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## RESEARCH ARTICLE



# *In vitro* inhibition of beta-hematin formation and *in vivo* effects of *Diospyros mespiliformis* and *Mondia whitei* methanol extracts on chloroquine-susceptible *Plasmodium berghei*-induced malaria in mice

JOHN OLUDELE OLANLOKUN , JANET ADENIKE  
ADETUTU, and OLABODE OLUFUNSO OLORUNSOGO

Laboratories for Biomembrane Research and Biotechnology, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria

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## ABSTRACT

**Background and aim:** This study was carried out to investigate antiplasmodial activities of *Diospyros mespiliformis* (DM) and *Mondia whitei* (MW) in *Plasmodium berghei*-infected mice. **Materials and methods:** Air-dried stem of DM and root of MW were soaked in methanol, decanted and concentrated to give extracts. Parts of these extracts were partitioned successively to give dichloromethane, ethylacetate and methanol fractions. Mice ( $18 \pm 3$  g) were infected with *Plasmodium berghei*-infected erythrocytes from a donor mouse and were treated with the extracts, while the drug control group received 10 mg/kg body weight of artesunate and the parasitized control received the vehicle (5% v/v DMSO). Percentage parasitemia and clearance were estimated from thin films of blood smear. Hematological parameters were determined using standard methods. Both extracts were also tested on *in vitro* inhibition of  $\beta$ -hematin formation. **Results, discussion and conclusion:** Results showed that DM had the least percentage parasitemia (0.67%) and highest percentage parasite clearance (84.7%) while the MW had 0.89% percentage parasitemia and 79.7% clearance at the highest dose used after the seventh day relative to untreated control. The cell free antiplasmodial activity of the fractions and extracts of both DM and MW revealed that DM significantly inhibited  $\beta$ -hematin formation than MW. The packed cell volume, white blood cell count, Lymphocyte, Eosinophil, Monocyte and Neutrophil significantly increased in the treated groups compared with the control. The results showed that the DM had higher antiplasmodial activity.

## KEYWORDS

Protoporphyrin, Malaria, *Mondia whitei*, *Diospyros mespiliformis*,  $\beta$ -hematin, Blood cells

## INTRODUCTION

Malaria is a mosquito-borne infectious parasitic disease caused by protozoans of the genus *Plasmodium* that are transmitted to humans after a bite from infected, female *Anopheles* mosquitoes. Despite advances in the control and prevention of the disease in the past years, malaria continues to be one of the world's major transmissible and deadly diseases due to the fact that the major human malaria parasites *Plasmodium falciparum* Welch and *Plasmodium vivax* Grassi and Feletti are acquiring resistance to the most important drugs, the quinoline antimalarials and artemisinin derivatives. Also, environmental conditions favour the reproduction, propagation and survival of malaria vectors: mosquitoes of the genus *Anopheles* Meigen. These and several other factors contribute immensely to the high malaria morbidity and mortality rates especially among children and pregnant women.

\*Corresponding author.

E-mail: jodel72000@yahoo.com



Due to the acquisition of resistance of malaria parasites to most important drugs, the use of plants for the treatment of malaria is gaining popularity. Resistance to antimalarial drug and because of provision as a cheap source of natural medicine, plants have been selected and used empirically as drugs for centuries, initially as traditional preparations peculiar to African and Asian continents, and later as an acceptable source of medicine [1]. Medicinal plants have a promising future because there are about half a million plants around the world and most of their medicinal properties have not yet been investigated [2]. With the available scientific research findings to prove their antimalarial efficacy, plant medicinal properties could be decisive in treatment of diseases either now or in the future. Green medicine is found to be safe and more dependable than the costly synthetic drugs, many of which have adverse side effects [2].

In tropical regions where malaria is endemic and per capita income is low, alternative therapies based on traditionally used antimalarial plants are used [3]. New drugs introduced into the therapeutic arsenal are mostly derived from natural products [4]. Plants provide natural products that are useful for the treatment of protozoan diseases such as malaria [5], leishmaniasis, and African and American trypanosomiasis [6]. Traditionally-used antimalarial plants are the origin of the alkaloid quinine (isolated from species of *Cinchona*) and the sesquiterpene artemisinin (isolated from *Artemisia annua* L.) that gave rise to the synthetic quinoline antimalarials (e.g., chloroquine) and semi-synthetic artemisinin derivatives (sodium artesunate). These two classes of drugs, especially the sesquiterpene lactone artemisinin and its derivatives are the basis of artemisinin-combined therapies (ACTs) now used worldwide to combat chloroquine-resistant strain of *P. falciparum*. Plants also used to prevent malaria by providing essential oils extracted from citronella and neem trees which have mosquito repellent and insecticidal properties. Solvent extracts and isolated chemicals (chrysanthemic acid, nicotine, etc.) from plants have given rise to the synthesis and production of pyrethroid, neonicotinoid and other insecticides and mosquito repellents [7]. In Nigeria, *Diospyros mespiliformis* (*DM*) stem is used in combination with *Enantia chlorantha* and *Alstonia boonei* stem barks to treat malaria [8], while in Namibia, *DM* is used alone to prepare antimalarial decoction, suggesting that *DM* may be worth screening for antimalarial properties [9]. A root decoction of *Mondia whitei* (*MW*) alone is used to treat malaria in countries like Benin and Nigeria [10]. This study was carried out in order to investigate the indigenous claims of the use of these two herbs; (*DM* and *MW*) in the treatment of malaria.

