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Original article

Antiplasmodial activity of *Morinda lucida* Benth. Leaf and bark extracts against *Plasmodium berghei* infected mice

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ABSTRACT

Ethnopharmacology relevance: *Morinda lucida* is an ethnopharmacologically important plant that has traditionally been used to treat malaria in the Southwest of Nigeria. The aim of this study is to look into the antiplasmodial properties of different solvent extracts of *Morinda lucida* bark and leaves.

Materials and methods: The antiplasmodial model, (or curative assay), was tested against *Plasmodium berghei* NK65, a chloroquine-sensitive *Plasmodium berghei* strain. In experimental mice, parasitaemia, percentage inhibition, weight changes, and packed cell volume were measured and compared to chloroquine (10 mg kg⁻¹). Standard phytochemical procedures were used to evaluate the extracts' chemo-profile.

Results and Discussion: Phytochemical analysis of the extracts revealed the presence of tannins, alkaloids, steroids, saponins, phenols, and alkaloids, among other metabolites. The highest quantities of total phenolic, total tannins, and total flavonoid content were found in 50% ethanolic extracts. There was significant decrease in the body weight of the mice after inoculation, however, after administration of crude extracts, an increase in weight was observed. A negative variation (-3.00 g) was observed in group without treatment. The ethanolic crude extracts (200 and 400 mg/kg) significantly increased the packed cell volume compared to other extracts. CQ treated experimental mice showed 100% inhibition with activity greater than extracts treated groups. The lowest inhibitory effect was observed in 200 mg/kg ethanolic bark extract treated group with activity of 72.16%. The antiplasmodial activities exhibited by these extracts could be linked to the chemical constituents investigated.

Conclusion: The findings of this study suggest the use of *M. lucida* leaves and bark as a medicinal agent for malaria treatment and as a potential source of effective antimalarial templates. Further research is needed to determine the safety and toxicological profile of these extracts *in vivo*.

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1. Introduction

Parasitic illnesses have long been one of the most serious global health problems in both tropical and sub-tropical countries (Nkiruka et al., 2021). It played a role in the recent rise in the number of

deaths in tropical areas. *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium berghei*, *Plasmodium vivax*, and *Plasmodium malariae* are spread through the bite of female Anopheles mosquitoes (White 2008; WHO, 2018). The uncontrollable proliferation of these parasite vectors in tropical African countries could be connected to climatic circumstances such as rainfall, temperature range, and environmental variables such as poor drainage and unregulated garbage dumping. In reality, several drugs have been successfully used to treat malaria infections; however, the development of *Plasmodium* species resistance to the available anti-malarial drugs is concerning. Chloroquine, artemisinin, quinoline, and antifolate resistance are all well-known (Dondorp et al., 2010). These have posed a significant challenge to researchers looking for potent (non-resistant) pharmacophores. To this end, research clusters or units have been relentless in their pursuit of

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safe, cost-efficient, and effective pharmacophores based on biore-sources (medicinal plants) as lead or beginning materials. In order to identify novel pharmacophores, the drug discovery technique unexpectedly enhanced ethnobotanical and ethnopharmacological investigations of medicinal plants. In comparison to other drug discovery procedures, this method is less expensive, safer, and does not require specialized equipment (Ekins et al., 2007).

Morinda lucida (benth.) also known as Brimstone tree is an ever-green medium-sized trees of the *Morinda* genus. *Morinda* is the most diverse genus classified in the family Rubiaceae. The genus is widely dispersed throughout the tropical and subtropical regions, with over 131 accepted species of flowering trees, shrubs and herbaceous plants (Zhang et al., 2018). It is widely known as Sagogo or Bondoukou alongua (Ivory Coast), Ewi or Konkroma (Ghana), Atak ake or Ewe amake (Togo) and Oruwo (Nigeria). Secondary metabolites such as anthraquinones, glycosides, alkaloids, terpenoids, iridoids, fatty acids, essential or volatile oils, tannins and flavonoids have been reported in *M. lucida* (Ogunlana et al. 2008; Lawal et al. 2012). The secondary metabolites present in crude extracts, fractions or isolates of Brimstone tree could be responsible for distinct anti-cancer, antidiabetic, antimicrobial, antitrypanosomal, anti-inflammatory, anti-oxidant, antipyretic and anti-plasmodial potencies of different parts of the plant (Adejo et al., 2015; Gemedede and Ratta, 2014; Igwilo et al. 2018; Kazeem et al. 2013). Several reports on the antiplasmodial activities of leaves, roots or bark of Brimstone tree have been studied, however, there are few or no reports comparing the antiplasmodial potency of leaves and bark via parameters such as effects of dosage on weight, packed cell volume and percentage inhibition. To bridge this gap, this study is aimed at investigating the antiplasmodial potencies of stem-bark and leaves of Brimstone via phytochemical and curative assays with ultimate goal of substantiating its folkloric usage and unearthing novel antiplasmodial bioactive plant-based resources.

