Phytochemical analysis, antioxidant and antiplasmodial (curative) activities of methanolic leaf extract of *Morinda lucida* (Ewe Oruwo) in male Swiss mice infected with *Plasmodium berghei NK65*

ABSTRACT

AIM: Medicinal plants have been used for the treatment of many infections and diseases including malaria. The study was conducted to determine the effect of in-vivo anti-plasmodial and antioxidant properties of methanolic leaf extract of *Morinda lucida* in male Swiss albino mice infected with *Plasmodium Berghei NK65*.

Study Design and Methodology: Phytochemical, GC-MS and AAS analyses were determined in the plant. Swiss albino mice were inoculated intraperitoneally with *Plasmodium berghei NK65*. Thirty-five (35) mice were grouped into seven groups, five per group. Group A were not infected with *P.berghei NK65.* Group B, C and D served as the negative and positive control groups while Group E, F and G mice were treated with 400, 600 and 800 mg/kg body weight of methanolic leaf extract of *M. lucida*. Hematological parameters were determined in the whole blood using BC-3200 Auto Hematology Analyzer. TP, MDA, CAT, SOD % inhibition, SOD unit and vitamin A were all determined in the liver homogenate using standard procedures**.**

Results: The GC-MS result of the *M. lucida* shows the presence of five bioactive compounds. It was also observed that the plant contains the following minerals: iron, magnesium, potassium, phosphorus and copper. Acute toxicity shows that the LD_{50}>5000mg/KgB.WT. The extract caused 30.96%, 32.93% and 67.24% reduction in parasitemia at 400, 600 and 800 mg/kg body weight respectively while chloroquine exerted 96.53% and artesunate exerted 92.03% reduction at 10 mg/kg body weight respectively. The Hematological parameters showed that the plant extract is not hematotoxic since it significantly ($P<0.05$) reduced WBC count, and increase RBC, HGB, and HCT values in the treated mice compared to the infected untreated mice. This study shows that the mean lipid peroxidation (MDA) level was significantly decreased in the malaria treated mice (group C, D, E, F and G) compared to the untreated mice (group B). There was also a significant increase in the total protein, catalase, SOD % inhibition, SOD unit and Vitamin A levels in the liver homogenate of animals treated with chloroquine, artesunate and extract of *M. lucida* compared to the untreated mice.

Conclusions: The study shows that *Morinda lucida* possess antiplasmodial activity in male Swiss mice infected with *Plasmodium berghei NK 65.*

Keywords: *Morinda lucida,* anti-Plasmodial activity, antioxidant properties, biochemical parameters and *Plasmodium berghei NK 65* infected Swiss mice.

INTRODUCTION

Malaria remains one of the world's most devastating human parasitic infections, afflicting more than 500 million people each year [1]. It has been shown that mortality caused by malaria is currently estimated over a million people per year, has risen in recent years, probably due to increasing resistance to antimlarial medicines [2].

Morinda lucida ***is*** *a* tree in the mahogany family *Rubiaceae*. It is one of the two species in the genus *Morinda*, and is native to India, Pakistan and Bangladesh growing in tropical and semi–tropical regions. It is a natural, well-known medicinal plant habitat in West Africa, for which toxicology study has shown that the leaf-extract is non-toxic to living organs [3]. The plant is called different names in different countries. It is known as Sangogo or Bondoukou alongua in Cote d' Ivoire; Twi, kon kroma or Ewe amake in Ghana; Ewe amake or atak ake in Togo and Oruwo in South Western Nigeria [4]. *Morinda lucida* is known as "Ugigo" by the Ebira people in Kogi State North-Central Nigeria, is used in the treatment of malaria; fever, amongst other ailments [5]. Different parts of the plants are used in different ways in different countries. Cold decoction of the plant leaves is used for the treatment of fever in Cameroon; the bitter water decoction of the plant bark root and leaf are used as bitter tonic and an astringent for dysentery, abdominal colic and intestinal worm infestation [4]. Koumaglo *et al.,* 1992 and Obih *et al.,* 1985 documented in-vitro antimalarial activity of *Morinda lucida* leaf extract against *Plasmodium falciparum* and antimalarial activity of *Morinda lucida* against *Plasmodium berghei berghei* in mice [6,7]. Methanolic extract of *Morinda lucida* leaf have been reported to possess trypanocidal activity [8].
This research is designed to explore the antiplasmodial potential (curative) of the leaf extracts of *M. lucida* against chloroquine resistant strain of *Plasmodium berghei* and its effect on some biochemical parameters.

METHODOLOGY

Collection and Identification of Plant Material

The leaves of *M.lucida* were obtained from Ikorodu in Lagos State, Nigeria. The plant was authenticated by a botanist at University of Lagos with a Voucher number of 6947. The plant name corresponds to the official botanical plant name in "The Plant List" (www.theplantlist.org).

Preparation of methanolic leaf extract of *M.lucida*

The leaves of *M.lucida* were washed, air dried under shade in the Biochemistry Laboratory, pulverized to coarse powder using blender. Extraction was carried out by dispersing 200g of the grounded *M.lucida plant* material in 1L of 80% methanol and shaking was done with GFL shaker for 72 hours. This was followed by vacuum filtration and concentrated by rotary evaporator at a temperature not exceeding 40^{o}C. The concentrated extract was dried to complete dryness in an aerated oven at 40^{o}C for 48 hours. The extract was later stored in a refrigerator at 4^{o}C.

