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Full Length Research Article

Antimalarial and Antioxidant Properties of Ethanolic Leaf Extract of *Entada africana* in *Plasmodium berghei*-Infected Mice

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ABSTRACT
Entada africana is one of the most exploited medicinal plants as an anti-inflammation, antibacterial and anti-angiogenic among others, using its various parts and mostly roots. This study was aimed at exploring the ameliorative potential of the ethanol leaf extract of *Entada africana* (ELEA) via *in vivo* antimalarial activities. Phytochemical analysis conducted for ELEA revealed the presence of flavonoids, glycosides, saponins, alkaloids, terpenes, phenols, and tannins. Healthy Swiss albino mice of male sex weighing 17-23g were used to determine the acute toxicity of the extract which was found to be 2121 mg/kg. Mice were inoculated with chloroquine-sensitive *Plasmodium berghei* strain (NK 65) while the infected mice were then grouped into 100, 200, and 400 mg/kg b. wt.ELEA with chloroquine 5 mg/kg b. wt. as the reference drug. In the antimalarial study, rectal temperature, antioxidant, and hematological parameters were determined. The tested extracts mostly exhibited dose-dependent efficacy patterns in antimalarial, rectal temperature, blood indices, and antioxidant parameters determined. The efficacy was mostly found with 400 mg/kg b. wt. ELEA in the majority of the indices. *Entada africana* leaves could therefore be recommended as a source of promising molecules which could be of phenolics family for the combat with malaria parasites.
Keywords: *Entada africana* leaves, acute toxicity, antimalarial, hematological parameters, rectal temperature, antioxidant.

INTRODUCTION
Malaria is a tropical disease that has affected every aspect of life in humans (Sachs and Malaney, 2002). Many cases and deaths of this disease have been reported mainly of children aged under five and pregnant women (Adefioye *et al*., 2007). Malaria is caused by the parasite called plasmodium which is transmitted by female anopheles mosquito through bites from an infected person to a new victim. Human infection involves species *Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae, and Plasmodium vivax* (WHO, 2015). Among the species of parasites is *P. vivax*, which has the highest distribution worldwide and its infection is common in Asian countries (Hay *et al*., 2004). It was reported that the most lethal of the disease is the type caused by the specie *P. falciparum* and the occurrence is mainly in sub-Saharan Africa (Bruce-Chwatt, 1986). *P. falciparum* and *P. malariae* are peculiar to Nigerian malaria cases (Molineaux and Gramiccia, 1980). Variation in bioclimatic zones signifies a major susceptibility factor in the incidence of favourable survival of various species of the implicated parasites (Molineaux and Gramiccia, 1980). Recent prevalence of world malaria has shown that there were reductions in the incidences in 108 endemic countries, including sub-Saharan Africa from 243 million in 2000 to 231 million in 2015 but later increased to 249 million in 2022 (WHO, 2023). There was a marginal reduction in the mortality rate of the malaria epidemic from 839,000 in 2000 to 608,000 in 2022 (WHO, 2023). Despite the observed reduction, Africa still accounts for more than 90% of both global malaria cases and deaths. Of the African prevalence, Nigeria as the world most endemic country accounted for over 66 million of the global 249 million reported incidences and over 180,000 of the global mortality reported for 2022 (WHO, 2023).

The occurrence of Nigerian malaria is a result of infection from bites of the large population of the vectors (female anopheles mosquitoes) harboring *P. falciparum* and *P. malariae*. During the infection, the parasite intoxicates red blood cells, as sporozoites, then enters into liver, multiplies into merozoites, and re-enters the blood to attack red blood cells (erythrocytic stage), causing hematological disorders and eventually leads to a series of symptoms of malaria. This shows that plasmodium parasites have two different life cycles within organisms; mosquito and animal (rat/man). The life cycles lead to modified reactions in the blood and eventually in organs like the liver and kidney with consequent induction of free radical generation. Free radicals; reactive oxygen species (ROS) or reactive nitrogen species (RNS) include superoxide anions, hydroxyl radicals, peroxides, and nitric oxide (Guha *et al*., 2006) which have both beneficial and detrimental effects. Overproduction of free radicals or imbalance between the production of Free radicals and antioxidants have been implicated in the initiation and progression of various diseases (Oloyede *et al.*, 2015; Jacob and Burri, 1996).

Resistances of mosquitoes to insecticides and parasites to antimalarial drugs especially to the current most effective and newest artemisinin derivatives (Silva *et al.*, 2011). Currently, multi-drug resistance has become one of the most important problems impeding malaria control efforts (Htut, 2009; Sendagire *et al*., 2005). This has led to attempts to discover other antimalarial agents. For decades, medicinal plants have been used by various traditional medical practitioners (TMPs) for the treatment

and cure of malaria (Asase *et al.,* 2005). These plants have been used singly as well as in various combinations with others in the form of recipes for malarial control and also as sources of free radical scavengers due to their low tendency of contraindication. Secondary metabolites of plant origin have been shown to have antiplasmodial activity (Saxena *et al*., 2003). Medicinal plants commonly used in Nigeria and neighboring countries as traditional medicine for the treatment of malaria include *Alstonia boonei* (Apocynaceae)and *Azadirachta indica* (Meliaceae), *Khaya senegalensis* (Meliaceae)*, Entada africana* (Fabaceae) *among others.*

Entada africana Guill. & Perr. is a small tree of 4 to 10 meters in height and 90 cm in girth, with local names as 'Dorot' in Arabic and 'Ayunre banabana' in Yoruba, Nigeria (Mbatchou *et al*., 2011). It belongs to the phylum Magnoliophytas and family Fabaceae. The stem bark is said to have abortive effects, while a root decoction is a stimulating agent and tonic (Orwa *et al*., 2009). The plant is said to have antidote effects against various toxic agents because of its emetic properties (Orwa *et al*., 2009). Mbatchou *et al.* (2011) reported the ethanolic extract of the stem bark of *E. africana* inhibited the growth of some bacteria such as *S. typhi.* The leaves of this plant (Plate 1) are widely prepared as concoctions and considered ethnobotanically effective against malarial in the Western part of Ekiti State. In this study, we report the antimalarial effects of ethanolic leaf extract of *E. africana* in *P. berghei*-infected mice in parameters that include parasitemia, anemia, oxidative stress and hypothermia.

Plate 1: *Entada africana* leaves

MATERIALS AND METHODS

Plant Material Collection and Authentication

The fresh leaves of *Entada africana* were obtained in Agba dam area, Ilorin, Kwara State, Nigeria. Authentication of the plant was done by Mr. Bolu Ajayi in the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. Voucher number (UILH/004/960) was assigned while the specimen was deposited in the Herbarium.