2. Materials and methods

2.1. Drugs and reagents

Chloroquine (Sigma-Aldrich, Germany), 5% Dimethyl sulphoxide acid (DMSO), Giemsa (Science Lab, Gaithersburg, MD, USA), methanol, ethyl acetate, chloroform and n-hexane (all of analytical grade) were procured from certified suppliers. Rutin, apigenin, quercetin and Kaempferol were acquired from Sigma Chemical Co (USA).

2.2. Collection of plant material

Fresh and healthy leaves and stem-bark of *Morinda lucida* were collected from Ogbomoso, Oyo State, Nigeria in June 2020 (Latitude 8° 08' N, Longitude 4° 15' E). It was identified by a taxonomist and the voucher specimen number (UIH/001/913/2021) was deposited in the herbarium of the Department of Botany, University of Ilorin, Nigeria.

2.3. Preparation and extraction

The bark and leaves of *M. lucida* was rinsed under running tap water to remove dirt and other extraneous constituents. The plants materials were air dried for 42 days, pulverized and sieved through mesh size of 20 mm. About 1.5 kg pulverized leaves sample was macerated in n-hexane (2 × 4.5 L) for 48 h on a shaker. The residue obtained was further extracted using ethyl acetate and then, hydro-ethanol (50:50) (2 × 4.5 L). The filtrates obtained for each solvent was concentrated under reduced pressure using rotary

evaporator (25 °C – 35 °C). The method was repeated for the extraction of secondary metabolites from bark samples. The respective crude extracts were collected and store for further phytochemical appraisals (4 °C).

2.4. Phytochemical screening

2.4.1. Qualitative phytochemical screening

The qualitative phytochemical screenings of *M. lucida* bark and leaves extracts were carried out to detect the presence of some secondary metabolites such as flavonoids, tannins, steroids, saponins, alkaloids and terpenoids (Oladeji et al., 2020a; Zimudzi et al., 2012).

2.4.2. Quantitative phytochemical screening

2.4.2.1. Determination of Total phenolic content. The total phenolic content (TPC) of each extracts was assessed via Folin Ciocalteu technique using gallic acid calibration curve (6.25, 12.50, 25.00, 50.00 and 100 mg·mL⁻¹) (Singleton et al., 1999). Accurately weighed 50 µL crude extract and 250 µL Folin-Ciocalteu reagent were sequentially dissolved in 3 mL of distilled water for 5 min. 750 µL of 20% sodium carbonate was added, shaken for 2 – 4 mins. This was incubated at room temperature for 30 mins. The absorbance was measured at 760 nm via UV-Visible spectrophotometer. The assessment was done in triplicate and expressed as mg GAE/100 g.DM.

2.4.2.2. Determination of Total flavonoid content. Total flavonoid content (TFC) of each extracts was determined via aluminium chloride colorimetric assay using quercetin calibration curve (6.25, 12.50, 25.00, 50.00 and 100 mg·mL⁻¹). To 500 µL of crude extract, 15 mL ethanol (99%), 100 µL potassium acetate (1 M), 100 µL aluminium chloride (10%) and distilled water (3 mL) were shaken together for 10 min, kept in dark and was incubated at room temperature for 30 min. The absorbance was measured at 415 nm. The analysis was carried out in triplicate and expressed as µg QE/100 g DM) (Zhishen et al., 1999).

2.4.2.3. Determination of Total tannins content. Total tannin content (TTC) in the crude extracts was determined by the Folin-Ciocalteu assay using gallic acid calibration curve (6.25, 12.50, 25.00, 50.00 and 100 mg·mL⁻¹). The extract, distilled water (5 mL), Folin-Ciocalteu reagent (500 µL) and Na₂CO₃ (35%) were agitated for 15 min. This was left on standing at room temperature for 30 mins. The absorbance for standard solutions was measured against blank at 725 nm and expressed as mg of GAE/100 g.DM (Singleton et al., 1999).

2.5. Animals

A total of 65 healthy Swiss albino mice (20 to 27 g) were obtained from the Institute of Advanced Medical Research and Training (IAMRAT), University of Ibadan, Ibadan, Nigeria. The animals were housed in plastic cages at room temperature to acclimatize for 8 days. They were maintained on 12 h light-dark cycle and nurtured on standard animal pellets and water ad libitum.

2.6. Infection with malaria parasite.

Chloroquine-sensitive *Plasmodium berghei berghei* (NK65) (CQ-S) was obtained from IAMRAT, University of Ibadan, Nigeria. This was maintained in mice (donor). The inoculum consisted of 5 × 10⁷ *P. berghei berghei* parasitized erythrocytes per millilitre. This was prepared by determining both the percentage parasitaemia and erythrocytes count of donor mouse, after which the blood is diluted with isotonic saline in proportions indicated by

Table 1
Grouping of experimental mice.

Group	I	II	III	IV	V	VI
Dose (mg)	200 ML _{HL}	400 ML _{HL}	200 ML _{EAL}	400 ML _{EAL}	200 ML _{ETOHL}	400 ML _{ETOHL}
Group	VII	VIII	IX	X	XI	XII
Dose (mg)	200 ML _{HB}	400 ML _{HB}	200 ML _{EAB}	400 ML _{EAB}	200 ML _{ETOHB}	400 ML _{ETOHB}

Group XIII (CQ- treated), Group XIV (No treatment).