GC-MS analysis of the leaf of *Morinda lucida*

GC-MS analysis of the plant was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD). Ms model is agilent technology 5975 ms, the column used is HP-5MS agilent technology, length of the column is 30 m, internal diameter 0.320 mm, thickness of 0.25 μm. Volume of sample injected is 1 µL. Oven temperature program with initial temperature of 80°C to hold for 2 minutes at 10°C/min to final temperature of 240°C to hold for 6 minutes with injector temperature of 250°C. The mobile phase is helium gas while the stationary phase is the column.

Detection of Components

Analysis of mass spectrum GC-MS was conducted by the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unidentified component was compared with the spectrum of the identified components stored

in the NIST library. The names, molecular weight, structure of the components in the test material were ascertained [9-11].

Mineral determination in the plant using Atomic absorption spectrometry (AAS)

The minerals component of the plant was determined using AAS in University of Lagos, Nigeria. The mineral composition of the plant was analyzed on aliquots of dry-ashing. 2g of the *M. lucida* leaf, was separately weight into 250ml conical flasks, 10ml of aqua regia was added (HNO_3 and HCl in the ratio 1:3), the mixture was heated on porcelain crucible until the brown fumes disappeared leaving white fumes. It was later filtered with whatman filter paper into universal bottle; the mineral elements in the samples were determined by Atomic Absorption Spectrophotometer (Model PerkinElmer AAnalyst 400).

Phytochemical analysis

Phytochemical test were carried out on the methanolic leaf extract of *M.lucida* for the quantitative determination of phytochemical constituents using standard procedures for detecting the presence of alkaloids, tannins, saponin e.t.c [12-14].

Experimental Animal

Experimental Swiss albino mice

Seven (10) weeks old male Swiss albino mice weighing 22-32g were obtained from Ratzmattazz Nigeria enterprises, 21 insurance estate satellite town Lagos, Lagos, Nigeria. They were acclimatized for two weeks to Laboratory condition of 23 ±2ºC., housed in plastic cages with saw dust as beddings; food and water were given *ad libitum.* The mice were used in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

Acute toxicity test

The acute toxicity test of *M. lucida m*ethanolic extract was carried out using method described by Momoh and modified Lorkes method [15,16]. Eighteen Swiss mice weighing 23 to 32 grams were randomized into three groups of six mice each and were given 1600, 2900 and 500mg/Kg body weight of the extract orally. They were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. The oral median dose LD_{50} was calculated.

Animal grouping for infection and treatment

The parasites were kept alive by continuous intraperitoneal inoculation of known amount of the parasites into Swiss mice. Iml of blood was taken from donor mice and diluted with 5ml phosphate buffer; such that 0.1ml contained standard inoculum of 1×10^7 infected red blood cells [17]. Thirty five acclimatized male Swiss albino mice were randomly selected and thirty Swiss mice were inoculated intraperitoneally from the same source to avoid variability in parasitemia. The mice were randomly distributed into six groups of five per group as shown below:

Group A: (Normal control) Healthy Uninfected Swiss mice
Group B: (Negative control) Infected mice with *P.berghei NK65* received no treatment
Group C: (Positive control) Infected mice with *P.berghei NK65* + 10mg/kg B,WT of chloroquine.
Group D: Infected mice with *P.berghei NK65* + 10mg/kg B,WT of Artesunate
Group E: Infected mice with *P. berghei NK65* + 400mg/kg B.WT of *M.lucida* extract.

Group F: Infected mice with *P. berghei NK65* + 600mg/kg B.WT of *M.lucida* extract.
Group G: Infected mice with *P. berghei NK65* + 800mg/kg B.WT of *M.lucida* extract.

Anti-Plasmodium Study

Curative test

The Curative test of methanolic leaf extract of *Morinda lucida* on fresh infected Swiss albino mice were carried out according to the method described by Ryley and Peters [18]. The mice were injected intraperitoneally with standard inoculum of 1×10^7 *Plasmodium berghei NK 65* infected erythrocytes on the first day (day 0). Seventy two hours later, thirty-five mice were divided into seven groups of five mice per group as shown above. The groups were orally treated with 10mg/kg B.WT of chloroquine; artesunate and *Morinda lucida* leaf extract (400, 600 and 800mg/kg B.WT respectively). The treatment was carried out once daily for 5 days, on each day of the treatment, blood was collected from the mice tail and smeared onto microscope slide to make thin and thick films. The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 for 10 minutes and examined microscopically to monitor the parasitemia level. The parasite density was calculated for each group over a period of six days.

$$\text{Parasites per microlitre} = \frac{\text{Number of parasite counted} \times 8000}{\text{Number of leucocytes}}$$

Percentage suppression of parasitemia was calculated as:

$$\frac{\text{Parasitemia of negative control - parasitemia of test}}{\text{Parasitemia of control}} \times 100$$

Collection of blood Samples

The albino rats were sacrificed by cervical decapitation after 24 hours fasting. Blood were collected from the male albino rats by ocular puncture into EDTA tubes for hematological analysis and the remaining blood were collected in an heparinised tubes and centrifuge at 3000 rpm for 20 minutes using centrifuge and the plasma stored at -20°C

Determination of Hematological Parameters

The hemoglobin concentration (HGB), total red blood cell (RBC), white blood cell count (WBC), Hematocrit (HCT), and other hematological parameters were determined in the whole blood using BC-3200 Auto Hematology Analyzer in University of Lagos Teaching Hospitals (LUTH) in Idi-araba, Lagos, Nigeria.

Preparation of liver homogenate

The Liver tissues of some of the sacrificed albino rats were excised and the liver samples were cut into small pieces and homogenized with phosphate buffer saline (PBS) to give a 10% (w/v) liver homogenate. The homogenates were then centrifuged at 12,000 rpm for 50 minutes. The supernatant obtained was later used for the assay of total protein and few oxidative stress parameters.

