

## **Antimalarial Activity of Ethanolic Leaf Extract of *Maesobotrya barteri* and Effects on Some Hematological and Biochemical Parameters of Mice**

**Dikioye Emmanuel Peters<sup>1\*</sup>, Emmanuel Onyebuchi Ezendiokwere<sup>1\*</sup>,  
Uche Chinedu Njoku<sup>1</sup> and Matthew Owhonda Wegwu<sup>1</sup>**

<sup>1</sup>University of Port Harcourt, Choba, Rivers State, Nigeria.

### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors DEP and MOW designed the study and supervised it. Author EOE managed the literature searches, wrote the protocol and the first draft of the manuscript. Author UCN performed the statistical analysis. All authors read and approved the final manuscript.*

### **Article Information**

DOI:10.9734/JALSI/2017/32992

#### Editor(s):

(1) Shahira M. Ezzat, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

#### Reviewers:

(1) Ogundolie Olubusola Omolara, Federal University of Technology Akure, Nigeria.

(2) Aina, Oluwagbemiga. Olanrewaju, Nigerian Institute of Medical Research, Nigeria.

Complete Peer review History: <http://www.sciencedomain.org/review-history/19954>

**Original Research Article**

**Received 27<sup>th</sup> March 2017**

**Accepted 22<sup>nd</sup> April 2017**

**Published 8<sup>th</sup> July 2017**

### **ABSTRACT**

**Aim:** This study was conducted to determine the antimalarial activity of ethanolic leaf extract of *Maesobotrya barteri*, and effects on some hematological and biochemical parameters.

**Methodology:** Twenty eight albino mice were selected and twenty four of these were injected intraperitoneally with  $1 \times 10^6$  *Plasmodium berghei* (NK65 strain) parasitized red blood cells in 0.5 ml of distilled water on the first day. After 72 hours, the animals were placed in seven groups having four animals each. Group 1 was uninoculated and untreated. Group 2 (inoculated and untreated) served as negative control. Group 3 was treated with 25 mg/kg bwt chloroquine and served as the positive control. Group 4 was treated with 4 mg/kg body weight of artesunate. 500 mg/kg, 800 mg/kg and 1000 mg/kg body weight of the extract were administered to groups 5, 6 and 7 respectively for four (4) days. On the fifth day post administration, the animals were anesthetized and blood obtained by cardiac puncture for hematological and biochemical investigation. The liver from the mice were harvested after sacrifice on the fifth day and subjected to histological

\*Corresponding author: E-mail: [petersidikioye@ymail.com](mailto:petersidikioye@ymail.com), [emmanuel\\_ezendiokwere@uniport.edu.ng](mailto:emmanuel_ezendiokwere@uniport.edu.ng);

techniques in order to generate permanently stained sections.

**Results:** *Maesobotrya barteri* showed a significant parasitemia reduction. For all three doses of the extract administered to infected mice, there was a significant reduction in the mean number of parasitized red blood cells during the four days of treatment. The concentration of 1000 mg/kg body weight of the plant extract had the highest antimalarial activity with a parasite inhibition of 97.2%. There was a significant increase in PCV level and haemoglobin concentration. The WBC count showed an increase which was not statistically significant. The ESR level was lowered for the treated groups when compared to the negative control. The liver markers assay revealed a significant lowering in the levels of ALT, AST, ALP and total bilirubin for all treated groups when compared to the negative control. The levels of the liver enzymes for the positive control was similar to the values obtained for the extract treated groups. The histology of the infected, untreated group showed endothelial cell derangement in the central vein (CV) and hepatic necrosis. The histological section of groups treated with standard drugs and 1000 mg/kg bwt extract showed the impression of normal histology.

**Conclusion:** This study has established the antiplasmodial activity of *Maesobotrya barteri*. The study also confirmed that the plant has hematopoietic potential and hepato-enhancing property. These findings support the folkloric use of the plant in the treatment of malaria.

**Keywords:** *Maesobotrya barteri*; *Plasmodium berghei*; antimalarial activity; hematological parameters; Biochemical indices.

## ABBREVIATIONS

*M.barteri* : *Maesobotrya barteri*;  
*PCV* : *Packed Cell Volume*;  
*Hb* : *Haemoglobin*;  
*ESR* : *Erythrocyte sedimentation rate*.

## 1. INTRODUCTION

Phytochemicals are naturally occurring bioactive substances. They are found in different parts of plants in different concentrations. They are deemed valuable for their various functions [1] amongst which are: They play important protective and disease preventive role in plants and other organisms including humans, some of them attract pollinating agents, whereas others prevent predators. Due to the therapeutic properties of phytochemicals, most are now employed for culinary uses [2]. Recently, phytochemicals have attracted much interest from researchers in various fields because of their various applications [1]. They are valuable biosource of drugs used frequently in traditional systems of medicine, modern medicine, pharmaceutical intermediates and as leads in drug synthesis [3]; They have been reported to be responsible for the antimicrobial, antibiotic, anticancer, antihelminthic and antisickling properties of many agents [4]. Significant bioactive components found in plants include; alkaloids, flavonoids, tannins, phenolic compounds and others [5]. The extraction of these phytochemicals and their application has been the focus of advances in science of late [4].

The knowledge derived from their application is being harnessed daily in health care provision.

Malaria is a mosquito transmitted infectious disease caused by plasmodium species with symptoms such as fever, headache, shivering, nausea, anemia, hepatomegaly etc [6]. The most severe form of the disease is caused by *Plasmodium falciparum* [7]. This disease is common in the tropics, being prominent in regions like the Central and Latin America, sub Saharan Africa and some parts of Asia. Parham et al. [8] reported that they are a major cause of morbidity and contribute significantly to the total recorded death in these areas. The socioeconomic impact of malaria endemicity has cost implication which include money value of lost workdays, reduced productivity owing to absenteeism and morbidity and under-performance at the job [9]. There is a wide gap in economic prosperity between malarious and non-malarious nations citing the disparity in purchasing power as an example [10]. There has been concerted efforts towards the control and prevention of malaria. These have come in various forms like the use of mosquito nets, indoor residual spraying and the development of vaccines [11-14].

Despite these efforts, a number of factors are greatly facilitating the spread and persistence of malaria. The high cost of orthodox medicine and particularly, the current resistance to available antimalarial drugs by the parasite are among the major factors [15] Drugs like chloroquine,

quinine, primaquine and mefloquine have become ineffective in the treatment of malaria and there has been reported cases of emergence of resistance to certain artemisinin class of drugs in some areas [16].

*Plasmodium berghei* is a protozoan parasite that causes malaria in certain rodents. It is commonly used in the study of human malaria due to its ability to infect rodents and relative ease of genetic engineering. It is similar to other species of plasmodium which causes human malaria, has similar life-cycle to these species and causes disease in mice with signs alike to that seen in human malaria [17].

