International Journal of Biochemistry Research & Review
12(4): 1-9, 2016, Article no.IJBCRR.21645
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

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 (Nk*65) Infected Mice

M. Oguche[1] and H. C. Nzelibe[1*]

[1]Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author HCN designed the study, wrote the protocol and supervised the work. Author MO carried out all laboratories work and performed the statistical analysis. Author HCN managed the analyses of the study. Author MO wrote the first draft of the manuscript. Author MO managed the literature searches and edited the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2016/21645
Editor(s):
(1) Richard A. Manderville, Departments of Chemistry and Toxicology University of Guelph, Canada.
Reviewers:
(1) Jessie Ndem, University of Uyo, Uyo, Nigeria.
(2) Aina Oluwagbemiga Olanrewaju, Nigerian Institute of Medical Research, Yaba, Nigeria.
(3) Daniel Arrieta-Baez, National Polytechnic Institute, Nano Sciences and Micro and Nano Technologies Center, Mexico.
(4) Isiaka Ogunwande, Lagos State University, Lagos, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/15215

Original Research Article

Received 27th August 2015
Accepted 25th May 2016
Published 30th June 2016

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 (Nk*65) 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 18-28 g were randomly divided into thirteen

**Corresponding author: E-mail: zeechuks6@gmail.com;*

(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 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/gl), (0.36±0.21 mg/gl), alkaloids (0.12±0.04 mg/gl), (0.67±0.01 mg/gl), tannins (0.73±0.36 mg/gl), (0.51±0.22 mg/gl), and glycoside had the highest concentration of (1.15±0.10 mg/gl), (0.97±0.33 mg/gl) 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. 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. 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.

Keywords: Antiplasmodial activity; Diospyros mespiliformis; P. berghei berghei; methanol fractions.

1. 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.

Malaria mortality rate has reduced to an estimated 584 000 death between the range of 367 000 to 755 000 mostly among African children [3]. Drugs such as quinine related drugs, antifolate combination drugs and artemisinin and its derivatives are antimalarial drugs used for effective treatment [4]. 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 [5].

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 [6]. 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 [7]. *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 [8]. Bark and roots are used for

serious infections such as malaria, pneumonia, syphilis, leprosy and dermatomycoses, as an antihelmintic and to facilitate delivery [9]. 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' [10]. 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 [11]. Studies of *D. mespiliformis* showed valuable medicinal biological properties such as anti-diabetic, antihelmintic, analgesic, antioxidant and anti-inflammatory activities [12]. 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.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 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.

2.1.2 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 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.

2.2 Methods

2.2.1 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 (250 ml) was placed in a separatory funnel and 250 ml each of ethylacetate and *n*-butanol solvents were added sequentially as a 1:1 (v/v) solution and rocked vigorously [13]. 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 ethylacettate, *n*-butanol as well as the aqueous residue fractions were concentrated over a water bath maintained at 45^{o}C. The concentrated fractions were kept in sealed containers and refrigerated at 2-4^{o}C until required for analysis.

2.2.2 Parasites

The choloquine sensitive *Plasmodium berghei berghei* 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. [14,15].

Plasmodium berghei berghei infected red blood cells were intraperitoneally injected into the mice from the blood diluted with Phosphate Buffered Saline (PBS) so that each 0.2 ml administered per kg body weight contains approximately 10^7 infected red cells.

2.2.3 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 [16,17,18].

2.2.4 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 [19]. Adult Swiss mice weighing between 18 to 28 g were inoculated by intraperitonial injection with standard inoculum of *Plasmodium berghei bergheiNK65* 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 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.

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

APC = Average Parasitaemia in the Negative Control.
APT = Average Parasitaemia in the Test group.

2.2.5 Haematological assays

Packed cell volume (PCV) and haemoglobin concentration (Hb) were determined using the cyanomethaemoglobin and PCV by micro-haematocrit methods [20,21].

2.2.6 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 [10]. 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. [22]

$$\text{Percentage (\%) parasiteamia} = \frac{\text{No of parasitized RBC}}{\text{Total RBC}} \times \frac{100}{1}$$

2.3 Data Analysis

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

3. RESULTS

Table 1 shows the presence of these phytochemicals while Cardiac glycoside anthraquinones and alkaloids were absent in the stem bark extract.