## MATERIALS AND METHODS

### Collection of Plant Materials

The woody stem of *DM* and the roots of *MW* were obtained from Oje Herbal market, Ibadan, Nigeria. Samples were authenticated and identified at the Department of Botany,

Ekiti State University as *D. mespiliformis* (Hochst ex A.DC) and *Mondia whitei* (Hook F) skeels, respectively. The *DM* and *MW* were assigned the University Herbarium, Ado-Ekiti number as UHAE2017/062 and UHAE2017/063, respectively by Mr. Omotayo, F.O. of the University Herbarium Unit, Ekiti State University, Ado-Ekiti.

### Extraction

The powdered samples were extracted separately by soaking in methanol at room temperature for 72 h. Each crude methanol extract concentrate was concentrated using rotary evaporator (Stuart Brand, United Kingdom) at 40°C under reduced pressure. The brown slurry residues were further concentrated into solvent free extract in a water bath at 40°C. These samples were then kept in the refrigerator until used.

### Partitioning of the Crude Methanol Extract

Methanol extract of each sample (20 g) was pre-adsorbed with silica gel on ratio 1:1 basis. A sintered funnel was packed with 30 g of silica gel and connected to a conical flask and vacuum pump for a Vacuum Liquid Chromatography (VLC) set up. After packing the gel with dichloromethane, the pre-adsorbed sample was added to the column and eluted with dichloromethane until exhaustion. The remaining marc was further eluted with ethylacetate and methanol successively to obtain their respective fractions. These fractions were concentrated using the rotary evaporator and further concentrated in the water bath at 40°C to obtain the dichloromethane, ethylacetate and methanol fractions (DCMF, EF and MF) respectively. The solvent-free fractions were stored in the refrigerator until used.

## QUANTITATIVE PHYTOCHEMICAL ANALYSIS

### Determination of Total Flavonoid

Total flavonoid content of methanol extracts of *MW* and *DM* was carried out according to the method of Park et al. [11]. Samples of Methanol extracts of *MW* and *DM* (3, 4, and 5 mg/mL) were mixed with 3.4 mL of 30% methanol, 0.15 mL of NaNO<sub>2</sub> (0.5 M) and 0.15 mL of AlCl<sub>3</sub> (0.3 M). After 5 min, 1 mL of NaOH (1 M) was added to the reaction mixture. The solutions were vortexed and the absorbance of the samples was read against reagent blank at 506 nm. Total flavonoid was calculated from a standard curve using quercetin (1 mg/mL) and expressed as µg per g of the extracts.

### Determination of Total Phenolic Content

Total soluble phenolics in the methanol extracts of *MW* and *DM* were determined using Folin-Ciocalteu reagent and gallic acid as standard [12]. Graded concentrations (20, 40 and 80 mg/mL) of methanol extracts of *MW* and *DM* were diluted with distilled water and made up to 1 mL. Then, 1 mL of 0.1% Folin-Ciocalteu reagent was added and the content mixed thoroughly. Three minutes later, 3 mL of 2%



$\text{Na}_2\text{CO}_3$  was added and the mixture was allowed to stand for 90 min, vortexed and the absorbance was measured at 760 nm using 752N UV–visible spectrophotometer. The concentration of total phenolics in the samples was obtained from a gallic acid standard curve.

### Determination of Alkaloids

Total alkaloid was determined according to standard procedure [13]. Five mL of 60%  $\text{H}_2\text{SO}_4$  was added to graded concentrations (2, 4 and 6 mg/mL) of *MW* and *DM* methanol extracts in separate test tubes and after 5 min, 5 mL of 0.5% solution of formaldehyde in 60%  $\text{H}_2\text{SO}_4$  was added. The mixture was allowed to stand for 3 h and absorbance was read at 568 nm. Pure alkaloid was used as standard.

### Determination of Saponin

Saponin in the graded concentrations (200, 400 and 800 mg/mL) of methanol extracts of *MW* and *DM* samples was determined using Association of Analytical Communities Method [13]. Olive oil (a drop) was added to each of the sample tube and the intensity of the cloudiness was measured at 620 nm. The saponin content in each sample was estimated from a saponin standard curve.

### Determination of Tannins

Tannin in the samples was estimated using a modified method of Van Buren and Robinson [14]. Varied concentrations (200, 400 and 800 mg/mL) of methanol extracts of *MW* and *DM* samples were made up to 1 mL and 1 mL each of 0.1 M  $\text{FeCl}_3$  and 0.1 M HCl was added to the medium. Thereafter, 0.1 mL of 0.008 M potassium ferrocyanide was added and the mixture vortexed. The absorbance was read at 670 nm within 10 min. Tannin concentration was extrapolated from a tannic acid standard curve.

## ETHICAL CONSIDERATION, EXPERIMENTAL ANIMALS AND THEIR INFECTION

All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and National Institute of Health. Male Wistar strain mice ( $18 \pm 3$  g) were obtained from the Animal House section of the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan. The animals were kept in well ventilated plastic cages and acclimatized for two weeks. The animals were given free access to food and water *ad-libitum*. They were thereafter infected with  $10^7$  inoculum size of erythrocytes infected with chloroquine-sensitive strain of *Plasmodium berghei* from a donor mouse. Parasitemia was confirmed after 72 h of infection and infected animals were sorted out for grouping.

