

In-vivo antiplasmodial activity of Aqueous, N-butanol and Ethylacetate fractions of Leaf and Stem Bark Methanol Extracts of *Diospyros mespiliformis* on *Plasmodium berghei berghei* (Nk65) Infected Mice.

Abstract

Aim: To determine the in-vivo antiplasmodial activity of aqueous, N-butanol and ethylacetate fractions of leaf and stem bark methanol extracts of *Diospyros mespiliformis* on *Plasmodium berghei berghei* (Nk65) infected mice.

Place and Duration of Study Sample: Biochemistry Department. Ahmadu Bello University Zaria and Pharmacy Department. Ahmadu Bello University Zaria. For a period of 6 months.

Methodology: A total of 130 mice weighing between 18-28g were randomly divided into thirteen (13) groups each (leaf extract=65 mice, stem bark extract =65 mice) of five (5) mice per group. Leaf extract at doses of 100, 200 and 400 mg/kg body weight and stem bark extract at doses of 50, 100 and 200 mg/kg body weight of ethylacetate, n-butanol and aqueous fractions, chloroquine (5 mg/kg) and Artemisinin-based combination therapy (Artesunate) 10 mg/kg were administered orally for four days. Qualitative, quantitative, parasitemia, packed cell volume and relative body weight analysis of the mice were monitored.

Results: The phytochemical studies revealed the presence of carbohydrates, free anthraquinone, cardiac glycosides, glycosides, saponins, tannins, alkaloids and flavonoids in the crude leaf extract and absence of cardiac glycosides, flavonoids, free anthraquinone and alkaloids in the stem bark extract. The quantitative phytochemical analysis of the fractions of leaf and stem bark extract of *Diospyros mespiliformis* showed that, saponins (0.57 ± 0.06 mg/g), (0.36 ± 0.21 mg/g), alkaloids (0.12 ± 0.04 mg/g), (0.67 ± 0.01 mg/g), tannins (0.73 ± 0.36 mg/g), (0.51 ± 0.22 mg/g), and glycoside had the highest concentration of (1.15 ± 0.10 mg/g), (0.97 ± 0.33 mg/g) respectively. At the end of the four (4) days suppressive test, packed cell volume and haemoglobin concentration showed significant ($P < 0.05$) decrease in the negative control group and significance ($P < 0.05$) increase in the infected but treated with chloroquine. Relative organ weight in the negative control group showed significant ($P < 0.05$) increase in n-butanol and aqueous fractions.

Conclusion: The present work establishes the antiplasmodial activity of the methanol extract of *Diospyros mespiliformis* which have shown potent parasite suppressive effects on *P. berghei* infected Swiss albino mice in a dose related fashion. Leaf and stem bark extract of *Diospyros mespiliformis* have shown potent parasite suppressive effects on *P. berghei* infected mice in a dose related fashion which is in agreement with previous studies of Ayodele,

(1979).The extracts treated groups did not show a significant decrease in PCV ($P > 0.05$) when compared to the negative control group. This is suggestive that the extract may contain some substances that either increase appetite or blood quality to the animals, in addition to its anti-plasmodial activity (Abebe *et al.* 2003) Therefore, the extracts showed a potential source of new chemotherapeutic agent. The mechanism of action of the leaf and stem bark methanol extracts exhibited competitive and non-competitive pattern of inhibition respectively.

Key words: Antiplasmodial activity, *Diospyros mespiliformis*, *P. berghei berghei*, Methanol fractions.

Introduction

Human malaria is caused by five different species of the protozoan parasite Plasmodium: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium knowlesi* and *Plasmodium malariae*, transmitted by the female anopheles mosquito^[1]. Although all the five species of malaria parasite can infect and cause illness. Only the malaria caused by *Plasmodium falciparum* is known to be potentially life threatening in humans. Infection with *P. falciparum* is therefore a medical emergency^[2]. The severity of *P. falciparum* infections (48hrs) has been reported to be due to high percentage of red blood cells (RBCs) that are infected by this particular Parasite. In extreme infections, up to 80% of RBCs can be parasitized and destroyed.