Synthetic compounds are used in the treatment of malaria [18]. Some of these drugs are effective both as curative and preventive agents. Various agents are effective against different forms of the parasite and at different life stages of the parasite [19]. The common drugs used in malaria therapy like chloroquine, mefloquine, halofantrine, lumefantrine, primaquine and artesunate belong to various drug classes like 4-aminoquinolines, arylaminoalcohols, 8-aminoquinoline and artemisinin [20-24]. Traditional plants have been used to treat malaria for thousands of years and are the source of the two main groups of modern antimalarial drugs, artemisinin and quinine derivatives, both from *Artemisia annua* and *Cinchona spp* respectively [25]. A research finding reported that about 160 families of plants consisting of 1200 plant species have been documented as possessing both antiplasmodial and antipyretic activities and are extensively used in three continents. Among these families were those of Annonaceae, Anacardiaceae, Crassulaceae, Fabaceae and Euphorbiaceae [25]. One of such plants that form part of African rich medicinal flora, is *Maesobotrya barteri*. It is a member of the Euphorbiaceae family [26] and is widely spread. In Nigeria, it is well distributed and known by various names such as mmiriogu among the Ibos, Uvune in Etche land and Oruru in Benin. As of its pharmacological relevance, various communities use the plant to treat several health conditions like diarrhea, stomach ache, venereal infections, malaria etc [27,28]. It was reported that the plant contained the following bioactive compounds- tannins, saponins, cardiac glycosides, deoxysugar and terpenes [29]. Anemia is a common symptom associated with malaria. In anemic conditions, the total amount of red blood cells or the hemoglobin concentration in whole blood is decreased, hence oxygen carrying capacity of

the blood is lowered [30]. Hemolytic anemia is witnessed in malaria infection due to the destruction of red blood cells. Following this, there is increased level of bilirubin in the blood and also liver enzymes like aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase levels are elevated [31].

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

Fresh leaves of *Maesobotrya barteri* were collected in Chokocho community, Etche Local Government Area, Rivers State, Nigeria. The plant sample was identified and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State.

### 2.2 Sample Preparation

The leaves of the plant were obtained and dried under shade. They were ground into fine powder using a pulverizer. The powder (2.4 kg) was macerated in absolute ethanol (99.9%) at room temperature for 72 hours. It was then filtered using a filter paper and the filtrate was condensed in a rotary evaporator. The filtrate was further evaporated to dryness in a water bath at 50°C. A greenish residue weighing 36 g was obtained. The extract was kept in air tight sample bottles in a refrigerator until needed.

### 2.3 Phytochemical Screening

The crude leaf extract was phytochemically screened for the presence of alkaloids, tannins, glycosides, flavonoids, anthraquinones, saponins and cardenolides employing standard procedures and tests [32].

Quantification of the phytochemical constituents was done using a BUCK M910 Gas chromatography equipped with a flame ionization detector (FID).

### 2.4 Acute Toxicity Test

The safety of the leaf extract of *M. barteri* was determined using a standard method [33]. The animals were administered orally with doses of the leaf extract ranging from 1000 mg/kg to 5000mg/kg body weight. The animals were all kept under the same conditions and observed for toxicity signs and mortality for 24 hours for a seven (7) days period. LD<sub>50</sub> values were

calculated as geometric mean of the dose that resulted in 0 and 100% lethality.

## 2.5 Experimental Design

### 2.5.1 *In vivo* antimalarial studies

Twenty-eight (28) albino mice weighing 20-25 g were selected, and used for this study. The study was carried out after approval by the Animal Welfare Research Ethics Committee of the University of Port Harcourt, Rivers State Nigeria. Animal experiments were conducted in accordance with the internationally accepted principle for laboratory animal use and care [34]. Chloroquine sensitive strain of *Plasmodium berghei* (NK 65) was obtained from the Malaria Research and Phytotherapy Centre, University of Port Harcourt, Port Harcourt.

Twenty four (24) of the mice were injected intraperitoneally with  $1 \times 10^6$  *Plasmodium berghei* (NK 65 strain) parasitized red blood cells in 0.5ml of water. After 72 hours, the infection was confirmed by viewing the Giemsa stained thin blood films made with drops of blood from the tail of the infected mice and studied under the microscope (magnification  $\times 100$ ) [35]. The animals were distributed into seven groups and received oral treatments as given below:

- Group 1: Normal mice fed *ad libitum* with normal feed and distilled water.
- Group 2: Infected mice treated with 10 ml/kg bwt distilled water daily.
- Group 3: Infected mice treated with 25 mg/kg bwt chloroquine.
- Group 4: Infected mice treated with 4 mg/kg bwt artesunate.
- Group 5: Infected mice treated with 500 mg/kg bwt *M. barteri*.
- Group 6: Infected mice treated with 800 mg/kg bwt *M. barteri*.
- Group 7: Infected mice treated with 1000 mg/kg bwt *M. barteri*.

Group 2 served as the negative control, and group 3 was the positive control. Thin blood films were made daily for the 4 days of administration with blood obtained from the tail of the animals to determine the number of parasitized red blood cells (parasite density). On the fifth day, the animals were sacrificed after subjecting to mild anaesthesia using chloroform. Blood was collected by cardiac puncture and transferred to EDTA and heparin bottles which were used for hematological and biochemical tests

respectively. The liver was harvested for histological studies.

The percentage parasitemia was determined by microscopic examination using the formula:

$$\% \text{ Parasitemia} = \left[ \frac{\text{No of parasitized RBC}}{\text{No of Total RBC}} \times 100 \right]$$

The average percentage parasite inhibition was determined using the formula:

$$\text{Av \% inhibition} = \left[ \frac{\text{Av. Parasitemia in Negative Control} - \text{Av. Parasitemia in treated group}}{\text{Av. parasitemia in Negative control}} \times 100 \right]$$

## 2.6 Hematological Investigations

The haematocrit was determined using the method of Green and Ezilo [36]. Haemocytometer method was used to estimate the number of white blood cells. Leukocyte differential count was determined using the formula of Osim et al. [37]. The haemoglobin concentration was determined by haemometric method. The erythrocyte sedimentation rate (ESR) was determined by Westergreen method.

## 2.7 Biochemical Assays

### 2.7.1 Liver enzymes

The activity of the enzymes, aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were determined by kinetic methods with Randox Kits [36].

### 2.7.2 Total bilirubin (T.Bil) concentration

To determine the total bilirubin level, the method of Jendrassik and Grof [37] was used.

## 2.8 Histological Techniques

To generate permanently stained sections, small portions of the liver were fixated in formolsaline, dehydrated in various concentrations of alcohol, cleared with xylene and embedded. They were sectioned using the microtome and stained with haematoxylin and eosin [38].

## 2.9 Statistical Analysis

The data obtained from the experiment were expressed as mean  $\pm$  SEM. One way analysis of

variance (ANOVA) was used to analyze the data. Multiple comparison was done using post-Hoc. The values were considered to be statistically significant at  $p < 0.05$  [39].