### Animal Grouping and Treatment

The method of established infection according to Ryle and Peters [15] was used for this assay. Forty-five mice that were infected with *P. berghei* were randomly assigned to nine groups of five animals in each group. This included the parasitized treated control group that was treated with 10 mg/kg body weight of artesunate, parasitized untreated control group that received equivalent volume of the vehicle; dimethyl sulphoxide (5% v/v DMSO) and groups treated with methanol extracts of *DM* and *MW*. The groups treated with *DM* and *MW* were given graded dosages of 100, 200 and 400 mg/kg of each methanol extract, respectively. To determine the effects of the vehicle on the hematological parameters, the unparasitized vehicular control (VC) groups were treated with the vehicle only while the normal unparasitized control (NC) group were treated with distilled water only.

### Percentage Parasitemia and Percentage Clearance

Thin film slides were made from blood smears collected from the tails of the animals and were fixed in absolute methanol. These were air-dried and stained with Giemsa stain, rinsed with buffered water, dried again, and viewed under microscope using the  $\times 100$  objective (oil immersion). Both infected and uninfected erythrocytes were counted; percentage parasitemia and clearance were calculated as follows:

Percentage Parasitemia

$$= \text{Number of infected Red Blood Cells} \frac{\text{counted}}{\text{Total}} \\ \times \text{Number of Red Blood cells counted} \times 100$$

$$\text{Percentage Clearance} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

## DETERMINATION OF HEMATOLOGICAL PARAMETERS

### Collection of Blood Samples

The animals were sacrificed through cervical dislocation and blood was collected from the heart into sample bottles coated with anticoagulant (EDTA). The blood samples were swirled gently to ensure thorough mixing.

### Packed Cell Volume

Packed Cell Volume (PCV) was determined by micro-hematocrit technique using capillary tube [16]. Blood samples were aspirated into non-heparinized tubes, sealed with plasticin and centrifuged in hematocrit centrifuge at 2,000 rpm for 5 min. The PCV was read out using hematocrit reader.



### Total White Blood Cell Count (WBC)

Total WBC was determined according to the method of Brown [17]. Whole blood sample (20  $\mu$ L) was pipette into 380  $\mu$ L of Turk solution, mixed and allowed to settle for 2 min. The samples were thereafter loaded into Newbauer counting chamber and put inside humid chamber for 1 min. It was then observed under the microscope using  $\times 10$  objective.

### Platelet Count

Whole blood sample (20  $\mu$ L) was pipette into 380  $\mu$ L of ammonium oxalate solution, allowed to settle for 2 min and then loaded into counting chamber and put inside humid chamber for 1 min. Counting was done using  $\times 40$  objective.

### Differential WBC Count for Lymphocytes, Monocytes, Eosinophils and Neutrophils

Differential WBC count was determined as formerly described [18]. A thin film of whole blood was made on a grease-free slide, air-dried and stained with Leishman stain for 5 min. The stain was thereafter diluted on the slide with phosphate buffered saline and the slide was further allowed to be stained for 10 min, rinsed, mopped, air-dried and read under microscope using oil immersion objective.

## IN VITRO ANTIPLASMODIAL CELL FREE ASSAY

The methanol extracts and fractions of *DM* and *MW* were screened for their cell free antiplasmodial activity by using the modified method of Afshar et al. [19]. Varying concentrations (0.125–4 mg/mL) of both extracts and fractions of *DM* and *MW* were dissolved in dimethylsulphoxide (DMSO). The reaction mixtures were incubated with 50  $\mu$ L of 60 mM hemin chloride, 250  $\mu$ L of 40 mM oleic acid, and 250  $\mu$ L of 4 M HCl. The final volume was adjusted to 1.0 mL volume using varied volumes of sodium acetate buffer, pH 5, for 18 h at 37°C in a shaker water bath. Artesunate and Chloroquine diphosphate were used as positive controls. After incubation, the samples were centrifuged (14,000 rpm, 10 min, at 21°C) and the hemozoin pellet obtained was repeatedly washed with incubation (15 min at 37°C with regular shaking) in 2.5% (w/v) SDS in phosphate buffered saline followed by a final wash in 0.1 M sodium bicarbonate until the supernatant was clear (usually 3–8 washes). After the final wash, the supernatant was discarded and the pellets were dissolved in 1.0 mL of 0.1 M NaOH before determining the hemozoin content by measuring the absorbance at 400 nm using 752N BOSCH UV–VIS spectrophotometer. The results were recorded as percentage inhibition (I%) of heme crystallization compared to negative control (DMSO) using the following equation:

$$I\% = [(AN - AS)/AN] \times 100$$

where, AN is absorbance of negative control and AS is absorbance of test samples.

### Statistical Analysis

Data were presented as mean  $\pm$  SD of five animals per group ( $n = 5$  per group). The values were analyzed using one way analysis of variance followed by the *post hoc* Duncan's multiple range test using Graphpad prism (5.0). The level of significance was set at  $P < 0.05$ .

## RESULTS

Figure 1 shows the quantitative phytochemical screening of the methanol extracts of *DM* and *MW*. The results show that the values for total phenol, flavonoid, alkaloid, saponin and tannin were significantly higher ( $P < 0.001$ ) in *DM* than *MW*. The most abundant phytochemicals in *DM* are the phenolic compounds while saponin is least abundant in *MW* (Fig. 1A and C, respectively).

Figures 2A and B show that there are significant ( $P < 0.05$ ) increases in the percentage parasitemia of the negative control with increasing number of days when compared with the standard antimalarial drug which show a significant decrease in the percentage parasitemia throughout the experiment. Zero parasitemia was obtained on days five and seven in the artesunate treated group. These figures also show that the two extracts have significant reduction in the percentage parasitemia in a dose dependent manner ranging from 100 through 200 to 400 mg/kg. Methanol extract of *DM* has the highest antimalarial effect with the least percentage parasitemia when compared with *MW*.