It has been estimated that approximately 198 million clinical cases of malaria occur in the year 2014 with 82% of these cases occurring in Africa.^[3] Malaria is estimated to kill nearly one million people annually, with most of the deaths occurring in children under 5 years of age^[4]. Drugs such as quinine related drugs, antifolate combination drugs and artemisinin and its derivatives are antimalarial drugs used for effective treatment^[5]. Clinical resistance to antimalarial combination drugs has been recently reported, suggesting that *Plasmodium falciparum* parasite have already developed the ability to grow in the presence of these drugs. The discovery of new and effective antimalarial drugs based on new mechanisms of action or with new structures, is urgently needed to overcome the problem of rapid emergence of drug resistance and achieve long-term clinical efficacy^[6].

Despite the substantial progress made in the treatment of parasitic diseases, malaria remains a significant therapeutic challenge especially because of the wide spread resistance of malaria parasites to currently available anti-malarial agents. These have stimulated the search for new pharmacologically active agents^[7]. The plant kingdom remains a major target in the search of lead compounds and new drugs to treat this debilitating parasitic disease. *Diospyros mespiliformis*, also known as African Ebony is a large deciduous tree found mostly in the savannas of Africa^[8].

Diospyros mespiliformis has been reported to have wide applications in traditional medicine which include the use of leaf decoction as a remedy for fever, whooping cough and for wounds^[9]. Bark and roots are used for serious infections such as malaria, pneumonia, syphilis, leprosy and dermatomycoses, as an antihelmintic and to facilitate delivery^[10]. In Nigeria, a leaf infusion is taken as a mild laxative and as a vermifuge, for fever, dysentery and is applied to wounds as a haemostatic. People prefer to use medicinal plants over allopathic medicine for various reasons; relatively low cost, effectiveness, perceived safety and minimal side effects^[11]. Studies have indicated that some of the plant's secondary metabolites are potent to human diseases as well. Therefore, plants are widely used in traditional medicines and many natural medicinal products are derived from ethno medicinal plants. *D. mespiliformis* is used in areas where malaria is endemic, where individuals might possess at least some degree of immunity in which relief may in addition be symptomatic^[12]. Studies of *D. mespiliformis* showed valuable medicinal biological properties such as anti-diabetic, antihelmintic, analgesic, antioxidant and anti-inflammatory activities^[13]. From the foregoing, it is necessary to subject these plants to detailed scientific studies, to determine its antimalarial activities and its efficacy in the treatment of malaria.

MATERIAL AND METHODS

Material

Plant material

Fresh leaf and stem bark of *Diospyros mespiliformis* were collected from Zango village, in Sabo-Gari Local Government Area of Kaduna State, in the month of June 2013 and authenticated by Gallah U. J. a taxonomist, in the herbarium unit, Department of Biological sciences, Ahmadu Bello University Zaria where a voucher specimen with voucher number 901431 was deposited.

Experimental animals

A total of a hundred and thirty (130) mice (leaf extract=65 mice, stem bark extract =65 mice) weighing between 18-28g were purchased from the animal house, Department of Pharmacology, Ahmadu Bello University, Zaria. The animals were housed in well-ventilated cages and allow to acclimatized under standard laboratory conditions for a period of two weeks before commencement of the experiment. The mice were randomly divided into thirteen (13) groups, with five animals per group: Normal control group, Negative control group (Infected but not treated), Infected and treated with standard chloroquine (5 mg/kg), Infected and treated with Artemisinin-based Combination

therapy (Artesunate) 10 mg/kg , Infected and treated orally with low, medium and high dose (100, 200 and 400 mg/kg) of ethylacetate, n-butanol and aqueous fractions of leaf methanol extract, Infected and treated orally with low, medium and high dose (50, 100, and 200 mg/kg) of ethylacetate, n-butanol and aqueous fractions of stem bark methanol extract.