### 3. RESULTS AND DISCUSSION

Table 1 shows the preliminary phytochemical screening of the ethanolic extract of *Maesobotrya barteri*. The result reveals that the plant contained alkaloids, flavonoids, steroids, carbohydrates, cardenolides and saponins. Flavonoids was the most abundant with kaempferol having a concentration of 45.5ug/g, rutin, a concentration of 27.ug/g, and epicatechin and catechin present in the concentrations of 7.4 ug/g and 25.2 ug/g respectively. (Table 2). This finding is supported by the report of Ogwuche et al. [40] which stated that the aerial parts of *M.barteri* had these secondary metabolites in addition to tannins. According to Ojewole [41], the pharmacological relevance of ethnomedicinal plants is due to their phytochemical composition. As such, the active ingredients present in the plant under study is highly implicated in its pharmacological property. For instance, there has been reports of the antiplasmodial property of alkaloids [42]. The presence of triterpenoids, saponins and glycosides was reported to be responsible for the antiplasmodial activity of the root extract of *Haematostaphis barteri*[43]. The steroids tumacone and tumacoside have been shown to inhibit the growth of *P. falciparium* [44]. The result of the acute toxicity studies conducted is contained in Table 3. The result shows that the plant extract did not cause mortality when administered orally at concentrations of 5,000 mg/kg, 3000 mg/kg, 2000 mg/kg and 1000 mg/kg body weight with the calculated median lethal dose of about 2250 mg/kg body weight. The plant could be deemed safe for administration without concern for hepatic or cellular damage. It is in keeping with the findings of Hodge and sterner [45] and Ghosh [46] who reported that with such concentration as median lethal dose, a plant could be considered relatively safe.

The mean number of parasitized red blood cells for each group of animals is presented in Table 4. The result revealed that there was a significant parasitemia reduction ( $P < 0.05$ ) when compared to the negative control. The reduction in parasitemia level at the three doses of the extract were similar to those observed for the reference drugs-25mg/kg chloroquine and 4mg/kg artesunate. The 1000 mg/kg bwt extract had the

highest parasitemia reduction by the fifth day post administration. The average percentage parasite inhibition/clearance were calculated for the three doses of the extract to be 95.5%, 95.3% and 97.2% (Table 5).The treatment with the plant extract showed a lowering of the parasite density in all extract treated groups. *M.barteri* belonging to the family Euphorbiaceae have been previously reported to have antimicrobial activity [28]. The research finding of the study conducted by Ogwuche et al. [39] revealed that the triterpene B-amyrin isolate from the aerial parts of *M.barteri* exhibited biological activity against various microbial culture growth. Its use in the traditional treatment of malaria has been reported although the scientific basis has not been established. From the results of the present study, *M. barteri* showed a significant antimalarial activity against *Plasmodium berghei in vivo*. The antimalarial action is dose dependent since the highest dose of the plant extract has the greatest parasite clearance. (Table 5). The lower and median doses had similar percentage parasite inhibition. Deharo et al. [47] states that *in vivo* antimalarial activity of plant extracts can be categorized as moderate, good and very good if the plant extract showed 50% or more chemosuppression/ inhibition at 500,250 and 100 mg/kg/day extract dose respectively. This suggests that the plant's antimalarial property can be rated as being moderate. Similar reports have rated other plants having antimalarial potential based on this scale [48,49]. *Languas galangal* and *Agelanthus dodoneifolius* have been shown to have similar suppressive action as *M. barteri* [50]. The standard drugs showed a chemosuppression/inhibition that could be deemed very good and this is in keeping with previous reports [51]. Chloroquine is reported to act by interfering with the parasites iron metabolic machinery [52]. *Artemisia annua*, *Annona senegalensis* and *Addhathoda schimperiana* have been suggested to follow same mechanism of action, owing to the fact that their bioactive compounds like alkaloids are the parent compound for synthetic products like chloroquine [53,54]. Flavonoids have been detected in the artemisia species and have been reported to show significant anti-material activity against *P. falciparium* [53]. These set of compounds (alkaloids and flavonoids) were identified in the plant, hence the allusion that the presence of these secondary metabolites could be the reason for the plant's therapeutic action.

**Table 1. Qualitative phytochemical composition of *M. barteri***

Phytochemical	Status
Alkaloids	+
Flavonoids	+
Tannins	-
Anthraquinones	-
Triterpenoid / Steroids	+
Fixed oils	-
Carbohydrates	+
Cardenolides	+
Saponins	+

Key: + = Present, - = Absent

**Table 2. Quantitative phytochemical composition of ethanolic leaf extract of *M. barteri***

Component	Subclass	Concentration (ug/g)
Flavonoid	Kaemferol	45.49
Flavonoid	Rutin	27.85
Flavonoid	Catechin	25.18
Flavonoid	Epicatechin	2.54
Saponin	-	21.32
Phenol	-	7.42
Alkaloid	Ribalidine	1.75
Oxalate	-	1.50
Phytate	-	0.57

**Table 3. Result of acute toxicity test of ethanolic leaf extract of *M. barteri***

Group	Dose (mg/kg BW)	No of deaths	Mortality %
1	1000	0/3	0
2	2000	0/3	0
3	3000	0/3	0
4	5000	0/3	0

(LD<sub>50</sub> = 2250mg/kg)

The effect of the plant extract on some hematological indices is shown in Table 6. The effect of different concentrations of the plant on PCV shows a significant increase (P<0.05) for the treated groups when compared to the negative control; the PCV levels for the treated groups were similar to the value for the positive control. Comparing the ESR of the treated groups to the negative control showed a decrease which was not statistically significant (P>0.05). The haemoglobin concentration for the treated groups showed a significant (P<0.05) increase relative to the negative control; the WBC count for the treated groups showed an increase when compared to the negative control; the levels of eosinophils and basophils were elevated in the treated groups whereas the Neutrophil level was significantly (p<0.05)

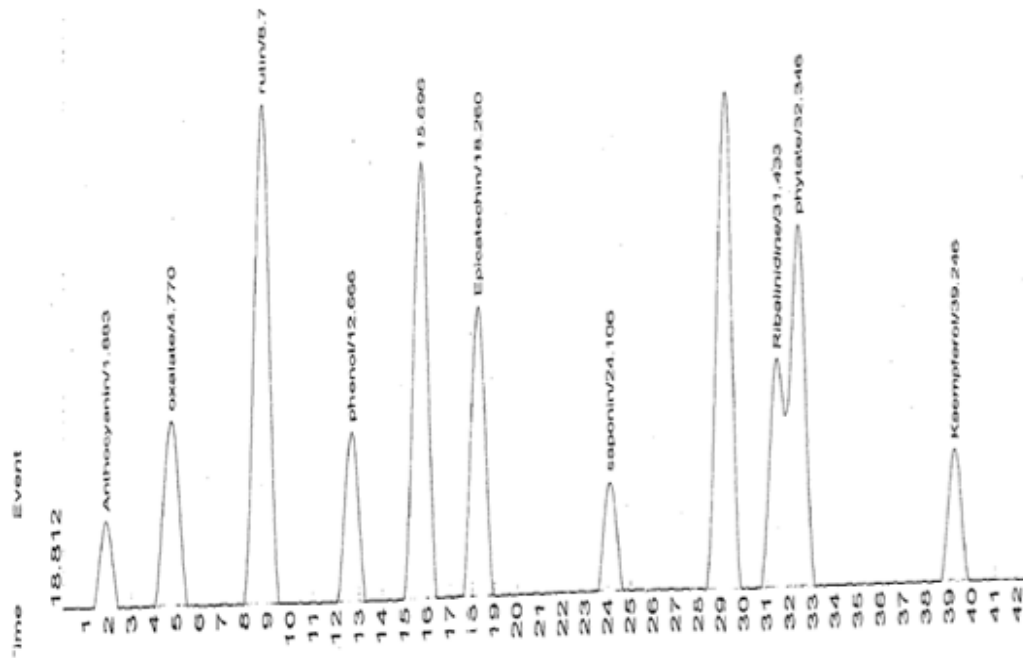
**Table 4. Average percentage parasitemia obtained from mice in each group in the curative test**