ML (Morinda lucida), HL (n- hexane leaf), EAL (ethyl acetate leaf), ETOHL (50% ethanolic leaf), HB (n- hexane bark), EAB (ethyl acetate bark), ETOHB (50% ethanolic bark).

both determinations. On day 0, each experimental mice was inoculated with whole blood taken from the donor mouse. Prior to this, it was diluted in normal saline treated with trisodium citrate so that each 0.2 mL aliquot contain 1×10^7 infected RBCs. During the antiplasmodial screenings, experimental mice were inoculated intraperitoneally (Dibessa et al., 2020).

2.7. Experimental animal grouping

Seventy Swiss albino mice were grouped into fourteen each containing five mice. The experimental animals were dole out into groups as follows (See Table 1):

2.8. In vivo antimalarial assay of crude extracts.

2.8.1. Monitoring of changes in body weight

The weight of Swiss Albino mice were obtained using an electronic balance (Ohaus Parsippany, New Jersey, USA). Weight before inoculation (D_0) and post-inoculation, that is, treatment period (D_4 to D_8) were recorded (Table 3).

2.8.2. Monitoring of changes in packed cell volume

Packed cell volume of experimental Swiss albino mice was monitored throughout the assay starting from D_0 (before inoculation) and post-inoculation, that is, treatment period (D_4 to D_8). Blood sample was taken from the tail snip of each test organism was filled up in heparinized microhaematocrit capillary tubes sealed with plasticizer. The tubes were centrifuged in microhaematocrit centrifuge (HAEMATOSPIN 1400, England) at 11,000 rpm for a constant period of 5 min. The PCV values of each groups were calculated using the formula below. This assay was repeated on D_0 (before inoculation) and post-inoculation, that is, treatment period (D_4 to D_8).

$$PCV = \frac{\text{Volume of erythrocytes in a given blood} \times 100}{\text{Total blood volume}}$$

2.8.3. Evaluation of established infection (Rane's test)

The antiplasmodial or curative potency of crude extracts of leaves and stem-bark of *M. lucida* was studied using curative or Rane's test according to the method of Ryley and Peters (1970). An aliquot or standard inoculum (1×10^7) of parasitized red blood cell collected from donor mice was intraperitoneally injected into each experimental mice on day 1 (D_0). The parasitized was allowed to establish for four days (96 h) (D_4). A drop of tail blood was taken from each mice every 24 h through treatment period ($D_4 - D_8$). On day four (D_4), tail blood was taken before dosage of plant extracts were administered. The parasitized blood was fixed and stained with methanol and Giemsa on a slide. Each mice in group I to XII were treated with 200 or 400 mg kg^{-1} crude extract (0.2 mL) while group XIII and XIV were treated with 0.2 mL chloroquine and distilled water respectively ($D_4 - D_8$). The amount of parasitaemia in blood samples before or during treatment were counted using 100x oil immersion lens of a Zeiss Standard 20 microscope (Carl Zeiss LTD, Welwyn Garden City, UK). The average parasitaemia

and mean percentage inhibition were calculated using the formula below:

$$\text{Average \% parasitaemia} = \frac{\text{Number of parasitized erythrocytes} \times 100}{\text{Total number of erythrocytes}}$$

$$\% \text{ Inhibition} = \frac{\% \text{ parasitaemia control} - \text{parasitaemia treated group} \times 100}{\text{parasitaemia in control group}}$$

2.9. Statistical analysis

All data were presented as the Mean \pm SEM (Standard Error of the Mean) and analysed using IBM SPSS version 20. One-way analysis of variance (ANOVA) ($p < 0.05$) was used to compare the levels of parasitaemia, change in body weight change and PCV of the *P. berghei* infected mice between the control and extract treated groups at a set time.

3. Result

3.1. Qualitative phytochemical screening

Phytochemical screening of hexane, ethyl acetate and hydro-ethanol extracts of *M. lucida* bark and leaf showed the presence of tannins, alkaloids, steroids, saponins, phenols and alkaloids (Table 2). Secondary metabolites such as tannins, alkaloids, saponins and phenols were not present in leaves and bark hexane extracts. All phytochemicals analysed were present in 50% ethanolic leaves extracts however, saponins, tannins, phenols and alkaloids were in abundance. Similarly, 50% ethanolic bark extract showed the presence of all the metabolites analysed except steroids which was below detectable limit.

3.2. Quantitative phytochemical screening

3.2.1. Total phenolic content

Total phenolic content of leaves and bark of *M. lucida* extracts was evaluated from gallic acid calibration curve (Table 3). The concentration was expressed as mg GAE/100 g of dry material. According to Table 3, 50% ethanolic leaves extract showed concentration of $67.905 \pm 0.0153 \text{ mg}\cdot\text{mL}^{-1}$ followed by $26.802 \pm 0.0006 \text{ mg}\cdot\text{mL}^{-1}$ (50% ethanolic bark extract). Low concentrations of 14.7 ± 0.0010 and $10.648 \pm 0.0006 \text{ mg}\cdot\text{mL}^{-1}$ were estimated in n-hexane bark and leaves extracts respectively (Fig. 1).

