Pomaquero C, Villarroel S, Zurita A, Calvache C, Celi K, Contreras T, Corrales D, Naciph MB, Peña J, Caicedo A. Key points for the development of antioxidant cocktails to prevent cellular stress and damage caused by reactive oxygen species (ROS) during manned space missions. *NPJ Microgravity*. 2021; 7(35): 1–19.

[9] Arrey Tarkang AP, Nwachiban Atchan AP, Kuiate JR, Okalebo FA, Guantai AN, Agbor GA. Antioxidant potential of a polyherbal antimalarial as an indicator of its therapeutic

value. *Adv Pharmacol Sci.* 2013; Article ID 78458.

[10] Hagazy K, Sibhat GG, Karim A, Tekulu GH, Periasamy G, Hiben GM. Antimalarial activity of *Meriandra dianthera* leaf extracts in *Plasmodium berghei*-infected mice. *Evid Based Complement Altern Med*. 2020; Article ID 8980212.

[11] Oche O, Uche SN, John O, Samuel MR, Esin EE, David AD, Catherine EO. GC–MS profiling and antimalarial activity of *Khaya grandifoliola* on *Plasmodium berghei*-infected mice. *J Herbs Spices Med Plants.* 2018; 25(1): 21–32.

[12] Lawal B, Shittu OK, Kabiru AY, Jigam AA, Umar MB, Berinyuy EB, Aloziuwa BU. Potential antimalarials from African natural products: a review. *J Intercult Ethnopharmacol.* 2015; 4(4): 318–343.

[13] Sofowora EA. Medical plant and traditional remedies in African. 2nd ed. Ibadan: University of lle-lfe Press, 1993.

[14] Beentje HJ. Kenya trees, shrubs and lianas. Nairobi: National Museums of Kenya, 1994.

[15] Iwueke AV, Nwodo OFC, Okoli CO. Evaluation of the anti-inflammatory and analgesic activities of *Vitex doniana* leaves. *Afr J Biotechnol.* 2006; 5(20): 1929–1935.

[16] Okpe O, Ibrahim S, Njoku GC, Ndidi US, Atabo S. Pancreatic islet regeneration and some liver biochemical parameters of leaf extracts of *Vitex doniana (Verbenaceae)* in normal and STZ induced diabetic albino rats. *Asian Pac J Trop Biomed.* 2014; 4(2): 124–130.

[17] Yakubu OE, Ojogbane E, Nwodo OFC, Nwaneri-Chidozie VO, Dasofunjo K. Comparative antioxidant and hypoglycaemic effects of aqueous, ethanol and n-hexane extracts of leaf of *Vitex doniana* on streptozotocin-induced diabetes in albino rats. *Afr J Biotechnol*. 2013; 12(40): 5933–5940.

[18] Ibrahim S, Okpe O, Njoku G, Ndidi US. Antihyperglycaemic and antihyperlipidaemic effects of ethanolic and aqueous leaf extract of *Vitex doniana* (*Verbenaceae*) on streptozotocin-induced diabetic Albino rats. *Res J Med Plant.* 2014; 8(4): 178–186.

[19] Sasidharan S, Chen Y, Saravanan D, Sundran KM, Yoga Y. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr J Tradit Complement Altern Med.* 2011; 8(1): 1–10.

[20] Aliyu AB, Achika JI, Adewuyi JA, Gangas P, Ibrahim H, Oyewale AO. Antioxidants from Nigerian medicinal plants: What are the evidence? [Accessed 2022] Available from: https://www.intechopen.com/chapters/67162.

[21] Mahalik G, Jali P, Sahoo S, Satapathy K. Ethnomedicinal, phytochemical and pharmacological properties of *Mangifera indica* L. *Int J Botany.* 2020; 59(2): 1–5.

[22] Chowdhury A, Alam MA, Rahman NS, Hassan MA, Rashid MA. Antioxidant, antimicrobial and cytotoxic activities of *Corypha taliera* Roxb. *Lat Am J Pharm.* 2020; 29(7): 1231–1234.

[23] Kavimani S, Saminathan K, Senthil KR. Antioxidant and free radical scavenging activities of *Dolichandrone atrovirens* using various in vitro assay models. *Int J Phytopharm.* 2014; 5(4): 293–300.

[24] Gulcin I, Huyut Z, Elmastas M, Aboul-Enein HY. Radical scavenging and antioxidant activity of tannic acid. *Arab J Chem.* 2010; 3(1): 43–53.

[25] Benzie IF, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem.* 1996; 239(1): 70–76.