Figures 3A and B show the effects of the methanol extracts of *DM* and *MW* on percentage parasite clearance, respectively. This shows that the percentage clearance of the negative control is significantly reduced to zero starting from day one up to day seven when compared with the standard drug that shows a significant ( $P < 0.05$ ) increase in the percentage clearance with 100% efficiency on day five. Percentage clearance increased dose dependently in both *MW* and *DM* while the latter has the highest parasite clearance maximally at 400 mg/kg. The percentage clearance of extracts of both plants increased in a dose dependent manner. This indicates that these plants have antiplasmodial activities with *DM* having the highest effect. Again, these results reflect that the percentage parasitemia of *DM* decreased towards zero, similar to the standard drug, while the percentage parasitemia of *MW* tends to increase; an indication that the antiplasmodial activity of *MW* may be overwhelmed overtime due to increase in parasite load. The antimalarial activity of *DM* is significantly ( $P < 0.05$ ) higher than the antimalarial activity of *MW* at the highest dose used.

Figure 4A shows the WBC of the two extracts (*DM* and *MW*). The WBC value of the treated groups increased at low dose (100 mg/kg body weight) when compared with the parasitized control. However, the WBC decreased at high dose with significant reduction which was noticed at the



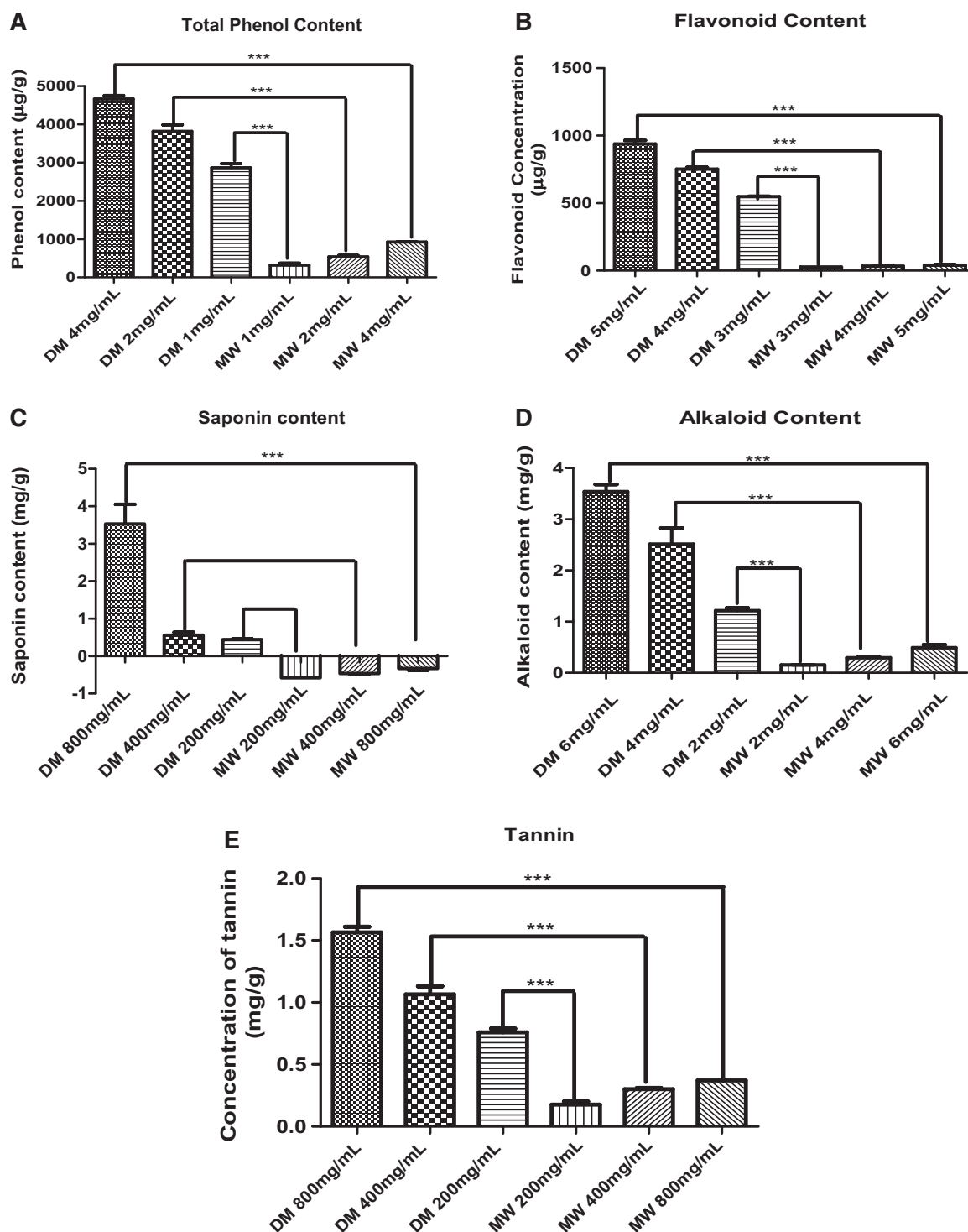


Figure 1 A–E: Quantitative phytochemical screening of methanol extracts of *Mondia whitei* and *Diospyros mespiliformis*. The concentrations of total phenol (A), flavonoid (B), saponin (C), alkaloid (D) and tannin (E) are significantly ( $P < 0.001$ ) higher in the methanol extract of *D. mespiliformis* (DM) than in the methanol extract of *M. whitei* (MW)