3.2.2. Total tannin content

Total tannin content of leaves and bark of *M. lucida* extracts was evaluated from gallic acid calibration curve (Table 3). The concentration was expressed as mg GAE/100 g of dry material. According to Table 3, 50% ethanolic leaves and bark extracts showed concentrations of 76.933 ± 0.0000 and $22.959 \pm 0.0023 \text{ mg}\cdot\text{mL}^{-1}$ while concentrations of 18.183 ± 0.0010 and $4.593 \pm 0.0006 \text{ mg}\cdot\text{mL}^{-1}$ for evaluated in n-hexane bark and leaves respectively (Fig. 2).

Table 2
Secondary metabolites in bark and leaves extracts of *M. lucida*.

Phytochemical/ extract	ML _{HL}	ML _{EAL}	ML _{ETOHL}	ML _{HB}	ML _{EAB}	ML _{ETOHB}
Saponins	-	+	++	-	-	+
Tannins	-	+	++	-	+	++
Terpenoids	+	-	+	++	++	+
Steroids	+	+	+	+	+	-
Phenol	-	+	++	-	+	++
Flavonoids	++	+	+	+	+	+
Alkaloids	-	-	++	-	-	+

+ (present), ++ (abundance), - (absent).

Table 3
The total phenolic content analysed in the solvent extracts of leaf and bark of *M. lucida*.

Extract(s)	TPC (mg·mL ⁻¹)	TFC (mg·mL ⁻¹)	TTC (mg·mL ⁻¹)
ML _{HL}	10.648 ± 0.0006	515.748 ± 0.0071	4.593 ± 0.0006
ML _{EAL}	26.341 ± 0.0016	684.369 ± 0.0040	24.817 ± 0.0010
ML _{ETOHL}	67.905 ± 0.0153	1348.966 ± 0.006	76.933 ± 0.0000
ML _{HB}	14.700 ± 0.0010	492.872 ± 0.0025	18.183 ± 0.0010
ML _{EAB}	24.469 ± 0.0020	653.103 ± 1.0935	19.978 ± 0.0047
ML _{ETOHB}	26.802 ± 0.0006	919.541 ± 0.0110	22.959 ± 0.0023

An = Mean absorbance (n = 3).

3.2.3. Total flavonoids content

Total flavonoids content of leaves and bark of *M. lucida* extracts was evaluated from Rutin calibration curve (Table 3). The concentration was expressed as mg GAE/100 g of dry material. According to Table 3, 50% ethanolic leaves and bark extracts showed concentrations of 1348.966 ± 0.006 and 919.541 ± 0.0110 mg·mL⁻¹ while concentrations of 515.748 ± 0.0071 and 492.872 ± 0.0025 mg·mL⁻¹ was evaluated in n-hexane leaves and bark respectively (Fig. 3).

3.3. In vivo antiplasmodial assay of crude extracts.

3.3.1. Body weight assessment of the extracts

The body weight of experimental Swiss albino mice were assessed before inoculation with parasitaemia (NK65) and

throughout treatment period (D₄ – D₈) (Table 4). According to Table 4, administration of chloroquine and plant extracts (200 and 400 mg/kg) contributed to variation in body weights of mice during treatment period (p > 0.05). The response or variation in weight is dose-dependent with a slight increase in body weights of mice treated with chloroquine and *M. lucida* stem-bark and leaves extracts. A negative trend in body weight was displayed by untreated group. 50% ethanolic treated groups exhibited a significant increase in weight during treatment period compared to other extracts administered. The variation exhibited by chloroquine treated group (+3.6000 g) is slightly higher than ethanolic treated group (+3.100 g). At p < 0.05, weight variations exhibited by leaves extracts were statistically higher than weights observed in bark extracts at the same dosage.

3.3.2. Effect of the extracts on packed cell volume (PCV)

At p > 0.05, there is a variation in packed cell volume (PCV) of mice treated with chloroquine and *M. lucida* stem-bark and leaves extracts (200 and 400 mg/kg). The variation shown is dose dependent when PCV of treatment period was statistically compared, with a slight increase observed in 400 mg/kg compared to 200 mg/kg groups (Table 5). 50% ethanolic treated groups exhibited a significant increase in PCV during treatment period compared to other extracts administered. The variation exhibited by chloroquine treated group (+3.60%) is slightly higher than ethano-