Table 1. Qualitative phytochemical analysis of leaf and stem bark extract of *Diospyros mespiliformis*

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

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

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 [23]. The presence of these secondary metabolites in *Diospyros mespiliformis*may be responsible for their anti-*plasmodium* activity. Anti-plasmodial screening of plant substances have been shown to be caused by alkaloids, tannin and flavonoids [24,25]. 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 [26]. 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 [27]. Therefore, this justifies its usage in the management of malaria in Nigeria [28]. Tables 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 parasiteamia 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 Tables 4 and 5. The chlooroquine group almost cleared the parasite at dose of 5 mg/kg body weight which exhibited higher suppressive antiplasmodial activities by the extent of inhibition of parasitemia [19]. Kiseko et al. 2000 showed that when a standard anti-malarial drug is used in mice infected with *P. berghei berghei*, it suppresses the parasiteamia to a non-detectable level which is in line with our study [29]. The effect of oral administration of leaf and stem bark fractions of *Diospyros mespiliformis* on packed cell volume and heamoglobin level is shown in Figs. 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 [30]. The significant decrease (*P*<0.05) may be due to intravascular haemolysis, impaired

Table 2. Quantitative phytochemical analysis of fractions of leaf extract

Phytochemicals (mg/g)	Aqueous fraction	N-butanol fraction	Ethylacetate 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}

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

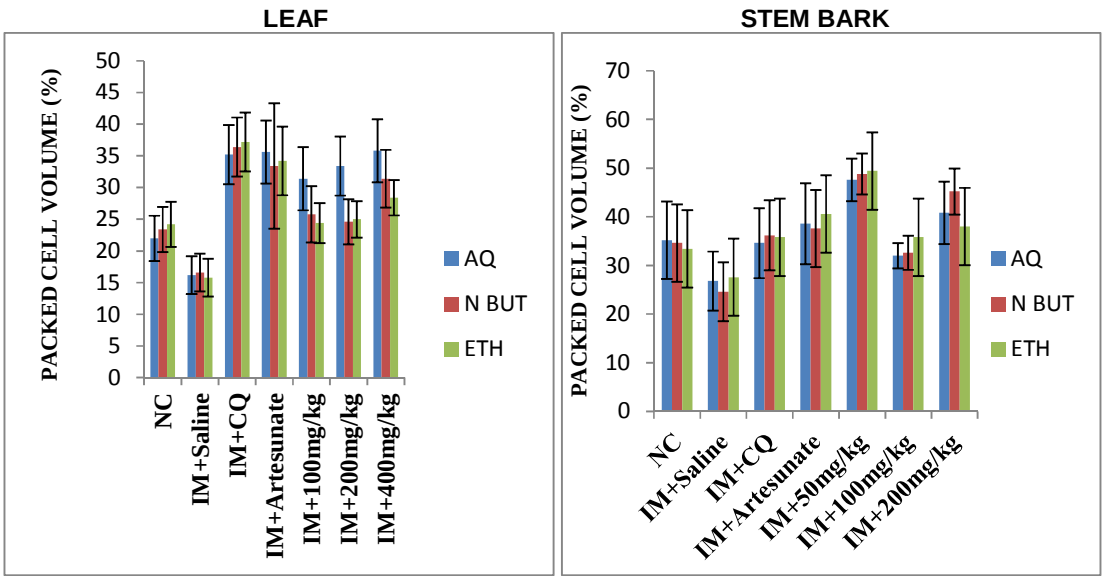

Fig. 1. Effect of different fractions of leaf and stem bark extracts of *Diospyros mespiliformis* on Packed Cell Volume (PCV)

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, Artesunate=10 mg/kg

Table 3. Quantitative phytochemical analysis of fractions of stem bark extract

Phytochemicals (mg/g)	Aqueous fraction	N-butanol fraction	Ethylacetate 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 ± Standard Deviation (n=3). Values with different superscript in the row differ significantly (P<0.05)

Table 4. *In-vivo* parasiteamia level and percentage suppression of different fractions of leaf extract