Estimation of Lipid peroxidative (LPO) indices

Lipid peroxidation as evidenced by the formation of TBARS was measured in the homogenate by the method of Niechaus and Sameulsson as described by Jiang *et al.*[19].

Estimation of superoxide dismutase (SOD)

The SOD activity was estimated by its capacity of inhibiting the pyrogallol autooxidation in alkaline medium. One SOD unit (U) was considered the quantity of enzyme that was able to promote 50% inhibition. The liver homogenate was assayed for the presence of SOD by utilizing the technique described by Mccord and Fridovich [20].

Estimation of catalase (CAT)

The liver homogenate was assayed for catalase colorimetrically at 620 nm and expressed as μmoles of H_2O_2 consumed/min/mg protein as described by Venugopal *et al*., [21].

Estimation of Vitamin A

Vitamin A was determined in the liver homogenate using the method described by Rutkowski and Grzegorczyk [22].

Data analysis

Data analysis was done using the Graph Pad prism computer software. Student's'- T test and two-way analysis of variance (ANOVA) were used for comparison. A P-value < 0.05 was considered statistically significant.

RESULTS

Table 1. Phytocomponents identified in the methanolic leaves extract of *M. lucida* analyzed by GC-MS.

SN	Retention Time	Name of the compound	Molecular Formulae	Molecula Weight	Peak Area (%	Activity
1	13.198	Pentacyclo [6.6.6.0(2,7).0 (9,14).0	$C_{20}H_{14}O_2$	286.33 g/mol	18.35	NF
2	13.266	1H-Pyrazole, 3,4,5 -tribromo-	$C_4H_3Br_3N_2$	318.79 g/mol	17.59	Antimicrobial activity against *E.coli* etc [23].
3	13.324	2-Benzyl-6-methyl-1,2,3,4-tetrahydrodipyrido[1,2-a:4,3-d]pyrimidin-11-one	$C_{22}H_{18}N_4O_2S_3$	466.592 g/mol	13.97	NF
4	13.341	2,5-Dibromobenzo trifluoride	$C_7H_2Br_2ClF_3$	338.35 g/mol	10.06	NF
5	13.398	1H-Imidazole, 2,4,5- tribromo-	$C_4H_2Br_3ClN_2$	353.24 g/mol	34.04	Antimicrobial activity against *E.coli* etc [23].

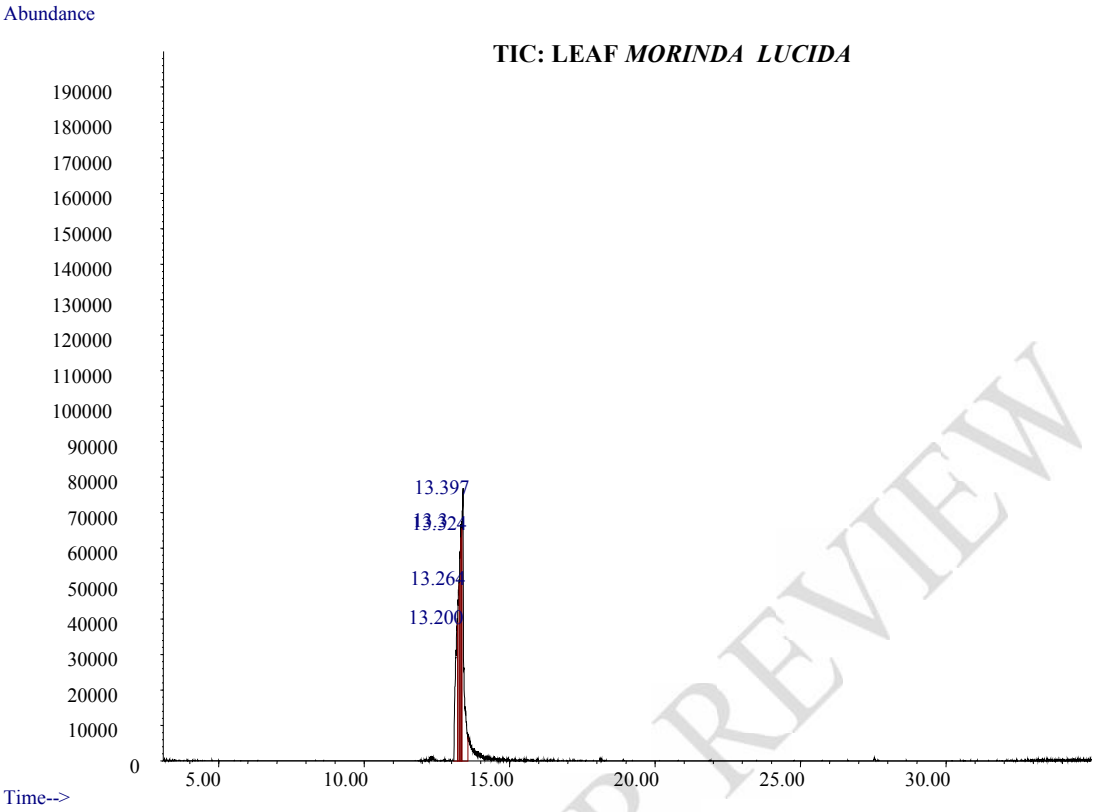
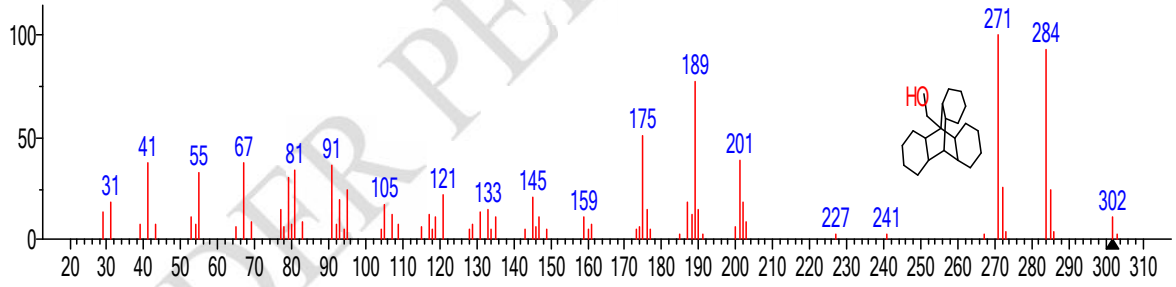
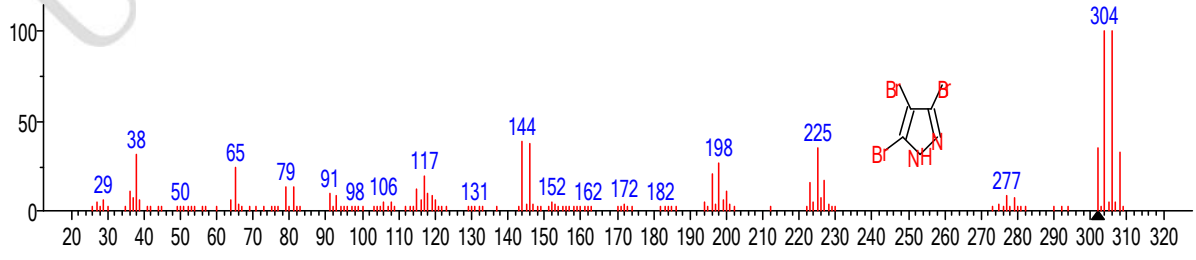