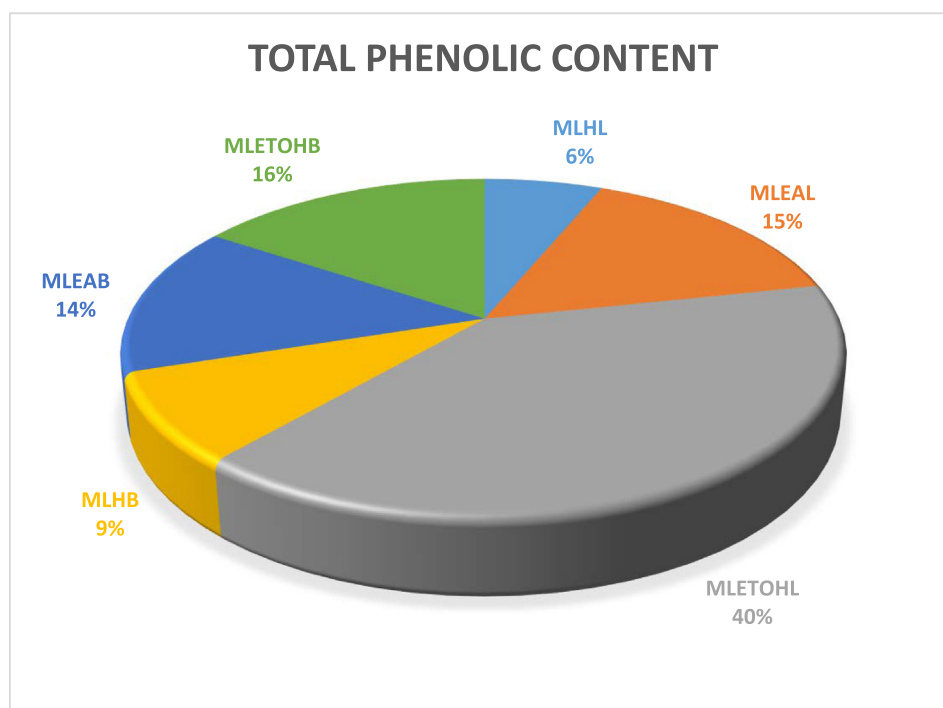


Fig 1. Total phenolic contents of leaves and bark of *M. lucida* extracts.

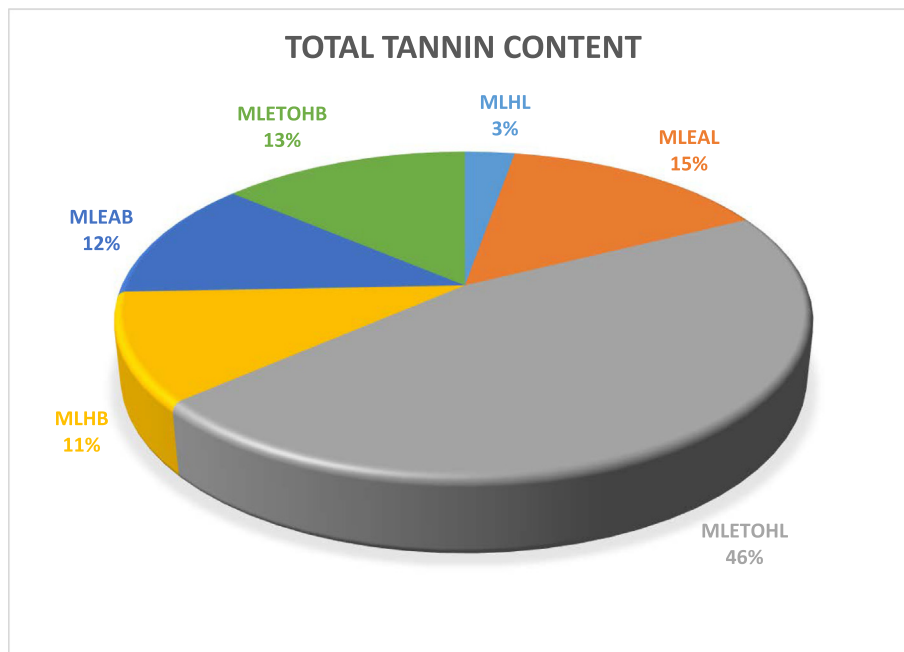


Fig 2. Total tannin contents of leaves and bark of *M. lucida* extracts.

lic treated group (+2.60%). At $p < 0.05$, PCV variations exhibited by bark extracts were statistically higher than weights observed in leaves extracts at the same dosage.

3.3.3. Effect of the extracts on level of parasitaemia

At $p > 0.05$, there is a difference in percentage parasitaemia in chloroquine and extract treated groups. The parasitaemia reduction in chloroquine treated group is statistically higher than extracts treated. Chloroquine considerably reduced the para-

sitaemia with no infected or parasitized red blood cells after treatment (Table 6). The effects of crude extracts on level of parasitaemia is not dose dependent nor depend on polarity of extracts. According to Table 5, at $p > 0.05$, there is a slight significant difference between values obtained in n-hexane and ethyl acetate extracts. Though, 50% ethanolic extracts significantly reduced level of parasitaemia after treatment with 200 and 400 mg/kg crude extracts, however, these activities were slightly lower than values obtained in chloroquine treated groups.