Test groups	Day 1	Day 2	Day 3	Day 4	Day 5
Uninfected, Untreated	0.00	0.00	0.00	0.00	0.00
Negative Control	10.1±1.62	11.4±1.84 <sup>a</sup>	12.9±2.25 <sup>a</sup>	17.6 ±.18 <sup>a</sup>	30.0 ± 4.48 <sup>a</sup>
Positive Control	7.26±1.41	1.10±0.30 <sup>a</sup>	1.53±0.12 <sup>a</sup>	1.36±0.29 <sup>a</sup>	0.30±0.05 <sup>a</sup>
4 mg/kg Artesunate	8.73±2.03	2.56 ± 0.44 <sup>a</sup>	1.23 ± 0.28 <sup>a</sup>	1.33 ± 0.26 <sup>a</sup>	0.20 ± 0.11 <sup>a</sup>
500 mg/kgbw of extract	13.9± 2.98	4.20 ± 1.34 <sup>a</sup>	2.60 ± 0.61 <sup>a</sup>	1.60 ± 0.40 <sup>a</sup>	1.36 ± 0.39 <sup>a</sup>
800 mg/kgbw of extract	10.1± 3.78	2.13 ± 0.31 <sup>a</sup>	2.13 ± 0.31 <sup>a</sup>	1.63 ± 0.41 <sup>a</sup>	1.40 ± 0.30 <sup>a</sup>
1000 mg/kgbw of extract	7.06± 0.34	2.66 ± 0.61 <sup>a</sup>	2.53 ± 0.67 <sup>a</sup>	1.56 ± 0.12 <sup>a</sup>	0.83 ± 0.33 <sup>a</sup>

Data are Mean ± SEM of four determinations. Values found in a column and bearing common superscript letter a, are significantly different (p<0.05) when compared to the negative control while values without superscript a, are not significantly different (p>0.05) in comparison to the negative control.

**Table 5. Average percentage inhibition of *Plasmodium berghei***

Drugs	Av % parasitemia	Av % inhibition
Negative Control	30.0 ± 4.48	0.00
Positive Control	0.30 ± 0.05	99
4mg/kg Artesunate	0.20 ± 0.11	99.3
500mg/kg bw of extract	1.36 ± 0.39	95.5
800mg/kg bw of extract	1.40 ± 0.30	95.3
1000mg/kg bw of extract	0.83 ± 0.33	97.2



**Fig. 1. Chromatogram of the phytochemical composition of *M. barteri***

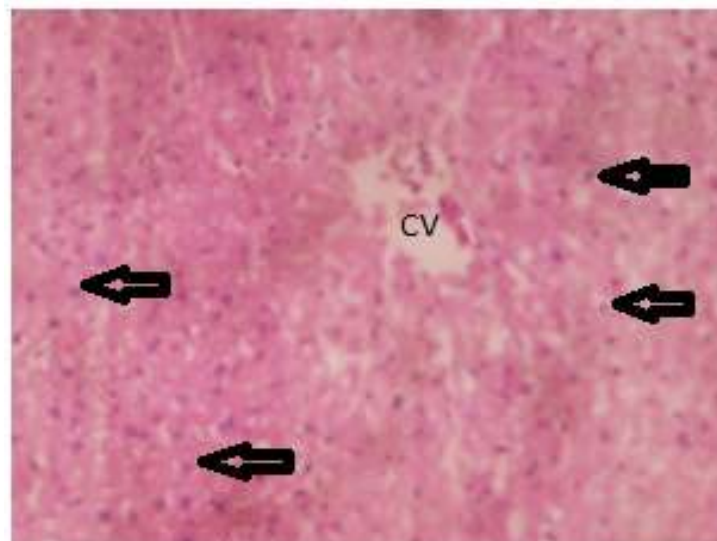
lowered relative to the negative control. The decreased WBC count in the infected, untreated group has been attributed to the damaging effect of the parasite in hematopoietic stem cells and hence the progression of the disease [55], this is because white cells are first line of defence in the incidence of infection [56]. The extract increased WBC count suggestive of the ability of the extract to increase the production of the cells of the immune system, thereby increasing the ability of the mice to combat the infection. The increase in levels of eosinophil and basophil is consistent with the findings of Bakhubaira [57]. The lowering of the neutrophil level on day 5 post administration could be suggestive of normalization of immune response following recovery; this is noteworthy because leukocytosis is indicative of pathological condition [58]. Upon administration of plant extract, PCV level was elevated, implying the amelioration of the hemolytic condition occasioned by infection. This is indicative of the hematopoietic potential of the plant extract. This is in line with findings of Ovuakporaye [59] which stated that plants with antimalarial property always increased the levels of the blood elements. The mechanism of action has been suggested as being by a direct effect on the reticuloendothelial system or by directly eliminating parasites via radical generation [60]. In the infected and untreated group, the haemoglobin concentration was lowered; this is

consistent with the finding of George and Ewelike-Ezeani [61]. The reason has been given as due to the destruction of the red blood cells. Extract administration elevated the concentration of haemoglobin, suggesting that the extract may have had stimulatory effect on red cell production (erythropoiesis). The findings of this study agrees with the report of a previous study stating that ESR level increase during attack with malaria [62]. In a bid to effect cure, the plant extract caused alteration in haematological indices, an observation which has been made in a previous report [63].