Treatment /dose	Aqueous fraction		N-butanol fraction		Ethylacetate fraction	
(n=5)	Parasiteamia level	% suppression	Parasiteamia level	% suppression	Parasiteamia 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 (5 mg/kg)	0.22±0.21[a]	88.66	0.22±0.21[ab]	88.66	0.22±0.21[b]	88.66
IM+ Artesunate	0.37±0.22[ab]	80.93	0.37±0.22[bc]	80.93	0.37±0.22[bc]	80.93
IM + 100 mg/kg	0.84±0.47[c]	56.70	0.62±0.31[cd]	68.04	0.42±0.81[c]	78.35
IM+ 200 mg/kg	0.77±0.27[c]	60.30	0.48±0.26[bc]	75.25	0.85±0.21[d]	56.19
IM+ 400 mg/kg	0.73±0.52[bc]	62.37	0.81±0.21[d]	58.24	0.54±0.92[c]	72.16

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, Artesunate=10 mg/kg.

Table 5. *In-vivo* parasiteamia level and percentage suppression of different fractions of stem bark extract

Treatment /dose	Aqueous fraction		N-butanol fraction		Ethylacetate fraction	
(n=5)	Parasiteamia level	% suppression	Parasiteamia level	% suppression	Parasiteamia 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 (5 mg/kg)	0.22±0.21[a]	88.66	0.22±0.21[ab]	88.66	0.22±0.21[b]	88.66
IM+ Artesunate	0.37±0.22[ab]	80.93	0.37±0.22[bc]	80.93	0.37±0.22[bc]	80.93
IM + 50 mg/kg	0.46±0.22[b]	76.29	0.69±0.22[d]	64.43	0.29±0.28[b]	85.05
IM+ 100 mg/kg	0.11±0.09[a]	87.5	0.62±0.13[d]	68.04	0.53±0.30[c]	72.68
IM+ 200 mg/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, Artesunate=10mg/kg

haematopoiesis and bone marrow depression. This is similar to the finding of Mbajiorgu et al. [28]. Relative organ weight of different fractions of the leaf and stem bark methanol extracts of *Diospyros mespiliformis* is shown in Tables 6 and 7. The results showed that there were no significant difference (p<0.05) in relative change of the liver and kidney weight.

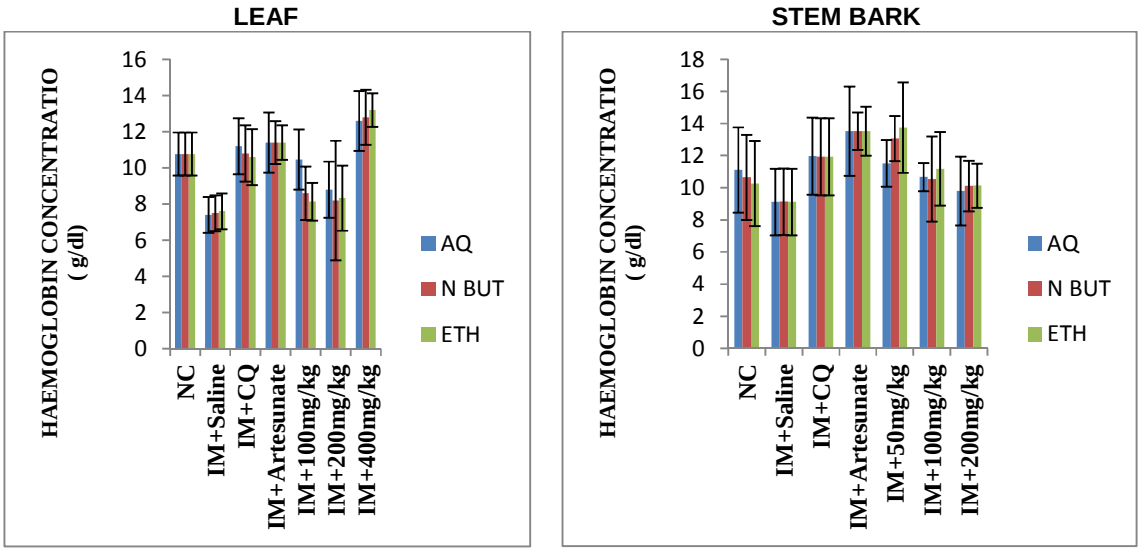

Fig. 2. Effect of different fractions of leaf and stem bark extracts of *Diospyros mespiliformis* on haemoglobin level (Hb)

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, Artesunate=10mg/kg.