Experimental Animals

Seventy-two healthy Swiss albino mice of male sex weighing 17 – 23 g (6 – 8 weeks old) were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin. They were randomized into six groups and were housed within clean plastic cages under standard conditions to acclimatize (12-hour light/dark cycle, ambient temperature, 28.0 ± 2.0 ^{o}C, and humidity 46%). All the mice were allowed access to food (growers mash) and water *ad libitum* throughout the experimental period. Good hygiene was maintained by constant cleaning and removal of feces and spilled feed from cages daily.

Ethical Clearance

The commencement of the experimental part of this work was based on the authorization given by the University of Ilorin Ethical Review Committee (UERC). Ethical clearance for the study with approval number UERC/ASN/2018/1318 was obtained in this regard. All the rules concerning the guide for the care and use of laboratory animals were strictly adhered to.

Chemicals and Reagents

Chemicals

Chemicals used for determinations were from Merck KGaA Co., Ltd., Germany. All the chemicals were of analytical grades and were all prepared according to standard specifications.

Biochemical Assay Kits

All biochemical assay kits such as superoxide dismutase (SOD) and total antioxidant capacity (TAC) were purchased from Randox laboratories Co-Artrimy, United Kingdom.

Preparation of Plant Material

The fresh leaves of the *Entada africana* were washed and air-dried at room temperature for seven days (Sofowora 1982). The dried plant materials were ground into powder using an electric mill. The pulverized leaf (250 g) was soaked in 2.5 L of ethanol then placed on a rotary shaker set at 190-220 rpm for 24 h and after which the mixture was filtered. The filtrate was concentrated in a vacuum and dried at 45°C for ethanol removal, and the extracts were kept in sterile bottles at 4^{0}C.

Plasmodium Strain Inoculation

Chloroquine sensitive plasmodium parasite, *P. berghei berghei* (NK 65) was selected for this study and was obtained from the Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, Ibadan. The parasites were maintained weekly through passage in mice. Three animals at a time were used as infected donors and as parasite reservoirs. The donor mice were monitored for signs of infection which include lethargy, anorexia, ruffled appearance, shivering, and heat-seeking behavior. A standard inoculum of 0.2 ml containing 1x10^{7} chloroquine-sensitive *P. berghei*-infected erythrocytes was prepared by drawing blood from the donor mouse's infected tail (the reservoir for the parasites) (Chung *et al*., 2009). This blood was then diluted with isotonic 0.88 % saline. The experimental mice were intraperitoneally injected with 0.2 ml of infected erythrocytes using a 1 ml syringe and a ½ inch 23-gauge needle (Odeghe *et al*., 2012).

Phytochemical Screening
Qualitative Phytochemistry was conducted on the aqueous leaf extracts to test for the presence of Flavonoids, alkaloids, tannins, saponins and phenolics, following methods described by Sofowora (1993) and Edeoga *et al*. (2005).

Determination of Oral Acute Toxicity
The oral acute toxicity study of Ethanolic extract of *E. africana* leaves was carried out on mice using a modified Lorke (1983) method. The study was performed in two phases consisting of 12 mice in each. In the first phase, the acclimatized mice were randomized into 4 mice in each group and administered 10, 100 & 1000 mg/kg ELEA. The mice were administered the extract doses orally and were observed for physical abnormalities and death for the first 6 hours and then daily for 7 days. In the second phase, as a result of the outcome of the first phase, fresh 12 per group of 4 acclimatized mice were orally administered with 1500, 3000, and 4500 mg/kg of the ethanolic extract of *E. africana* leaves and were observed just like in the first phase. The LD_{50} was calculated as a product of the minimum lethal dose and maximum non-lethal dose using the formula;
$LD_{50}=\sqrt{}$lowest toxic dose × highest tolerated dose

Dose Determination
Doses for in vivo evaluation were determined from extrapolations made from the results of the oral acute toxicity as reported by Tibiri *et al*. (2007). The doses in this work for the treatment groups were modified from 10% of LD_{50} of the acute oral toxicity (Tibiri *et al*., 2007).

Experimental Design
Evaluation of Antimalarial Activity in Late Infection (Curative or Rane's Test)
The curative potential evaluation of the extract was done using the method of Ryley and Peter (1970). Standard inoculums of 1x107 *P. berghei*-infected erythrocytes were injected intraperitoneally on the mice, on the first day (Day 0). Seventy-two hours later (day 3) the mice were divided into six groups of 8 mice each. The groups were orally administered with *Entada africana* leaves extract (100, 200, 400 mg/kg), chloroquine (5mg/kg) was given to the positive control group, and an equal volume of normal saline to the negative control group. The drug and extract were all administered orally once daily for 5 days. Thin films were stained with 10% Giemsa at pH 7.2 for 10 min and parasitemia was examined microscopically daily to monitor parasitemia.

Parasitemia and Mean Survival Time Determinations
Parasitemia was determined using the light microscopy method as described by Peters and Robinson (1992). Briefly thick blood smear (obtained from the mice's tail) was fixed with methanol. The fixed smear was stained with 10% freshly prepared Giemsa at pH 7.2 and left for 10 minutes. The parasitemia was examined microscopically by counting the number of erythrocytes and the percentage of parasitemia was calculated using the formula below.

$$\%\ \text{Parasitemia} = \frac{\text{No.of infected RBCs}}{\text{Total No.of RBCs counted}} \times 100$$

Mean survival time (MST) was determined by finding the average survival time in days of mice in a test group starting from the first day of infection with *P. berghei* for a total period of 30 days (D0 to D29) using the formula below (Belay *et al*. 2018):

$$MST = \frac{\text{total survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

Rectal Temperature Determination
The rectal temperature was determined as described by Basir *et al*. (2012). Each mouse was carefully hand-held to an appropriate and convenient position for the use of a digital thermometer. The probe of a BIOSEB (BIO9882) rectal thermometer was inserted approximately 1.5 cm past the anal sphincter into the colon of the mice. The colonic/rectal temperature was read ahead of infection, 4 hours following infection, and then daily throughout the study period.

Determination of *In vivo* Antioxidant Activity of the Ethanolic Leaf Extract of *E. africana*
Serum and liver homogenate obtained from each mouse were used to assess the following antioxidant parameters: superoxide dismutase (SOD) activities (Misra and Fridovich 1972), catalase (CAT) activities (Sinha 1972) and Total antioxidant capacity, TAC (Miller *et al*., 1993).

Heamatological Assays
Heparin-coated capillary tubes were employed for blood collection from the tail of each mouse. Red blood cells (RBCs) and Hemoglobin (Hb) contents were determined, followed by Packed cell volume (PCV). For PCV, blood-filled tubes (up to 3-quarter of the volume) were sealed with sealing clay and then placed in a microhematocrit centrifuge, with the sealed end outwards followed by centrifugation for five minutes at 11,000 rpm. Finally, tubes were removed from the centrifuge and PCV was evaluated via a standard microhematocrit reader. Other blood indices such as Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were therefore calculated from the above-mentioned parameters (Belay *et al*., 2018).