Methods

Preparation and fractionation of plant extract

Fresh leaf and stem bark of *Diospyros mespiliformis* were rinsed in clean water and air dried at room temperature for two weeks. The dried leaf and stem bark were pulverized using Thomas-Wiley laboratory mill (Model 4) U.S.A. 500 g of the pulverized plant leaves and stem bark were suspended each in 2.5 L of methanol and the solution was left standing for 48 hours in large amber bottles with intermittent shaking. At the end of the extraction, the crude methanol extract was filtered and then evaporated to dryness using a rotatory evaporator. The crude methanol extract of *Diospyros mespiliformis* was subjected to liquid-liquid partition to separate the extract into different fractions. The reconstituted extract (250ml) was placed in a separatory funnel and 250ml each of ethylacetate and *n*-butanol solvents were added sequentially as a 1:1 (v/v) solution and rocked vigorously^[14]. The sample was left standing for 30 minutes for each solvent on the separating funnel until a fine separation line appear clearly indicating the supernatant from the sediment before it was eluted. The process was repeated thrice in order to get adequate quantity for each fraction. The ethylacetate, *n*-butanol as well as the aqueous residue fractions were concentrated over a water bath maintained at 45 °C. The concentrated fractions were kept in sealed containers and refrigerated at 2-4 °C until required for analysis.

Parasites

The choloquine sensitive *Plasmodium bergheberghei* used in this study was obtained from, Nigerian Institute for Medical Research (NIMR) Yaba, Lagos, Nigeria. The parasite was maintained by sub-passaging into healthy mice on a weekly basis throughout the duration of this study using the method described by Peter *et al* and David *et al*,^{[15][16]}. *Plasmodium bergheberghei* infected red blood cells were intraperitoneally injected into the mice from the blood diluted with Phosphate Buffered Saline (PBS) so that each 0.2ml administered per kg body weight contains approximately 10⁶-10⁷ infected red cells.

Qualitative Preliminary Phytochemical Screening

Qualitative Phytochemical screening of the leaf and stem bark methanol extract of *Diospyros mespiliformis* were carried out according to standard methods [17, 18 and 19].

Antiplasmodial Activity (4-Days Suppressive Test)

In-vivo study of antimalarial property of the plant was evaluated by determining its suppressive antiplasmodial properties by using the method of Peter^[20]. Adult Swiss mice weighing between 18 to 28g were inoculated by intraperitoneal injection with standard inoculum of *Plasmodium berghei berghei* NK65 with 1×10^7 infected red blood cells. The mice were divided into thirteen groups as shown above and treated for 4 consecutive days with 5 mg/kg body weight of Chloroquine, 10 mg/kg body weight of Artemisinin Combination-based therapy (Artesunate), 100, 200, and 400 mg/kg (leaf extract), 50, 100, 200 mg/kg (stem bark extract) body weight of *Diospyros mespiliformis* extract orally daily. On day 5 of the experiment, blood was collected from the tail of each mouse and smeared onto microscope slide to make a film.

$$\% \text{ Suppression} = \frac{\text{APC} - \text{APT}}{\text{APC}} \times \frac{100}{1}$$

APC = Average Parasitaemia in the Negative Control.

APT = Average Parasitaemia in the Test group.

Haematological assays

Packed cell volume (PCV) and haemoglobin concentration (Hb) were determined using the cyanomethaemoglobin and PCV by microhaematocrit methods [21, 22].

Estimation of parasitemia

Parasitemia was monitored in all the groups for 14 days starting from day 1 using thin smears of blood films made from tail vein puncture of mice^[11]. The smears were stained with 10% Giemsa stain at pH 7.2 for 15 min and examined under the microscope. The Percentage parasitemia was calculated by Giemsa-stained thin blood films using the blood collected from the tail of each mouse in all the groups. The mean % parasitemia were recorded for each mouse and for each group as described by Iwalewa et al^[23]

139 $\text{Percentage (\% parasitemia)} = \frac{\text{No of parasitized RBC}}{\text{Total RBC}} \times 100$ _____

140 **Total RBC**

141 **Data analysis**

142 The results obtained from the present study were analyzed by the analysis of variance (ANOVA) and expressed as
143 mean $\pm$ Standard Deviation (SD) except where otherwise stated. P value less than 0.05 were regarded as significant
144 ($P < 0.05$).