highest dose of DM administered. The levels of PCV in both control and treated groups are shown in Fig. 4B. The PCV value of the untreated control significantly ( $P < 0.05$ ) decreased when compared with the groups treated with artesunate and extracts of DM and MW. The artesunate group has the highest PCV values but not significantly higher than the DM groups. Again, it is interesting to note

that the PCV increased in a dose dependent manner for the two extracts with the DM having the highest effect. Figure 5A shows the platelet counts for the treated animals. It is observed that the values for the platelet count in the DM treated groups are significantly higher ( $P < 0.05$ ) than other treated groups. Percentage neutrophils, as shown in Fig. 5B is significantly ( $P < 0.05$ ) higher in the untreated control



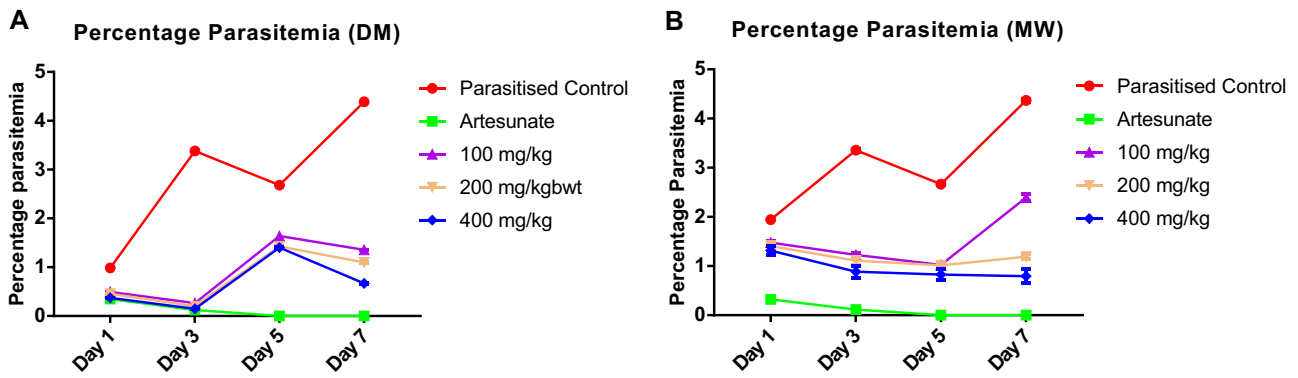


Figure 2 A–B: Effects of methanol extracts of *Diospyros mespiliformis* (A) and *Mondia whitei* (B) on the percentage Parasitemia of *Plasmodium berghei* induced malaria in mice. PC = Parasitized control, ART = Artesunate, 100, 200 and 400 mg/kg body weights of both *D. mespiliformis* and *M. whitei*

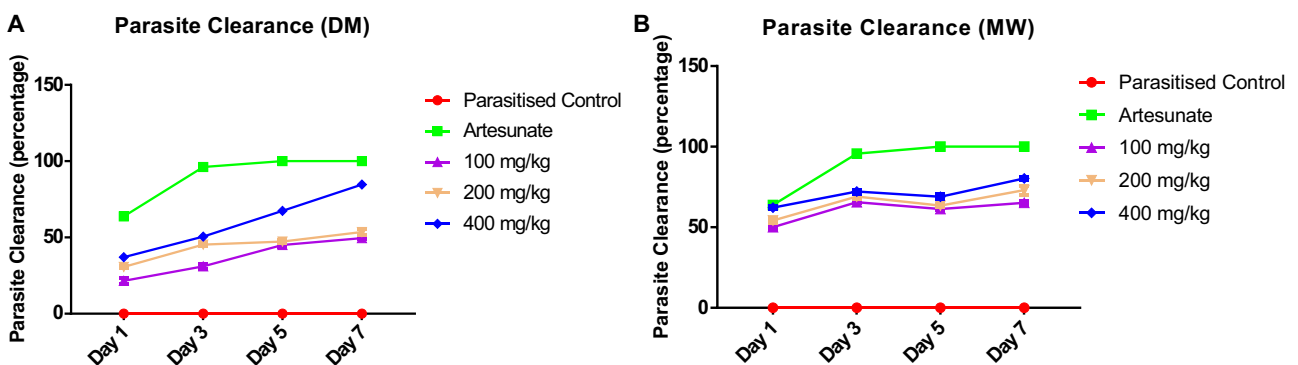


Figure 3 A–B: Effects of methanol extracts of *Diospyros mespiliformis* (A) and *Mondia whitei* (B) on the percentage clearance of *Plasmodium berghei* induced malaria in mice. PC = Parasitized control, ART = Artesunate, 100, 200 and 400 mg/kg body weights of both *D. mespiliformis* and *M. whitei*