[26] Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity- a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia.* 1999; 70(3): 251–257.

[27] Ibezim A, Ofokansi MN, Ndukwe X, Chiama CS, Obi BC, Isiogugu ON, Ikechukwu PE, Onwuka AM, Ihim SA, Asegbeloyin JN, Nwodo NJ. Evaluation of anti-malarial potency of new pyrazole hydrazine coupled to Schiff base derivatives. *Malar J.* 2022; 21(243): 1–9.

[28] Iwalokun BA. Enhanced antimalarial effects of chloroquine by aqueous *Vernonia amygdalina* leaf extract in mice infected with chloroquine resistant and sensitive *Plasmodium berghei* strains. *Afr Health Sci.* 2008; 8(1): 25–35.

[29] Connerty HV, Briggs AR. Determination of serum calcium by the means of sodium alizarin sulfonate. *Clin Chem.* 1965; 11(7): 716–728.

[30] Mgbemena IC, Ifeyinwa C. The efficacy of antioxidant (vitamin C) in the treatment and management of malaria. *Adv Biotech Micro.* 2020; 15(4): 97–103.

[31] Gazdik Z, Krska B, Adam V, Saloun J,

Pokorna T. Electrochemical determination of the antioxidant potential of some less common fruit species. *Sensors*. 2008; 8(12): 7564–7570.

[32] Soare JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activity of some extracts of *Thymus zygis*. *Free Radic Res*. 1997; 26(5): 469–478.

[33] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine, 4th ed. Oxford: Claredon Press, 2007.

[34] Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (Ka-Kiniha-Cha). *Food Chem.* 2005; 89(4): 569–575.

[35] Dorman HJD, Peltoketo A, Hiltunen R, Tikkanen MJ. Characterization of the antioxidant properties of deodorized aqueous extracts from selected Lamiaceae herbs. *Food Chem.* 2003; 83(2): 255–262.

[36] Igbokwe NA. A review of the factors that influence erythrocyte osmotic fragility. *Sokoto J Vet Sci.* 2018; 16(4): 1–23.

[37] Sharma B, Rai DK, Rai PK, Rizvi SI, Watal G. Determination of erythrocyte fragility as a marker of pesticide-induced membrane oxidative damage. *Methods Mol Biol.* 2010; 594: 123–128.

[38] Safeukui I. Retention of *Plasmodium falciparum* ring-infected erythrocytes in the slow, open microcirculation of the human spleen. *Blood*. 2008; 112(6): 2520–2528.

[39] Lew VL, Macdonald L, Ginsburg H, Krugliak M, Tiffert T. Excess haemoglobin digestion bymalaria parasites: a strategy to prevent premature host cell lysis. *Blood Cells Mol Dis.* 2004; 32(3): 353–359.

[40] Buffet PA. The pathogenesis of *Plasmodium falciparum* malaria in humans: insights from splenic physiology. *Blood*. 2011; 117(2): 381–392.

[41] Anosike CA, Obidoa O, Ezeanyika LUS. Membrane stabilization as a mechanism of the anti- inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). *DARU J Pharm Sci*. 2012; 20(1): 1–7.

[42] Amin C, Shofiul A, Mohammed AJ, Kazi OF, Atiqul I. Antibacterial activities and in vitro anti- inflammatory (membrane stability) properties of methanolic extracts of *Gardenia coronaria* leaves. *Int J Microbiol.* 2014; Article ID 410935.

[43] Ogbomade RS, Asara AA, Eboh AS. *In vitro* anti-inflammatory properties of leaf extract of *Polyalthia longifolia* extract. *Pharm Chem J.* 2019; 29(6): 112–115.

[44] Mathieu B, Oliver B. Calcium signalling in malaria parasites. *Mol Microbiol.* 2016; 100(3): 397–408.

[45] Golvan J. Elements in medical parasitology. 4th ed. Paris: Flammarion Médecine-Sciences, 1983.

[46] Carafoli E. Intracellular calcium homeostasis. *Annu Rev Biochem.* 1987; 56: 395–433.

[47] Glennon MC, Bird GJ, Kwan CY, Putney JW. Actions of vasopressin and the Ca^{2+}-ATPase inhibitor, thapsigargin, on Ca^{2+} signaling in hepatocytes. *J Biol Chem.* 1992; 267(12): 8230–8233.

Abbreviations

VDE: *Vitex doniana* extract; VDF: *Vitex doniana* fraction; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; SPSS: statistical product and service solutions; ANOVA: analysis of variance; RBCs: red blood cells; HRBCs: human red blood cells; NIH: National Institutes of Health