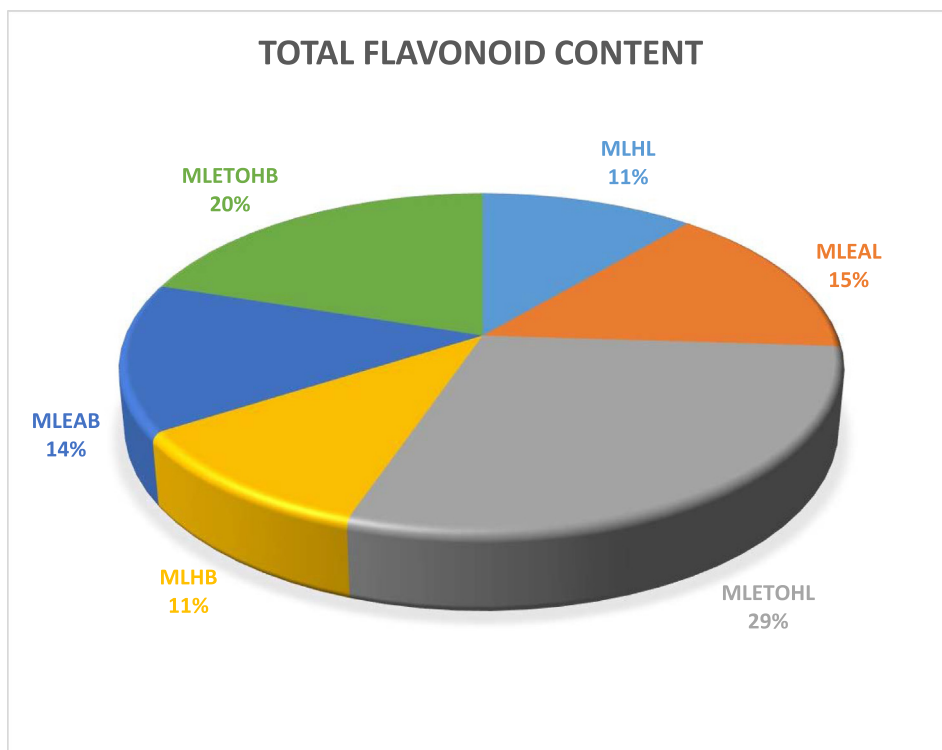


Fig 3. Total flavonoid contents of leaves and bark of *M. lucida* extracts.

Table 4
The body weight of experimental mice before and during treatment.

Extract	D ₀	D ₄	D ₅	D ₇	D ₈	Δ(D ₈ -D ₀)
I	22.80 ± 2.5884	20.80 ± 1.9235	21.00 ± 0.8165	21.50 ± 0.1213	22.00 ± 2.1213	1.2000 ^b
II	21.20 ± 2.3875	20.00 ± 2.5495	20.40 ± 1.1408	20.50 ± 1.2910	21.25 ± 2.0615	1.2500 ^c
III	21.80 ± 2.3877	20.00 ± 2.1213	21.80 ± 3.0332	21.75 ± 1.2583	22.00 ± 1.4142	2.0000 ^d
IV	21.00 ± 2.000	19.80 ± 1.4832	20.60 ± 2.6077	21.56 ± 1.1547	22.00 ± 1.0000	2.2000 ^d
V	23.40 ± 2.1909	21.60 ± 1.1402	23.80 ± 1.9235	24.20 ± 1.9235	24.60 ± 2.1909	3.0000 ^e
VI	22.00 ± 1.8908	20.80 ± 1.6432	22.00 ± 1.4142	23.33 ± 1.5275	23.90 ± 1.5275	3.1000 ^e
VII	21.40 ± 1.1402	20.00 ± 1.4142	20.00 ± 1.4142	20.40 ± 2.1909	21.00 ± 2.1602	1.0000 ^b
VIII	23.00 ± 1.8708	20.80 ± 0.8367	21.75 ± 2.2174	22.00 ± 1.0000	22.67 ± 1.1547	1.8700 ^f
IX	21.80 ± 1.9235	20.20 ± 1.3038	22.80 ± 1.0954	23.00 ± 1.0000	23.00 ± 1.0000	1.8000 ^f
X	22.00 ± 2.8284	19.80 ± 2.0494	21.40 ± 2.3022	21.60 ± 2.4083	21.60 ± 2.4083	1.8000 ^f
XI	21.80 ± 1.7889	20.00 ± 1.2247	22.20 ± 1.6432	22.40 ± 1.0954	22.60 ± 1.1402	2.6000 ^g
XII	23.00 ± 1.2247	21.00 ± 0.7071	23.40 ± 1.5166	23.60 ± 2.0736	23.75 ± 1.5166	2.7500 ^g
XIII	20.60 ± 2.1909	19.20 ± 1.6432	21.40 ± 1.3416	22.60 ± 1.1402	22.80 ± 1.1402	3.6000 ^h
XIV	23.40 ± 2.3022	20.00 ± 1.5811	19.25 ± 1.5000	17.50 ± 0.7071	17.00 ± 0.0000	-3.0000 ^a

Values are expressed as mean ± SEM. n = 5; * = p < 0.05 compared to negative control.

Table 5
The packed cell volume of experimental mice before and during treatment.

