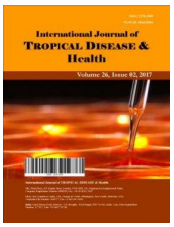

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Phytochemical Analysis and Antiplasmodial (curative) Activities of Methanolic Leaf Extract of *Morinda lucida* (Ewe Oruwo) in Male Swiss Mice Infected with *Plasmodium berghei NK65*

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Authors' contributions

This work was carried out in collaboration among all authors. Author MJO designed the methodology and study, wrote the protocol and the first draft of the manuscript and contributed to the discussions and corrections. Authors DOA and AAO edit the introduction and explained the interpretation of the GC-MS, AAS and the medicinal functions of the phytocomponents found in the plant. Author BAO is involved in the collation of data, statistical analysis and contributed to the discussions. Authors AOM and BNO carried out the laboratory analyses. Author MAA carry out the analysis and the interpretation of the hematological parameters. All authors read and approved the final manuscript.

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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 the 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*. Haematological 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} >000mg/Kg b.wt. The extract caused 30.96%, 32.93% and 67.23% 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 Haematological parameters showed that the plant extract is not haematotoxic 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; AAS, phytochemical analysis; biochemical parameters and Plasmodium berghei NK 65 infected Swiss mice.

1. 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 grow in tropical and semi–tropical regions. It is a natural, well-known medicinal plant habitat in the West Africa, in 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.

2. METHODOLOGY

2.1 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 the 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).

2.2 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 hrs. This was followed by vacuum filtration and concentrated by rotary evaporator at a temperature not exceeding 40°C. The concentrated extract was dried to complete dryness in of an aerated oven at 40°C for 48 hrs. The extract was later stored in a refrigerator at 4°C.

2.3 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 *μ*m. The Volume of sample injected is 1 µL. Oven temperature program with an initial temperature of 80°C to hold for 2 minutes at 10°C/min to the final temperature of 240°C to hold for 6 minutes with an injector temperature of 250°C. The mobile phase is helium gas while the stationary phase is the column.

2.4 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].

2.5 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. 2 g of the *M. lucida* leaf, was separately weight into 250 ml conical flasks, 10 ml 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 a universal bottle; the mineral elements in the samples were determined by Atomic Absorption Spectrophotometer (Model Perkin Elmer AAnalyst 400).

2.6 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 etc [12-14].

2.7 Experimental Animal

2.7.1 Experimental Swiss albino mice

Seven (10) weeks old male Swiss albino mice weighing 22-32 g were obtained from Ratzmattazz Nigeria enterprises, 21 insurance estate satellite town Lagos, Nigeria. They were acclimatized for two weeks to Laboratory condition of 23 ±2°C., 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).

2.7.2 Acute toxicity test

The acute toxicity test of *M. lucida m*ethanolic extract was carried out using a 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 500 mg/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 the LD_{50} was calculated.

2.7.3 Animal grouping for infection and treatment

The parasites were kept alive by continuous intraperitoneal inoculation of a known amount of the parasites into Swiss mice. Iml of blood was taken from donor mice and diluted with 5ml phosphate buffer; such that 0.1 ml 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* **+** 10 mg/kg b.wt of chloroquine.

Group D : Infected mice with *P. berghei NK65* **+** 10 mg/kg b.wt of Artesunate

Group E : Infected mice with *P. berghei NK65* + 400 mg/kg b.wt of *M. lucida* extract.

Group F : Infected mice with *P. berghei NK65* + 600 mg/kg b.wt of *M. lucida* extract.

Group G : Infected mice with *P. berghei NK65* + 800 mg/kg b.wt of *M. lucida* extract.

2.8 Anti-Plasmodium Study

2.8.1 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 the 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 10 mg/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.

Parasites per microlitre = {(Number of parasite counted / Number of leucocytes) × 8000}

Percentage suppression of parasitemia was calculated as:

{(Parasitemia of negative control ‐ Parasitemia of test / Parasitemia of control) x 100}

2.8.2 Collection of blood samples

The albino rats were sacrificed by cervical decapitation after 24 hrs 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

2.8.3 Determination of haematological parameters

The haemoglobin content (HGB), total red blood cell (RBC), white blood cell count (WBC), Hematocrit (HCT), and other haematological 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.

2.8.4 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.

2.8.5 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].

2.8.6 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].

2.8.7 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].

2.8.8 Estimation of Vitamin A

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

2.9 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.

3. RESULTS

Phytochemical screening of methanolic leaf extracts 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.