Figure 1. GC-MS chromatography of methanolic leaf extract of *M. lucida*.



(mainlib) pentacyclo[6.6.6.0(2,7).0(9,14).0(15,20)]icos-1-ylmethanol

Figure 2. Mass spectrum of pentacyclo[6.6.6.0(2,7).0(9,14).0 structure (18.35%, RT13.198)



(mainlib) 1H-Pyrazole, 3,4,5-tribromo-

Figure 3. Mass spectrum of 1H-Pyrazole, 3,4,5-tribromo- structure (17.59%, RT13.266)

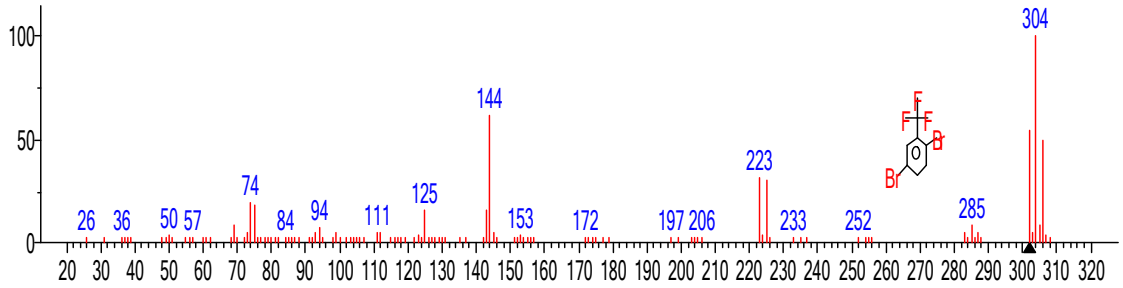


Figure 4. Mass spectrum of 2,5-Dibromobenzotrifluoride structure (10.06%, RT13.341)

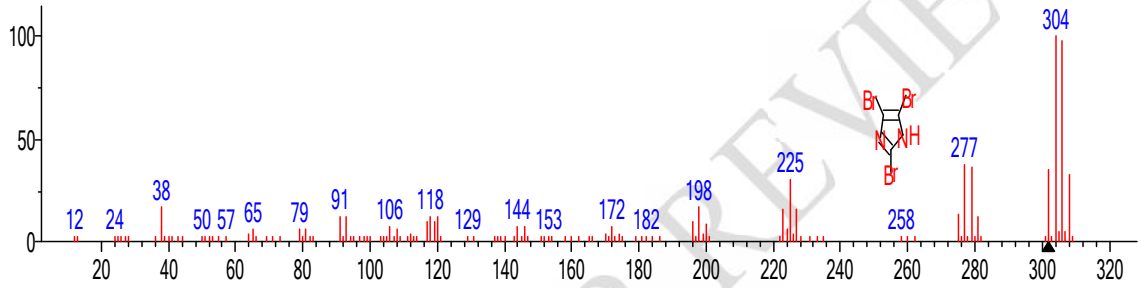


Fig.ure 5. Mass spectrum of 1H-Imidazole, 2,4,5-tribromo- structure (19.97%, RT13.324)

Table 2: Results of mineral constituents found in *M. lucida.* plant

Inorganic Constituent	Potassium (K)	Copper (Cu)	Iron (Fe)	Magnesium (Mg)	Phosphorus (P)
Concentration (mg/L)	8.6572	0.0882	34.8304	9.4532	7.2617

Phytochemical screening of methanolic leaf extract of *Morinda lucida* shows the present of secondary metabolite like tannins, anthocyanine, steroid, anthraquinones, terpenoids and saponin (Table 3). The presence of these secondary metabolites in *Morinda lucida* may be responsible for its anti-plasmodial activity.