Extract	D ₀	D ₄	D ₈	Δ(D ₈ -D ₀)
I	48.6 ± 2.8809	44.00 ± 1.5275	45.17 ± 1.5275	1.17 ^b
II	51.00 ± 2.8284	42.40 ± 2.3022	44.00 ± 1.4142	1.60 ^c
III	50.60 ± 2.9665	45.5 ± 2.3805	47.50 ± 1.7321	2.00 ^d
IV	50.8 ± 4.2071	45.40 ± 2.4083	46.67 ± 0.5774	1.27 ^b
V	48.60 ± 2.7019	44.80 ± 1.7889	46.40 ± 0.5477	1.60 ^c
VI	53.60 ± 2.1909	48.00 ± 1.6329	50.25 ± 1.7078	2.25 ^d
VII	51.60 ± 2.8809	46.25 ± 1.5000	47.75 ± 2.0616	1.50 ^c
VIII	50.06 ± 3.6469	42.75 ± 2.2174	45.10 ± 2.8868	2.35 ^d
IX	54.20 ± 1.7889	47.4 ± 2.3022	50.20 ± 1.3038	2.80 ^e
X	49.80 ± 2.5884	45.60 ± 2.9665	47.00 ± 1.2247	1.40 ^b
XI	49.80 ± 2.5884	45.60 ± 2.9665	47.40 ± 2.0736	1.80 ^c
XII	51.00 ± 3.0822	42.80 ± 1.6432	45.40 ± 0.5477	2.60 ^d
XIII	51.00 ± 0.0000	42.80 ± 1.6432	46.40 ± 0.5477	3.60 ^f
XIV	49.60 ± 3.6469	44.33 ± 2.3094	43.67 ± 3.7859	-0.66 ^a

Values are expressed as mean ± SEM. n = 5; * = p < 0.05 compared to negative control.

Similarly, parasitaemia level in untreated group increased from inoculation to post four-day treatment (D₈).

The percentage inhibitory effects of the extracts at different dosage and chloroquine were presented in Table 7. At p > 0.05, there is no significant difference between the activity of chloroquine and 50% ethanolic extracts. According to Table 7, inhibitory of 96.39% was evaluated in 400 mg/kg 50% ethanolic bark extract (group), 96.24% in 200 and 400 mg/kg 50% ethanolic (leaves) and ethyl acetate (leaves) extracts respectively. Chloroquine significantly cleared all parasitized cells (100%). The lowest inhibitory effect was observed in 200 mg ethanolic bark extract treated group with activity of 72.16%.

4. Discussion

4.1. Phytochemical screening

The systematic procedure for discovery of innovative and drug resistant therapeutic substances from bioresources is based on gathering information on folkloric uses of medicinal plants (Oladeji et al., 2021). The use of medicinal plants could be dated back to the Stone Age where plants serve as the key therapeutic source to mankind. The utilization of herbal drugs from medicinal plants is well documented in Asia and Africa continents, however, its therapeutic potency have been reported in South America and Europe. The therapeutic or curative potency of medicinal plants could be linked to the presence of small or large organic molecules known as secondary metabolites or phytochemicals (Oladeji et al., 2019a). Secondary metabolites constitutes a fundamental part of

medicinal plants and are linked to numerous therapeutic properties exhibited. Several studies have documented the roles of medicinal plants as curative agents in the treatment of acute or chronic parasitic or infectious diseases. The folkloric belief and scientific reports established that medicinal plants exhibited strong inhibitory activity against different plasmodium strains (Oladeji et al., 2020; Orabueze et al., 2017).

The identification of a vast range of secondary metabolites defined by distinct structural moieties has been substantially aided by comprehensive phytoconstituents studies of medicinal plants (Oladeji et al., 2021). Phytochemical screenings of different parts of *M. lucida* revealed the presence of alkaloids, flavonoids, ter-

Table 6
The percentage parasitaemia of experimental mice before and after treatment.

Extract	D ₄ (%)	D ₈ (%)
I	0.7377 ± 0.1631	0.0923 ± 0.0006 ^c
II	1.8453 ± 0.3986	0.1543 ± 0.1071 ^d
III	2.1257 ± 0.0906	0.0943 ± 0.0035 ^c
IV	1.4180 ± 0.2113	0.0927 ± 0.0006 ^c
V	1.4760 ± 0.1611	0.0927 ± 0.0006 ^c
VI	1.4727 ± 0.1582	0.1940 ± 0.1058 ^d
VII	1.1986 ± 0.4899	0.3950 ± 0.2193 ^e
VIII	0.3697 ± 0.0006	0.3380 ± 0.1417 ^e
IX	0.3080 ± 0.1065	0.0923 ± 0.0006 ^c
X	1.5697 ± 0.0015	0.1580 ± 0.0572 ^d
XI	1.2887 ± 0.0042	0.6960 ± 0.0676 ^f
XII	2.1543 ± 0.0982	0.0903 ± 0.0046 ^c
XIII	0.5230 ± 0.2659	0.0000 ± 0.0000 ^b
XIV	1.2073 ± 0.0006	2.5000 ± 1.0629 ^a

Table 7

The percentage inhibition of the extracts and chloroquine on the level of parasitaemia in experimental mice after treatment