Preparation of Serum and Tissue
Serum
After the treatment period, the model animals will be made unconscious with diethyl ether, to receive venous blood into EDTA sample bottles, for hematological study and plain sample bottles for biochemical analysis (Adebayo *et al*., 2013; Balogun *et al*., 2014)

Tissue
Liver was collected from the sacrificed mice. The organ was made free of blood, blotted with tissue paper, then put in ice-cold 0.25M sucrose solution to maintain the integrity of the organ. Then, 0.8 g was cut thinly with sterile scalpel blade and homogenized in 4 ml ice-cold 0.25 M sucrose solution (1:5 w/v) (Akanji and Yakubu, 2000). The homogenates were then frozen overnight to ensure the maximum release of the enzymes located in the cell before being used for the enzyme assay (Ngaha *et al*., 1989; Balogun *et al*., 2014).

Statistical Analysis
Data was expressed as the mean ± SEM of at least three determinations. Values were analyzed using One-way analysis of variance followed by Tukey's post hoc test. Graph pad prism version 6.0 was used for statistical analyses. Statistical significance was set at P<0.05.

RESULTS

Preliminary Phytochemical Screening
The phytochemical analysis showed appreciable quantities of alkaloids, flavonoids, phenolics, and tannins among others as presented in Table 1.

Table 1: Phytochemical Screening results of ethanolic leaf extract of *Entada africana.*

PHYTOCHEMICALS	QUANTITY
Alkaloids	+
Flavonoids	++
Phenolics	+++
Tannins	+
Glycosides	+
Saponins	+
Terpenoids	+

+ - mild, ++- moderate, +++ - abundant

Oral Acute Toxicity
As revealed from the oral toxicity study, the extract caused no death in mice within the first 24 hours and 14 subsequent days at 1000mg (1g) in the first phase and at 1500 mg of the second phase. The physical and behavioral observations showed no signs of toxicity such as a decrease in appetite, tremors, hair erection, diarrhea, and salivation. This may indicate that the oral LD_{50} of *E. africana* leaves extract is higher than 1500 mg/kg. A death and two deaths occurred respectively at the doses 3000mg and 4500mg of the second phase of the toxicity study, following physical and behavioral signs of toxicity in doses above 1500mg/kg.LD_{50} formula was then used to obtain LD_{50} of 2121mg/kg b. wt.

***In vivo* Antimalarial study**
Effect of Ethanolic Leaf Extract of *Entada africana*on *P. berghei*-Infected Mice in a Curative Test
The ethanolic leaf extract of *E. africana*(ELEA) exhibited a partial dose-dependent reduction in parasitemia levels in the extract-treated groups similar to the reference drug in the positive control group. It could be noted that 200 mg/kg b.wt. ELEA caused a significant (p<0.05) reduction in parasitemia but still lesser than the effect of 5 mg/kg b. wt. of chloroquine. The average percentage suppression of the parasitemia of the extract-treated group is 74.94, 92.61, and 89.48 respectively for doses 100, 200, and 400 mg/kg/day on day 5 while 93.92% was exerted by 10 mg/kg b.wt. chloroquine (Table 2).

Effects of Ethanolic Leaf Extract of *Entada Africana* on Rectal Temperatureof *P. berghei*-Infected Mice
The extract (ELEA) at the highest dose (400mg/kg) caused a significant (p<0.05) increase in the rectal temperature of *P. berghei*-infected mice. Likewise, the reference drug (chloroquine 5mg/kg) showed a considerable (p< 0.001) increase in rectal temperature in the experimental mice (Table 3). Experimental mice infected with murine malarial parasites undergo hypothermia during malarial infection. Any potent antimalarial agent elicits a remarkable upward adjustment of the body temperature of *P. berghei*-infected mice.

Antioxidant Study
Effects of Ethanolic Leaf Extract of *E. africana* on Liver Antioxidant Parameters of *P. berghei*-Infected Mice
The ethanolic extract was administered to the various test groups of mice at concentrations of 100, 200, and 400 mg/kg b.wt. ELEA was evaluated for *in vivo* antioxidant activity via indices such as superoxide dismutase, catalase, and total antioxidant capacity in liver samples of *P. berghei*-infected mice. The efficacy of ELEA in mopping up available systemic free radicals via liver SOD was manifested in 400 mg/kg b. wt. ELEA with no significant difference (p<0.05) in 5 mg/kg chloroquine-treated and non-parasitized groups. On the other hand, 100 mg/kg and 200 mg/kg b. wt ELEA elicited activities that were not significantly different (p<0.05) to saline-treated parasitized mice. The activities of liver CAT and TAC in all the test groups as well as the non-parasitized group exhibited a significant difference (p<0.05) as compared with the saline-treated parasitized (Table 4).

Effects of Ethanolic Leaf Extract of *E. africana* on Serum Antioxidant Parameters of *P. berghei*-Infected Mice
Various concentrations at 100, 200 and 400 mg/kg b.wt. ELEA were evaluated for *in vivo* antioxidant activity via superoxide dismutase, catalase, and total antioxidant capacity in serum samples of *P. berghei-infected* mice. There were no significant differences (p<0.05) in serum SOD activities in the parasitized mice treated with 200 mg/kg b.wt., 400 mg/kg b.wt. and saline-treated non-parasitized mice.

On the other hand, 5 mg/kg b. wt. Cq. and 100 mg/kg b. wt. ELEA exhibited no significant difference (p<0.05) in serum SOD activities as compared with saline-treated parasitized mice (Table 5). In the serum CAT activities, 400 mg/kg b. wt. ELEA, 5 mg/kg b. wt. Cq. and saline-treated non-parasitized groups exhibited a significant

difference ($p<0.05$) as compared with 100 mg/kg b. wt., 200 mg/kg b. wt. ELEA and saline-treated parasitized mice. The activities of serum TAC in all the test groups as well as the non-parasitized group exhibited a significant difference ($p<0.05$) as compared with the saline-treated parasitized showing 400 mg/kg b. wt. ELEA eliciting TAC activity is not significantly different ($p<0.05$) from % mg/kg b. wt. Cq. and saline on the parasitized mice in their respective groups (Table 5).

Table 2: Parasitemia and Mean Survival time of *P. berghei*-infected mice treated with ethanolic leaf extract of *E. africana* in a curative test.