145 **3.0 RESULTS**

146 **TABLE 1: Qualitative Phytochemical Analysis (mg/kg) of Leaf and Stem Bark Extract of *Diospyros***
147 ***mespiliformis***

Phytoconstituents	Leaf	Stem bark
Carbohydrate	+	+
Glycoside	+	+
Saponin	+	+
Cardiac glycoside	+	-
Flavonoids	+	+
Tannin	+	+
Anthraquinone	+	-
Alkaloids	+	-

148 (+ve)=present; (-ve)=Absent

149

150 **TABLE 2: Quantitative Phytochemical Analysis of Fractions of Leaf Extract**

PHYTOCHEMICALS (mg/g)	AQUEOUS	N-BUTANOL	ETHYLACETATE
FRACTION		FRACTION	FRACTION
Saponins	0.22±0.03 ^{ab}	0.17±0.03 ^a 0.24±0.04 ^b	
Glycosides	0.45±0.03 ^b	0.34±0.03 ^a 0.35±0.03 ^a	
Alkaloids	0.04±0.01 ^a	0.06±0.01 ^a 0.06±0.02 ^a	
Tannins	0.14±0.04 ^a	0.37±0.05 ^c 0.25±0.04 ^b	

151 Values are mean ± Standard Deviation (n=3) .Values with different superscript in the row differ significantly

152 ($P<0.05$).

153 **TABLE 3: Quantitative Phytochemical Analysis of Fractions of Stem Bark Extract**

PHYTOCHEMICALS	AQUEOUS	N-BUTANOL	ETHYLACETATE
(mg/g)	FRACTION	FRACTION	FRACTION
Saponins	0.24±0.03 ^b	0.08±0.04 ^a 0.06±0.02 ^a	
Glycosides	0.46±0.03 ^c	0.38±0.03 ^b 0.23±0.04 ^a	
Alkaloids	0.03±0.02 ^a	0.04±0.02 ^a 0.05±0.03 ^a	
Tannins	0.16±0.03 ^a	0.14±0.03 ^a 0.25±0.04 ^b	

Values are mean $\pm$ Standard Deviation (n=3) .Values with different superscript in the row differ significantly ($P<0.05$).

TABLE 4: In-Vitro Parasitemia Level and Percentage Suppression of Different Fractions of Leaf Extract

TREATMENT/DOSE	AQUEOUS FRACTION		N-BUTANOL FRACTION		ETHYLACETATE FRACTION	
(n=5)	PARASITEAMIA	%	PARASITEAMIA	%	PARASITEAMIA	%
	LEVEL	SUPPRESSION	LEVEL	SUPPRESSION	LEVEL	SUPPRESSION
NC	0.00 $\pm$ 0.00 ^a	0	0.00 $\pm$ 0.00 ^a	0	0.00 $\pm$ 0.00 ^a	0
IM+ normal saline	1.94 $\pm$ 0.08 ^d	0	1.94 $\pm$ 0.08 ^e	0	1.94 $\pm$ 0.08 ^e	0
IM+ CQ(5mg/kg)	0.22 $\pm$ 0.21 ^a	88.66	0.22 $\pm$ 0.21 ^{ab}	88.66	0.22 $\pm$ 0.21 ^b	88.66
IM+ ACT(10mg/kg)	0.37 $\pm$ 0.22 ^{ab}	80.93	0.37 $\pm$ 0.22 ^{bc}	80.93	0.37 $\pm$ 0.22 ^{bc}	80.93
IM + 100mg/kg	0.84 $\pm$ 0.47 ^c	56.70	0.62 $\pm$ 0.31 ^{cd}	68.04	0.42 $\pm$ 0.81 ^c	78.35
IM+ 200mg/kg	0.77 $\pm$ 0.27 ^c	60.30	0.48 $\pm$ 0.26 ^{bc}	75.25	0.85 $\pm$ 0.21 ^d	56.19
IM+ 400mg/kg	0.73 $\pm$ 0.52 ^{bc}	62.37	0.81 $\pm$ 0.21 ^d	58.24	0.54 $\pm$ 0.92 ^c	72.16

Values are Means $\pm$ SD (n=5). Values with different superscript down the columns are significantly different ($P<0.05$).

NC=Normal Control, IM=Infected Mice CQ=Chloroquine, ACT=Artemisinin-based combination therapy (Artesunate).