Table 3: The phytochemical constituents of methanolic leaf extract of *M.lucida.*

Phytochemical components	Inference
Tannins	+
Saponins	+
Antraquinone	+
Alkaloids	+
Steroids	+
Terpenoids	+
Phenolic compound	+
Anthocyanine	+
Reducing sugar	+

+ indicate present

Acute toxicity test result

No death occurred during toxicity test at all the dose level used which shows that the extract does not have any toxic effect that can lead to the death of the animals. The median lethal dose LD_{50} was estimated to be greater than 5000 mg/Kg body weight. Behavioural signs of toxicity like: reduced activities, salivation, paw licking, and stretching were observed.

Curative test

There was a dose dependent reduction in the level of parasitemia in the treated group mice compared to the untreated group mice (Group B) in which there are consistent increase in the blood parasite density.

TABLE 4: Curative effect of methanolic leaf extract of *M. lucida* against *P. berghei NK65* in Swiss male mice.

Groups	Dose (mg/kg)	Mean parasitemia density (D6)	% Suppresion
A	NHC	NIL	NIL
B	NC	115, 428.57	NIL
C	10	4000*	96.53*
D	10	9,200.00*	92.03*
E	400	79,692.3*	30.96*

F	600	77,419.94*	32.93*
G	800	37,818.18*	67.23*

NHC = Normal healthy control group, NC = Negative control. *Significantly different (P<0.05) between group B and other groups using Student's'- T test. A P-value < 0.05 was considered statistically significant.

TABLE 5: Curative test showing the effect of methanolic leaf extract of *M. lucida, artesunate* and chloroquine on hematological parameters in Swiss albino mice infected with *P. berghei NK65*.

Hematological Parameters	Groups						
	A	B	C	D	E	F	G
WBC ($\times10^9$/L)	8.4**	18.4	8.2*	13.0*	15.6*	13.6*	13.0*
PLT ($\times10^9$/L)	361**	781	530*	555*	655*	539*	519*
PCT (%)	0.363	0.677	0.454	0.487	0.641	0.458	0.432
MPV (fL)	7.3	7.0	7.5	8.3	9.4	9.1	8.9
PDW	15.1	15.7	15.2	15.4	15.6	15.8	15.6
HGB (g/dl)	14.6**	10.8	13.0*	13.3*	12.5*	13.5*	12.5*
RBC ($\times10^{12}$/L)	9.10**	5.20	7.83*	8.33*	7.32*	7.12*	7.11*
HCT (%)	48.3**	30.5	42.2*	41.8*	33.5	35.5	43.5*
MCV (fL)	54.22	53.5	72.1	62.6	54.8	50.8	54.8
MCH (Pg)	15.7	13.5	16.5	15.0	13.8	12.5	11.8
MCHC (g/dl)	29.10	25.4	24.9	22.0	25.3	22.3	25.3
RDW-CV (%)	17.70	15.90	21.8	18.0	17.2	17.2	17.2
RDW-SD (fL)	30.40	31.2	30.8	33.9	35.5	32.2	31.1

The values are expressed in Mean value for five mice in each group. * indicate Significant difference (P <0.05) when the treated groups (Groups C, D, E, F and G) mice were compared to negative control mice (group B).** indicate significant difference (P <0.05) when comparing negative control mice and group A mice . Hemoglobin (HGB), Red blood count (RBC), Hematocrit (HCT), Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV), Red Blood Cell Distribution Width Standard Deviation (RDW-SD). Platelet count (PLT), Mean platelet volume (MPV), platelet Distribution Width (PDW) and Plateletcrit (PCT). A P-value < 0.05 was considered statistically significant.

TABLE 6: The effect of Methanolic leaf extract on *M. lucida* on oxidative stress parameter.

Oxidative Stress parameters	Groups						
	A	B	C	D	E	F	G
TP	6.19 ±0.18**	1.63 ±0.02	2.87 ±0.02	3.133 ±0.07*	4.13 ±0.08*	6.63 ±0.11*	7.15 ±0.16*
MDA (nmol/1)	3.10 ±0.007**	11.72 ±0.36	5.31 ±0.08*	5.36 ±0.14*	4.04 ±0.19*	4.68 ±0.13*	3.85 ±0.23*
Catalase (μmol/min/mg)	40.16 ±1.13**	10.56 ±0.25	49.43 ±1.38*	51.63 ±1.42*	34.76 ±0.73*	23.23 ±0.32*	46.84 ±0.53*
SOD % inhibition	91.75 ±3.96**	64.28 ±2.88	81.06 ±4.03*	76.59 ±3.32*	78.89 ±3.17*	79.17 ±3.75*	84.43 ±4.26*
SOD Unit	12.83 ±0.37*	1.80 ±0.003	4.83 ±0.03*	3.67 ±0.01*	4.33 ±0.02*	4.77 ±0.03*	5.18 ±0.03*
Vitamin A	9.12 ±0.29*	2.007 ±0.005	3.40 ±0.02	3.81 ±0.22*	4.98 ±0.11*	4.55 ±0.04*	6.92 ±0.12*

The values are expressed in Mean ± SD value for five mice in each group.* indicate Significant difference when the treated groups (Groups C, D, E, F and G) mice were compared to negative control (group B) ($P < 0.05$). mice and ** indicate significant difference ($P < 0.05$) when comparing negative control mice and group A mice. A P-value < 0.05 was considered statistically significant.

DISCUSSION

Malaria continues to pose a serious threat to human population especially in the tropical and subtropical regions of the world. The appearance of drug resistant strains of malaria parasite has worsened the danger of the parasite, and this has become a major stumbling block to economic development and tourism in the affected areas [24].