GROUPS (mg/kg b. wt.)	PARASITEMIA (%)		SUPPRESSION (%)	MEAN SURVIVAL TIME (MST)
	D_0	D_4		
PM + Saline	15.23± 0.05	21.38± 0.62^{c}		8.00 ± 0.32^{a*}
PM + ELEA 100	15.43 ± 0.96	5.36 ± 0.95^{b}	74.94%	9.80 ± 1.24^{c*}
PM + ELEA 200	16.55 ± 1.00	1.58 ± 0.40^{a}	92.61%	16.40 ± 3.80^{ab}
PM + ELEA 400	14.85 ± 1.10	2.16 ± 0.40^{a}	89.48%	10.60 ± 1.20^{ba}
PM + Cq 5	16.56 ± 1.17	1.30 ± 0.61^{a}	93.92%	29.20 ± 0.58^{ab}

NB.: PM- Parasitized mice; ELEA-Ethanolic leaf extract of *Entada africana;*Cq- Chloroquine; D_0- Day 0; D_4-Day 4
N.B. Values are expressed as Mean ± S.E.M. for n=5. Values not sharing common superscripts differ significantly at $p<0.05$. a, b = significant decrease at $p<0.05$, n = 5 when compared with negative control group (Parasitized mice + saline). a*, c* = significant decrease at $p<0.05$, n = 5 when compared with 200 mg/kg b. wt. ELEA, 400 mg/kg b. wt. ELEA and 5 mg/kg b. wt. Cq. c = significant increase at $p<0.05$, n = 5 when compared with 100, 200, and 400 mg/kg b. wt. ELEA and 5 mg/kg b. wt. Cq.

Table 3: Effects of ethanolic leaf extract of *E. africana* leaves on rectal temperature of *P. berghei*-infected mice in a curative test.

GROUPS (mg/kg b. wt.)	TEMPERATURE (^{0}C)			
	D_0	D_4	Δ	%Δ
PM + Saline	37.20 ± 0.360	35.40 ± 0.46^{a}	- 1.80^{a}	4.83^{a}
PM + ELEA 100	37.16 ± 0.15	35.85 ± 0.49^{a}	- 1.31^{a}	3.53^{a}
PM + ELEA 200	36.67 ± 0.24	36.48 ± 0.19^{b*}	- 0.19^{b}	0.52^{b}
PM + ELEA 400	37.19 ± 0.14	37.25 ± 0.11^{b}	+0.06^{b}	0.16^{b}
PM + Cq 5	36.95 ± 0.26	37.36 ± 0.23^{b}	+0.06^{b}	0.16^{b}

NB.: PM- Parasitized mice; ELEA:Ethanolic leaf extract of *Entada africana;*Cq- Chloroquine; D_0- Day 0; D_4- Day 4; Δ- Change; %Δ-Percentage change. Values are expressed as Mean ± S.E.M. for n=5. Values not sharing common superscripts differ significantly at $p<0.05$. a = significant decrease at $p<0.05$, n = 5 when compared with 200 mg/kg b. wt. ELEA, 400 mg/kg b. wt. ELEA and 5 mg/kg b. wt. Cq. b = significant increase at $p<0.05$, n = 5 when compared with negative control group (Parasitized mice + saline) and 100 mg/kg b. wt. ELEA. b* = increase at $p<0.05$, n = 5 when compared with negative control group (Parasitized mice + saline) and 100 mg/kg b. wt. ELEA.

Table 4: Antioxidant parameters of Livers of *P. berghei*-infected mice treated with various doses of ethanolic leaf extract of *E. africana* in a curative test.

GROUP/DOSE (mg/kg b. wt.)	LIVER SOD Umg^{-1} protein	LIVER CAT µmole min mL^{-1}	LIVER TAC mmolL^{-1}
PM + Saline	53.30± 6.59^{a}	55.83±23.11^{a}	73.70±3.89^{a}
PM +100 ELEA	98.26 ±3.05^{b*}	115.5±5.86^{b}	98.68±2.11^{b}
PM +200 ELEA	110.1 ±18.75^{b*}	127.5±5.81^{b}	106.7±3.22^{b}
PM + 400 ELEA	130.8 ±14.34^{b}	120.8±2.85^{b}	109.2±4.71^{b}
PM + 5 Cq	127.4 ±7.98^{b}	86.63±5.34^{b*}	100.6±0.42^{b}
NPM + Saline	139.0 ±23.98^{b}	133.4±5.43^{b}	99.60±1.14^{b}

NB.:PM- Parasitized mice; ELEA: Ethanolic leaf extract of *Entada africana;* Cq- Chloroquine; NPM- Non parasitized mice. Values are expressed as Mean ± S.E.M. for n=5. Values not sharing common superscripts differ significantly at $p<0.05$. b & b* = significant increase at $p<0.05$, n = 5 when compared with negative control group (Parasitized mice + saline). a = significant decrease at $p<0.05$, n = 5 when compared with groups of 100, 200, and 400 mg/kg b. wt. ELEA, 5 mg Cq, and non-parasitized mice. b* is a decrease at $p<0.05$, n = 5 when compared with b

Haematological Study

Effects of Ethanolic Leaf Extract of *E. africana* on Hematological Indices of *Plasmodium berghei*-infected Mice

The crude extract of *E. africana* leaves in the various tested doses significantly ($p<0.05$) prevented the hydrolysis of blood cells by plasmodium as manifested in the indices. Red blood cells, as well as haemoglobin, were protected by the crude extract in a no dose-dependent manner and even concerning the reference drug and uninfected groups. The protection led to the prevention of reduction in hematocrit (HCT), as well as in the calculated hematological indices such as MCH, MCHC, and RDWCV with an exception in MCV where dose 400 mg/kg b. wt. ELEA slightly failed ($p<0.05$) to ameliorate the status of the index (Table 6).

DISCUSSION

Malaria is an infectious disease that has remained a major devastating health factor to the health of children around age 5 and adults in general (Winter *et al*., 2006). The prevalence of malaria continually rises, having failed among the MDGs and SDGs, incidences and mortality have been reported to be 241 million and 627,000 respectively in 2020 (WHO, 2021). Attempts in the management and eradication of malarial cases have posed a great threat to economic stability in sub-Saharan African countries and every other nation throughout the world (Iyiola *et al*., 2011). Plasmodium species which are responsible for this infection have frustrated the efficacies of single and combinational conventional antimalarial agents due to cases of resistance. In Nigeria, *P. falciparum* is the specie that is largely responsible for malaria. There have been several incidences of *P. falciparum* resistance to antimalarial drugs in Africa (Ariey *et al*., 2014) and this has demanded alternative therapy from medicinal plants in the folklores (Odugbemi *et al*., 2007). Numerous medicinal plants have been explored for their antimalarial phytoconstituents, including *Entada africana* as sources of novel molecules for malarial therapy.