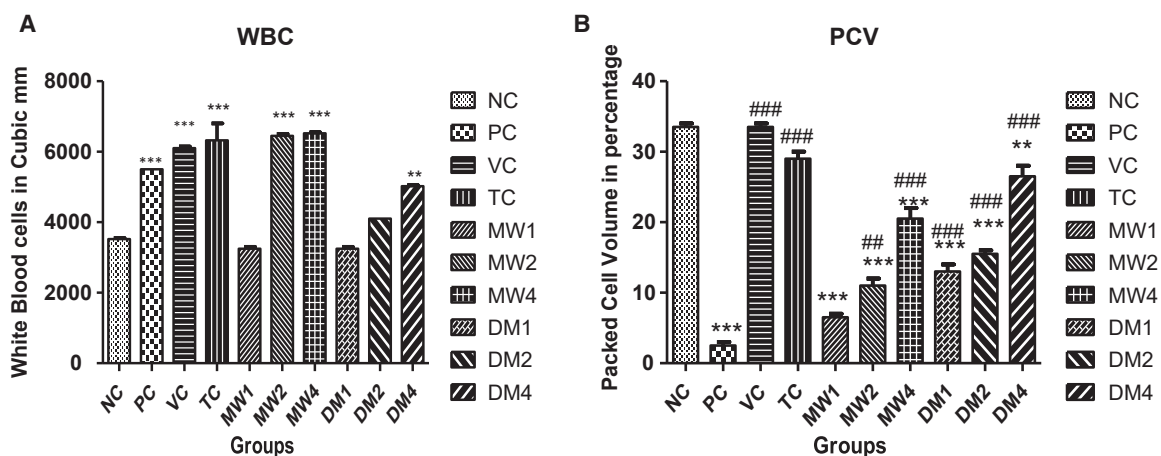


Figure 4 A–B: Percentage white blood cells (A) and packed cell volume (B) of the various treatment groups. NC = Normal control, PC = Parasitized control, TC = Treated control, MW1–MW4 = *Mondia whitei* 100, 200 and 400 mg/kg body weight, DM1–DM4 = *Diospyros mespiliformis* 100, 200 and 400 mg/kg body weight. Data are mean  $\pm$  SD ( $n = 5$ ; \*\*\* $P < 0.05$  vs normal control in both figures, ### $P < 0.05$  vs parasitized control in B)

group and the groups treated with MW while it is significantly ( $P < 0.05$ ) lower in the DM treated groups at the highest dose.

Figure 6 (A–C) shows percentage lymphocytes, monocytes and eosinophils, respectively for the groups treated with the two plant extracts. There is a significant ( $P < 0.05$ )

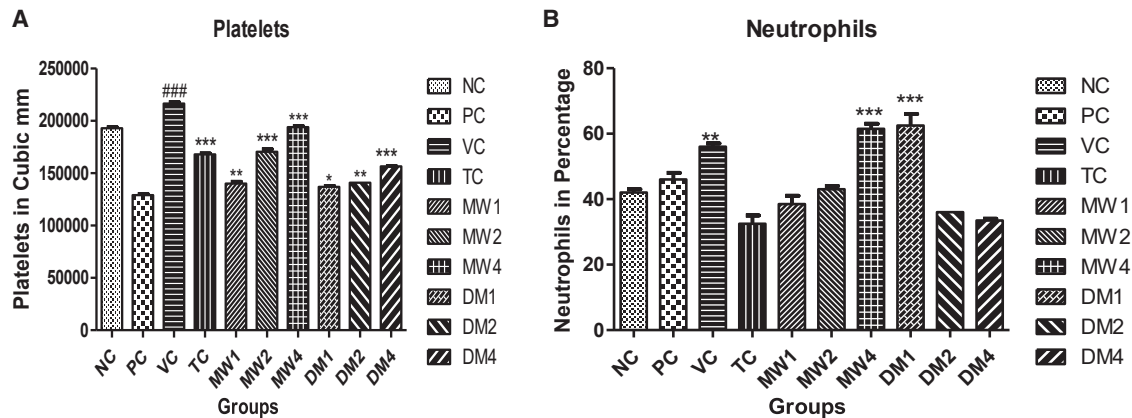


Figure 5 A–B: Cumulative platelet count (A) and neutrophils (B) of the various treatment groups. NC = Normal control, PC = Parasitized control, TC = Treated control, MW1–MW4 = *Mondia whitei* 100, 200 and 400 mg/kg body weight, DM1–DM4 = *Diospyros mespiliformis* 100, 200 and 400 mg/kg body weight. Data are mean  $\pm$  SD ( $n = 5$ ; ### $P < 0.05$  vs normal control; \*, \*\*, \*\*\* $P < 0.05$  vs parasitized control in A; \*\*, \*\*\* $P < 0.05$  vs normal control)