TABLE 5: In-Vitro Parasitemia Level and Percentage Suppression of Different Fractions of Stem Bark Extract

TREATMENT/DOSE	AQUEOUS FRACTION		N-BUTANOL FRACTION		ETHYLACETATE FRACTION	
(n=5)	PARASITEAMIA	%	PARASITEAMIA	%	PARASITEAMIA	%
	LEVEL	SUPPRESSION	LEVEL	SUPPRESSION	LEVEL	SUPPRESSION

NC	0.00±0.00 ^a	0	0.00±0.00 ^a	0	0.00±0.00 ^a	0
IM+ normal saline	1.94±0.08 ^d	0	1.94±0.08 ^e	0	1.94±0.08 ^e	0
IM+ CQ(5mg/kg)	0.22±0.21 ^a	88.66	0.22±0.21 ^{ab}	88.66	0.22±0.21 ^b	88.66
IM+ ACT (10mg/kg)	0.37±0.22 ^{ab}	80.93	0.37±0.22 ^{bc}	80.93	0.37±0.22 ^{bc}	80.93
IM + 50mg/kg	0.46±0.22 ^b	76.29	0.69±0.22 ^d	64.43	0.29±0.28 ^b	85.05
IM+ 100mg/kg	0.11±0.09 ^a	87.5	0.62±0.13 ^d	68.04	0.53±0.30 ^c	72.68
IM+ 200mg/kg	0.50±0.20 ^c	94.33	0.56±0.19 ^{cd}	71.13	0.22±0.07 ^b	88.66

Values are Means ± SD (n=5).Values with different superscript down the columns are significantly different (P<0.05).

NC=Normal Control, IM= Infected Mice , CQ=Chloroquine, ACT=Artemisinin-based Combination therapy (Artesunate)

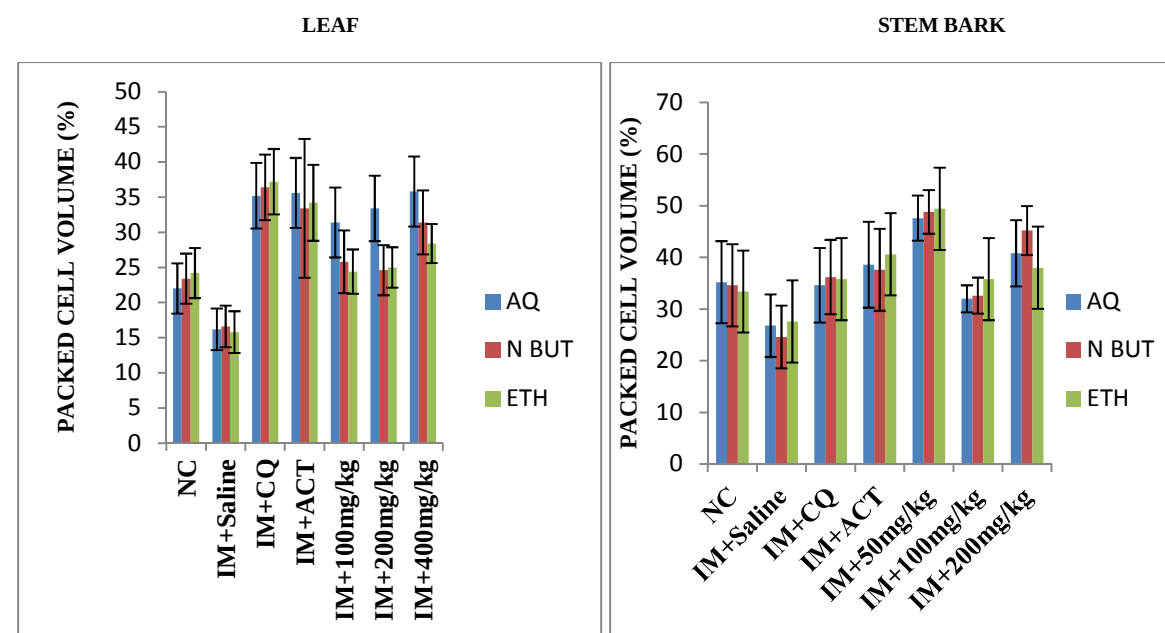


FIGURE 1: Effect of Different Fractions of Leaf and Stem Bark Extracts of *Diospyros Mespiliformis* on Packed Cell Volume (PCV).