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±0.22^{ab}	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^{de}	2.12±0.97^{c}	4.65±1.17^{c}	2.12±0.97^{c}	4.65±1.17^{c}	2.12±0.97^{b}
IM+ CQ (5 mg/kg)	2.29±0.53^{abc}	1.82±0.58^{ab}	2.29±0.53^{b}	1.82±0.58^{ab}	2.29±0.53^{b}	1.82±0.58^{ab}
IM+ Artesunate	1.55±0.54^{ab}	1.54±0.76^{ab}	1.55±0.54^{ab}	1.54±0.76^{ab}	1.55±0.54^{ab}	1.54±0.76^{ab}
IM + 100 mg/kg	1.84±0.65^{ab}	1.77±0.85^{ab}	1.86±0.30^{ab}	1.39±0.21^{ab}	3.74±3.03^{bcd}	2.00±0.40^{b}
IM+ 200 mg/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+ 400 mg/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, Artesunate=10 mg/kg.

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 (5 mg/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+ Artesunate	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 + 50 mg/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+ 100 mg/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+ 200 mg/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, Artesunate=10 mg/kg

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 ≤ 400 mg/kg and ≤ 200 mg/kg body weight suppresses *Plasmodium berghei 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 ploted 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mu J, Myers RA, Jiang H, Liu S, Ricklefs S, Waisberg M, Su XZ. *Plasmodium falciparum* genome-wide scans for positive selection, recombination hot spots and resistance to antimalarial drugs. Nature Genetics. 2010;42(3):268-271.
2. Jambou R, El-Assad F, Combes V, Grau EG. *In vitro* culture of *Plasimodium berghei*-ANKA maintains infectivity of mouse erythrocytes inducing cerebral malaria. Malaria J. 2011;10:346.
3. Vivas LL, Rattray LB, Stewart BL, Robinson B, Fugmann RK, Haynes W, Peters, Croft SL. Antimalarial efficacy and drug interactions of the novel semi-synthetic endoperoxide artemisone *in vitro* and *in vivo*. Journal of Antimicrobial Chemotherapy. 2007;59(4):658-665.
4. Sneh S, Saumyadripta C, Patrick LS, Neelima M, Nalini S, Joseph KD, Ravindran, Jane MC, Alex E. Chloroquine efficacy confirmed on drug susceptibility of *Plasmodium vivax* in Chenni, India. Malaria Journal. 2014;13:129.
5. Ebbo AA, Mammam M, Suleiman MM, Ahmed A, Bello A. Preliminary phytochemical screening of *Diospyros mespiliformis*. Anat Physiol. 2014;4(10): 156.
6. Ali AJ, Usman T, Abdulrazaq, Rukkaya S. Suleiman, Patience S. Kali. Effects of sub-chronic administration of *Diospyros mespiliformis Hochst* (Ebenaceae) root extracts on some biochemical parameters in mice. Journal of Applied Pharmaceutical Science. 2012;2(5):60-64.
7. *In vitro* antiplasmodial activity and phytochemicals screening of ethnomedicinal plants used to treat malaria associated symptoms. A Thesis Submitted in Fulfillment of the Requirement of the Degree of Master of Science of The University of Namibia by Sylvia Ndeshihafela Nafuka Dr. D R. Mumbengegwi, (Multidisciplinary Research Center, University of Namibia) Co-supervisor: Dr. R. Böck, (Department of Biological Science, University of Namibia).
8. Watt JM, Brandwijk BM. The medicinal and poisonous plants of southern and Eastern African. 2nd ed. Livingstone Edinburgh. 1962;369.
9. Irvine FR. Woody plants of Ghana. Oxford University Press London; 1961.
10. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives. 2001;109(1):69.
11. Bulus, Oluwakanyinsola AS. Screening *Diospyros mespiliformis* extract for antimalarial potency. Int. J. Biol. Chem. Sci. 2009;3(2):271-276.
12. Adzu B, Amos S, Dzarma S, Muazzam I, Gamaniel KS. Pharmacological evidence favouring the folkloric use of *Diospyros mespiliformis* Hochst in the relief of pain and fever. J. Ethnopharmacol. 2002;82: 191-195.
13. Peter LT, Anatoli VK. The current global malaria situation. Malaria parasite biology, pathogenesis and protection. ASM press. Washington DC. 1998;11-22.