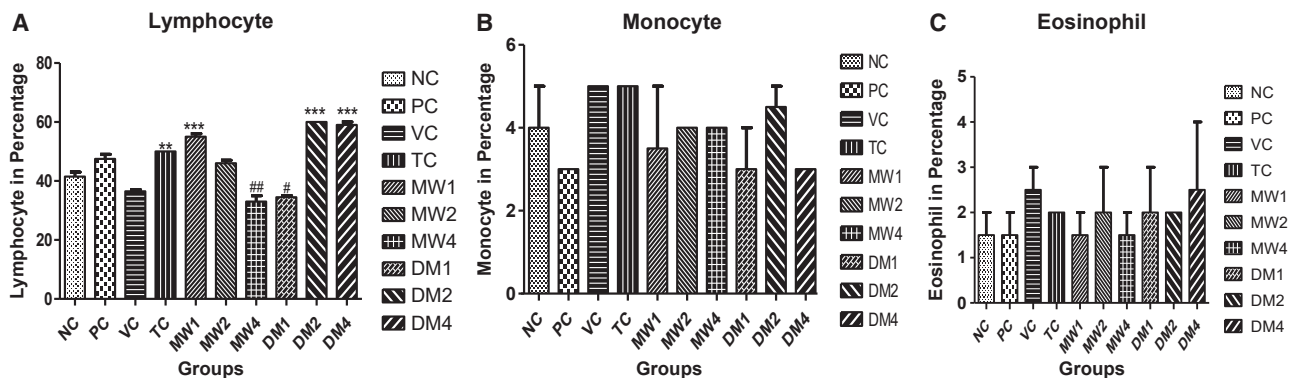


Figure 6 A–C: Percentage lymphocyte (A), monocyte (B) and eosinophil (C) of the various treatment groups. NC = Normal control, PC = Parasitized control, TC = Treated control, MW1–MW4 = *Mondia whitei* 100, 200 and 400 mg/kg body weight, DM1–DM4 = *Diospyros mespiliformis* 100, 200 and 400 mg/kg body weight. Data are mean  $\pm$  SD ( $n = 5$ ; #, ## $P < 0.05$  vs normal control; \*, \*\*, \*\*\* $P$  vs normal control in A)

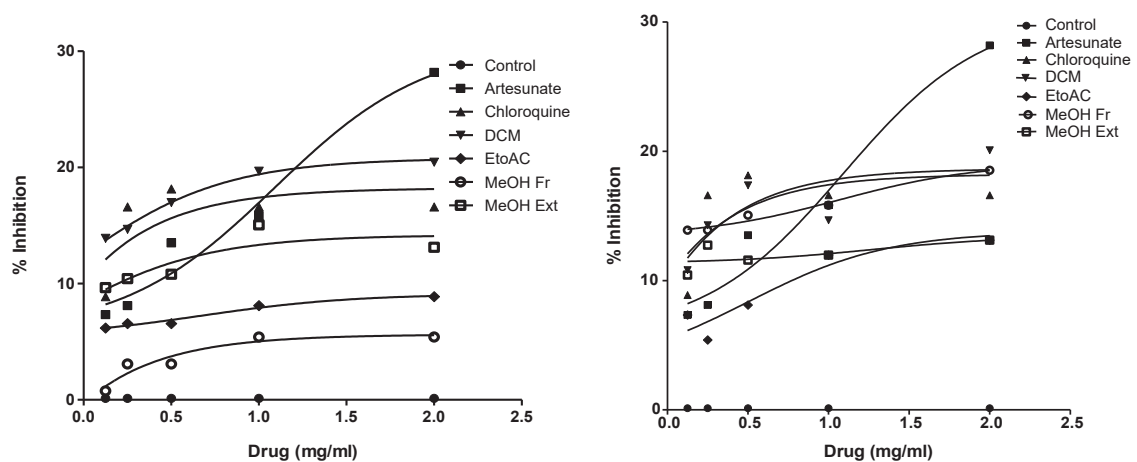


Figure 7. Percentage inhibition of the formation of  $\beta$ -hematin formation by extracts and fractions of *Diospyros mespiliformis* and *Mondia whitei*

Table 1. The IC<sub>50</sub> values of the extracts and fractions of *Mundia whitei* and *Diospyros mespiliformis*

IC <sub>50</sub> (mg/mL)	<i>Mundia whitei</i>	<i>Diospyros mespiliformis</i>
Artesunate	0.086	0.086
Chloroquine	0.2673	0.2673
DCM	0.9744	0.7863
EtoAC	4.625	3.182
MeOH Fr	11.68	0.5540
MeOH Ext	18.43	0.5669

decrease in the percentage lymphocyte in MW treated groups as the dose increased when compared with the normal control (Fig. 6A). Interestingly, lymphocyte values significantly ( $P < 0.05$ ) increased with a corresponding increase in of DM treated groups. Monocyte and eosinophil values increased albeit insignificantly ( $P > 0.05$ ) in both DM and MW treated groups when compared with the parasitized untreated control (PC). The hematological parameters of the unparasitized vehicular control (VC) group did not vary significantly with the unparasitized normal control (NC) group that were treated with only the vehicle.

Figure 7 showed the cell free antiplasmodial assay of the extracts and fractions of the two plants. This figure shows that the methanol extract of DM has the highest inhibitory effect on  $\beta$ -hematin formation. Interestingly, the solvent fractions of DM had the highest inhibitory effect on  $\beta$ -hematin formation when compared with the solvent fractions of MW as shown by the IC<sub>50</sub> values shown in Table 1.

## DISCUSSION

In folkloric medicine, DM stem is used for the treatment of malaria, so also, the extract of the roots of MW plant. In spite of this usefulness, there had not been any scientific fact to substantiate the indigenous claim for the use of MW for malarial treatment, we therefore present the scientific proof for the antiplasmodial effects of this plant and its possible mechanism as antimalarial herb for the first time.

Quantitative determination of the phytochemicals of both plants has shown that they contain a plethora of secondary metabolites such as phenols, flavonoids alkaloids and saponins with high antimalarial potentials. Phenolic compounds from *Parkia biglobosa* [20], *Erythrina crista-galli* [21] and alkaloid from cinchona [22] had been shown to have anti-malarial properties. In addition to this, phenolic compounds and flavonoids are known antioxidants that are needed to reduce oxidative damage in malarial infected patients.