Group	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	CQ
Inhibition (% [^])	96.31 ^a	94.00 ^b	96.24 ^a	96.29 ^a	96.29 ^a	96.24 ^a	84.20 ^e	86.48 ^d	96.31 ^a	93.68 ^c	72.16 ^f	96.43 ^a	100.00 ^a

n = 5; * = p < 0.05 compared to positive control.

penoids, saponins and tannins. Malaria contributes to loss of appetite in animals, however, this could be overcome by administration of saponins or saponins-supplements. This metabolite is reported to enhance food intake or appetite in mammals (Agbor et al., 2011; Liu, 2014). Polyphenolic, saponins and alkaloids are well documented for a wide range of medicinal applications such as antidiabetic, antioxidant, anticancer, anti-inflammatory and antimicrobial (Hostettmann and Marston, 1995). Some secondary metabolites such as terpenoids, alkaloids, rutin and gallic acid have significantly reduce parasitaemia level in experimental mice, however, these metabolites may not be enough to fully elucidate the total effect of plant extracts (Alson et al., 2018; Ganesh et al., 2012; Khasanah et al., 2017). The secondary metabolites present in the extracts show close proximity with previous study by Adebayo et al., (2017). Hence, ethnobotanical medicine offers a colossal or wide range of unexploited metabolites with potential source of new drugs (Habtemariam, 2011).

4.2. Antiplasmodial screenings

In tropical and subtropical regions, outbreak of parasitic infections could be linked to ever-increasing resistance build-up of these parasites to available therapeutic substances (Cock et al., 2019; Oladeji et al., 2020b; Oladeji and Oyebamiji, 2020a; Oladeji et al., 2020b). To curb this state of affairs, several medicinal plants have been studied as a means of unearthing novel or non-drug resistant pharmacophores which could function as preventive, suppressive and curative antiplasmodial therapies. The antiplasmodial potency of solvent extracts of *M. lucida* leaves and bark were investigated in plasmodium infected experimental mice, and factors such as body weight, packed cell volume and level of parasitaemia were investigated. The parasitized red blood cell in studied experimental mice significantly reduced the body weight and packed cell volume. The two factors studied are two common symptoms of malaria infection which could be linked to the spread or growth of plasmodium infections in the examined organisms. From observation, a significant increase in packed cell volume and body weight could be due to some secondary metabolites such as terpenoids, alkaloids and saponins analysed. The trend was observed throughout treatment period which is concomitant to the ability of the extracts to cure parasitized red blood cells which were responsible for the loss in weight and red blood level (Chen and Chen, 2003; Olanlokun et al., 2020). The weight and packed cell volume rebuilding potency of Brimstone tree extracts depends on dose concentration and polarity of extracts administered (Langhorne et al., 2002; Mzena et al., 2018).

The antimalarial properties of *M. lucida*'s leaves, bark, fruit, and roots have been widely documented in traditional medicine (Ade-wole et al., 2021; Awe and Makinde, 1998). The antimalarial potency of *M. lucida* has been described in several research papers, leading to the isolation of certain secondary metabolites (Cimanga et al., 2006). In Africa, patients with symptoms including headaches, bodily pains, and fever are sometimes misdiagnosed as having malaria, leading to the prescription of plant materials to treat the symptoms. It's possible that medicinal plants have precursors that need to be activated by the host in order to have an antiplasmodial effect (Clarkson et al., 2004; Shuaibu et al., 2008). Plant extracts' antiplasmodial efficacy may be linked to the polarity of the solvent utilized, as previously discussed. The findings of this

investigation supported this notion, albeit there were some exceptions to the action of n-hexane extracts (Lima et al., 2015). Because some metabolites in polar extracts exhibit their activity only when they are combined with other metabolites in the crude extract, they showed less activity against plasmodium infections than non-polar extracts (Debaro and Ginsburg, 2011; Ezenyi et al., 2020).

The bark of vascular plants is made up of numerous tissues as well as phytoconstituents such polysaccharides, lignin, and tannins (Raven et al., 1981). Bark extracts were found to have considerable inhibitory effects on parasitized red blood cells and to raise packed cell volume in plasmodium-infected mice in this investigation. This could be related to the presence of condensed tannins in *M. lucida* bark, which are thought to impede cell or tissue breakdown (Vane et al., 2006). The most notable chemical events, such as photosynthetic reactions, occur in the leaves of medicinal plants. Furthermore, the leaves of medicinal plants carry a variety of phytoconstituents or bioactive chemicals, which contribute to the strong inhibitory activity of the leaf extracts used in this investigation. Phytochemistry, on the other hand, has made tremendous success in uncovering antimalarial (artemisinin compounds) molecules from medicinal plants (Oladeji et al., 2022). The study confirmed and justified folklore applications of Brimstone tree leaves and bark in the treatment of malaria infections, and found that these activities are linked to secondary metabolites.

5. Conclusion

This study provide evidence that solvent extracts of *Morinda lucida* leaves and bark have antiplasmodial potential. Further studies are on-going to isolate the specific active metabolites.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

All authors contributed equally.

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