Values are Means ± SD. (n=5).Values with different superscript down the columns are significantly different (P<0.05).

NC=Normal Control, IM=Infected Mice, CQ=Chloroquine, ACT=Artemisinin-based Combination therapy (Artesunate).

LEAF

STEM BARK

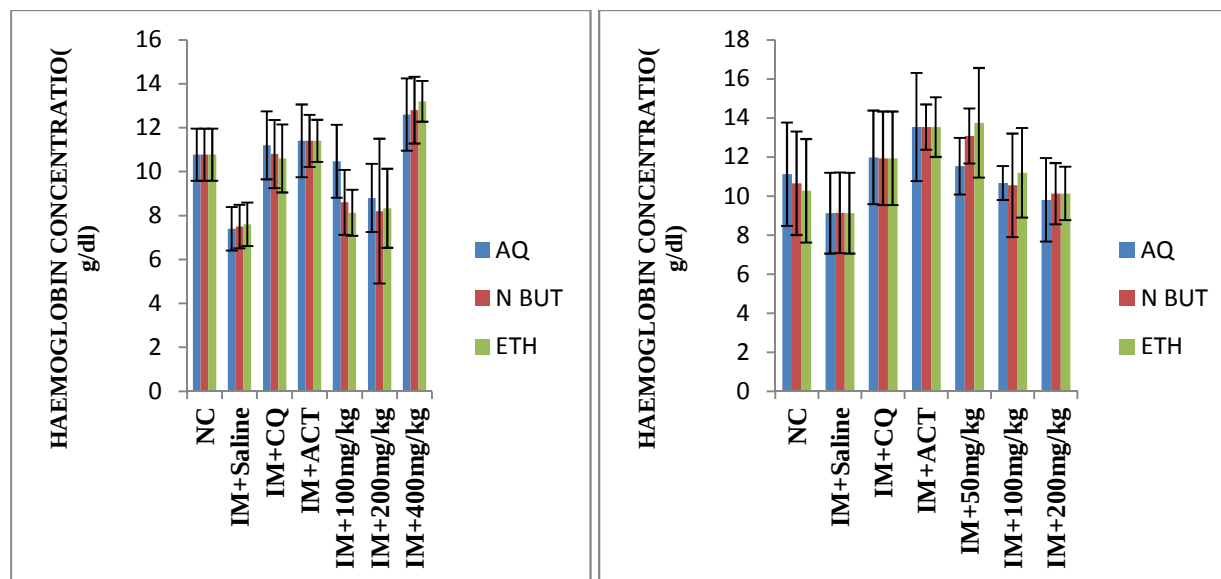


FIGURE 2: Effect of Different Fractions of Leaf and Stem Bark Extracts of *Diospyros Mespiliformis* on Haemoglobin Level (Hb).

Values are Means $\pm$ SD. (n=5). Values with different superscript down the columns are significantly different ($P < 0.05$).

NC=Normal Control, IM=Infected Mice, CQ=Chloroquine, ACT=Artemisinin-based Combination therapy (Artesunate).

TABLE 6: Effect of Different Fractions of Leaf Extract of *Diospyros Mespiliformis* on Relative Liver and Kidney Organ Weight.