The level of the liver enzymes (AST, ALT and ALP) were significantly lowered ( $p < 0.05$ ) when compared to the negative control. The total bilirubin concentration was significantly ( $p < 0.05$ ) elevated in the negative control when compared to the treated groups. The concentration of total bilirubin for the treated groups were similar to the value observed for the positive control (Table 7). The biochemical tests can be checked to follow hepatocellular integrity and liver injury, especially in cases of infection. The levels of these enzymes provide valuable confirmatory and suggestive basis for assessing liver status [64], [65]. The administration of the plant extract caused a decrease in the activities of AST, ALT and ALP. There is a liver stage in the life cycle of the parasite in the course of the progression of the disease. The elevated levels of these

enzymes in the infected, untreated group may be attributed to damage to the liver via oxidative stress upon infection; the lowering of the activity of these enzymes could be said to be as a result of the healing of the tissue damage by the extract. Hillwell [66] explained that plants containing flavonoids have good anti-oxidant property. It's reasoned that the healing was achieved via mopping up of the free radicals by flavonoids contained in the plant extract. The total bilirubin concentration was lowered upon extract administration, further establishing the positive effect of the extract on the liver. This is important, recalling that the lysis of red cells in malaria is largely responsible for increased plasma concentration of total bilirubin. The histopathology results are shown in Figs. 2-7. Fig. 2 (histology of the negative control) shows the central vein with cords of hepatocytes radiating away from the central veins. Hepatocytes have microvesicular steatosis. These indicate hepatic necrosis. The standard drugs treated groups histological sections are presented in Figs.3 and 4. The photomicrograph reveals a normal portal triad (hepatic arteriole, portal venule and bile duct) and normal cords of hepatocytes. This impression is a normal histology. Figs. 5 and 6 shows the histological sections of mice treated with 500 mg/kg and 800 mg/kg bwt of extract. The photomicrographs reveal central veins and hepatocytes with microvesicular steatosis and these were

indicative of disruption of the architectural integrity of the liver. Fig. 7 is the histology of mice treated with 1000 mg/kg bwt extract. The features include central vein which is congested and normal hepatocytes. The impression is a normal histology. The endothelial cell derangement in the central vein (CV) and hepatic necrosis evidenced in the negative control can be attributed to the exoerythrocytic stage of the parasite's life cycle in the liver. This is in agreement with the report by Prudencio [67] which states that motile sporozoites migrate and infect hepatocytes forming parasitophorous vacuoles. The disruption in the plasma membrane of the hepatocytes induces spontaneous necrosis of the affected liver cells. Partial or total blockage of hepatic arteries and lactic acid generation also contribute to the cellular derangement. For the extract treated groups, the group treated with 1000 mg/kg bwt of the extract showed no evidence of hepatic necrosis. The histology was similar to that of the standard drug treated groups. The parasitophorous vacuoles closing up indicates the healing of the liver by the plant extract [68]. It can be inferred that the extract mopped up most free radicals generated by the presence of *Plasmodium berghei* and helped stabilize the plasma membrane of the hepatocytes thereby reducing any possible necrotic effect caused by the parasite.



**Fig. 2. Histological Section of Liver of Negative Control**  
**Photomicrograph depicting hepatocytes with microvesicular steatosis. Magnification ×400.**  
**Haematoxylin and Eosin stained**



**Table 6. Effect of ethanolic leaf extract of *M.barteri* on some haematological parameters**

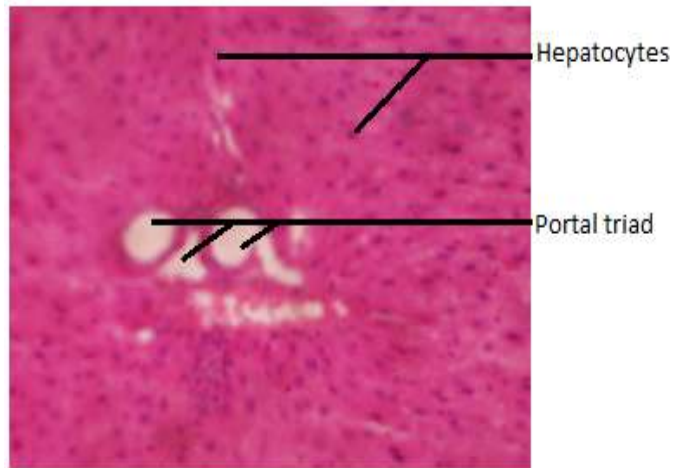
Test Groups	PCV(%)	ESR(mm/h)	HB(g/dl)	WBC( $10^3$ /ul)	EOS(450/ul)	BAS(200/ul)	NEU(/ul)
Uninfected, untreated.	43.5±1.21 <sup>b</sup>	6.0±1.03	14.4±0.90 <sup>b</sup>	5.7±1.85	7.5±4.15 <sup>b</sup>	3.5±2.50 <sup>b</sup>	28.5±2.56 <sup>b</sup>
Negative Control	36.3 ±0.33 <sup>ab</sup>	8.33 ±1.33	12.4 ±0.20 <sup>ab</sup>	4.66 ± 0.33	10.6 ± 0.66 <sup>b</sup>	7.00 ± 2.00 <sup>b</sup>	108.0 ± 3.00 <sup>ab</sup>
Positive Control	43.6 ± 1.66 <sup>a</sup>	5.66 ± 0.33	14.3 ± 0.10 <sup>a</sup>	6.33 ± 0.33	13.6 ± 2.66 <sup>b</sup>	14.6 ± 2.66 <sup>b</sup>	90.6 ± 2.66 <sup>ab</sup>
4 mg/kg Artesunate	51.0 ± 0.00 <sup>a</sup>	7.66 ± 0.33	14.6 ± 0.13 <sup>a</sup>	7.33 ± 0.66	19.0 ± 3.00 <sup>b</sup>	14.3 ± 1.33 <sup>b</sup>	55.3 ± 10.3 <sup>ab</sup>
500 mg/kg bw extract	49.0 ± 1.00 <sup>a</sup>	4.66 ± 0.66	14.2 ± 0.10 <sup>a</sup>	5.66 ± 0.66	28.6 ± 15.6 <sup>b</sup>	8.33 ± 2.33 <sup>b</sup>	47.6 ± 9.66 <sup>ab</sup>
800 mg/kg bw extract	42.0 ± 1.00 <sup>a</sup>	6.33 ± 1.33	14.3 ± 0.16 <sup>a</sup>	7.00 ± 0.00	22.6 ± 10.6 <sup>b</sup>	9.66 ± 0.66 <sup>b</sup>	58.0 ± 4.00 <sup>ab</sup>
1000 mg/kg bw extract	44.3 ± 0.33 <sup>a</sup>	6.66 ± 0.66	14.4 ± 0.16 <sup>a</sup>	7.00 ± 1.00	23.3 ± 8.33 <sup>b</sup>	11.3 ± 3.33 <sup>b</sup>	90.0 ± 1.00 <sup>ab</sup>

Data are expressed as Mean ± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ( $p<0.05$ ) when compared to the negative control. Values with superscript b, are significantly different ( $p<0.05$ ) relative to the uninfected untreated group.