Table 1 shows the GC-MS result of *M. lucida*. Five compounds were identified; their retention time, peak area, molecular formulae, molecular weight, and their activities or properties are shown in the Table 1 above. All these compounds are important in the formulation of different medicines. The compounds found in the plant are : pentacyclo[6.6.6.0(2,7).0(9,14).0(18.35%), 1H-Pyrazole,3,4,5 tribromo-(17.59%), 2-Benzyl-6-methyl-1,2,3,4-tetrahydrodipyrido[1,2-a:4,3-d]pyrimidin-11-one, 2,5Dibromobenzotrifluorid(10.06%) and 1H-Imidazole,2,4,5-tribromo- (34.04%). The GC-MS analysis was based on the computer evaluation of mass spectra of samples through NIST by direct comparison of peaks and retention time with those for standard compounds, with four peak index and computer matching with the NIST. Besides that, the characteristic fragmentation patterns greatly helped in the identification of a particular class of compounds. These compounds were identified through mass spectrophotometer attached with gas

chromatography. The GC-MS study of the leaves of *M.lucida* had shown the presence of many phytochemicals which might contribute to the medicinal activity of that plant. This study shows the formulae and structures of active compounds which may be used in the synthesis of drugs. Kirstein *et al.,* study shows that 1H-Pyrazole, 3,4,5-tribromo- and 1H-Imidazole, 2,4,5- tribromo- have anti-microbial activity against *E .coli, S. aureus* and *P. aeruginosa.* [23]

The AAS analysis indicates the presence of micro and macro nutrients found in M. *lucida.* They include*:* Copper (Cu = 0.0882 mg/L), Phosphorus (P = 7.2617 mg/L), Potassium (K = 8.6572 mg/L), Magnesium (mg = 9.4532 mg/L) and Iron (Fe = 34.8304mg/L). Element like Fe is responsible for the formation of red blood cells and in the prevention of anemia and other related diseases [25], phosphorous is used for the formation of strong bones and teeth. Increases phosphorous intake also has potential to lower blood pressure [26]. Magnesium and potassium are used for proper functioning of enzymes. Magnesium and potassium are important in the prevention and treatment of hypertension and their high intake may reduce coronary heart disease and stroke [27]. According to Andreini *et al.* [28], transition metals like iron, zinc, manganese, and copper are essential for life through their function as both structural and catalytic cofactors for proteins.

Phytochemicals constitute is an integral part of medicinal plants and are responsible for their numerous bioactivities. The study shows that the phytochemicals present in *M. lucida* includes: saponin, tannin, alkaloids, steroids, anthocyanine, terpenoid etc. Different plants contain a wide variety of phytochemicals or secondary metabolites as their bioactive principle having antiplasmodial activities [29,30]. The presence of flavonoids and other bioactive constituents in *M.lucida* is believed to have contributed to the observed antiplasmodial activity of *Morinda lucida* leaf extracts [5].

According to Bruce [31,32] and American Society for Testing and Materials [33], any chemical substance with LD_{50} estimated to be greater than 2000 – 5000 mg/kg/oral route could be considered of having low toxicity and safe for consumption. This study shows that methanolic leaf extract of *M. lucida* has an LD_{50} >5000 mg/Kg B.WT. This study was supported by research work carried out by Asuzu and Chineme. In their study, intraperitoneal LD_{50} value of 2000 mg/kg was obtained for 50% methanol extract of the dried leaves of *M. lucida* [8]. Adeneye and Agbaje`s study shows that acute oral toxicity of *M. lucida* is nonlethal at 2000 mg/kg body weight [4].

P. berghei has been used in studying the activity of potential antiplasmodials in-vivo in rodents [34,35], and it produces diseases similar to those of human plasmodial infection [36,37]. *Plasmodium berghei* parasite is used in predicting treatment outcomes of any suspected antimalaria agent due to its high sensitivity to chloroquine making it the appropriate parasite for this study.. In this study, chloroquine was used as one of the standard antimalarial drug. Chloroquine has been used for suppressive, curative and prophylactic antiplasmodial study. In early and established infection, chloroquine interrupts with the heme polymerization by forming a FP-chloroquine complex. This complex is responsible for the disruption of the parasite's cell membrane function and ultimately leads to auto digestion.

This study revealed that *P. berghei* chloroquine sensitive strain infected animals, have significant increase ($P<0.05$) mean parasite density compared to the treated mice . Since the *P. berghei* is highly sensitive to chloroquine, the chloroquine treated mice has the highest suppression of 96.53% followed by artesunate with 92.03%. For the mice administered with the plant extract, they produce percentage suppression in a dose dependent manner, with the highest concentration of 800 mg/kg B.WT having 67.23% suppression and the lowest concentration of 400 mg/kg BWT having 30.96% suppression. The results obtained are in line with other study who showed that the plant extract reduces plasmodium parasite in a dose dependent manner [38-40].

The study shows that, there were significant decrease (P<0.05) in the level of WBC and a significant increase (P<0.05) in HGB and RBC of the healthy animals and mice treated with chloroquine, artesunate and different concentrations of the leaf extract of *M. lucida* compared to the untreated group mice (group B). All other hematological parameters (MCV, MCH, MCHC, PCT, RDW-CV, RDW-SD, MPV and PWD) showed no significant differences (P>0.05) in the entire different group. One of the effects of malaria on the blood of infected host is the reduction in the number of RBC leading to an anemic conditions, this is due to low production and increased destruction of RBC during malaria infection [41]. It was observed in this study as shown in Table 5 that *P. berghei* chloroquine resistant strain infected animals treated with the extracts of *Morinda lucida* (800mg/Kg B.WT), chloroquine and artesunate significantly increase the (P<0.05) HCT level in the treated Swiss mice. This finding agrees with what was reported by Akindele and Busayo on the ability of *M. pruriens* in stabilising PCV [42]. Idih *et al.* also show in their study that *M.lucida* significantly maintain PCV in animal induce with *P. berghei*. [38].