TREATMENT/DOSE	AQUEOUS FRACTION		N-BUTANOL FRACTION		ETHYLACETATE FRACTION	
(n=5)	LIVER(g)	KIDNEY(g)	LIVER(g)	KIDNEY(g)	LIVER(g)	KIDNEY(g)
NC	1.33 $\pm$ 0.22 ^{ab}	0.96 $\pm$ 0.06 ^a	1.33 $\pm$ 0.22 ^a	0.96 $\pm$ 0.06 ^a	1.33 $\pm$ 0.22 ^a	0.96 $\pm$ 0.06 ^a
IM+ normal saline	4.65 $\pm$ 1.17 ^{de}	2.12 $\pm$ 0.97 ^c	4.65 $\pm$ 1.17 ^c	2.12 $\pm$ 0.97 ^c	4.65 $\pm$ 1.17 ^c	2.12 $\pm$ 0.97 ^b
IM+ CQ (5mg/kg)	2.29 $\pm$ 0.53 ^{abc}	1.82 $\pm$ 0.58 ^{ab}	2.29 $\pm$ 0.53 ^b	1.82 $\pm$ 0.58 ^{ab}	2.29 $\pm$ 0.53 ^b	1.82 $\pm$ 0.58 ^{ab}
IM+ ACT (10mg/kg)	1.55 $\pm$ 0.54 ^{ab}	1.54 $\pm$ 0.76 ^{ab}	1.55 $\pm$ 0.54 ^{ab}	1.54 $\pm$ 0.76 ^{ab}	1.55 $\pm$ 0.54 ^{ab}	1.54 $\pm$ 0.76 ^{ab}
IM + 100mg/kg	1.84 $\pm$ 0.65 ^{ab}	1.77 $\pm$ 0.85 ^{ab}	1.86 $\pm$ 0.30 ^{ab}	1.39 $\pm$ 0.21 ^{ab}	3.74 $\pm$ 3.03 ^{bcd}	2.00 $\pm$ 0.40 ^b

IM+ 200mg/kg	1.72±0.31 ^{ab}	1.31±0.26 ^{ab}	1.01±0.27 ^a	1.20±0.30 ^a	3.80±1.45 ^{bcd}	1.97±0.95 ^b
IM+ 400mg/kg	0.92±0.16 ^a	1.74±0.18 ^{ab}	1.85±0.78 ^{ab}	1.53±0.37 ^{ab}	4.00±0.39 ^{cd}	2.43±0.74 ^b

Values are Means ± SD. (n=5). Values with different superscript down the columns are significantly different (P<0.05).

NC=Normal Control, IM=Infected Mice, CQ=Chloroquine, ACT=Artemisinin-based Combination therapy (Artesunate).

TABLE 7: Effect of Different Fractions of Stem Bark Extract of *Diospyros Mespiliformis* on Relative Organ

Weight

TREATMENT/DOSE	AQUEOUS FRACTION		N-BUTANOL FRACTION		ETHYLACETATE FRACTION	
(n=5)	LIVER(g)	KIDNEY(g)	LIVER(g)	KIDNEY (g)	LIVER(g)	KIDNEY(g)
NC	1.33±0.22 ^a	0.96±0.06 ^a	1.33±0.22 ^a	0.96±0.06 ^a	1.33±0.22 ^a	0.96±0.06 ^a
IM+ normal saline	4.65±1.17 ^{bc}	2.12±0.97 ^a	4.65±1.17 ^{cd}	2.12±0.97 ^b	4.65±1.17 ^c	2.12±0.97 ^{abc}
IM+ CQ (5mg/kg)	2.29±0.53 ^{ab}	1.82±0.58 ^a	2.29±0.53 ^{ab}	1.82±0.58 ^{ab}	2.29±0.53 ^{ab}	1.82±0.58 ^{ab}
IM+ ACT (10mg/kg)	1.55±0.54 ^a	1.54±0.76 ^a	1.55±0.54 ^a	1.54±0.76 ^{ab}	1.55±0.54 ^a	1.54±0.76 ^{abc}
IM + 50mg/kg	1.93±0.34 ^a	2.15±1.35 ^a	6.21±2.10 ^d	1.04±0.29 ^a	2.26±1.45 ^{ab}	2.11±0.61 ^{abc}
IM+ 100mg/kg	8.43±4.10 ^d	2.13±0.96 ^a	3.94±1.00 ^{cd}	1.45±0.46 ^{ab}	4.20±1.42 ^{bc}	2.00±1.44 ^{abc}
IM+ 200mg/kg	6.69±3.00 ^{cd}	1.48±0.52 ^a	6.30±2.23 ^d	1.71±0.40 ^{ab}	5.42±2.91 ^c	2.68±0.88 ^{bc}

Values are Means ± SD (n=5). Values with different superscript down the columns are significantly different (P<0.05).