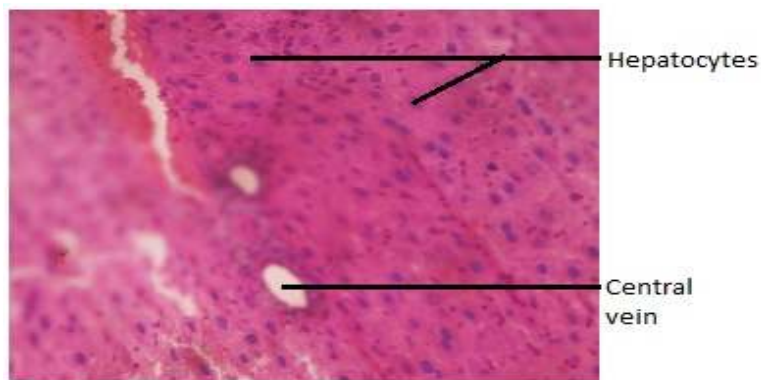
**Table 7. Effect of Ethanolic Leaf Extract of *M. barteri* on Some Biochemical Parameters of Mice**

Test groups	ALT(U/L)	AST(U/L)	ALP(U/L)	TB(umol/l)
Uninfected, Untreated	6.9±0.89 <sup>b</sup>	6.5±0.39 <sup>b</sup>	72.5±1.42 <sup>b</sup>	5.4±0.58 <sup>b</sup>
Negative Control	23.0 ± 2.00 <sup>ab</sup>	19.6 ± 0.66 <sup>ab</sup>	225.0 ± 16.0 <sup>ab</sup>	14.7 ± 0.63 <sup>ab</sup>
Positive Control	11.0 ± 1.00 <sup>a</sup>	9.66 ± 1.33 <sup>a</sup>	107.3 ± 4.04 <sup>a</sup>	9.63 ± 0.43 <sup>a</sup>
4 mg/kg Artesunate	12.3 ± 0.66 <sup>a</sup>	8.33 ± 0.33 <sup>a</sup>	98.3 ± 1.33 <sup>a</sup>	4.66 ± 0.66 <sup>a</sup>
500 mg/kgbw extract	13.0 ± 1.00 <sup>a</sup>	12.0 ± 1.00 <sup>a</sup>	115.6 ± 4.66 <sup>a</sup>	9.20 ± 0.90 <sup>a</sup>
800 mg/kgbw extract	8.66 ± 0.66 <sup>a</sup>	7.33 ± 0.66 <sup>a</sup>	97.6 ± 4.66 <sup>a</sup>	9.30 ± 0.46 <sup>a</sup>
1000 mg/kgbw extract	12.6 ± 3.33 <sup>a</sup>	5.00 ± 2.00 <sup>a</sup>	74.6 ± 14.6 <sup>a</sup>	5.86 ± 0.76 <sup>a</sup>

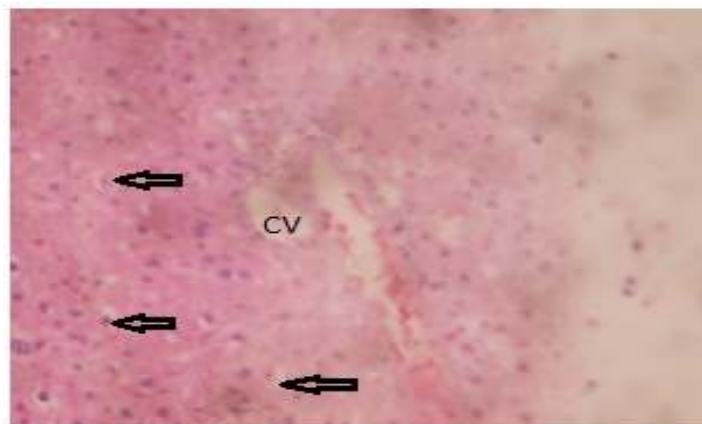
Data are Mean± SEM. n=4. Values found in a column with common superscript letter a, are significantly different ( $p<0.05$ ) when compared to the negative control. Values with superscript b, are significantly different ( $p<0.05$ ) relative to the uninfected untreated group.



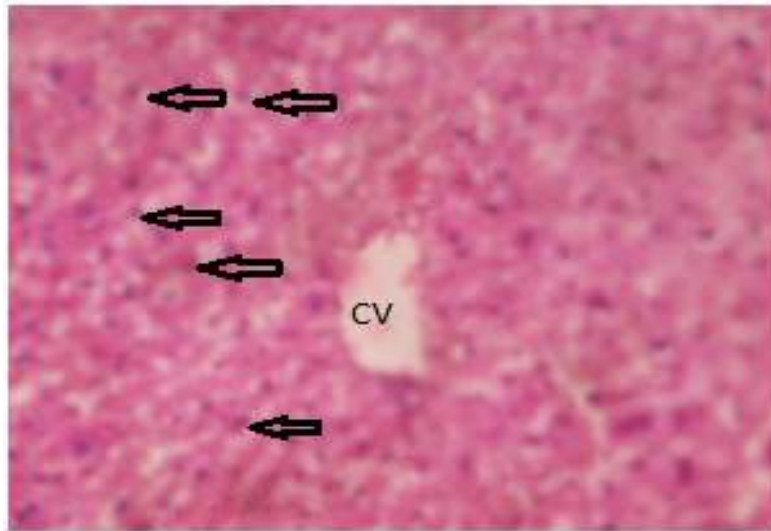
**Fig. 3. Histological Section of Liver for Group treated with 25mg/kg bwt chloroquine Photomicrograph showing normal histology, no fatty degeneration. Magnification ×400. Haematoxylin and Eosin stained**



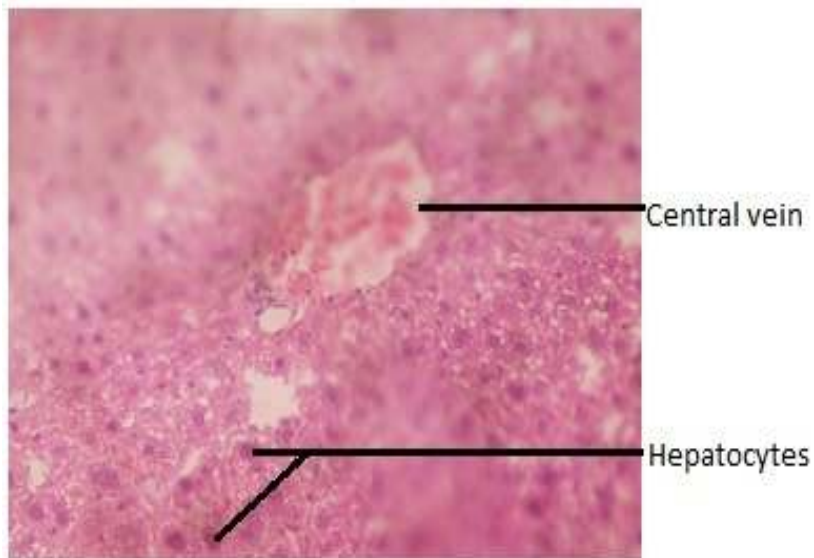
**Fig. 4. Histological Section of Liver of Group treated with 4mg/kg bwt Artesunate Photomicrograph depicting no area of necrosis and fatty degeneration. Magnification ×400. Haematoxylin and Eosin stained**



**Fig. 5. Histological section of liver of group treated with 500 mg/kg bwt extract PhoTomicrograph showing hepatocytes with microvesicular steatosis. Magnification ×400. Haematoxylin and Eosin stained**



**Fig. 6. Histological Section of Liver of Group treated with 800mg/kgbw extract**  
Photomicrograph depicting hepatocytes with microvesicular steatosis. Magnification  $\times 400$ .  
Haematoxylin and Eosin stained.