Liver is the major organ used for removing xenobiotic substances from the body and as such it is subjected to many substances causing oxidative stress. Oxidative stress is caused by the presence of reactive oxygen species (ROS) in excess of the available of antioxidant buffering capacity [43]. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage biological cells. The malaria parasite could be responsible for the upsurge increase in the oxidative stress in the infected mice due to reduction in antioxidant enzymes activity like catalase and superoxide dismutase. The parasite enhanced the production of large quantity of reactive oxygen species (ROS). Lipid peroxidation is a well-established mechanism of cellular injury in humans and is used as an indicator of oxidative stress in cells and tissues. MDA level is widely utilized as a marker of lipid peroxidation in states of elevated oxidative stress. This study shows that the mean lipid peroxidation (MDA) levels were significantly decrease (P<0.05) in the malaria treated mice compared to the infected untreated mice an indication of oxidative stress in group B animals as shown in Table 6 above. SOD is an effective defence enzyme that catalyses the dismutation of superoxide anions into hydrogen peroxide [43]. Catalase catalyses the conversion of hydrogen peroxides into oxygen and water and protects the tissue from oxidative damage by highly reactive oxygen free radicals and hydroxyl radicals [44]. Protein sulfurhydryls serves as sacrificial antioxidants, preventing plasma lipid peroxidation as well as being targets for oxidative damage. There were significant increase (P<0.05) in the total protein, catalase, SOD % inhibition, SOD unit and Vitamin A level of chloroquine, artesunate and extract treated mice compared to that of the negative control group mice (Group B).

CONCLUSION

The study shows that 400, 600 and 800 mg/kg body weight of methanolic leaf extract of *Morinda lucida* suppresses *Plasmodium berghei NK65* and could be used in the management of malaria.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCE

1. WHO. The World Health Organization World Malaria Report 2005. Geneva: World Health Organization; 2005.

2. World Health Organization. World Malaria Report 2011: the successes, existing challenges and the way forward. Geneva: World Health Organization; 2011.

3. Lawal HO, Etatuvie SO, Fawehinmi AB. Ethnomedicinal and pharmacological properties of *Morinda lucida* . J Nat Prod. 2012; 5: 93-99.

4. Adeneye AA. and Agbaje EO. Pharmacological Evaluation of Oral Hypoglycaemic and Antidiabetic Effects of Fresh leaves Ethanol Extract of *Morinda lucida* Benth in Normal and Alloxan Induced Diabetic Rats. African J. Biomedical Research. 2008; Vol. 11(1): 65-71. ISSN 1119 – 5096 © Ibadan Biomedical Communications Group.

5. Maimuna BU, Emmanuel OO, Josephine YI, Oluwakanyinsola AS, Adeniyi YT, Ibrahim MH. Antiplasmodial Efficacy Of Methanolic Root And Leaf Extracts Of *Morinda lucida* Journal of Natural Sciences Research. 2013; ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online) Vol.3, No.2.

6. Koumaglo KM, Gbeassor O, Nikabu C, de Souza, Werner W. Effects of three compounds extracted from *Morinda lucida* on *Plasmodium falciparum*. Planta Med. 1992; 58: 533-534.

7. Obih, PO., JM. Makinde and JO. Laoye, 1985. Investigations of various extracts of *Morinda lucida for antimalaraial actions on Plasmodium berghei berghei* in mice. Afr. J. Med. Med Sci. 14: 45-49.

8. Asuzu IU, Chineme CN. Effects of *Morinda lucida* leaf extract on *Trypanosoma brucei brucei* infection in mice. J. Ethnophamacol. 1990; 30: 307-331.

9. Principe P. Monetising the pharmacological benefits of plants. US Environmental Protection Agency, Washington, D.C. 2005.

10. Stenhagen E, Abrahamson S, McLafferty F. Registry of spectral data. J. Wiley and Sons, New York, NY; 1974.

11. Jennings W, Shibamoto T. Quality of flavour and fragrance volatiles by glass capillary gas chromatography. Academic Press, New York, NY; 1980.

12. Harborne JB. Phytochemical methods. Chapman and Hall Ltd., London. 1973; 49-188.

13. Sofowora A. Medicinal plants and traditional medicines in Africa. Spectrum Book Ltd., Ibadan, Nigeria. 1993.

14. Trease GE, Evans WC. (Pharmacognosy. 14th ed. London: W.B. Sanders Company. 1985.

15. Momoh J,.Aina OO,. Akoro, SM, Ajibaye O, 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 BergheiNK 6.* Nigerian Journal of Parasitology ISSN 1117 4145 Vol. 35 [1-2] 2014; 109-116

16. Lorke D.. A new approach to practical acute toxicity test. Arch. Toxicol. 1993; 54:275-286.

17. Maegraith BG, Deegan T, Sherwood EJ. Suppression of malarial (*P. berghei*) by milk. *British Medical Journal*. 1952; 2 (4799): 1388.

18. Ryley JF, Peters W. The anti-malarial activity of some quinolone esters. Ann.Trop. Med. Parasitol. 1970; 84: 209-222.

19. Jiang ZY, Hunt JY, Wolff SP. Detection of lipid hydroperoxides using the 'Fox method'. Anal Biochem. 1992; 202: 384-389.