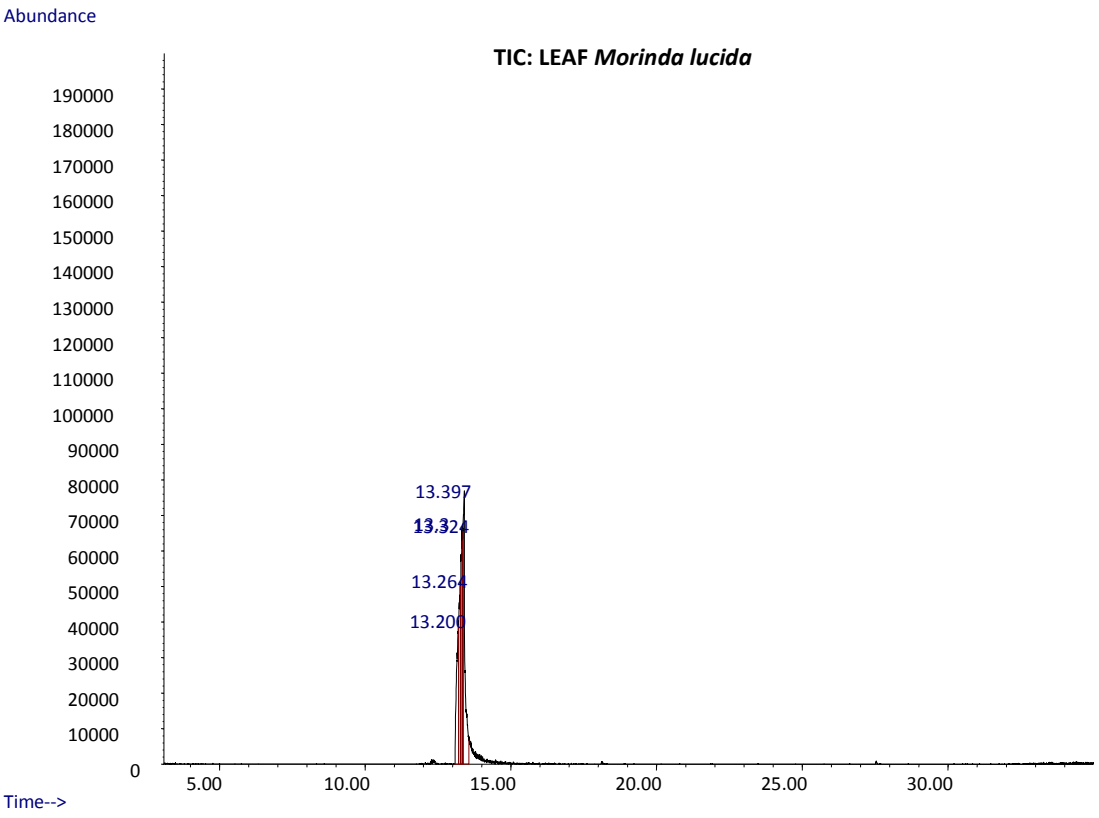

Fig. 1. GC-MS chromatography of methanolic leaf extract of *M. lucida*

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	Molecular 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].

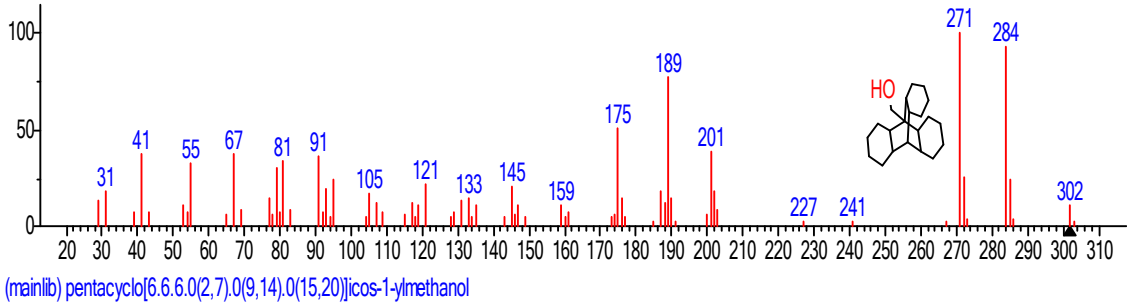

Fig. 2. Mass spectrum of pentacyclo[6.6.6.0(2,7).0(9,14).0 structure (18.35%, RT13.198)