14. David FA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: Efficacy models for compound screening. Nature Reviews Drug Discovery. 2004;3(6):509-520.
15. Brain KP, Turner TD. The practical evaluation of pharmaceuticals. Wright Science Technical Bristol. 1975;44:81.
16. Sofowora EA. Traditional medicine methods and techniques. John Wiley and Son Ltd., New York. 1982;2626-2253.
17. Trease GE, Evans WC. Pharmacognosy. (12th), Bailliere and Tindale, London. 1983;774-775.
18. Peters W. Drug resistance in *Plasmodium berghei*. Chloroquine Resistance. Exptl. Parasitol. 1965;17:80-89.
19. Alexander RR, Grifiths JM. Haemoglobin determination by the cyanome-thaemoglobin method. Basic biochemical methods. New York: John Wiley and Sons. 1993;118:61-73.
20. Bulus A, Joseph A, Habiba V, Karniyus G. Studies on the use of *Cassia singueana* in malaria ethnopharmacology. Journal of Ethnopharmacology. 2003;88:261–267.
21. Iwalewa EO, Lege Oguntoye L, Rai PP, Iyaniwura TT. *In vivo* and *In vitro* antimalarial activity of two crude extracts of *Cassia occidentals* leaf. Nigeria Journal of Pharmaceutical Science. 1997;5:23-28.
22. Poongothai A, Sreena KP, Sreejith K, Uthiralingam M, Annapoorani S. Preliminary phytochemical screening of *Ficus racemesa* Linn. Bark. International Journal of Pharma and Bio Sciences. 2011;2:431-434.
23. Momoh JOO, Aina SM, Akoro O, Ajibaye, Okoh HI. *In vivo* anti-plasmodial activity and the effect of ethanolic leaf extract of *Rauvolfia vomitoria* on hematological and lipid parameters in Swiss mice infected with *Plasmodium berghei* NK 65. Nigerian Journal of Parasitology. 2014;35(1,2):109-116.
24. Dangoggo SM, Hassan LG, Sadiq IS, Manga SB. Phytochemical analysis and antibacterial screening of leaves of *Diospyros mespiliformis* and *Ziziphus spina-christi*. Journal of Chemical Engineering. 2012;1(1)31-37.
25. Kantamreddi VS, Wright CW. Investigation of Indian *Diospyros* species for antiplasmodial properties. Evidence-based Complementary and Alternative Medicine. 2008;5(2):187-190.
26. Kiseko K, Hiroryuki M, Syun-ichi F, Ryuiichi F, Tomotaka K, Seiji M. Anti-malarial activity of leaf extract of *Hydrangea macrophyla,* a common Japanese plant. Acta Med. Okoyama. 2000;54(5):227-232.
27. Mohamed I.E, El Nur EE, Choudhary MI, Khan SN. Bioactive natural products from two Sudanese medicinal plants *Diospyros mespiliformis* and *Croton zambesicus*. Records of Natural Products. 2009;3(4): 198-203.
28. Mbajiorgu EF, Aire TA, Vlok W, Alberts M, Debusho LK. Haematological profile of male rats treated with ethanol and/or chloroquine and fed normal or low protein diet. Internet. J. Haematol. 2007;1:1-20.
29. Ibrahim MA, Njoku GC, Sallau AB. *In vivo* activity of stem bark aqueous extract of *Khaya senegalensis* against *Trypanosoma brucei*. *Afr.* J. Biotech. 2008;7(5):661-663.
30. Abdelgadir EH, Ahmed RH, Adam SLY, Husein AM. Evaluation of toxicological activity (acute and sub-chronic toxicities) of *Lawsonia innermis* seeds on Wister rats. J Pharmacol. Toxicol. 2010;5(7):324-333.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15215