20. McCord, J, Fridovich I. Superoxide dismutase, an enzymic function for erythrocuprin. J. Biol. Chem. 1969; 244, 6049–6055.

21. Rajagopalen R, kode A, Penumathsa SV, kailikat NR, Venugopal, PM. Comparative effects of Curcumin and an analog of Curcumin on alcohol and PUFA induced oxidative stress. J. Pharm Pharmaceut Sci. 2004; 7(2): 274-283.

22. Rutkowski, M., Grzegorczyk, K., (2007). Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic Practice. *Acta Sci. Pol., Technol. Aliment.* 6(3) 2007; 17-28.

23. Kirsten B, Ramesh P, John BB. 2,4,5-tribromo-1H-Imidazole in the egg masses of three muricid molluscs. natural product research, vol. 18, no. 5. 2004; 427–431.

24. Motlaleputa M. Antimalarial medicines from the medicinal plants of Southern Africa project. Quarterly Report 2003.

25. Oluyemi EA, Akilua AA, Adenuya AA, Adebayo MB. Mineral contents of some commonly consumed Nigerian foods. *Science Focus,* vol. 11. 2006; 153-157.

26. Elliot P, Kesteloot, H, Appel LJ, Dyer AR, Ueshima H, Chan Q, Brown IJ, Zhao L, Stamler, J. Dietary phosphorous and blood pressure International study of macro- and micro-nutrients and blood pressure. Hypertension. 2008; 51: 669-675.

27. Houston MC, Harper KJ. Potassium, magnesium and calcium: Their role in both the cause and treatment of hypertension. The Journal of Clinical Hypertension. 2008; 10 (7): 3-11.

28. Andreini C, Bertini I, Cavallaro G, Holliday, GL, Thomton JM.. Metal ions in biological catalysis: from enzyme databases to general principles. Journal of Biological Inorganic Chemistry. 2008; 13: 1205-1218.

29. Matur BM, Mathew T, Ifeanyi CIC. Analysis of the phytochemical and in vivo antimalarial properties of Phyllanthus fraternusn Webster extract. New York Sci. J. 2009; 2: 12-19.

30. Alshawsh SM, Mothana RA, Al-Shamahy HA, Alsllami SF, Lindequist U. Assessment of antimalarial activity against Plasmodium falciparum and phytochemical screening of someYemeni medicinal plants. eCAM 2007; 6: 453-456.

31. Bruce RD. An Up-and-Down Procedure for Acute Toxicity Testing. Fundamental Applied Toxicology. 1985; 5: 151-157.

32. Bruce RD. A confirmatory study of Upand- Down Method of Acute Oral Toxicity Testing. Fundamental Applied Toxicology. 1987; 8: 97 – 100.

33. American Society for Testing and Materials. Standard Test Method for Estimating Acute Oral Toxicity in Rats. American Society for Testing and Materials. 1987; E 1163 87, Philadelphia, U.S.A.

34. Thomas AM, Van Der Wel AM, Thoma AW, Janse CJ, Water AP. Transfection systems for animal models of malaria. Parasitol Today. 1998;14: 248-249.

35. Pedroni HC, Bettoni CC, Spalding SM, Costa TD. Plasmodium berghei Development of an irreversible experimental malaria model in wistar rats. Exp Parasitol. 2006; 113: 193-196.

36. English MC, Waruiri C, Lightowler C, Murphy SA, Kirigha G, Marsh K. Hyponatraemia and dehydration in severe malaria. Arch Dis Childhood. 1996; 74: 201-205.

37. Kumar KA, Singn S, Babu PP. Studies on the glycoprotein modification in erythrocyte membrane during experimental cerebral malaria. Exp Parasitol. 2006; 114: 173-179.

38. Idih FM, Ighorodje-Monago CC, Ezim OE. Antiplasmodial Effect of Ethanol Extract of *Morinda lucida* and *Mucuna pruriens* Leaves on *NK65* Chloroquine Resistant Strain of *Plasmodium berghei* in Mice. Clin Exp Pharmacol. 2017; 7: 234. doi: 10.4172/2161-1459.1000234.

39. Bello IS, Oduola T, Adeosun OG, Omisore NOA, Raheem GO, Ademosun AA. Evaluation of Antimalarial Activity of Various Fractions of Morinda lucida Leaf Extract and Alstonia boonei Stem Bark. Global J. Pharmacol. 2009; 3 (3): 163-165.

40. Umar MB, Ogbadoyi EO, Ilumi JY, Salawu OA, Tijani AY, et al. Antiplasmodial efficacy of methanolic root and leaf extracts of *Morinda lucida.* J Nat Sci Res. 2013

41. Menendez C, Fleming AF, Alonso PL. Malaria-related anaemia. Parasitol Today. 2000; 16: 469-476

42. Akindele AJ, Busayo FI. Effects of the hydroethanolic extract of Mucuna pruriens (L.) DC (Fabaceae) on haematological profile in normal and haloperidol treated rats. Nig Q J Hosp Med. 2011; 21: 93-98.

43. Momoh JO, Adeniyi MO, Aderele OR. Experimental and Mathematical Model for the Hepatoprotective Effect of Methanolic Extract of *Moringa oleifera* Leaf against CCl_4-induced Hepatotoxicity in Sprague Dawley Male Albino Rats. *JAMMR, 26(5): 1-14, 2018; Article no.JAMMR.32062 ISSN: 2456-8899*

44. Salvi M, Battaglia V, Brunati AM, LaRocca N, Tibaldi E, Pietrangeli P, Marcocci L, Mondovi B, Rossi CA, Toninello A. Catalase takes part in rat liver mitochondria oxidative stress defense. J Biol Chem. 2007; 282: 24407–24415.