NC=Normal Control, IM=Infected Mice, CQ=Chloroquine, ACT=Artemisinin-based Combination therapy (Artesunate).

4. DISCUSSION

The presence of pharmacologically active phytochemical such as, saponin, alkaloids, tannin, cardiac glycosides, anthraquinone, glycosides and flavonoids are present in the leaf extract and absence of cardiac glycosides, flavonoids, free anthraquinone and alkaloids in the stem bark extract (Table 1). Phytochemicals constitute an integral part of medicinal plants and are responsible for their numerous bioactivities^[24]. The presence of these secondary metabolites in *Diospyros mespiliformis* may be responsible for their antiplasmodium activity. Anti-plasmodial screening of plant substances have been shown to be caused by alkaloids, tannin and flavonoids^[25, 26].

These compounds could be acting singly or in synergy with one another to exert the anti-plasmodial activity observed in this study. Leaf and stem bark extract of *Diospyros mespiliformis* have shown potent parasite suppressive effects on *P. berghei* infected Swiss albino mice in a dose related fashion. It was observed from these results that in the untreated group, there was a significant increase in the parasite count ($P < 0.05$) compared to the treated groups. This is in agreement with previous studies (Ayodele, 1979). Meanwhile, the extracts treated groups did not show a significant decrease in PCV ($P > 0.05$) when compared to the negative control group. This is suggestive that the extract may contain some substances that either increase appetite or blood quality to the animals, in addition to its anti-plasmodial activity (Abebe *et al.* 2003). Therefore, this justifies its usage in the management of malaria in Nigeria^[27]. Table 2 and 3 showed significantly higher level of glycosides, saponin and tannins content compared to the stem bark extract. At the end of the four (4) days suppressive test, there were significant increase ($P < 0.05$) in parasitaemia level in the negative control group and infected but treated with chloroquine group compared to the normal control and treated groups which is shown in Table 4 and 5. The chloroquine group almost cleared the parasite at dose of 5mg/kg body weight which exhibited higher suppressive antiplasmodial activities by the extent of inhibition of parasitemia^[20]. Kiseko *et al.*, 2000 showed that when a standard anti-malarial drug is used in mice infected with *P. berghei*, it suppresses the parasitaemia to a non-detectable level which is in line with our study^[28]. The effect of oral administration of leaf and stem bark fractions of *Diospyros mespiliformis* on packed cell volume and haemoglobin level is shown in Figure 1 and 2. The result showed that, the packed cell volume (PCV) and haemoglobin (Hb) level in the negative control group showed significant decrease ($P < 0.05$) and significant increase ($P < 0.05$) in the infected but treated with chloroquine^[29]. The significant decrease ($P < 0.05$) may be due to intravascular haemolysis, impaired haematopoiesis and bone marrow depression. This is similar to the finding of Mbajorgu *et al.*, 2007. Relative organ weight of different fractions of the leaf and stem bark methanol extracts of *Diospyros mespiliformis* is shown in Table 6 and 7. The results showed that there were no significant difference ($p < 0.05$) in relative change of the liver and kidney weight.

5. CONCLUSION

The results of this study have scientifically validated the traditional use of *Diospyros mespiliformis* in the management and treatment of malaria. The results indicate that leaf and stem bark methanol extracts of *Diospyros mespiliformis* are relatively safe at doses $\leq 400\text{mg/kg}$ and $\leq 200\text{mg/kg}$ body weight suppresses *Plasmodium berghei* NK 65 and could be used in the management of malaria. The body weight changes serve as a sensitive

indication of the general health status of animal. The observed decline in food consumption and water intake in the infected groups may have contributed to the observed reduction in body weight. The mechanism of inhibition against Cyseine protease extracts from *Plasmodium berghei* was determined at different substrate concentrations and at a fixed concentration of inhibitor. Line weaver-Burk plots were plotted in the presence and absence of an inhibitor. The mode of action of the leaf and stem bark methanol extracts exhibited competitive and non-competitive pattern of inhibition respectively. It is therefore concluded that, the fractions of leaf and stem bark methanol extracts of *Diospyros mespiliformis* may have active principle with antimalarial potential and has opened up a fresh line of research into discoveries of new drugs.

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