**Fig. 7. Histological Section of Liver of Group treated with 1000 mg/kgbw extract**  
Photomicrograph showing normal hepatocytes. No area of necrosis and no fatty degeneration.  
Magnification  $\times 400$ . Haematoxylin and Eosin stained

#### **4. CONCLUSION AND RECOMMENDATION**

This study has shown that *Maesobotrya barteri* possesses antimalarial activity. The plant also increased the levels of haemoglobin, packed cell volume and white blood cells while lowering the levels of AST, ALT, ALP and total bilirubin,

implying that the plant has both hematopoietic and hepatic-enhancing functions. These effects further establishes its antimalarial potential. However, further studies should be carried out to understand how the combination of the plant with other plants with known antimalarial activity can boost its therapeutic potency.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge the input of the laboratory staff of malaria and phytotherapy research laboratory and pharmacognosy laboratory, University of Port Harcourt towards this research work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Onoja S. Phytochemical analysis and proximate composition of *Nauclea latifolia*. Postgraduate research project. 2009; 7-13.
2. Okwu DE. Evaluation of the chemical composition of indigenous spices and flavouring agent. Journal of Science. 2004;7:455-459.
3. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology. 2008;7(12):1797-1806.
4. Preshant T, Bimlesh K, Mandeef K, Gurpreet K, Harken H. Phytochemical screening and analysis. A review. International Pharmaceutical Scientia. 2011;1(1):98-106.
5. Hill AF. Economic botany. A textbook of useful plants and plant products. 2<sup>nd</sup> ed. New York: Mc Graw-Hill Book Company Inc. 1952;10-13.
6. Maude RJ, Hassan MU, Beare NA. Severe retinal whitening in an adult with cerebral malaria. American Journal of Tropical Medicine and Hygiene. 2009;80(6): 881.
7. Jung-Mi K, Sung-Ung M, Jung-Yeon K, ShinHyeong C, Khin L. and Woon-Mok S. Genetic polymorphism of merozoite surface protein-1 and merozoite surface protein-2 in *Plasmodium falciparum* field isolates from Myanmar. Malaria Journal. 2010;9:131.
8. Parham PE, Christiansen-Jucht C, Pople D, Michael E. Understanding and modeling the impact of climate change on infectious diseases In: Blanco, J. and Kheradmand H. Climate Change-Socioeconomic Effects. 2011;43-66.
9. Center for disease control and prevention. Impact of Malaria Worldwide. CDC 24/7; 2012. Available:<http://www.cdc.gov/a:ariaworldwide/impact.html>
10. Sachs J. Malaney P. Economic and social burden of malaria: A review article. Nature. 2002;415:680-685.
11. Bidla G, Titanji VP, Joko B, Ghazali GE, Bolad A, Berzins K. Antiplasmodial activity of seven plants used in African folk medicine. Indian Journal of Pharmacology. 2002;36(4):245-246.
12. Gbeassor M, Kedjagni AY, Koumaglo K, de Souza C, Agbo K, Aklikokou K, Amegbo KA. *In vitro* antimalarial activity of six medicinal plants. Phytotherapy Research. 1990;4(3):115-117.
13. Hill AV. Vaccines against malaria. Philosophical Transactions of the Royal Society. 2011;366(1579):2806-14.
14. Riley EM, Stewart VA. Immune mechanisms in malaria: New insights in vaccine development. Nature Medicine. 2013;19(2):168-78.
15. Schlitzer M. Malaria chemotherapeutics part 1: History of antimalarial drug development, currently used therapeutics and drugs in clinical development. Chemistry Enabling Drug Discovery. 2007; 7:944-986.
16. Arjen MD, Francois N, Poravuth Y, Debashish Aung P, Joel T. Resistance in *Plasmodium falciparum* malaria. New English Journal of Medicine. 2009; 361:455-467.
17. Craig AG, Grau GE, Janse C, Kazura JW, Milner D, Barnwell JW, Turner G, Langhorne J. The role of animal models for research on severe Malaria. PLOS Pathogens. 2012;8(2):e1002401.
18. Bruce-Chwatt LJ. Essential malariology. William Heinemann Medical Books Ltd. 1985;55-59.
19. Programme for appropriate technology in health. Malaria. Health Technology Directives. 1990;18:1154-1155.
20. David J, Sullivan J, Ilya Y, Gluzman David G, Russell, Daniel E. On the molecular mechanism of chloroquine's antimalarial action. Proceedings of the National

- Academy of Sciences, USA. 1996; 93:11865-11870.
21. Srinivas. Malaria prophylaxis: Malaria control in Mangaluru; 2015.  
Available:<http://www.malariasite.com/prophylaxis/>
  22. Grewal RS. Pharmacology of 8-aminoquinolines. Bulletin of the World Health Organization. 1981;59(3):397-406.
  23. Meshnick SR. Artemisinin: mechanisms of action, resistance and toxicity. International Journal for Parasitology. 2002;32(13): 1655-1660.
  24. Gemma L, Steve A, Paul M, Giancarlo A. Inhibitors of the plasmodium mitochondrial respiratory chain. In Encyclopedia of Malaria (Eds: Marcel H and Peter, G); 2015.  
DOI :10.1007/SpringerReference-372319
  25. Merlin L, Gerard B. Traditional herbal medicines for malaria. Clinical Review. 2004;329:1156-9.
  26. Ogbuagu MN, Agu B. Fruit nutritive composition of *Maesobotrya barteri*, an underexploited tropical African tree. Fruits. 2008;63(6):357-361.
  27. Dalziel JM. The useful plants of West Tropical Africa. London UK: The Crown Agents for the Colonies.1994;612.
  28. Uduak A Essiett, Kola K, Ajibesin. antimicrobial activities of some Euphorbiaceae plants in the traditional medicine of Akwa Ibom State of Nigeria. Ethnobotanical leaflet. 2010;14:654-64.
  29. Akan, N.E. Phytochemical analysis and *in vitro* antimicrobial studies of ethanolic leaf extract of *Maesobotrya barteri*. An unpublished B. Sc thesis. Department of Chemistry, University of Uyo, Uyo Nigeria; 2014.
  30. Rodak F. Hematology: Clinical principles and applications. 3<sup>rd</sup> ed. Philadelphia: Saunders. 2007;220.
  31. Higgins C. Neonatal jaundice, breast milk and Gilbert's syndrome. Biomedical Scientist. 2002;2(2):1-7.
  32. Trease GE, Evans MC. Textbook of pharmacognosy. 13<sup>th</sup> ed. London: Bailliere, Tindall. 1989;683-684.
  33. British toxicology society working party on toxicity. Special report: A new approach to the classification of substances and preparations on the basis of their acute toxicity. Human Toxicology. 1984;3:85-92.
  34. National Institute of Environmental Health Sciences- NIEHS. Respect for life. NIH Publication. 1985;85-93.  
Available:<http://www.niehs.nih.gov>
  35. Riley JF, Peters W. The antimalarial activity of some quinolone esters. American Tropical Medical Parasitology. 1970;84:209-222.
  36. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamate oxaloacetate and pyruvate deaminases. American Journal of Clinical Pathology. 1957;28:56-68.
  37. Jendrassik L, Grof P. Estimation of total serum bilirubin level spectrophotometrically in serum and plasma. Biochem Zeitschrift. 1938;297:81-89.
  38. Sidheshwar PS. Manual of practical histology. 1<sup>st</sup> ed. Aba Nigeria: AAU Vitalis Book Co. 1993;7-15.
  39. Duncan RC, Knapp RG, Miller MC. Test of hypothesis in population means. In introductory biostatistics for the health sciences. New York: John Wiley and Sons Incorporation. 1977;71-96.
  40. Ogwuche CE, Amupitan JO, Ndukwe IG and Ayo RG. Isolation and biological activity of the Triterpene B-Amyrin from the aerial plant parts of *Maesobotrya barteri*. Medicinal Chemistry. 2014;4(11):729-733.
  41. Ojewole JA. Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllum pinnatum* (Crassulaceae) leaf aqueous extract. Journal of Ethnopharmacology. 2005;99:13-19.
  42. Tylor J, Arnold W, Thomas J, Saleh M. Antimalarial alkaloids isolated from *Annona squamosa*. Phytopharmacology. 2011; 1(3):49-53.
  43. Johnson N. *In vivo* antiplasmodial, anti-inflammatory and analgesic properties and safety profile of root extracts of *Haematostaphis barteri* Hook F. (Anacardiaceae). Journal of Parasitology Research. 2015;15:1-9.
  44. Arora R, Gill NS, Kaur S, Jain AD. Phytopharmacological evaluation of ethanolic extract of the seeds of *A. Precatorius* linn. Journal of pharmacology and toxicology. 2011;6(6):580-588.