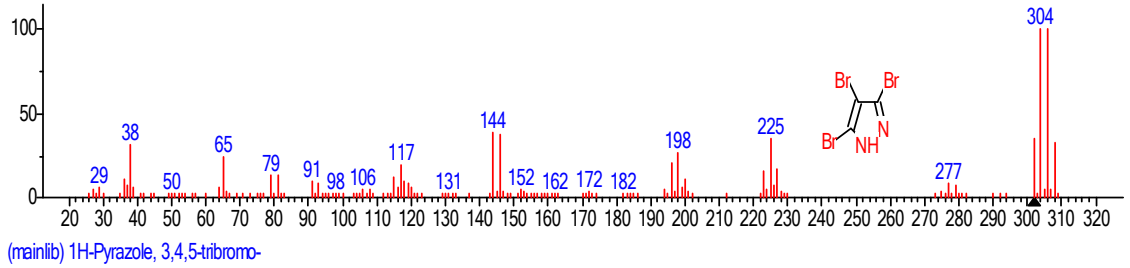

Fig. 3. Mass spectrum of 1H-Pyrazole, 3,4,5-tribromo- structure (17.59%, RT13.266)

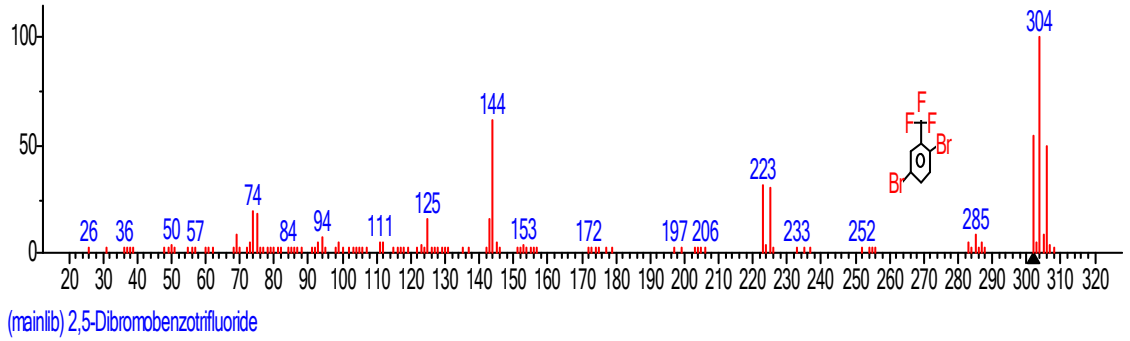

Fig. 4. Mass spectrum of 2,5-Dibromobenzotrifluoride structure (10.06%, RT13.341)

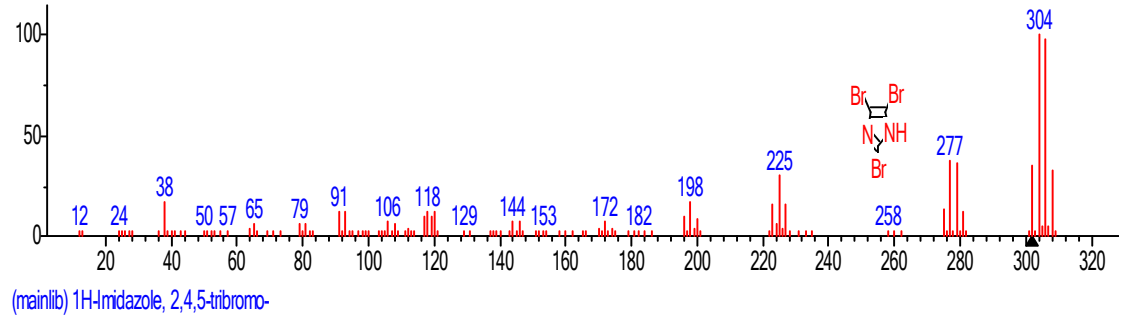

Fig. 5. Mass spectrum of 1H-Imidazole, 2,4,5-tribromo- structure (19.97%, RT13.324)

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

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

3.1 Acute Toxicity Test Result

No death occurred during toxicity test at all the dose levels 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 LD50 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.

3.2 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.

4. 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 (13.97%), 2,5Dibro moben-zotrifluorid(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. [23], revealed 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].

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

Group	Dose (mg/kg)	Mean parasitemia density (D8)	% 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	58.6	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 a significant difference (P <0.05) when comparing negative control mice and group A mice. Haemoglobin, (HGB); Red blood count, (RBC); Haematocrit, (HCT); Mean cell volume, (MCV); Mean corpuscular haemoglobin, (MCH); Mean corpuscular haemoglobin 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 the negative control (group B) (P <0.05) mice and ** indicate a significant difference (P <0.05) when comparing negative control mice and group A mice. A P-value < 0.05 was considered statistically significant*

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). The 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 the 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 a 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 reported 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 a 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 b.wt 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 haematological 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 in 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* (800 mg/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. revealed 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 availability 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 decreased ($P<0.05$) 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 in oxidative damage. There were significant increased ($P<0.05$) 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).

5. 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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