A preliminary phytochemical assay of the ethanolic leaf extract of *Entada africana* revealed the presence of alkaloids, flavonoids, phenolics, tannins, glycosides, saponins, and terpenoids (Table 1). This result is consistent to a very large extent of those reported by Ifemeje *et al*.(2014) and Mbatchou *et al*.(2011). Various medicinal purposes ethnopharmacologically attributed to each of these phytoconstituents have been reported as antiviral (Lu *et al*., 2004) and antibacterial (Akiyama *et al*., 2001; Silva *et al*., 1996) for its tannins. It could also be deduced from Table 1 that phenolic compounds appear to be one of the major principles in the leaves of *E. africana*. This showed an indication to the report published by Tibiri *et al*.(2010) about the abundance of total phenolics in different parts of the *E. africana* plant and it might be a key player in antimalarial activity.

The acute toxicity signs were observed in the mice used in this determination. The first of the two phases utilized in the determination with doses up to 1g (1000 mg) showed no toxicity signs. In general, if the lethal dose (LD_{50}) of the test substance is three times more than the minimum effective dose (MED), the substance is considered a good candidate for further studies (Carol *et al* 1995). In the dose of 1000 mg in the first phase up to 1500 mg in the second phase, the physical and behavioral observations showed no signs of toxicity such as a decrease in appetite, tremors, hair erection, diarrhea, and salivation. This may indicate that the oral LD_{50} of *E. africana* crude leaves extract is higher than 1500 mg/kg. These signs became prominent as the dose increased which gave few deaths in phase two. With the formula, LD_{50} was determined to be 2121 mg/kg b. wt.

The *in vivo* antimalarial study to evaluate the efficacy of the ethanolic extract of *E.africana* was carried out using the murine strain of plasmodium, *P. berghei*, inoculated into mice (Deharo *et al*., 1996). Even though rodent malaria could not be directly extrapolated to human malaria, it serves the same purpose as the initial steps of screening the activities of several antimalarial candidates. The choice of *P. berghei* NK 65 with C57BL/6 mice for this study was mainly due to the duration of infection of about 3 weeks of hyperparasitemia before death and anaemia as compared to *P. berghei* ANKA of a maximum of 8 days post-infection, justifying how ANKA makes a good model in experimental cerebral malaria (Lanar, 1990) and immunological experiment (Schofield and Grau, 2005). *P. berghei* NK65 infection leads to a greater number of 'latent merozoites' as against *P. berghei* ANKA, meaning merozoites are in the blood for an extended period before invading a new RBC (Beaute-Lafitte *et al*., 1994). NK65 possesses a strong preference to invade immature RBC (reticulocytes); a greater probability to multiply and infect the same cell and the ability to produce more ring forms early in infection (Deharo *et al*., 1996), resulting to a less synchronous infection than the ANKA strain (Goodman *et al*., 2013).

Three methods are widely used for screening antimalarial candidates; Curative (Rane's test), chemosuppressive, and prophylactic tests. Rane's test and 4-day suppressive test are the two methods universally accepted in the screening. In the two methods, suppression percentage estimation is a very important parameter (Kalra *et al*., 2006). In this current study, the *in vivo* antimalarial study was evaluated with a curative test together with rectal temperature and *in vivo* antioxidant status of the model animal.

Table 5: Antioxidant parameters of Serum of *P. berghei*-infected mice treated with various doses of ethanolic leaf extract of *E. africana* in a curative test.

GROUP/DOSE (mg/kg b. wt.)	SERUM SOD Umg^{-1} protein	SERUM CAT µmole min mL^{-1}	SERUM TAC $mmolL^{-1}$
PM + Saline	45.40 ±7.83^{a}	46.91 ±2.50^{a}	57.73±5.60^{a}
PM +100 ELEA	105.20 ±5.50^{b*}	87.35±17.65^{a*}	81.97±7.00^{b}
PM + 200 ELEA	114.8 ±7.52^{b}	106.8±12.97^{a*}	91.99±5.17^{b}
PM + 400 ELEA	135.7 ±11.61^{b}	128.20±25.67^{b}	104.8±1.00^{bc}
PM + 5 Cq	111.0 ±17.21^{b}	113.9±6.90^{b}	99.74±0.58^{bc}
NPM + Saline	118.9 ±10.56^{b}	118.20±12.14^{b}	112.3±0.63^{bc}

NB.: PM- Parasitized mice; ELEA: Ethanolic leaf extract of *Entada africana;* Cq- Chloroquine; NPM- Non parasitized mice. Values are expressed as Mean ± S.E.M. for n=5. Values not sharing common superscripts differ significantly at p<0.05. b, b*& bc = significant increase at p<0.05, n = 5 when compared with negative control group (Parasitized mice + saline). a* = increase at p<0.05, n = 5 when compared with negative control group (Parasitized mice + saline).

TABLE 6: Hematological parameters of *P. berghei*-infected mice treated with various doses of ethanolic leaf extract of *E. africana* in a curative test.

GROUPS/TREATMENTS (mg/kg b. wt.)	RBC ($\times10^{6}$/µl)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (g/dl)	MCHC (g/dl)
PM + Saline	6.77±0.69^{b*}	11.00±0.28^{c}	35.18±0.99^{c}	54.53±6.50^{c}	13.80±1.37^{b*}	25.62±0.77^{c}
PM+100 ELEA	7.03±0.56	12.40±0.01	38.52±1.23	56.13±4.45^{a}	17.76±1.27	32.49±1.17
PM+200 ELEA	6.79±0.70	14.74±1.18^{a}	40.94±6.25	56.00±4.19^{a}	22.77±3.39	41.49±10.46^{a}
PM+400 ELEA	9.56±0.61^{a}	15.32±0.46^{b}	49.94±1.27^{a}	53.01±3.20	16.35±1.34^{a}	30.79±1.40^{b}
PM+5Cq.	8.74±0.45	14.52±0.34^{a}	47.62±1.35^{ab}	55.25±3.91^{b}	16.77±0.77^{a}	30.62±1.17^{b}
NPM + Saline	9.91±0.35^{a}	15.24±0.66^{b}	54.52±0.52^{a}	55.36±2.50^{b}	16.21±0.70^{a}	29.13±0.27^{b}

NB.:PM- Parasitized mice; ELEA:Ethanolic leaf extract of *Entada africana;*Cq- Chloroquine; NPM- Non parasitized mice. Values are expressed as Mean ± S.E.M. for n=5. Values not sharing common superscripts differ significantly at p<0.05. a, b, ab = significant increase at p<0.05, n = 5 when compared with negative control (Parasitized mice + saline). b*,c= significant decrease at p<0.05, n = 5 when compared with all ELEA and 5 mg Cq treatments.