45. Hodge A, Sterner B. Toxicity classes. In: Canadian Center for Occupational Health and Safety; 2005.  
Available:<http://www.ccohs.ca/oshanswers/chemicals/id50.htm.on3/5/2010>
46. Ghosh MN. Fundamentals of experimental pharmacology, 2<sup>nd</sup> ed. Calcutta: Scientific Book Agency; 1984.
47. Deharo E, Bourdy G, Quenevo C, Munoz V, Ruiz G, Sauvain MA. Search for natural bioactive compounds in Bolivia through multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. Journal of Ethnopharmacology. 2001;77:918.
48. Al-Adhroey A, Nor Z, Al-Mekhlafi H, Mahmud R, Median Lethal Dose. Antimalarial activity, phytochemical screening and radical scavenging of methanolic *langas galangal* rhizome extract. Molecules. 2010;15:8366-76.
49. Builders MI, Uguru MO, Aguiyi C. Antiplasmodial potential of the African Mistletoe: *Agelanthus didoneifolius*-Polli and wiens. Indian Journal of Pharmaceutical Sciences. 2012;74:223-9.
50. Oreagba A, Usman S, Aina O, Ogunkorede T, Olawale T, Olayemi S. Evaluation of the antimalarial effects of the leaf extract and fruit juice of *Annona muricata* against *Plasmodium berghei* infection in Mice. University of Lagos Journal of Basic Medical Sciences. 2013; 1(2):1-6.
51. Saxena S, Pant N, Jain DC, Bhakuni RS. Antimalarial agents from plant sources. Current Science. 2003;85:1314-29.
52. Dharani N, Rukunga G, Yenesew A, Mbora A, Mwaura L, Jamnadass R. Common antimalarial trees and shrubs of East Africa. World Agroforestry Centre, United Nations Avenue, Gigiri, P.O Box 30677-00100, Nairobi, Kenya; 2008.
53. Chanphen R, Thebtaranonth Y, Wanuppathamkul S, Yuthavong Y. Antimalarial principles from *Artemisia indica*. Journal of Natural Products. 1998; 61:1146-1147.
54. Yakubu MI, Akanji MA, Oladiji AT. Hematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia argrestis* stem. Pharmacology Magazine. 2007;3(9): 34-38.
55. Nwanjo HU, Opara AU. Effects of falciparum malaria infection on some haematological indices and renal functions. Journal of Medical Laboratory Science. 2005;14:6-10.
56. Maina RN, Douglas W, Charla G, Gordon H, John W, Lucas O, Danel J, Bernhard RO. Impact of *Plasmodium falciparum* infection on hematological parameters in children living in Western Kenya. Malaria Journal. 2010;9(3):54-61.
57. Bakhubaira S. Hematological parameters, severe complicated *Plasmodium falciparum* malaria among adults in Aden. Turkish Journal of Hematology. 2013; 30:394-399.
58. Fidele NK, Pascal AO, Lydia LL, Jean CN. The potential of antimalarial compounds derived from African medicinal plants, part 2a Pharmacological evaluation of non-alkaloids and non-terpenoids. Malaria Journal. 2014;13:18-23.
59. Ovuakporaye SI. Effect of malaria parasite on some haematological parameters: Red blood cell count, packed cell volume and haemoglobin concentration. Journal of Medical and Applied Bioscience. 2011; 3:45-57.
60. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery; Efficacy models for compound screening. National Review on Drug Discovery. 2004; 3:509-20.
61. George IO, Ewelike-Ezeani CS. Haematological changes in children with malaria infection in Nigeria. Journal of medicine and medical science. 2011;2(4): 760-771.
62. Supcharoen O, Widjaja H, Ali KB, Kitayaporn D, Pukrittayakamee S, Wilairatana P, Sathawarawong W. A study of erythrocyte sedimentation rate in malaria. Journal of Infectious Antimicrobial Agents. 1992;9(4):193-199.
63. Ajagbonna OP, Esaigun PE, Alayande NO, Akinloye AO. Anti-malarial activity and hematological effect of stem bark water extract of *nuclealatifolia*. Bioscience Research Communication. 2002;14(5): 481-486.
64. Nicholas CP, Lewis S. Clinical concept of enzymology. In: Fundamentals of enzymology. 2<sup>nd</sup> ed. New York: Oxford University Press. 1989;450-477.

65. Dufour DR, Lott JA, Nottle FS, Seff LB. Diagnosis and monitoring of hepatic injury in performance characteristic of laboratory tests. *Clinical Chemistry*. 2000;46:2027-2049.
66. Hillwell B. Free radicals, antioxidant and human disease: Curiosity, cause or constipation? *Lancet*. 1994;244:721-724.
67. Prudencio M, Rodriguez A, Mota MM. Silence path to thousands of merozoites; The plasmodium liver stage. *International Journal of Parasitology*. 2006;36:1283-1293.
68. Haines T. Do sterols reduce proton and sodium leaks through lipid bilayers? *Progress in Lipid Research*. 2001;40:299-324.

© 2017 Peters et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<http://sciencedomain.org/review-history/19954>