An assessment of the percentage parasitemia and parasite clearance of methanol extracts of the two plants showed that there was a decrease in the parasite level and an increase in the parasite clearance in the treated groups in dose dependent manner. The percentage parasite clearance was low during the first three days of the treatment with the

methanol extract of both MW and DM but increased as the treatment progressed. However, it is interesting to note that the 400 mg/kg dose of the two extracts was found to be the most effective of all the doses used. This is an indication that these extracts at the highest dose are potential sources for new antimalarial drugs. DM had less parasite level at the highest dose when compared with MW at the same dose. The gradual increase in the parasite clearance and the concomitant reduction in the parasite load at the highest dose administered showed that the daily dose administered had antiplasmodial effect. Artesunate significantly showed antimalarial activity against chloroquine sensitive *P. berghei* infection in mice as evidenced by the clearance of the parasite on days five and seven. Although, to the best of our knowledge, this research report is the first scientific assessment of the folkloric claims of MW's antimalarial potency, previous phytochemical screening indicated that secondary metabolites that are of therapeutic importance had been characterized in it [23].

It is interesting to note that continuous decrease in the percentage parasitemia with concomitant increase in the parasite clearance is an indication that as the treatment progressed, the parasite load tapered, showing that the parasites were susceptible to the test drug candidates administered and thus shortening the parasite clearance time.

Anaemia is a common index and one of the parameters of severity of malarial infection. This is usually assessed by evaluating the packed cell volume (PCV), haemoglobin (Hb), and red blood cell (RBC) count [24] in malaria patients.

Infection of the erythrocytes by the malarial parasites and the feeding of the parasites on the blood meal leads to the rupture of the erythrocytes, a reduction in the red blood cells and eventually leads to hemolytic anemia. Our study evaluated the changes in haematological parameter in mice treated with the extracts after infection with *P. berghei*. The results obtained show a significant decrease in PCV of the untreated group when compared with the treated and unparasitized control groups. This is one of the features of malarial infection. Low hemoglobin concentration is indicative of anaemia in most cases [25]. Hemolytic lysis of erythrocytes invaded by malaria parasites might have caused low PCV in the untreated group. This effect was reduced by the antiplasmodial activity of the extracts of both DM and MW especially at the highest dose in which both extracts had increased PCV values. The clearance or destruction of infected red blood cells, the clearance of uninfected red blood cells, erythropoietic suppression and dyserythropoiesis have also been implicated in human and in mouse malarial anaemia [26]. The PCV was used as a measure of the efficacy of the extracts of the two plants in preventing hemolysis as a resultant effect of malarial infection. Various studies had confirmed that haematological abnormalities in malaria infection are common. In clinical studies for example, leucopenia was frequently seen in the malaria-infected patients [27].

Analysis of WBC counts in all the treated and control groups showed that there was an insignificant increase in





WBC counts in all the treated groups compared with the untreated control group. The increase in the levels of WBC in the treated group might not be unconnected to the pathogen eliciting this increase in WBC as a first defense mechanism due to parasite invasion. Previous studies have shown that an increase in WBC is linked to severe malaria [28].

Platelet abnormalities in malaria are both qualitative and quantitative change. The results obtained in this study have shown that platelet counts were reduced in the untreated control group compared with other treated groups. This observation may imply that thrombocytopenia may be a marker of plasmodium infection. The association of thrombocytopenia with plasmodium infection has previously been reported [29]. In a clinical study carried out both in Kenya and Nigeria, there is a direct correlation between thrombocytopenia and anemia and our report directly agree with this finding [30]. A dose dependent increase in the levels of platelets between the groups treated with *MW* is a therapeutic advantage because platelets are known to destroy parasitized erythrocytes ultimately reducing the parasite load when compared with *DM* treated groups [31, 32]. Levels of lymphocytes, monocytes and eosinophils increased in the groups treated with *MW* and *DM* methanol extracts when compared with the untreated parasitized control. Levels of both lymphocytes and monocytes significantly increased in the group treated with the extracts and artesunate than the normal control. Lymphocytes and monocytes are mononuclear leucocytes that are involved in both innate and adaptive immune response system. Eosinophils and neutrophils are granulocytes that contain enzymes that damage or digest pathogens. Eosinophils primarily deal with parasitic infections. There increased levels after the administration of the extracts produced immune response to the parasite invasion and increased digestion of the parasitized erythrocytes.

At the erythrocytic stage of malaria parasite survival, it encounters the unique problem of disposing of toxic heme derived from the digestion of host hemoglobin after blood meals. This problem is surmounted by crystallizing the free heme as insoluble hemozoin presented as malarial pigments in infected individuals. Important antimalarial drugs are believed to inhibit this conversion process thus the malaria parasite becomes susceptible to the toxic effects of heme. Previous studies have proposed that heme is the target of chloroquine [33, 34]. Our results show that both artesunate and chloroquine have inhibitory effects on  $\beta$ -hematin formation. Previous experiments have shown that inhibition of  $\beta$  hematin formation by artesunate had been observed [35] although this claim is highly controversial [36, 37] but our results have shown that indeed, inhibition of  $\beta$  hematin can as well be one of the possible mechanisms of action of both artesunate and chloroquine. In view of this, more detailed investigation into these claims is highly necessary. Methanol extract, dichloromethane and methanol fractions of *DM* had higher inhibitory effects that other fractions and extract of *MW*. Taking together, this showed that potent antimalarial constituents of the two plants may possess medium polarity and that *DM* demonstrated the most potent activity.

The results obtained in this study, no doubt, have shown that the two plants used have antiplasmodial activity and further work on these plants extracts is highly necessary to purify the active principles present in them. Purification and characterization of the active compounds is also essential in order to identify the natural product responsible for this activity and to possibly optimize such bioactive compound(s).

*Conflict of interest:* There is no conflict of interest.

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