The 5-day curative test evaluated was carried out with the ELEA on *Plasmodium berghei*-infected mice. It is important to note that the percentage of parasitemia determined revealed the *in vivo* antiplasmodial activity of the extract as exhibited by the ability ofELEA to significantly reduce ($p<0.05$) the parasitemia and also increase the mean survival time of *P. berghei*-infected mice in a non-dose dependent manner. The exhibited parasitemia reduction was estimated to be percentage suppression of 74.94, 92.61, and 89.48 respectively of 100, 200, and 400 mg/kg b. wt. ELEA (Table 2). The effects observed may indicate that this important medicinal plant possesses some bioactive and promising molecules. The antiplasmodial activity elicited by this extract could be attributed to the phytochemicals present, most especially, phenolic compounds in the extract. It could be recalled from the phytochemical results of the plant that total phenolics manifested as one of the major compounds present. Phenolics have been proven as the second leading non-alkaloidal candidate in antimalarial drug search. This major active compound class, phenolics has been substantiated as reported by Builders (2019) that natural phenolic products have in recent times traditionally provided most of the antimalarial drugs in use. Several investigations have reported a wide range of phenolic compounds with various modes of action that result in antimalarial activities (Mukherjee, 2002).

The antiplasmodial activity of *E. africana* in the current study also significantly ($p<0.05$) averted other typical features of murine malaria. The features in mice in addition to parasitemia are hypothermia, oxidation due to free radicals generated by heme metabolism, anaemia, and other hematological disorders (Langhorne *et al*, 2002). Pyrexia is universally accepted in human malaria as against hypothermia in murine malaria. Rectal temperature values were determined to monitor the mice temperature, showing detrimental falling values due to established infections by *P. berghei* which could lead to death in mice (Jennings and Elia, 1987). The antimalarial effect of the ELEA was able to inhibit significantly ($p<0.05$) the hypothermic progression of the infection in a dose-dependent version (Table 3).

Antioxidant property of ELEA was significantly ($p<0.05$) exhibited in the ability to scavenge the generated free radicals during the infection. In the studies carried out by Millogo-Kone*et al*. (2000), it was reported that a plant possessing phenolics as major compounds could be good as antioxidants. It could therefore be concluded that the free radicals scavenging properties of ELEA might be attributed to the phenolic constituents of the plant (Kim *et al*., 2003) which might also be due to their possession of redox properties in the termination of devastating activities of singlet oxygen, free radicals or peroxides. The *in vivo* antioxidant parameters estimated in the study include serum and liver values of superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC). The crude extract exhibited non dose-dependent ameliorative effects on the estimated antioxidant indices. The test extract at 400 mg/kg dose was able to significantly ($p<0.05$) prevent the decrease in the liver (Table 4) and serum (Table 5). SOD, CAT, and TAC except in liver catalase where 200 mg/kg dose was the most potent.

Malarial infection possesses a great number of hematological features resulting from the disorders brought about by various developmental stages of plasmodial attack. The parasite proliferation in the erythrocytic stage usually leads to hemolysis due to erythrocyte attack by merozoites. This devastating attack subsequently results in anemia due to low packed cell volume (PCV), also known as hematocrit (HCT), hence the measurement of red blood cells, hemoglobin, and hematocrit (hct) values among other blood indices. Very low levels of red blood cells (RBC), haemoglobin (Hb), and PCV can predispose to anaemia (Muhammad and Oloyede, 2009). Hematological parameters evaluated in the study revealed the efficacy of 400 mg/kg b. wt. dose as the most potent among other doses. The text extract showed a significant ability ($p<0.05$) to maintain normalcy in all the indices studied except in MCV where 100 mg/kg as well as MCHC and RDWCV where 200 mg/kg b. wt. exhibited the greatest potency among other doses. From these results, it could be deduced that ethanolic leaf extract of *Entada africana* showed a non-dose dependent efficacy in protecting and restoring integrity of red blood cellsmembrane (Table 6).

From the parasitemia results of the curative test, it could be concluded that the suppressive activity of the test extract (ELEA) gives 74.94%, 92.61%, and 89.48% corresponding to 100 mg/kg b. wt., 200 mg/kg b. wt. and 400 mg/kg b. wt. doses, respectively. It could be denoted that ELEA is effective in the treatment of malaria. In standard screening tests, potential antimalarial candidates are the ones that give ≥ 30% suppressive effects and consequently prolong the survival date of the experimental animal administered with the test extract (Adugna *et al*., 2014). The efficacy might be attributed to a great content of antioxidant phenolic compounds which contribute to the exhibited antimalarial effects. The mechanism of action might be due to the ability of the phenolics to promote the oxidation of red blood cells and inhibition of the protein synthesis in plasmodium and also to counteract the oxidative damage induced by malaria parasites (Builders *et al*., 2010). The ameliorative effects elicited in the evaluated parameters by administration of test extracts in antimalarial studies such as with ELEA could be an indication of the upregulated immune system of the *P. bergehi* - infected mice (Abubakar and Oloyede, 2020).

CONCLUSION

Entada africana is a medicinal plant that possesses a moderate antimalarial potency as manifested in the results of the study. Phytochemical evaluation revealed phenolic compounds as the major constituents of the leaves of *E. africana*. This is clear proof that in vivo

antiplasmodial activity with ethanolic extract may be attributed to the phenolic constituent of the *E. africana* leaves. It is important to conclude that this important plant may contribute promising antimalarial molecule(s) to the struggle for malaria eradication. Further work is therefore recommended for structure elucidation and more importantly, molecular docking.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Abubakar,F. A. and Oloyede, H.O. B. (2020). *Prosopis africana* stem bark extract: effects on parasitemia and haematological parameters of *Plasmodium berghei* -infected mice.*Biokemistri*© 2020 *Nigerian Society for Experimental Biology,* ***32***(3):177 - 184

Adebayo, J. O., Balogun, E. A., Malomo, S. O., Soladoye, A. O., Olatunji, L. A., Kolawole, O. M., Oguntoye, O. S., Babatunde, A. S. And Akinola, O. B., Aguiar, A. C. C., Andrade, I. M., Souza, M. B. and Krettli, A. U. (2013). Antimalarial activity of Cocos nucifera husk fibre: further studies. Evidence-Based Complimentary and Alternative Medicine.; 742476.

Adefioye, O. A., Adeyeba, O. A., Hassan, W. O. and Oyeniran, O. A.(2007). Prevalence of Malaria Parasite Infection among Pregnant Women in Osogbo, Southwest, Nigeria. *American-Eurasian Journal of Scientific Research,* **2**(1): 43-45.

Adugna, F. T. M., Taddese, W. and Admasu, P. (2014). "In vivo antimalarial activity of crude extract of aerial part of *Artemisia abyssinica* against P. berghei in mice," *Global Journal of Pharmacology*, **8**(4): 557-565. Doi: 10.5829/idosi.gjp.2014.8.4.822.

Akanji, M.A. and Yakubu, M.T., (2000). Tocopherol protects against metabisulphite-induced tissue damage in rats. *Nigerian Journal of Biochemistry and Molecular Biology,* **15**:179–183.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T. and Iwatsuki, K. (2001). Antibacterial action of several tannins against *Staphylococcus aureus. Journal of Antimicrobial Chemotherapy*; **48**(4):487–91.

Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A. C., Khim, N., Kim, S., Duru, V., Bouchier, C., Ma, L., Lim, P., Leang, R., Duong, S., Sreng, S., Suon, S., Chuor, C. M., Bout, D. M., Ménard, S., Rogers, W. O., Genton, B., Fandeur, T., Miotto, O., Ringwald, P., Le Bras, J., Berry, A., Barale, J. C., Fairhurst, R. M., Benoit-Vical, F., Mercereau-Puijalon, O., Ménard, D. (2014). A molecular marker of artemisinin-resistant *Plasmodium falciparum* malaria. *Nature*, **505**(7481):50-5. doi: 10.1038/nature12876.

Asase, A., Alfred, A., Yeboah, O., Odamtten, G. T. and Simmonds, S. J. (2005). Ethnobotanical Study of some Ghanaian anti-malarial plants. *Journal of Ethnopharmacology*, **99**: 273-279.

Balogun, E. A., Malomo S. O., Adebayo, J. O., Isola, A. A., Soladoye, A. O., Olatunji, L. A., Kolawole, O. M., Oguntoye, O. S., Babatunde, A. S. and Akinola, O. B. (2014). *In vivo* antimalarial activity and toxicological effects of methanolic extract of *Cocos nucifera* (Dwarf red variety) husk fibre. *Journal of Integrated Medicine*, **12**(6): 504-511 doi: 10.1016/S2095-4964(14)60054-6.

Basir, R., Rahiman, S. F., Hasballah, K., Chong, W. C., Talib, H., Yam, M. F., Jabbarzare, M. Tie, T., Othman, F., Moklas, M., Abdullah, W. and Ahmad, Z. (2012). Plasmodium berghei ANKA Infection in ICR Mice as a Model of Cerebral Malaria. *Iranian Journal of Parasitology*, **7**(4), 62 – 74.

Belay, W. Y., Gurmu, A. E. and Wubneh, Z. B.(2018).*Evidence-Based Complementary and Alternative Medicine* Volume 2018, Article ID 4169397, 7 pages

Bruce-Chwatt, L. J. (1986). *Essential Malariology*. First ed. Williams Heinemann Medical Books ltd. London. pp.103-126 and 354.

Builders, M. I. (2019). Antimalarial Phenolic Compounds in Africa: A Review *European Journal of Biomedical and Pharmaceutical Sciences,* **6** (4): 6-11.

Builders, M.I., Wannang, N.N., Ajoku, G.A., Builders, P.F., Orisadipe, A. and Aguiyi, J.C. (2010). Evaluation of antimalarial potential of *Vernonia ambigua* Kotschy and Peyr (Asteraceae). *International Journal of Pharmacology*, 18(11), 1-9.

Chung, I. M., Seo, S. H., Kang, E. Y., Park, W. H. and Moon, H. I. (2009). Anti-malarial activity of 6-(8'Z-pentadecenyl)-salicylic acid from Viola websteri in mice. *Malaria Journal*, **8:**151 (2009). https://doi.org/10.1186/1475-2875-8-151.

Deharo, E., Coquelin, F., Chabaud, A. G. and Landau, I. (1996). The erythrocytic schizogony of two synchronized strains of plasmodium berghei, NK65 and ANKA, in normocytes and reticulocytes. *Parasitology Research*, **82**(2), 178–182.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 2005; **4**(7): 685-688.

Goodman, A. L., Forbes, E. K., Williams, A. R., Douglas, A. D., de Cassan, S. C., Bauza, K., Biswas, S., Dicks, M. D., Llewellyn, D., Moore, A. C., Janse, C. J., Franke-Fayard, B. M., Gilbert, S. C., Hill, A. V., Pleass, R. J. and Draper, S. J. (2013). The utility of Plasmodium berghei as a rodent model for anti-merozoite malaria vaccine assessment. *Scientific Reports*, **3**, 1706. doi: 10.1038/srep01706.

Guha, M., Kumar, S., Choubey, V., Maity, P., Bandyopadhyay, U. (2006). Apoptosis in liver during malaria: Role of oxidative stress and implication of mitochondrial pathway. *Federation of American Societies for Experimental Biology Journal*, **20**, E439–E449.

Hay, S. I, Guerra, C. A., Tatem, A. J., Noor, A. M. and Snow, R. W. (2004): The global distribution and population at risk of malaria: past, present, and future. *The Lancet infectious diseases*, **4**(6): 327-336.

Htut, Z.W. (2009).Artemisinin resistance in *Plasmodium falciparum* malaria. *England Journal of Medicine*, 361,1807–1808

Ifemeje, J. C., Egbuna, C., Udedi, S. C. and Iheukwumere, H. I. (2014).Phytochemical and *in vitro* Antibacterial Evaluation of the Ethanolic Extract of the Stem Bark of *Entada africana* Guill. & Perr and *Sarcocephalus latifolus International Journal of Biochemistry Research & Review, **4(***6):584-592.

Iyiola, O. A., Tijani, A. Y. and Lateef, K. M, (2011). Antimalarial Activity of Ethanolic Stem Bark Extract of *Alstonia boonei* in Mice*. Asian Journal of Biological Sciences, **4***:235-243.

Jacob, R. A. and Burri, B. J. (1996). Oxidative damage and defense..*American Journal of Clinical Nutrition,* **63**:985S–990S

Jennings, G. and Elia, M.(1987) Effect of E. coli endotoxin on temperature, oxygen consumption and brown adipose tissue thermogenesis in rats and mice," *Bioscience Reports*, **7**(6):517–523,.

Kalra,B. S.,Chawla, S., Gupta, P. and Valecha, N. (2006) "Screening of antimalarial drugs: an overview," *Indian Journal of Pharmacology*, **38**(1):5–12

Kim, K., Lee, S., Jung, S., Park, H., Shinkilt, Y. and Kim, B.K. (2003). Antioxidant activities of the extracts from the herbs of *Artemisia apiacea*. *Journal of Ethnopharmacology*. **85**: 69-72.

Lanar, D.E. (1990). Sequence of the circumsporozoite gene of *Plasmodium berghei* ANKA clone and NK65 strain. *Molecular & Biochemical Parasitology*, **39**(1):151-153.

Langhorne, J., Quin, S. J. and Sanni, L. A., (2002). "Mouse Models of Blood-Stage Malaria infections: Immune Responses and Cytokines Involved in Protection and Pathology," Chemical Immunology: 80: 204-228. Doi:10.1159/000058845.

Lorke, D. (1983). A new approach for acute toxicity testing. *Archives of toxicology*, **54**, 275-287.

Lü, L., Liu, S. W., Jiang, S. B. and Wu, S. G. (2004). Tannin inhibits HIV-1 entry by targeting gp41. *Acta Pharmacologica Sinica*; **25**(2):213–8.

Mbatchou, V. C., Ayebila, A. J. and Apea, O. B. (2011). Antibacterial activity of phytochemicals from *Acacia nilotica, Entada africana* and *Mimosa pigra L*. on Salmonella typhi. Journal of Animal and Plant Sciences., 10 (1): 1248-1258.

Miller, N. J., Rice–Evans, C. A., Davies, M. J., Gopinathan, V. and Milner, A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science*; 84:407–412.

Millogo-Kone, H., Lompo, M., Kini, F., Asimi, S., Guissou, I. P. and Naucoulma, O. (2000). Evaluation of flavonoids and total phenolics content of stem bark and leaves of *P.biglobosa* (Jacq) Benth (Mimosaceae), free radical scavenging and antimicrobial activities. *Phytotherapy Research*. **8**: 635-642.

Misra, H. P. and Fridovich, I., (1972). The role of anion in the auto-oxidation of Epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry,* **247**:3170-3175.

Muhammad, N. O. and Oloyede, O. B. (2009): Haematological parameters of Broiler chicks fed Aspergillus niger-fermented Terminalia catappa seed meal based diet. *Global Journal of Biotechnology and Biochemistry*; **4**(2): 169-178.

Molineaux, L. and Gramiccia, G. (1980). The Garki Project: Research on the epidemiology and control of malaria in the Sudan Savanna of West Africa. World Health Organization. p 311.

Mukherjee, P. R. (2002). Quality control of herbal drugs: an approach to evaluation of botanicals. 13th edition, New Delhi.: Business Edition; 100-103

Ngaha, E.O., Akanji, M.A. and Madusolumuo, M.A.(1989). Studies on the correlation between chloroquine-induced tissue damage and serum enzyme changes in the rats. *Experientia,* **45:**143–146.

Odeghe, O. B., Uwakwe, A. A. and Monago, C. C. (2012). Antiplasmodial Activity of Methanolic Stem Bark Extract of *Anthocleista grandiflora* in Mice. *International Journal of Applied Science and Technology*, **2**(4).142-148

Odugbemi, T. O., Akinsulire, O. R., Aibinu, I. E. and Fabeku, P. O. (2007). Medicinal Plants Useful for Malaria Therapy in Okeigbo, Ondo state, southwest Nigeria. *African Journal of Traditional Complementary and Alternative Medicines*, **4**(2): 191- 198.

Oloyede, H. O. B., Bello, T. O., Ajiboye, T. O. and Salawu, M.O. (2015). Antidiabetic and antidyslipidemic activities of aqueous leaf extract of *Dioscoreophyllum cumminsii* (Stapf). Diels in alloxan-induced diabetic rats. *Journal of Ethnopharmacology* **166**;313–322

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., and Simons, A. (2009). Agroforestree database: A tree reference and selection guide version 4.0. Available: http://www.worldagroforestry.org/af/treedb/.

Peters, W. and Robinson, B. L. (1992). The chemotherapy of rodent malarial. Studies on puronaridine and other manich base antimalarials. *Annals of Tropical Medicine and Parasitology*, 86: 455-465.

Ryley, J. F. and Peters, W. (1970). The antimalarial activity of some quinolone esters. *Annals of Tropical Medicine and Parasitology*, **84**: 209-222.

Sachs, J. and Malaney P.(2002). The economic and social burden of malaria. *Nature,* **415**(7):680-685

Saxena, S., Pant, N., Jain, D. C. and Bhakuni, R. S. (2003).Antimalarial agents from plant sources. *Current Science*. **9**: 1314-1329.

Schofield, L. and Grau, G.E. (2005).Immunological processes in malaria pathogen-nesis. *Nature Reviews Immunology*. 5(2005): 722-735.

Sendagire, H., Kyabayinze, D., Swedberg, G. and Kironde, F. (2005). Plasmodium falciparum: higher incidence of molecular resistance markers for sulphadoxine than for pyrimethamine in Kasangati, *Uganda. Tropical Medicine & International Health*, **10**: 537-543. https://doi.org/10.1111/j.1365-3156.2005.01414.x

Silva, J. R., Ramos-Ade, S., Machado, M., de Moura, D. F., Neto, Z., Canto-Cavalheiro, M. M., Figueiredo, P. do Rosário VE, Amaral AC, Lopes D. (2011). A review of antimalarial plants used in traditional medicine in communities in Portuguese-Speaking countries: Brazil, Mozambique, Cape Verde, Guinea-Bissau, Sao Tome and Prıncipe and Angola, *Memorias do Instituto Oswaldo Cruz/* **106***,* (Supplement 1):142–158.

Silva, O.,Duarte, A., Cabrita, J., Pimentel, M., Diniz, A. and Gomes, E. (1996).Antimicrobial activity of Guinea-Bissau traditional remedies. *Journal of Ethnopharmacology,*. **35**:12–16.

Sinha, A.(1972). Colorimetric assay of catalase. *Anal Biochem*;47:389–94.

Sofowora, S. (1982). Medical plants and Traditional Medicine in Africa, John Wiley and Sons Ltd. New York, p 214

Sofowora, S. (1993). Medicinal Plants and Traditional Medicine in Africa. 2nd ed: Spectrum Books Ltd., Nigeria. p 32

Tibiri, A., Sawadogo, R. W., Ouedraogo, N., Banzouzi, J. T., Guissou, I. P. and Nacoulma, G. O. (2010). Evaluation of antioxidant activity, total phenolic and flavonoid contents of Entada africana Guill. Et Perr. (Mimosaceae) organ extracts. *Research Journal of Medical Sciences*, **4**:81–87.

Tibiri, A., Banzouzi, J. T., Traore, A., Nacoulma, G. O., Guissou, I. P. and Mbatchi, B. (2007). Toxicological assessment of methanolic stem bark and leaf extracts of *Entada africana Guill.* And *Perr., Mimosacaeae*. *International Journal of Pharmacology,* **3**(5): 393-399.

Winter, R. W., Kelly, J. X., Smilkstein, M. J., Dodean, R., Bagby, G. C., Rathbun, R. K., Levin, J. I., Hinrichs, D. and Riscoe, M. K. (2006). Evaluation and lead optimization of anti-malarial acridones. Experimental Parasitology **114**(1):47-56. doi:10.1016/j.exppara.2006.03.014.

World Health Organization (2015). World malaria report 2015. Background-brief-malaria-vaccine/en/, accessed 16 November 2015)..

World Health Organization(2021). World malaria report 2021: Geneva: Licence: CC BY-NC-SA 3.0 IGO., 23-24

World Health Organization (2023). World malaria report 2023. Geneva: Licence: CC BY-NC-SA 3.0 IGO., p1-13.